Original Article

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Statistics Section

Assessment of Macro and Micronutrient Levels Associated with Gallbladder Cancer among Women of Eastern Uttar Pradesh and Western Bihar: A Case-control Study

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ABSTRACT

Introduction: Gallbladder Cancer (GBC) is highly fatal due to late-stage diagnosis and poor prognosis. Women are more prone to GBC than men. Imbalanced nutrient intake is cited as a risk factor for GBC. Association of nutrients with GBC incidence assessed using 24-hour recall method may mislead as food intake is altered in disease condition. The Food Frequency Questionnaire (FFQ) method that assesses usual nutrient intake pattern would be a better approach.

Aim: To evaluate the association of macro and micronutrients in women with GBC using the FFQ method.

Materials and Methods: This case-control study was carried out at a tertiary care hospital of Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, from September 2018 to August 2019. A total of 82 women with GBC, aged 40 years and above, considered as cases and 164 healthy controls were included in the study. Data was collected regarding food intake, following the FFQ method, which included breakfast, lunch, dinner, and other extra intakes. Macro and micronutrients were computed using a raw database of food. A multivariate statistical approach was adopted as the nutrients were correlated.

Results: The mean age of the controls (45.7±10.1 years) was significantly lower than the GBC cases (55.2±11.0 years). Preponderance in both cases and controls was of Hindus, rural and Other Backward Class (OBC) women. Illiterate patients were more than twice than the controls. Intake of 12 nutrients i.e., protein, fat, phosphorus, zinc, manganese, and carotene were higher and of calcium, potassium, selenium, vitamin-C, vitamin-E, and fibre were lower in cases than the controls. These 12 nutrients completely separated cases and controls. However, zinc, manganese, potassium, and fibre whose contributions were lowest when ignored, the separation ability to cases and controls remained the same as of 12 nutrients.

Conclusion: Protein, fat, phosphorus, carotene, calcium, selenium, vitamin-C and vitamin-E, were found to be associated with GBC risk. Therefore, further understanding the role of nutrients in bringing about the right intervention to reduce the incidence of GBC is needed.

Keywords: Food frequency, Imbalanced diet, Protein, Risk factor

INTRODUCTION

Globally, male to female ratio of GBC is 1:1.25; while in India male to female projected ratio is 1:1.49. The projected cases of GBC in India had shown rising trend from 37561 in 2015 to 55141 in 2020. The rise of GBC cases in five years was 1.40 times among males; while for females it was 1.51 times [1,2]. In India, GBC cases are much higher in northern and eastern Indian regions specifically in Uttar Pradesh, Bihar, Delhi, Orissa, Assam and West Bengal than any other region [3]. It is because of specific food habits in different parts of India that gives rise to the incidence of different types of gallstones which may result to GBC. Cholesterol gallstone incidence is higher in eastern, western, and northern Indian regions; while pigment type of gallstones is higher in the southern Indian region [4]. Among the cancers, GBC is highly fatal because of later-stage diagnosis and poor prognosis [5]. The role of dietary factors on the onset of GBC is well documented. Low consumption of fat and calories and higher consumption of vegetables, fruits and fibres that are rich in vitamin-C and vitamin-E play a protective role; while high intake of calorie, carbohydrate and oily foods are the risks to GBC [6-8]. Proteins and lipid, especially triglyceride and high-density lipoprotein, are also directly associated with GBC [9-12]. It was suggested that approximately 30% of cancers can be prevented by a healthy diet [13].

The role of nutrients on GBC are generally assessed by capturing information on foods and beverages using the 24-hour recall method. Food intake tends to alter drastically during diseased conditions hence, 24-hour recall method may mislead in establishing the true associations between GBC and nutrients. Moreover, single 24-hour recall method may not truly represent habitual diet [14]. The FFQ method that provides the usual food pattern would be a better approach than 24-hour recall method in assessing the usual intake pattern of nutrients and its effect on GBC. Though, FFQ method also suffers from recall bias, but reasonably good method reflecting usual long-term intake [15]. A recent study used FFQ method and reported that high-risk of GBC was associated with the less consumption of vegetables and fruits [16]. It was suggested to promote vegetarian diet with recommended calories, fats and high protein diet in individuals who are overweight or obese and having sedentary lifestyle. Further, they also recommended that total cholesterol, low density lipoprotein, and triglycerides lipids which are the independent risk factors for GBC should be assessed routinely in patients with the suspicion of Gall Stone Disease (GSD) [17]. The food pattern and lifestyle behaviour of Eastern Uttar Pradesh and Western Bihar population is very much different than the rest of region of India as they live in Gangetic basin. Although, studies in this region were carried but either compared intake of various vegetables and fruits between GBC and GSD cases or compared various macro and micronutrients in blood serum between GBC and GSD cases only [6,18]. These studies have not addressed the usual pattern of consumption of various macro and micronutrients that may influence GBC incidence.

Therefore, the present case-control study was designed for the women population to assess and compare macro and micronutrients in GBC with the healthy women population, also to evaluate those macro and micronutrients that can discriminate the cases from the controls for suggesting to prevent the incidence of GBC.

MATERIALS AND METHODS

This case-control study was conducted from September 2018 to August 2019, in the Department of Community Medicine in collaboration with the Department of Surgical Oncology at the tertiary care hospital of the Institute of Medical Science, Banaras Hindu University, Varanasi Uttar Pradesh, India. The study was approved by the Ethical Committee of the Institute of Medical Sciences, Banaras Hindu University (Letter No. Dean/2019/EC/1062 dated January 18, 2019).

Inclusion criteria: Women aged >40 years and above, with recently confirmed GBC (verified by Oncologist by symptomatic and clinical examination), without presence of any other co-morbidity conditions and consenting to participate in the study were included as cases. Female attendant of the patients of GBC or other female from outpatient department of SS Hospital on treatment for any minor illnesses and consenting to participate in the study were included as controls.

Exclusion criteria: Women aged <40 years, old female cases of GBC and already on treatment, presence of any other co-morbidity condition and having any severe or chronic illness or not consenting to participate in the study were excluded from the cases.

Sample size calculation: Sample sizes of cases and controls were computed by considering the variability of each macro and micronutrients from comparative studies carried out in past (Shukla VK et al., and Panda D et al.,) [6,8]. The formula used to compute the required sample size of cases and controls in the ratio of 1:2 was:

$$n=\frac{(Z_{\alpha/2}+|Z_{1-\beta}|)^2(1+1/k)\,S^2}{\Delta^2}$$

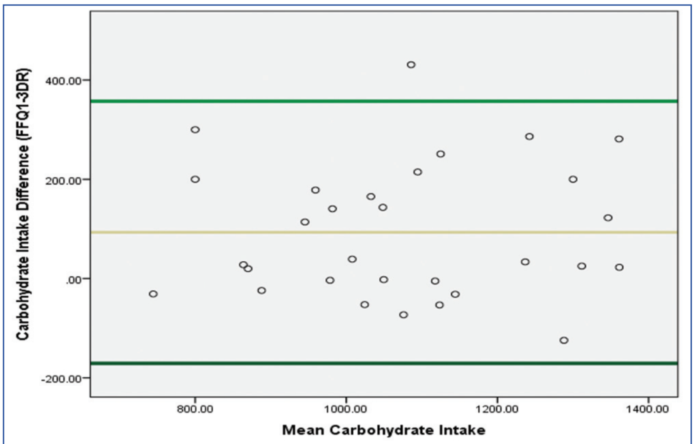
Where, S^2 is the pooled variance and Δ is the anticipated difference of means between cases and controls of the considered nutrients and k is the ratio of controls to cases to be taken. By considering cases and control in the ratio of 1:2, at α=0.05 and β=0.05 and anticipated difference of 5 units; vitamin-C with pooled variance of 94.90 yielded the maximum sample size from among all the macro and micronutrients which was 82 for cases and 164 for controls. Thus, 82 consecutive women of GBC from the Department of Surgical Oncology and 164 control either attendant of the patient of GBC or other outpatients from SS Hospital suffering with some other minor illnesses were enrolled in the study.

Data Collection and Evaluation of Nutrients

The data was collected personally with a semistructured questionnaire related to socio-demographic characteristics and food intakes following FFQ. One author (nutritionist), among the researchers, developed this semistructured FFQ with 87 items of food and beverages consumed in Eastern Uttar Pradesh and Western Bihar. Questionnaire included assessment of the frequency of consumption of the major food groups: cereals, pulses, green leafy vegetable, roots and tubers, other vegetables, fruits, milk and milk products, egg and flesh foods, sugar, salt and oils etc. The response on consumption of items was assessed by the frequency and the quantity for morning tea and breakfast, lunch, evening tea and snacks, dinner as well as any extra intake in between.

The response for each item consists of eight categories of intake frequency i.e., never; 1-3 times per month; once a week; 2-4 times per week; 5-6 times per week; once a day; 2-3 times per day; and ≥4 times per day. The data considered diet taken by the individuals in the last one month. Standardised utensils were used for each food item, such as a scoop, plate, bowl, cup, and tablespoon. Three-Day Dietary Record (3DR) was used as a criterion reference method for the validation of the FFQ. The 3DR was administered for three days (two weekdays and one weekend day). Participants were asked to provide detailed descriptions of the food and beverages consumed, the food preparation method, and the brand of the food and beverages consumed. The amounts and frequencies of food intakes recorded in the FFQ and 3DR were first converted to grams and then to nutrient intake (All macronutrients were measured in gm and all micronutrients and others were measured in mg except carotene and copper in µg).

The amount of daily food intake was calculated from the FFQ according to the following formula: (frequency of intake×serving size×total number of servings×weight of food in one serving). Carbohydrate, protein, fat, calcium, phosphorous, sodium, potassium, zinc, selenium, magnesium, manganese, iron, copper, vitamin-C, vitamin-E, vitamin-B6 and carotene were computed following Indian Food Composition Tables, National Institute of Nutrition [19]. For the reliability of the FFQ, questionnaire was administered at two times on the same subject named as FFQ1 and FFQ2. For the reliability, firstly Spearman's correlation was computed and thereafter, Intraclass Correlation Coefficients (ICCs) between them. The agreement between FFQ1 and 3 days recall (3DR) for micro and macronutrients intake was examined graphically using Bland-Altman Plot [20] and also by computing Cronbach's alpha values [15]. For example, Spearman's correlation for carbohydrate intake was 0.75 and ICC as 0.74 (95% CI: 0.53-0.87); while calculated Cronbach's alpha value was 0.852 [15]. These estimate, indicate a moderate to high reliability between the repeated FFQs. Also, the Bland-Atman method indicated good agreement FFQ, as almost all the differences between FFQ1 and 3DR lied within 95% confidence interval as shown in [Table/Fig-1].



[Table/Fig-1]: Bland Altman plot for carbohydrate Intake.

STATISTICAL ANALYSIS

The data was analysed by using Statistical Package for the Social Sciences (SPSS) version 19.0 [21] and each micro and macronutrient was summarised as mean±standard deviation (SD). The difference of means of macro and micronutrient between cases and controls was tested using student's t-test adjusted for Bonferroni correction. Since, micro and macronutrients were correlated, therefore, the difference in the vector of means between cases and control was tested using Hotelling's T^2 [22]. Thereafter, the relative importance and their contribution with statistical significance of each micro and macronutrient in presence of others was obtained. The relative importance of each micro and macronutrients was obtained by computing their standardised discriminant function coefficients. The contribution of each micro and macronutrients was obtained by subtracting the value of Hotelling's T^2 by excluding the one of interest from the value of Hotelling's T^2 by including the all. The statistical significance of the contribution of each micro and macronutrients was tested by using partial F test [23]. Further, the classification accuracy with significant contributors was obtained following Fisher's classification method. A backward elimination procedure was adopted to restrict to a minimum of nutrients in separating the cases and controls. The subset of macro and micronutrients was obtained by the successive deletion of the nutrients with the lowest contributions which continued till they drop in the classification accuracy was substantial [24]. The equations of discriminant functions to classify the cases and controls obtained were as:

For cases $c_1+a_1 X_1+a_2X_2+................+a_pX_p$

For controls $c'_1+a'_1 X_1+a'_2 X_2+................+a'_pX_p$

Where, X_i is the i[th] measured nutrient, c_1 and c_2 are the constants and a_i and a'_1 are the coefficients corresponding to i[th] nutrient for cases and controls, respectively.

RESULTS

The mean age of the controls (45.7±10.1 years) was significantly lower than the GBC cases (55.2±11.0 years). There was a preponderance of Hindus in both cases and majority of the participants in cases 56 (68.3%) and controls 88 (53.7%) belonged to Other Backward Caste (OBC). Illiterates were more among the cases compared to controls. Both cases and controls had more homemakers and patients had a sedentary lifestyle [Table/Fig-2].

Variables	Cases (n=82)	Control (n=164)	p-value
Age (years)	55.2±11.0	45.7±10.1	<0.001*
Religion			
Hinduism	76 (92.7)	155 (94.5)	0.572**
Islam	6 (7.3)	9 (5.5)	
Caste			
Un Reserved (UR)	15 (18.3)	44 (26.8)	0.181**
Other Backward Caste (OBC)	56 (68.3)	88 (53.7)	
Scheduled Caste (SC)	9 (11.0)	25 (15.2)	
Scheduled Tribe (ST)	2 (2.4)	7 (4.3)	
Area of residence			
Rural	70 (85.4)	119 (72.6)	0.025**
Urban	12 (14.6)	45 (27.4)	
Type of family			
Nuclear	47 (57.3)	111(67.7)	0.110**
Joint	35 (42.7)	53 (32.3)	
Education of participant			
Illiterate	63 (76.8)	54 (32.9)	<0.001**
Primary	5 (6.1)	14 (8.5)	
Middle school	6 (7.3)	14 (8.5)	
High school	3 (3.7)	12 (7.3)	
Intermediate	3 (3.7)	22 (13.4)	
Graduate/Postgraduate	2 (2.4)	48 (29.3)	
Occupation of participant			
Homemaker	63 (76.8)	131 (79.9)	0.581**
Working women	19 (23.2)	33 (20.1)	
Physical activity level			
Moderate	4 (4.9)	21 (12.8)	0.052**
Sedentary	78 (95.1)	143 (87.2)	

[Table/Fig-2]: Demographic profile of cases and controls.
*Independent t test; ** χ^2 test

In the univariate approach, statistically insignificant intakes of macro and micronutrients between cases and controls found for carbohydrate, zinc, magnesium, and iron. While in the multivariate approach, statistically similar intakes between cases and controls were reported in carbohydrate, sodium, magnesium, iron, copper, and vitamin B6. The significant findings of each nutrient are presented in a multivariate context only in differentiating cases and controls [Table/Fig-3].

Age difference between cases and controls was significant in univariate, but in a multivariate context, the contribution of age in separating the cases and controls was found insignificant. Among the macronutrients, intakes of protein and fat in cases compared to controls were statistically higher by 1.08 and 1.43 times, respectively. While among the micronutrients, the intake of calcium and vitamin-E in cases were significantly much less than the controls. The intake of potassium in cases was significantly lesser by 0.88 times of the controls. Phosphorus and manganese intakes were nearly 1.14 and 1.19 times higher among cases than in the controls; while intake of carotene was higher by 1.41 in cases than the controls. Zinc intake was also nearly 1.02 times higher in cases than in the controls; while fibre intake was lesser among cases compared to controls and was 0.85 of the controls [Table/Fig-3].

Eight macro and micronutrients, relatively of higher importance out of 12, were identified. These 12 macro and micronutrients classified all the cases as the GBC case and all the controls as the healthy controls. However, the classification remained unchanged with only eight macro and micronutrients i.e., protein, fat, phosphorus, calcium, selenium, vitamin-E, vitamin-C, and carotene after deletion following the backward elimination procedure. Thus, these eight identified macro and micronutrients were enough to separate the GBC cases and controls [Table/Fig-4].

Among these eight macro and micronutrients, the contributors to classifying the cases and controls were carotene, fat, protein, calcium, vitamin-E, vitamin-C, phosphorus and selenium. The intake was higher of carotene, fat, protein and phosphorus and lower of calcium, vitamin-E, vitamin-C, and selenium in GBC cases than in the controls [Table/Fig-5].

DISCUSSION

The present case-control study was confined to women only because women are at a higher risk of GBC compared to men. The study provides gender-specific dietary patterns that may be playing a role as a risk factor for the onset of gallstones. It also paves a thought into consideration of prophylactic cholecystectomy in women categorised as with high-risk with a history of their nutrient intake [25]. The present study was able to establish the association between various micro and macronutrients with the onset of GBC. Since macro and micronutrients are significantly correlated, and therefore the presence of a component of correlation be accounted for in the analysis. The age differential did not appear as the significant separator of cases and controls in a multivariate context. This has been established that the risk factors can be seen to have a key role even at a younger age of subjects [26]. Through the multivariate analysis which accounted for the correlation, it was found that carotene, fat, and protein were the most significant differentiating nutrients between cases and controls among eight different nutrients. It was also found that these eight nutrients were able to discriminate cases and controls with perfect precision.

Parameters	Univariate approach				Multivariate approach	
	Cases (n=82)	Controls (n=164)	Ratio of mean intake of cases to the controls	p*-value	p**-value	Rank of significant contributors[a]
	Mean±SD	Mean±SD				
Macronutrients						
Carbohydrate (gm)	932.39±189.9	946.34±201.94	0.99	0.603	0.182	--
Protein (gm)	182.30±35.30	169.00±36.53	1.08	**0.007**	**<0.001**	1
Fat (gm)	266.76±107.16	185.93±49.03	1.43	**<0.001**	**<0.001**	2

Micronutrients						
Calcium (mg)	634.66±230.06	966.58±260.01	0.66	**<0.001**	**<0.001**	3
Phosphorous (mg)	1146±244.96	1008.06±188.61	1.14	**<0.001**	**<0.001**	5
Sodium (mg)	5700.10±2321.04	4204.98±625.27	1.35	**<0.001**	0.092	--
Potassium (mg)	1569.25±336.49	1775.20±331.72	0.88	**<0.001**	**<0.001**	6
Zinc (mg)	10.46±7.69	10.21±3.73	1.02	0.789	**<0.001**	9
Selenium (mg)	35.44±6.86	46.24±10.52	0.77	**<0.001**	**0.002**	10
Magnesium (mg)	393.81±366.34	354.09±99.76	0.90	0.338	0.798	--
Manganese (mg)	4.799±3.07	4.02±1.71	1.19	**0.037**	**0.023**	12
Iron (mg)	11.23±2.45	11.61±3.23	0.97	0.300	1.000	--
Copper (μg)	1.13±0.21	1.28±0.27	0.88	**<0.001**	0.105	--
Vitamin-C (mg)	51.69±15.17	67.59±13.90	0.76	**<0.001**	**<0.001**	7
Vitamin-E (mg)	4.70±1.74	8.86±4.34	0.53	**<0.001**	**<0.001**	8
Vitamin-B6 (mg)	1.61±0.82	1.83±1.16	0.88	**<0.001**	1.000	--
Carotene (μg)	1422.98±781.75	1012.82±426.17	1.41	**<0.001**	**<0.001**	4
Others						
Fibre (gm)	26.65±5.92	31.5±10.04	0.85	**<0.001**	**0.011**	11

[Table/Fig-3]: Comparison of macro, micro and other nutrient intake between cases and controls.
*Independent t test and **Multivariate context including age (not presented in table) [a]Based on standardised discriminant coefficients; [a]Relative importance of macro and micronutrients in multivariate context

<table>
<tr><th colspan="4">Predicted group membership with 12 significant contributors</th><th>Total</th><th></th></tr>
<tr><td rowspan="3">Original group membership</td><td></td><td>Cases</td><td>Controls</td><td></td><td rowspan="3">Classification accuracy=100%</td></tr>
<tr><td>Cases</td><td>82</td><td>0</td><td>82</td></tr>
<tr><td>Controls</td><td>0</td><td>164</td><td>164</td></tr>
<tr><td colspan="6">*Protein, Fat, Calcium, Phosphorous, Potassium, Zinc, Selenium, Manganese, Vit-C, Vit-E, Carotene, Fibre</td></tr>
<tr><th colspan="4">Predicted group membership with only 8 relatively of higher importance</th><th>Total</th><th></th></tr>
<tr><td rowspan="3">Original group membership*</td><td></td><td>Cases</td><td>Controls</td><td></td><td rowspan="3">Classification accuracy=100%</td></tr>
<tr><td>Cases</td><td>82</td><td>0</td><td>82</td></tr>
<tr><td>Controls</td><td>0</td><td>164</td><td>164</td></tr>
<tr><td colspan="6">Protein, Fat, Phosphorus, Calcium, Selenium, vitamin-E, vitamin-C, Carotene</td></tr>
<tr><td colspan="6">Discriminant classification function</td></tr>
<tr><td>For cases</td><td colspan="5">– 28.897+ 0.359 Protein + 0.032 Fat – 0.004 Phosphorus – 0.019 Calcium – 0.306 Selenium – 1.739 vitamin-E + 0.320 vitamin-C + 0.001 Carotene</td></tr>
<tr><td>For controls</td><td colspan="5">– 32.787 – 0.123 Protein – 0.082 Fat + 0.042 Phosphorus + 0.014 Calcium + 0.268 Selenium + 0.133 vitamin-E + 0.661 vitamin-C – 0.014 Carotene</td></tr>
</table>

[Table/Fig-4]: Classification accuracy of the linear discriminant function with all 12 significant contributors of macro and micronutrients and with only 8 of relatively higher importance.

Macro and micronutrients	Contribution in separating cases and controls in presence of others	Rank
Carotene (μg)	1239.348	1
Fat (gm)	928.158	2
Protein (gm)	824.346	3
Calcium (mg)	625.086	4
Vitamin-E (mg)	586.956	5
Vitamin-C (mg)	514.386	6
Phosphorus (mg)	486.096	7
Selenium (mg)	287.328	8

[Table/Fig-5]: Relative importance and their contribution of macro and micronutrients in separating the cases and controls.

Micro and macronutrients derived from the dietary intake of a subject is an important risk factor in various studies [8,27-32]. These results were consistent with existing evidence from other studies which have also identified increased levels of copper, zinc and iron levels in diagnosed gallstones considered as the risk factor of GBC did not indicate the difference between cases and controls in present study [4,10]. The plausible reasons could be the analysis performed considering the covariance between the nutrients in the multivariate context accounting for the presence of the effect of other variables in the model. This study suggested elevated fat, phosphorous, manganese and carotene amongst GBC cases and increased levels of fat, carotene and cholesterol concentration in the diet were reported to be significant risk factors for GBC [33] .The present study was able to explicitly establish that nutrient contents of composite items of fruits and vegetables like green leafy vegetables, citrus rich fruits and cruciferous vegetables that are common dietary contents in Indian women had an inverse effect on GBC. This result substantiates the evidence previously established by studies on the diagnosis and treatment of GBC [9,10,32].

The multivariate approach encompasses the covariance between the micro and macronutrients, giving results holistically as a multifactorial risk factor. The results obtained from univariate and multivariate analysis were consistent for most nutrients as risk factors except sodium, zinc, copper and Vitamin B-6. Copper had a high correlation with most of the nutrients whereas zinc and sodium had a high correlation with carotene, suggesting that the effect seen in univariate analysis were influenced by the effects of these correlated variables. Vitamin B-6 showed peculiar characteristics as it did not have a significant correlation with other nutrients but as a differential factor was not significant in multivariate analysis. Studies report results after performing a multivariable analysis not duly accounting for the covariance [34,35]. The results obtained in the present study, therefore, have an edge over other evidence. Micro and macronutrients were used as a discriminatory factor to discriminate between cases and controls concerning GBC. It was seen that they had a perfect discriminatory ability to classify the subjects as cases and controls. Although studies exist as a prognostic model for predicting survival based on pretreatment nutritional index, very sparse literature exists as a screening tool for the onset of the disease [36,37].

Multivariate analysis to assess the potential risk factors associated with the onset of GBC can be considered a robust technique to understand the real effects of the nutritional values. It considers the intervariable dependency in understanding the role of a nutritional diet in alleviating the probability of GBC. The model can further be used to develop a screening tool to assess the risk of GBC amongst Indian women. External validation of the model with a wider population and larger sample size can further validate the model for generalisability. Consideration of other factors such as reproductive behaviour apart from the nutritional intake of these women can, further, enhance the quality of the model.

Limitation(s)

Recall bias on the response of long-term food consumption habit and pattern was the limitation of this study; but there is no alternative method available. Age matched controls could not be obtained as the guardian or the healthy care taken accompanying the patients were considered as controls.

CONCLUSION(S)

Protein and fat among macronutrients, while calcium and vitamin-E among micronutrients showed significant difference between cases and controls. Eight macro and micronutrients (protein, fat, phosphorus, calcium, selenium, vitamin-E, vitamin-C, and carotene) were of relatively higher importance out of 12 that were identified which could separate the GBC cases from controls. The intake of carotene, fat, protein, and phosphorus was higher in GBC cases; the intake of calcium, vitamin-E, vitamin-C, and selenium was lower in GBC cases than controls. Macro and micronutrients have been found to be a differential factor in the screening of risk for GBC. Therefore, further studies should try to understand their role to bring about the right intervention to improve the nutritional status of women to decrease the incidence of GBC.

REFERENCES

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68:394-24.

[2] Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santhappan S, et al. Cancer Statistics, 2020: Report from National Cancer Registry Programme, India. JCO Glob Oncol. 2020; 6:1063-75.

[3] Consensus Document for Management of Gallbladder Cancer, Indian Council of Medical Research. 2014.

[4] Ashok M, Rautray TR, Nayak PK, Vijayan V, Jayanthi V, Kalkura SN, et al. Energy dispersive X-ray fluorescence analysis of gallstones. J Radioanal Nucl Chem. 2003;257:333-35.

[5] GLOBOCAN. Estimated Cancer Incidence, Mortality and Prevalence Worldwide. International Agency for Research on Cancer (IARC). 2012; 1.0.

[6] Shukla VK, Adukia TK, Singh SP, Mishra CP, Mishra RN. Micronutrients, antioxidants, and carcinoma of the gallbladder. J Surg Oncol. 2003;84(1):31-35.

[7] Rai A, Mohapatra SC, Shukla HS. Correlates between vegetable consumption and gallbladder cancer. Eur J Cancer Prev. 2006;15(2):134-37.

[8] Panda D, Sharma A, Shukla NK, Jaiswal R, Dwivedi S, Raina V, et al. Gall bladder cancer and the role of dietary and lifestyle factors. Eur J Cancer Prev. 2013;22(5):431-37.

[9] Zatonski WA, La Vecchia C, Przewozniak K, Maisonneuve P, Lowenfels AB, Boyle P, et al. Risk factors for gallbladder cancer: A Polish case-control study. Int J Cancer. 1992; 51(5):707-11.

[10] Zatonski WA, Lowenfels AB, Boyle P, Maisonneuve P, Bueno de Mesquita HB, Ghadirian P, et al. 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. 1997; 89 (15): 1132-38.

[11] Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL. Prospective study of abdominal adiposity and gallstone disease in US men. Am J Clin Nutr. 2004;80(1):38-44.

[12] Hayata S, Hassanb Z, Changazia SH, Zahra A, Noman M, Abdin MZ, et al. Comparative analysis of serum lipid profiles in patients with and without gallstones: A prospective cross-sectional study. Ann Med Surg. (Lond). 2019;42:11-13.

[13] Heller MC, Keoleian GA, Willett WC. Toward a life cycle-based, diet-level framework for food environmental impact and nutritional quality assessment: A critical review. Environ Sci Technol. 2013;47(22):12632-47.

[14] Dietary Assessment Guideline: Strength and Weakness. https://www.nutritools.org/strengths-and-weaknesses. Accessed on 05-07-2018.

[15] Cronbach LJ. Coefficient alpha and the internal structure of tests. Psychometrika. 1951;16:297-34.

[16] Timmanpyati S, Mahajan P, Mehta S, Pati P, Talole S. Dietary intake and nutritional status of patients with gall bladder cancer. J Nut Res. 2015;3(1):54-61.

[17] Dhamnetiya D, Goel MK, Dhiman BR, Pathania OP. Gallstone disease and quantitative analysis of independent biochemical parameters: Study in a tertiary care hospital of India. J Lab Physicians. 2018;10(4):448-52.

[18] Pandey M, Shukla VK. Diet and gallbladder cancer: A case–control study. Eur J Cancer Prev. 2002;11(4):365-68.

[19] Longvah T, Ananthan R, Bhaskarachary K, Venkaiah K. Indian Food Composition Tables. National Institute of Nutrition (ICMR), Department of Health Research, Ministry of Health and Family Welfare, Government of India, Hyderabad, Telangana State, India. 2017.

[20] Bland JM, Altman DG. Statistical methods for assessing agreement between the two methods of clinical measurements. Lancet. 1986;1(8476):307-10.

[21] SPSS Version 19.0. IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.

[22] Mishra A, Harichandrakumar KT, Binu VS, Satheesh S, Nair NS. Multivariate approach in analyzing medical data with correlated multiple outcomes: An exploration using ACCORD trial data. Clin Epidemiology Glob Health. 2021;11:100785.

[23] Rencher AC. Methods of Multivariate Analysis, 2nd Ed, United States of America, A John Willy & Sons, Inc. Publication. 2002.

[24] Rencher AC. Contribution of individual variables to Hotelling's T2, Wilk's, and R2. Biometrics. 1993; 49 (2):479-89.

[25] Alvi AR, Siddiqui NA, Zafar H. Risk factors of gallbladder cancer in Karachi-A case-control study. World J Surg Oncol. 2011;9(1):164.

[26] Scragg RK, McMichael AJ, Baghurst PA. Diet, alcohol, and relative weight in gall stone disease: A case-control study. Br Med J (Clin Res Ed). 1984;288(6424):1113-19.

[27] Shin MH, Holmes MD, Hankinson SE, Wu K, Colditz GA, Willett WC, et al. Intake of dairy products, calcium, and vitamin D and risk of breast cancer. J Natl Cancer Inst. 2002;94(17):1301-11.

[28] Ghosh Y, Thakurdas B. Carcinoma gall bladder: Past, present, and future. Int J Biomed. 2014;4(4):198-03.

[29] Leitzmann MF, Tsai CJ, Stampfer MJ, Rimm EB, Colditz GA, Willett WC, et al. Alcohol consumption in relation to risk of cholecystectomy in women. Am J Clin Nutr. 2003;78(2):339-47.

[30] Simon JA, Hudes ES. Serum ascorbic acid and gallbladder disease prevalence among US adults: The Third National Health and Nutrition Examination Survey (NHANES III). Arch Intern Med. 2000;160(7):931-36.

[31] Cuevas A, Miquel JF, Reyes MS, Zanlungo S, Nervi F. Diet as a risk factor for cholesterol gallstone disease. J Am Coll Nutr. 2004;23(3):187-96.

[32] Jain K, Sreenivas V, Velpandian T, Kapil U, Garg PK. Risk factors for gallbladder cancer: A case-control study. Int J Cancer. 2013;132(7):1660-66.

[33] Lee DW, Gilmore CJ, Bonorris G, Cohen H, Marks JW, Cho-Sue M, et al. Effect of dietary cholesterol on biliary lipids in patients with gallstones and normal subjects. Am J Clin Nutr. 1985;42(3):414-20.

[34] Tamrakar D, Paudel IS, Adhikary S, Rauniyar B, Pokharel PK. Risk factors for gallbladder cancer in Nepal- A case control study. Asian Pac J Cancer Prev. 2016;17(7):3447-53.

[35] Strom BL, Soloway RD, Rios Dalenz JL, Rodriguez Martinez HA, West SL, Kinman JL, et al. Risk factors for gallbladder cancer. An International collaborative case–control study. Cancer. 1995;76(10):1747-56.

[36] Deng Y, Pang Q, Bi JB, Zhang X, Zhang LQ, Zhou YY, et al. A promising prediction model for survival in gallbladder carcinoma patients: Pretreatment prognostic nutrient index. Tumor Bio. 2016;37(12):15773-81.

[37] Wang J, Bo X, Li M, Nan L, Wang C, Gao Z, et al. Prediction efficacy for clinical outcome of prognostic nutritional index in patients with resectable biliary tract cancer depends on sex and obstructive jaundice status. Ann Surg Oncol. 2021;28(1):430-38.

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