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Role of demographic and dietary factors in predicting Gall Bladder Carcinoma: Findings of a case-control study

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Abstract---Introduction: Carcinoma of the gallbladder (GBC), is the most common cancer of the biliary tree. It is also one of the most highly malignant tumours. The aetiology of the carcinoma gall bladder is poorly understood. Chronic cholecystitis and gallstones, choledochal cysts, female gender, old age and exposure to carcinogens are some of the factors implicated in the causation of gall bladder cancer. The current changes in lifestyles of individuals about indulgence in unhealthy fat-rich food, lack of exercise, obesity, sedentary lifestyles and the high incidence of gall bladder carcinoma in Bihar, focused our attention on gall bladder carcinoma and its etiological factors. Methodology: An analytical case-control study was conducted to

study the various etiological factors causing carcinoma of the gall bladder in patients of various regions of Bihar coming to PMCH Patna. A total of 101 cases of gall bladder carcinoma and a total of 101 patients without any gall bladder disease in the control group were included in the study. Factors studied included demographic and dietary factors. Results: Age >40 years ($p = 0.008$), male : female ratio 1:2.88, mean age for male 55.08 years and SD +10.84 and for female 48.68 years & SD+ 15.26, residence in Gangetic districts (odds 2.88, $p = 0.0002$), literacy (odds ratio 0.45 and $p = 0.0049$), non-veg diets (odds ratio 2.45, $p = 0.0018$), consumption of fried food (odds ratio 2.61, $p = 0.0010$), missing breakfast (odds ratio 2.78, $p = 0.0004$), Consumption of fresh fruits and vegetables {Odds ratio 0.55, $p = 0.0339$ }, reuse of cooking oil (odds ratio 1.96, $p = 0.0311$), tobacco addiction (odds ratio 0.39, $p = \text{value } 0.002$) were found significant whereas family history of gall bladder disease and alcohol addiction came insignificant. On logistic regression age, gender, residence in Gangetic districts, non-veg diet, missing breakfast came out as independent etiological factors and consumption of fresh fruits and vegetables found to be protective. Conclusion: The present study shows results in association with other study results. Have certainly come out with factors other than gall stones as secondary etiological factors for carcinoma gall bladder. Keeping these factors in mind and utilizing them as a preventive factor may reduce the overall incidence of the carcinoma gall bladder.

Keywords---gall bladder carcinoma, etiological factors, dietary factors.

Introduction

The gallbladder is a surgically important organ of the body which may be affected by a variety of pathological processes. It has a wide spectrum of diseases ranging from congenital anomalies, and gallstones their complications, non-inflammatory, inflammatory to the neoplastic lesions. The prevalence of gall bladder diseases differs from nation to nation and in India, it is found to be 2-29%. [1,2] Among the complete gamut of gall bladder diseases, carcinoma of the gallbladder (GBC) has the worst prognosis. It is the fifth most common gastrointestinal neoplasm and the most common biliary tract malignancy in the world. [3] Due to its non-specific clinical presentation, it is seldom diagnosed preoperatively except in advanced cases. The aggressive biological nature of the carcinoma of the gallbladder (GBC) results in the rapid spread of tumours to adjoining vital structures and is often unresectable at presentation resulting in an overall dismal prognosis. [4] Chemotherapy, radiotherapy and immunotherapy are also not particularly curative.

The aetiology of the carcinoma gall bladder is poorly understood. Chronic cholecystitis and gallstones, choledochal cysts, female gender, age and exposure to carcinogens are some of the factors implicated in the causation of gall bladder cancer but a definite cause-effect relationship has yet to be established for any of these factors. [5]. The incidence of this malignancy is characterized by marked geographical and ethnic variations. [6] The overall incidence ranges from 0.1/10⁵ to 10.6/10⁵ in different cancer registries worldwide. [7] India accounts for 10% of the global burden of GBC. In India, the North, East, Northeast and Central region are among the high incidence areas in contrast to South and West India. [8] The incidence in North India is 10–22/per 100,000 population. [9] The age-standardized rate (ASR) for GBC in women of North and north-east India is 11.8/10⁵ population and 17.1/10⁵ population respectively. [10] The incidence has been steadily rising in India among women as well as men. The average age-adjusted rate among women has increased from 6.2/100,000 in 2001–2004 to 10.4/100,000 in 2012–2014. [11] The incidence of GBC in Bihar is high. Hospital-based cancer registry data in Bihar indicates GBC to be the third most common malignancy among the females after carcinoma cervix and carcinoma breast. [7]

Despite a large number of GBC patients in Bihar, it has not been reported to International Research Agencies because of the lack of a population-based cancer registry here and only a few epidemiologic studies done on GBC in Bihar so far. There is a need for studies on factors associated with gall bladder cancer in Bihar. This study was conducted in PMCH, Patna in the department of General Surgery to study the various etiological factors causing carcinoma of gall bladder in patients of various regions of Bihar coming to Patna Medical College.

Material and Methods

An analytical case-control study was conducted in a tertiary care centre of Patna among patients attending an out-patient clinic or emergency units of the surgery department. A case of gall bladder (GB) carcinoma confirmed histologically/ cytologically was enrolled in the study as a case after obtaining consent. Patients with any other major comorbid illness were excluded from the study. Hospital-based controls from OPD/ emergency of department of Surgery were chosen during the same time- period of cases. Controls were free from any gall bladder disease. The controls were matched regarding age (+10 years) and sex. A total of 101 cases and 101 controls were enrolled in this study. Approval for the study was obtained from the Institutional Research and Ethics Committee of Patna Medical College, and conducted after informed consent was obtained from the participants.

Every case and control were enquired about the demographic factors, diet history addiction history and presence of gall bladder disease based on the Ultrasound report. Demographic factors included age, gender, the residence of the Gangetic district, literacy, and family

history of Gall Bladder disease first-degree relative. Buxar, Bhojpur, Patna, Nalanda, Sheikhpura, Lakhisarai, Munger, Bhagalpur, Katihar, Khagaria, Begusarai, Samastipur, and Vaishali are included under Gangetic districts. Questionnaire about diet comprised of consumption on-vegetarian diet, fresh vegetables, missing breakfast and frequent reuse of cooking oil. Non-Vegetarians, consumption of fruits and fried food, and missing breakfast, were classified based on the frequency of consumption more than twice per week.

Missing breakfast: Missing breakfast was defined as 'Intake of fewer than 100 calories till noon in the noon. Reuse of oil was defined as 'the oil left after deep frying being reused for cooking' after being kept at room temperature. Tobacco addiction was defined as 'cigarette smoking per day for the past 5 to 10 years is considered as tobacco addiction in our study and Alcohol addiction as 'alcohol drinking > twice per week is considered as alcohol addiction in our study. Statistical analysis was carried out between Gall Bladder Carcinoma and healthy controls. Logistic regression was used for producing the odds ratio. Step-wise multiple logistic regression analysis was carried out with insertion of all the variables found to be significant on univariate analysis. A p-value of <0.05 was considered statistically significant. Statistical software Stata version 12.0 was used for statistical analysis.

Results

A total of 101 cases of gall bladder carcinoma and a total of 101 patients without any gall bladder disease in the control group were included in the study.

Socio-demographic and dietary exposure factors among cases and controls [Table 1]

The mean age among cases was 50.33, (SD +11.82) compared to 44.04 years, (SD +14.63) among controls. Thus, Gall bladder (GB) carcinoma was found to be higher in the >40 years age group in the current study (p-value 0.008). There was a clear demarcation in the ratio of males: to females in cases and control groups. The Male: Female ratio was 1:2.88 in cases while it was 1: 0.77 in controls. Also, a significant difference was noted in the age of presentation between male and female patients with GB carcinoma. The mean age for presentation among males was 55.08 (SD +10.84) while that for females, was 48.68 (SD+ 15.26) (p-value 0.0166). A significantly higher proportion of GB carcinoma resided in the Gangetic districts than in control groups. Thus, residence in Gangetic districts was associated with 2.88 times higher chances of developing GB carcinoma than those residing in non-Gangetic districts (p-value 0.0002). Similarly, a higher proportion of patients with GB carcinoma were illiterate compared to the controls (p-value 0.0049). The risk of developing GB carcinoma was 1.66 times higher among those who had a family history of GB disease and this difference was statistically significant (p <0.00).

Association between dietary factors and addiction history to GB carcinoma [Table 2]

In the current study, it was observed that a non-vegetarian diet, consumption of fried food, frequently missing breakfast, and frequent reuse of cooking oil was significantly associated with GB carcinoma. The non-vegetarian diet was associated with a 2.45 higher risk (p-value 0.0018), and consumption of fried food had a 2.61 higher risk of GB carcinoma (p-value 0.0010). Missing breakfast > twice a week was found more commonly in the case group as compared to the control group and the difference was significant with higher odds of 2.78 (p-value 0.0004). Similarly, frequent reuse of cooking oil was associated with 1.96 odds of developing GB carcinoma (p-value 0.0311). It was also found that consumption of fresh fruits and vegetables (> 2 days/week) lessened the risk of carcinoma 0.55 times (p-value 0.0399). It was observed that the controls (45.54%) were more addicted to tobacco than cases (24.75%). However, the cases comprised mainly of female patients and hence to nullify the confounding effect, stratification based on gender was done. It was found that the Odds ratio was 0.39 and the p-value 0.002. In this study, alcohol consumption did not come out as a significant variable. Fewer persons from both groups had an addiction to alcohol.

Table 1
Clinico-demographic features of Case of Gall Bladder Carcinoma (N=101) and Control (N= 101)

Clinico-Demographic characteristics	Cases n (%)	Control n (%)	Odds Ratio 95%CI	P-value
Age*	50.33	44.04		0.0009#
Age groups	(+11.81)	(+14.63)		
<=40 years	27 (26.73)	45 (44.55)	6.99 (1)@	0.008\$
>40 years	74 (73.27)	56 (55.45)		
Gender				
Male	26 (25.74)	57 (56.44)	19.65 (1)@	<0.00\$
Female	75 (74.26)	44 (43.56)		
Male to Female ratio	1:2.88	1:0.77		
Residence in Gangetic districts				
Gangetic Districts	67 (66.34)	41 (40.59)	2.88 (1.56- 5.33)	0.0002\$
Non- Gangetic Districts	34 (33.66)	60 (59.41)		
Literacy				
Literate	42 (41.58)	59 (58.42)	7.93 (1)@	0.005\$
Illiterate	62 (61.39)	39 (38.61)		
Family history of Gall Bladder Disease	24(23.76)	16 (15.84)	1.66 (0.78- 3.60)	<0.00\$
*Presented as mean (+ Std deviation) #student's t- test, \$ Chi- square, @ Pearson Chi square (degree of freedom)				

Table 2
Association of dietary factors and addiction history to Gall Bladder carcinoma

Factors	Cases n (%)	Control n (%)	Odds Ratio (95%CI)	P-value
Diet History				
Non- vegetarian diet	67 (66.34)	45 (44.55)	2.45 (1.34- 4.52)	0.0018
Consumption of fried food	71 (70.30)	48 (47.52)	2.61 (1.41-4.86)	0.001
Frequently misses breakfast	58 (57.43)	33 (32.67)	2.78 (1.51- 5.14)	0.0004
Consumption of fresh fruit and vegetables	48 (47.53)	63 (62.38)	0.55 (0.30- 0.90)	0.0339
Frequent reuse of cooking oil	78 (77.23)	64 (63.37)	1.96 (1.01-3.82)	0.0311
Addiction				
Tobacco addiction	25 (24.75)	46 (45.54)	0.39 (0.206- 0.745)	0.002
Addiction to Alcohol	17 (16.83)	16 (15.84)	1(0.446- 2.240)	0.976

Multivariate Logistic regression to identify independent risk factors associated with Gall Bladder Carcinoma [Table 3]

Multivariate logistic regression analysis was done entering all the exposure variables and out of 9 significant variables, only 6 came out to be independent associated etiological factors. The logistic regression model was statistically significant, $\chi^2(11) = 83.49$, $p < .0005$. The model explained 52.7% (Nagelker R²) of the variance in GB carcinoma and correctly classified 76.7% of cases. The seven predictors which were found significant were; female sex, increasing age, residence in Gangetic district, non-vegetarian diet, frequently skipping breakfast, consumption of fried food more than twice a week and family history of GB carcinoma. The presence of gall stone was not tested in the logistic regression as the control group was kept free from any gall bladder disease.

Table 3
Independent Predictors associated with Gall Bladder Carcinoma
[N= 212] [Final Model: LR Chi²: 83.49, P-value <0.00]

Predictors	Adjusted Odds Ratio	95% Confidence Interval	P-value
Age	1.05	1.025 1.085	0.000
Gender	4.08	1.912 8.704	0.000
Residents in Gangetic districts	2.53	1.260 5.088	0.009

Non-veg diet (>2/week)	3.07	1.475	6.394	0.003
Consumption of Fried food (>2/week)	2.12	1.027	4.356	0.042
Frequently misses breakfast(>2/week)	3.64	1.693	7.821	0.001
Addiction to Alcohol	6.19	1.952	19.634	0.002
Family history of GBC	1.05	1.025	1.085	0.000

Discussion

Gall Bladder Carcinoma is a highly malignant tumour. Identifying significant etiological factors is an important step in implementing preventive and surveillance strategies to decrease mortality from Gall Bladder Carcinoma. In the present case-control study, we investigated many etiological factors for Gall Bladder Carcinoma including those related to environmental influences, such as dietary factors, alcohol, smoking, geographical area, and host factors such as the family history of gall bladder disease in a comprehensive manner. This study gives a view of the profile of gall bladder carcinoma in Bihar, which was not yet found to be documented.

In our study, we tried to explore the demographic characteristics of GB carcinoma patients. The mean age of presentation in GB carcinoma patients was found 50.33 and it ranged from 22 to 76 years. Similarly, in a case-control study done in Uttar Pradesh, Shukla et al reported the mean age of the cancer gall bladder patient to be 50 years (range:40-60 years).[12] In Bihar, Varsha Singh et al (2004) reported a mean of 51.7 years (16 to 80 years range).[13] The mean age for males was 55.08 and SD +10.84. This implies that the carcinoma gall bladder is more prevalent in the elderly age group and delays in seeking medical care and diagnosis. Carcinoma of the gallbladder is predominantly a disease of elderly females. In our study female to male ratio was found to be 2.88:1 among cases. IARC reported a female to male ratio of 3.2:1. [10] Shukla et al in their series of 315 patients found a female to male ratio of 2.5: 1.[12] This implies that being female, was strongly associated with the cancer gall bladder.

Evidence suggests that the incidence of GBC was 10-fold higher in patients residing in Gangetic basin regions of India. These studies suggest the Ganges receives domestic, industrial and agricultural effluents which are loaded with heavy metals, insecticides and pesticides. This alters soil contents downstream. Also, the predominant rural cooking practices such as wood and coal burning and adulteration of mustard oil with sanguinarine, a chemical having potential genotoxic and carcinogenic features.[14–16] These might be some of the important contributing factors for such a geographical proclivity to Gall Bladder Carcinoma within the same country. It has also been reported that regions with higher arsenic content (>0.05mg/dl) in the soil had 1.45 higher odds of reporting GB cancer.[17] In our study majority of cases (66.34%) were from Gangetic

basin districts in comparison to the control group (40%). Gangetic belt emerged as an independent significant factor with an odd's ratio of 2.83.

Literacy was not independently associated with the disease in the multivariate analysis in our study, suggesting that various social factors directly associated with literacy and not considered in the study could be the indirect cause of this association. Illiteracy leads to unemployment, lower socioeconomic status and decreased livelihood capacity influencing preventive aspects of gall bladder carcinoma.[18] The study was significant but on logistic regression, it did not come out as an independent variable for causation of carcinoma gall bladder in our study.

The current study ascertains that a family history of gallstone disease increased the risk of GBC by 6.19 % (P 0.002). There are studies suggesting a genetic predisposition to develop gallbladder diseases including Gall stones and Gall bladder carcinoma. Kajal et al found an association between a family history of gall bladder disease with gall bladder carcinoma in their study in AIIMS, New Delhi.[20] Hsing et al (2007) also showed the association of a family history of gallstone disease with increased risk of biliary stones, GBC and bile duct cancer.[21] This finding emphasizes the genetic and environmental factors responsible for GBC.

Red meat increases the risk of gall bladder cancer while vegetables are protective.[22] In our study non-veg consumption more than twice a week came as an independent predictor (OR 2.84, 95% CI (1.475-6.394, P 0.003) of gall bladder cancer. Although insignificant, Tavani et al (2003) demonstrated a positive association between the consumption of red meat and several neoplasms.[23] Kajal Jain et al in their study in AIIMS, New Delhi have reported an association of increased risk of gall bladder cancer in persons having a non-veg diet more often in the week.[20] These findings were corroborated by Imran et al (2004) in their study.[24]

Findings in various studies on the consumption of vegetables indicate an inverse association with, Gall bladder cancer risk.[25] Consumption of vegetables in a higher amounts is associated with a reduced risk of many epithelial cancers of the alimentary tract.[26,27] A protective effect of vegetables and fruits on gall bladder cancer was also reported by Pandey et al (2002).[22] In our study consumption of fruits and vegetables >2 / week was found to be protective (unadjusted OR: 0.55 (0.30- 0.90)) and had an inverse relationship with gall bladder cancer. There are many possible reasons given as to how a diet with high consumption of vegetables and fruits prevents the occurrence of cancer. A large number of potentially anticarcinogenic agents i.e, carotenoids, vitamin C, vitamin E, selenium, folic acid, dietary fibres, indoles, phenols, flavonoids, protease inhibitors, allium compounds and plant sterols are found in these food sources. These have both

complementary and overlapping mechanisms of action which include the induction of detoxification of enzymes, inhibition of nitrosamine formation, provision of substrate for the formation of antineoplastic agents, dilution and binding of carcinogens in the digestive tract, alteration of hormone metabolism, stimulation of immune system, regulation of gene expression in proliferation and apoptosis and scavenging of oxidative agents.[28–30]

Among the dietary factors, fried food was found to be a risk factor for GBC. A study was done in AIIMS, Delhi by Kajal et al have also reported an increased risk of gall bladder carcinoma in persons having consumption of fried food >2/ week.[20] In our study, this also came out as a significant variable causing gall bladder carcinoma (OR 2.12, 95% CI (1.027-4.356, P 0.042). The risk of carcinoma increased by 2-fold with increased consumption of fried food. Also, an interaction between missing breakfast and fried food was observed in the study.

An elevated risk of gall bladder cancer is seen with missing breakfast (>2/week) in this study (OR 3.64, 95% CI (1.69- 7.821, P 0.001). A study conducted on 107 GB carcinoma patients in Karachi has found that missing dinner increased the odds of having GBC by 6.8%.[31] Prolonged fasting affects the size of the bile acid pool, increases the contact time of bile with the gallbladder mucosa and promotes biliary sludge formation. Stasis of bile in the gall bladder for longer hours irritates gall bladder mucosa by precipitation of bile salts.[32]

Repeated heating and keeping the oil at room temperature before using it again has shown a strong association with the cancer gall bladder. Oil containing polyunsaturated fatty acids like vegetable oils (cases and controls predominantly use vegetable oil for deep frying) produce HNE (4-hydroxy trans-2 nominal) when heated at frying temperature. Heating butter, ghee, olive oil or coconut oil produces HNE or other lipid peroxidation products in a lesser amount. HNE is a toxic compound that is easily absorbed from the diet. The toxicity arises because the compound is highly reactive with proteins, nucleic acids, DNA, RNA and other biomolecules. HNE was found in the bile of gall bladder cancer patients in more concentration than in the bile of healthy subjects.[33] This suggests that lipid peroxidation products may be carcinogenic in gall bladder cancer. Defective storage, especially in hot and humid climates promotes contamination with carcinogenic mycotoxin such as Aflatoxin B produced by *Aspergillus Flavus*. [34] In our state, it is a common practice in the majority of households to reuse cooking oil. Though it is more prevalent in rural and illiterate persons, the urban population and the literate groups are also not much behind. This can be seen by the fact that in about 77% of cases reuse of cooking oil was present whereas the control group with about 63% is also not much behind.

No association was seen between alcohol and smoking in the present study although tobacco came out to be a significant risk factor for

GBC. Earlier studies contradicted the effect of alcohol, smoking or tobacco on GBC.[35,36] Some studies suggested an increased risk, while others suggested that long-term use might play a role in reducing the cholesterol saturation of bile. Recent data however point more in favour of the conclusion that alcohol intake, smoking (cigarettes per day) and tobacco chewing per day are all independent etiological factors for gall bladder carcinoma.[18,37]

Conclusions

This overview of gallbladder carcinoma offers a summary of etiologic studies and a discussion of associated risk factors. The process of gallbladder carcinogenesis is usually related to a history of gall stones. Gall bladder carcinoma is found to be more commonly a disease in the elderly and more common in females. Further, the study is needed to study the factors related to females such as early menarche, high parity, hormonal factors etc., making them prone to gall bladder carcinoma. Dietary factors such as frequent non-veg diet, missing breakfast, frequent consumption of fried food and reuse of cooking oil should be discouraged and consumption of fresh fruits and vegetables should be encouraged. The present study shows results in association with other study results. This has certainly come out with factors other than gall stones as secondary etiological factors for carcinoma gall bladder. Keeping these factors in mind and utilizing them as a preventive factor may reduce the overall incidence of the carcinoma gall bladder.

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References

1. Stinton LM, Shaffer EA. Epidemiology of Gallbladder Disease: Cholelithiasis and cancer. Gut Liver [Internet]. 2012 Apr [cited 2022 Jun 11];6(2):172–87. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3343155/>
2. Zhang WJ, Xu GF, Zou XP, Wang WB, Yu JC, Wu GZ, Lu CL. Incidental Gallbladder Carcinoma Diagnosed During or After Laparoscopic Cholecystectomy. World J Surg [Internet]. 2009 Dec 1 [cited 2022 Jun 11];33(12):2651–6. Available from: <https://doi.org/10.1007/s00268-009-0218-9>
3. Furlan A, Ferris JV, Hosseinzadeh K, Borhani AA. Gallbladder Carcinoma Update: Multimodality Imaging Evaluation, Staging, and Treatment Options. American Journal of Roentgenology [Internet]. 2008 Nov [cited 2022 Jun 11];191(5):1440–7. Available from: <https://www.ajronline.org/doi/full/10.2214/AJR.07.3599>
4. Batra Y, Pal S, Dutta U, Desai P, Garg PK, Makharia G, Ahuja V, Pande GK, Sahni P, Chattopadhyay TK, Tandon RK. Gallbladder cancer in India: A dismal picture. Journal of Gastroenterology and Hepatology [Internet]. 2005 [cited 2022 Jun 11];20(2):309–14.

- Available from:
<https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1440-1746.2005.03576.x>
5. Dixit VK, Singh S, Shukla VK. Aetiopathogenesis of carcinoma gallbladder. *Trop Gastroenterol*. 2001 Jun;22(2):103–6.
 6. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. *Int J Cancer*. 2006 Apr 1;118(7):1591–602.
 7. PBCR_2001_04.pdf [Internet]. [cited 2022 Jun 11]. Available from: https://www.ncdirindia.org/All_Reports/PBCR_2001_04/PBCR_2001_04.pdf
 8. Mhatre SS, Nagrani RT, Budukh A, Chiplunkar S, Badwe R, Patil P, Laversanne M, Rajaraman P, Bray F, Dikshit R. Place of birth and risk of gallbladder cancer in India. *Indian Journal of Cancer* [Internet]. 2016 Apr 1 [cited 2022 Jun 11];53(2):304. Available from: <https://www.indianjcancer.com/article.asp?issn=0019-509X;year=2016;volume=53;issue=2;spage=304;epage=308;aulast=Mhatre;type=0>
 9. Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. *Clin Epidemiol* [Internet]. 2014 Mar 7 [cited 2021 Feb 9];6:99–109. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3952897/>
 10. Chapter1_Printed.pdf [Internet]. [cited 2022 Jun 11]. Available from: https://ncdirindia.org/ncrp/ALL_NCRP_REPORTS/PBCR_REPORT_2012_2014/ALL_CONTENT/PDF_Printed_Version/Chapter1_Printed.pdf
 11. Phadke PR, Mhatre SS, Budukh AM, Dikshit RP. Trends in gallbladder cancer incidence in the high- and low-risk regions of India. *Indian J Med Paediatr Oncol* [Internet]. 2019 Jan [cited 2022 Jun 11];40(1):90–3. Available from: http://www.thieme-connect.de/DOI/DOI?10.4103/ijmpo.ijmpo_164_18
 12. Shukla VK, Khandelwal C, Roy SK, Vaidya MP. Primary carcinoma of the gall bladder: A review of a 16-year period at the university hospital. *Journal of Surgical Oncology* [Internet]. 1985 [cited 2021 Feb 3];28(1):32–5. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/jso.2930280109>
 13. Singh DV. Factors associated with Gall Bladder cancer in Bihar-A case control study. :60.
 14. Gupta SK, Ansari MA, Shukla VK. What makes the Gangetic belt a fertile ground for gallbladder cancers? *J Surg Oncol*. 2005 Aug 1;91(2):143–4.
 15. Dixit R, Shukla VK. Why Is Gallbladder Cancer Common in the Gangetic Belt? In: R. Sudhakaran P, editor. *Perspectives in Cancer Prevention-Translational Cancer Research*. New Delhi: Springer India; 2014. p. 145–51.
 16. Unisa S, Jagannath P, Dhir V, Khandelwal C, Sarangi L, Roy TK. Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North India. *HPB* [Internet]. 2011 Feb 1 [cited 2022 Apr 12];13(2):117–25.

- Available from:
<https://www.sciencedirect.com/science/article/pii/S1365182X15303993>
17. Madhawi R, Pandey A, Raj S, Mandal M, Devi S, Sinha PK, Singh RK. Geographical pattern of carcinoma gallbladder in Bihar and its association with river Ganges and arsenic levels: Retrospective individual consecutive patient data from Regional Cancer Centre. *South Asian J Cancer* [Internet]. 2018 [cited 2022 Apr 12];7(3):167–70. Available from:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6069329/>
 18. Tyagi BB, Manoharan N, Raina V. Risk factors for gallbladder cancer: A population based case-control study in Delhi. *Indian Journal of Medical and Paediatric Oncology* [Internet]. 2008 Jan 1 [cited 2021 Feb 3];29(1):16. Available from:
<https://www.ijmpo.org/article.asp?issn=0971-5851;year=2008;volume=29;issue=1;spage=16;epage=26;aulast=Tyagi;type=0>
 19. Diehl AK. Gallstone Size and the Risk of Gallbladder Cancer. *JAMA* [Internet]. 1983 Nov 4 [cited 2021 Feb 3];250(17):2323–6. Available from: <https://doi.org/10.1001/jama.1983.03340170049027>
 20. Jain K, Sreenivas V, Velpandian T, Kapil U, Garg PK. Risk factors for gallbladder cancer: a case-control study. *Int J Cancer*. 2013 Apr 1;132(7):1660–6.
 21. Hsing AW, Bai Y, Andreotti G, Rashid A, Deng J, Chen J, Goldstein AM, Han TQ, Shen MC, Fraumeni JF, Gao YT. Family history of gallstones and the risk of biliary tract cancer and gallstones: A population-based study in Shanghai, China. *International Journal of Cancer* [Internet]. 2007 [cited 2021 Feb 3];121(4):832–8. Available from:
<https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.22756>
 22. Pandey M, Shukla VK. Diet and gallbladder cancer: a case-control study. *European Journal of Cancer Prevention* [Internet]. 2002 Aug [cited 2021 Feb 3];11(4):365–8. Available from:
https://journals.lww.com/eurjcancerprev/Abstract/2002/08000/Diet_and_gallbladder_cancer__a_case_control_study.8.aspx
 23. Tavani A, Vecchia CL, Gallus S, Lagiou P, Trichopoulos D, Levi F, Negri E. Red meat intake and cancer risk: A study in Italy. *International Journal of Cancer* [Internet]. 2000 [cited 2021 Feb 3];86(3):425–8. Available from:
<https://onlinelibrary.wiley.com/doi/abs/10.1002/%28SICI%291097-0215%2820000501%2986%3A3%3C425%3A%3AAID-IJC19%3E3.0.CO%3B2-S>
 24. Khan I, Panda N, Banerjee M, Das R. Epidemiological Factors in Gall Bladder Cancer in Eastern India-A Single Centre Study. *Indian J Surg Oncol* [Internet]. 2013 Mar 1 [cited 2022 Apr 12];4(1):67–72. Available from: <https://doi.org/10.1007/s13193-012-0203-x>
 25. Negri E, Vecchia CL, Franceschi S, D'Avanzo B, Parazzini F. Vegetable and fruit consumption and cancer risk. *International Journal of Cancer* [Internet]. 1991 [cited 2021 Feb 3];48(3):350–4. Available from:

- <https://onlinelibrary.wiley.com/doi/abs/10.1002/ijc.2910480307>
26. Moerman CJ, Demesquita HBB, Smeets FWM, Runia S. Consumption of Foods and Micronutrients and the Risk of Cancer of the Biliary Tract. *Preventive Medicine* [Internet]. 1995 Nov 1 [cited 2021 Feb 3];24(6):591–602. Available from: <http://www.sciencedirect.com/science/article/pii/S0091743585710948>
 27. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control* [Internet]. 1991 Sep 1 [cited 2021 Feb 3];2(5):325–57. Available from: <https://doi.org/10.1007/BF00051672>
 28. Dragsted LO, Strube M, Larsen JC. Cancer-Protective Factors in Fruits and Vegetables: Biochemical and Biological Background. *Pharmacology & Toxicology* [Internet]. 1993 [cited 2021 Feb 3];72(s1):116–35. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-0773.1993.tb01679.x>
 29. Pool-Zobel BL, Bub A, Liegibel UM, Lishaut ST van, Rechkemmer G. Mechanisms by which vegetable consumption reduces genetic damage in humans. *Cancer Epidemiol Biomarkers Prev* [Internet]. 1998 Oct 1 [cited 2021 Feb 3];7(10):891–9. Available from: <https://cebp.aacrjournals.org/content/7/10/891>
 30. Singh RP, Dhanalakshmi S, Agarwal R. Phytochemicals as Cell Cycle Modulators A Less Toxic Approach in Halting Human Cancers. *Cell Cycle* [Internet]. 2002 May 1 [cited 2022 Apr 12];1(3):155–60. Available from: <https://doi.org/10.4161/cc.1.3.117>
 31. RISK FACTORS FOR GALL BLADDER CANCER IN KARACHI [Internet]. [cited 2022 Apr 12]. Available from: <https://ayubmed.edu.pk/JAMC/PAST/15-3/talat.htm>
 32. Reshetnyak VI. Concept of the pathogenesis and treatment of cholelithiasis. *World J Hepatol* [Internet]. 2012 Feb 27 [cited 2022 Apr 12];4(2):18–34. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3295849/>
 33. Pandey M, Shukla VK, Singh S, Roy SK, Rao BR. Biliary lipid peroxidation products in gallbladder cancer: increased peroxidation or biliary stasis? *European Journal of Cancer Prevention* [Internet]. 2000 Dec [cited 2021 Feb 3];9(6):417–22. Available from: https://journals.lww.com/eurjcancerprev/Abstract/2000/12000/Biliary_lipid_peroxidation_products_in_gallbladder.7.aspx
 34. Norlia M, Jinap S, Nor-Khaizura MAR, Radu S, Samsudin NIP, Azri FA. Aspergillus section Flavi and Aflatoxins: Occurrence, Detection, and Identification in Raw Peanuts and Peanut-Based Products Along the Supply Chain. *Frontiers in Microbiology* [Internet]. 2019 [cited 2022 Apr 12];10. Available from: <https://www.frontiersin.org/article/10.3389/fmicb.2019.02602>
 35. Khan ZR, Neugut AI, Ahsan H, Chabot JA. Risk factors for biliary tract cancers. *The American Journal of Gastroenterology* [Internet]. 1999 Jan 1 [cited 2022 Apr 12];94(1):149–52. Available from: <https://www.sciencedirect.com/science/article/pii/S00029270980>

06674

36. Zatonski WA, Przewozniak K, Lowenfels AB, Boyle P, Maisonneuve P, Bueno de Mesquita HB, Moerman CJ, Ghadirian P, Simard A, Jain M, Howe GR, Baghurst P, McMichael AJ, Hsieh CC, Walker AM. Epidemiologic Aspects of Gallbladder Cancer: A Case-Control Study of the SEARCH Program of the International Agency for Research on Cancer. *JNCI: Journal of the National Cancer Institute* [Internet]. 1997 Aug 6 [cited 2021 Feb 3];89(15):1132–8. Available from: <https://doi.org/10.1093/jnci/89.15.1132>
37. Shukla VK, Chauhan VS, Mishra RN, Basu S. Lifestyle, reproductive factors and risk of gallbladder cancer. *Singapore Med J*. 2008 Nov;49(11):912–5.
38. Dwijayanti, N., Mufdlilah, M., & Suryaningsih, E. K. (2022). The role of midwives in the application of classroom services for pregnant women during the COVID-19 pandemic period. *International Journal of Health & Medical Sciences*, 5(3). <https://doi.org/10.21744/ijhms.v5n3.1918>
39. Widana, I.K., Dewi, G.A.O.C., Suryasa, W. (2020). Ergonomics approach to improve student concentration on learning process of professional ethics. *Journal of Advanced Research in Dynamical and Control Systems*, 12(7), 429-445.
40. Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, 29(3), 550–561. <https://doi.org/10.1002/cae.22202>