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Dietary Animal Protein vs. Plant Protein Sources in Relation to the Risk of Biliary Stone: A Case-Control Study

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ABSTRACT

Background: Dietary intake is one of the modifiable risk factors for biliary stones. In recent studies, plant protein intake is associated with a lower risk of biliary stones in comparison to animal protein intake; however, the results are contradictory. The current study aims to compare the effect of animal protein and plant protein on the formation of biliary stone in one of the gastroenterology and liver disease centers in Iran. **Methods:** 110 participants who had a history of biliary stone and 230 controls who were normal in terms of biliary diseases and referred to the medical center from November 2017 to October 2018 enrolled in this study. Food frequency questionnaire (FFQ) was used for the nutritional assessment; moreover, demographic and anthropometric data, habitual history and comorbidities were collected. Statistical analysis was conducted by SPSS software. **Results:** Plant protein consumption was significantly lower (P=0.03) and animal protein intake was significantly higher (P=0.02) among men in case compared to controls. Furthermore, the relationship between biliary stone disease and animal protein intake was significant in crude model for men (OR: 1.03, 95% CI=1.01-1.05). In addition, the risk of biliary stone was significantly lower in patients with higher consumption of plant protein (for women: OR: 0.94, 95% CI=0.89-0.99, for both sexes: OR: 0.96, 95%CI=0.93-0.99). **Conclusion:** The present study suggested that consumption of animal protein sources increased the risk of biliary stone, and intake of plant protein sources decreased the risk of biliary stone.

Keywords: *Biliary stone; Plant protein; Animal protein*

Introduction

Biliary stones are one of the most common disorders of the biliary system in the world with a 15-20% prevalence rate in western countries (Brighi *et al.*, 2018, Di Ciaula and Portincasa, 2018, Panpimanmas and Manmee, 2009, Seddighi *et al.*, 2018). It is also one of the most common

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diseases affecting emergency-room patients with epigastric pain, nausea, vomiting, abdominal pain, and loss of appetite (Chen *et al.*, 2012). The pathogenesis of biliary stones is believed to be multifactorial and probably develops from interactions between several genetic and environmental factors such as age, sex, ethnicity, family history, obesity, rapid weight loss, and pregnancy (Marschall and Einarsson, 2007, Tarantino *et al.*, 2017).

One of the modifiable risk factors for biliary stones is nutritional intake (Jessri and Rashidkhani, 2015, Lander *et al.*, 2016, Stinton and Shaffer, 2012). Dietary proteins have an influence on biliary cholesterol density and biliary stone formation (Catala *et al.*, 2000, Tsai *et al.*, 2004, Tseng *et al.*, 2000). Some studies have suggested that plant protein intake is associated with a lower risk of biliary stones in comparison to animal protein intake (Mahfouz-Cercone *et al.*, 1984, Tomotake *et al.*, 2006). In addition, some studies have observed no significant association between protein intake and biliary stones (Attili *et al.*, 1998, Misciagna *et al.*, 1999). Assessing the dietary habit in biliary stones patients can determine the relationship between protein intake and biliary stones incident (Hu, 2002).

The study aims to compare dietary animal protein vs. plant protein sources in relation to the risk of biliary stone.

Materials and Methods

Study design: This case-control study was conducted in Research Institute for Gastroenterology and Liver Diseases of Taleghani hospital (Tehran, Iran) from November 2017 to October 2018. The samples consisted of 110 cases (aged 21-91 years) who had a history of gallstone or common bile duct (CBD) stone confirmed by ultrasonography and/or history of cholecystectomy in a 6-month period before their admission to gastrointestinal disease clinic and after obtaining the consent form to participate in this study. Furthermore, the control group consisted of 230 subjects (aged 23-84 years) who were admitted to the other wards of the hospital for a wide spectrum of diseases, such as orthopedic problems, ear/nose/throat diseases or elective surgeries, and did not have history of dieting and did not have a history of biliary stones. Moreover, the control group was matched to the case group based on age (±5 years) and sex and the number of controls was twice that of the cases.

Data collection: To assess the dietary consumption of samples during 1 year before biliary stone determination in the case group or hospital admission in the control group, a validated food frequency questionnaire (FFQ) was used (Asghari *et al.*, 2012). Researchers asked the participants to explain the frequency of their dietary consumption on a daily, weekly, monthly, or yearly basis. Subsequently, dietary intakes were converted to daily frequency for each group of protein consumption. The data extracted from dietary intakes were analyzed by Nutritionist 4 software. The national food composition tables were used as a reference to analyze food intake.

Demographic data, anthropometric measurements, history of smoking, history of alcohol consumption and comorbidities were collected by a questionnaire. The physical activity questionnaire based on physical activity questionnaire (IPAQ) was used to measure the physical activity of the subjects. The validity and reliability of this questionnaire were previously confirmed (Cleland *et al.*, 2018). Metabolic equivalent of task (MET) scores were calculated via face to face interviews with all eligible participants. One MET is defined as 1 kcal/kg/hour, equivalent to the energy cost of sitting quietly while consuming 3.5 ml/kg/min of oxygen.

Furthermore, weight measurement was done in a standing position by digital scales (Soehnle®, Berlin, Germany) with an accuracy of 100 g. In addition, height was measured using a tape-meter which fixed to a wall with an accuracy of 0.5 cm. Body mass index (BMI) was calculated by dividing the weight by the square of height in meter.

Ethical considerations: This protocol of study was approved by Research Institute of

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Gastroenterology and Liver Diseases Ethics Committee (IR.SBMU.RIGLD.REC.1396.159).

Data analysis: Statistical analysis was conducted on the data by using SPSS software version 16 (SPSS Inc., Chicago, Illinois). Baseline characteristics and dietary intakes of the participants were compared between case and control groups by *t-test* or Mann-Whitney for quantitative variables and Chi-square test for qualitative variables. Logistic regression was used to calculate the odds ratio of biliary stone disease as the dependent variable in relation to the animal and plant protein as independent variables in 4 models: Crude model, model 2 (adjusted for energy intake and physical activity), model 3 (further controlled for BMI and history of diabetes) and, model 4 (additionally adjusted for dietary cholesterol and dietary fiber). The odds ratios of the outcomes were determined with a 95% confidence interval.

Results

Demographics: Baseline characteristics and dietary intakes of the participants are shown based on case and control group in **Table 1**. Individuals in the case group compared to those in the control group were higher in total energy intake and prevalence of type-2 diabetes and lower in physical activity, protein intake, dietary cholesterol, and dietary fiber ($P < 0.05$).

Binary analysis of variables: Dietary intake of animal and plant protein base on case and control group for men, women and both sexes are shown in **Table 2**. Men in the case group compared to men in the control group consumed higher animal protein and lower plant protein ($P<0.05$). In females, there was no significant difference between case and control groups. When both sexes considered, case group consumed lower plant protein compared to control group ($P<0.05$).

Logistic regression analysis: **Table 3** shows the odds ratios for the biliary stone disease for male, female and both sexes together in four models. In the crude model, there was a significant relationship between biliary stone disease and animal protein intake for males (OR: 1.03, 95% CI=1.01-1.05) and both sexes together (OR: 1.01, 95% CI=1.00 -1.01). In fact, patients with higher consumption of animal protein intake were significantly more likely to have the biliary stone disease. In model 2 (adjusted for energy intake and physical activity), model 3 (further controlled for BMI and history of diabetes), and model 4 (adjusted for dietary cholesterol and dietary fiber), these relationships were significant for males, females, and both sexes.

In the case of the plant protein intake, there was a significant relationship between biliary stone disease and plant protein intake for males in crude model (OR: 0.98, 95% CI=0.96-0.99) and model 2 (OR: 0.96, 95% CI=0.92-0.99). After adjustment for BMI and history of diabetes in model 3 and dietary cholesterol and dietary fiber in model 4, this relationship was not significant. For females and both sexes, although there was no significant relationship in crude and model 2, there was a significant relationship between biliary stone disease and plant protein intake in model 3 and model 4. Individuals with higher consumption of plant protein intake were significantly less likely to have the biliary stone disease (for females: OR: 0.94, 95% CI=0.89-0.99, for both sexes: OR: 0.96, 95% CI=0.93-0.99).

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Table 1. Baseline characteristics and dietary intakes of study participants based on the patients with biliary stone disease and control group.

Variables	Cases (n=110)	Controls (n=230)	P-value[a]
Age (year)	57.66 ± 16.39[b]	56.00 ± 10.64	0.072
Body mass index (kg/m^2)	27.04 ± 5.46	26.70 ± 4.01	0.884
Physical activity (MET)	29.47 ± 3.33	40.00 ± 9.35	<0.001
Total energy (kcal)	2448.28 ± 644.49	2302.27 ± 577.74	0.034
Carbohydrate (% of total energy)	48.27 ± 8.39	49.00 ± 6.67	0.079
Fat (% of total energy)	41.53 ± 9.02	40.91 ± 6.97	0.682
Protein (% of total energy)	12.59 ± 2.62	13.19 ± 2.12	0.004
Dietary cholesterol (mg/d)	236.99 ± 99.75	203.30 ± 89.65	0.001
Saturated fat (g/d)	25.49 ± 8.91	24.42 ± 5.76	0.142
Monounsaturated fat (g/d)	29.22 ± 12.69	29.74 ± 8.48	0.376
Polyunsaturated fat (g/d)	21.95 ± 9.75	22.79 ± 6.67	0.201
Dietary fiber (g/d)	36.08 ± 21.08	40.33 ± 11.21	0.001
Male	53 (48.2)[c]	129 (56.1)	0.172
Drank alcohol in past year	6 (5.2)	5 (2.2)	0.190
Diabetes type 2	18 (16.4)	20 (8.7)	0.036
Current smokers	29 (26.4)	41 (17.8)	0.069

[a]: Student *t-test* for quantitative variables and Chi-square *test* for qualitative variables. [b]: Mean ± SD; [c]: N (%); Dietary intakes (except total energy) were adjusted for total energy intake. **MET**: Metabolic equivalent task.

Table 2. Animal and plant protein dietary intakes of study participants based on the patients with biliary stone disease and control group.

Protein intake protein	Males		P-value[a]	Females		P-value[a]
	Cases (n=53)	Controls (n=129)		Cases (n=101)	Controls (n=57)	
Animal (g/day)	52.12 ± 34.84	40.37 ± 14.16	0.02	38.43 ±19.51	39.45 ± 14.79	0.32
Plant (g/day)	28.44 ± 36.19	38.56 ± 15.39	0.03	34.01 ± 21.35	32.97 ± 12.38	0.54

[a]: Student *t-test*

Table 3. Odds ratios of biliary stone disease for animal and plant protein intake a

	Males		Females		Both sexes	
Animal protein intake	OR	95% CI	OR	95% CI	OR	95% CI
Crude	1.03*	1.01- 1.05	0.99	0.98 - 1.02	1.01*	1.00 - 1.02
Model 2	1.04*	1.01 - 1.08	1.04*	1.01 - 1.08	1.03*	1.01 - 1.06
Model 3	1.04*	1.00 - 1.08	1.04*	1.00 - 1.08	1.03*	1.01 - 1.06
Model 4	1.05*	1.01 - 1.10	1.07*	1.01 - 1.13	1.05**	1.02 -1.08
Plant protein intake						
Crude	0.98*	0.96 - 0.99	1.00	0.98 -1.02	0.99	0.98 -1.01
Model 2	0.96*	0.92 - 0.99	0.94*	0.89 - 0.98	0.96*	0.94 - 0.99
Model 3	0.96	0.92 - 1.01	0.93*	0.88 - 0.98	0.96*	0.94 - 0.99
Model 4	0.96	0.92 - 1.01	0.94*	0.89 - 0.99	0.96*	0.93 - 0.99

A: Crude model; **B**: Model 2, multivariate adjusted for energy intake and physical activity; **C**: Model 3, further controlled for, BMI and history of diabetes; **D**: Model 4, additionally adjusted for dietary cholesterol and dietary fiber. Data are presented as the odds ratio (95%CI). (* significant P value<0.05, ** significant P value<0.01)

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Discussion

In this study, the relationship of nutritional habits, physical activity and protein consumption, and the incidence rate of biliary stones were investigated. Lower rate of physical activity, lower protein intake, fiber intake, and dietary cholesterol were associated with the higher risk of biliary stone. In addition, energy intake, and the rate of type 2 diabetes were higher in the biliary stone patients.

Studies examining the differences between animal protein vs. plant protein intake and biliary stones are conflicting. Primary observational studies including some case-control studies (Misciagna *et al.*, 1999, Pixley and Mann, 1988) and a prospective cohort study have shown total protein intake and biliary stones relationships, but not specific analyses for vegetable versus animal protein (Attili *et al.*, 1998). However, some previous studies have reported that plant-based protein may be more beneficial properties for decreasing the rate of biliary stones. For example, Maclure *et al.* conducted a prospective cohort of 88,837 women aged 34–59 years. Their analysis was energy-adjusted, but established risk factors were not included as covariates (Maclure *et al.*, 1989). According to their findings, biliary stones were less frequently among normal weight women (BMI < 25 kg/m^2) in the highest quintile of vegetable protein intake (≥ 20.5 g/d). In contrast, in a case-control study, Mathew *et al*. showed no associations between vegetable or animal protein intake and biliary stones (Mathew and Ko, 2015).

It is worth mentioning that, in this study, male case subjects significantly consumed higher amount of animal protein and lower amount of plant protein compared to control subjects, which led to high risk of biliary stones. In general, many studies have reported that women showed higher risk for biliary stone disease than men. However, this increased risk is dependent on many factors such as the number of pregnancies and sex hormones (Novacek, 2006, Sun *et al.*, 2009). Increased biliary cholesterol secretion can be brought about estrogen hormone, leading to super saturation of bile. A possible explanation for this finding of the present study may be that the main reason for higher rate of biliary stone among women is not related to high intake of animal protein. However, increased animal protein consumption can be considered as one of the main risk factors for biliary stone diseased among men.

The results of the present study must be considered in the larger context of several dietary and lifestyle risk factors for biliary stones. For example, the increased risk of biliary stones following animal-based protein may be related to exposures to other animal-based ingredients such as dietary saturated fat and cholesterol that are reflected in an animal-versus vegetable-rich diet. It is known that plant-based diets are associated with the lower intake of dietary triglycerides and cholesterol than animal-based diets (Lander *et al.*, 2016). Cholesterol stones, constituting as high as 80% of all gallstones (Reshetnyak, 2012), occurs when cholesterol formation continually exceeds the solubilizing capacity of bile (Lander *et al.*, 2016). Dietary saturated fatty acids increase cholesterol synthesis (Glatz and Katan, 1993). Therefore, higher intake of animal protein containing higher amount of cholesterol and saturated fats may elevate cholesterol to levels beyond bile solubilization capacity, a known pathogenesis of cholesterol gallstones (Feranchak *et al.*, 2007).

It has been observed that the dietary patterns have an effect on biliary stone formation; in the study of Jessri *et al.*, the rate of biliary stone was significantly lower in the individuals with healthy nutrition in comparison to unhealthy nutrition (Jessri and Rashidkhani, 2015). In addition, in the study of Goktas *et al*., the rate of biliary stone was significantly higher in patients who consumed liquid oil and did not consume milk (Goktas *et al.*, 2016). Furthermore, Ortega *et al.* suggested that the prevalence of biliary stone is significantly higher in patients with high energy intake, high intake of fat, low fiber intake, and low rate of physical activity (Ortega *et al.*, 1997). Moreover, in the study of Park *et al*., the rate of cholesterol biliary stone had a direct association with the high intake of lipid, meat, and fried food (Park *et al.*,

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2017). These results indicate the role of dietary pattern in the formation of biliary stone which is in line with the current study.

High animal protein intake significantly increased the biliary stone incidence in male sex in crude model, which remained significant after adjustment for energy intake, physical activity, BMI, history of diabetes, dietary cholesterol, and dietary fiber. However, high plant protein consumption led to significantly reduced rate of biliary stone in men in crude model and after adjustment for energy intake and physical activity.

In clinical investigations, high plasma level of triglyceride and low plasma level of HDL-C are correlated with higher risk of biliary stones (Méndez-Sánchez *et al.*, 2007). In addition, proteins have an effective role in biliary stones prevention by increasing the HDL-C and decreasing triglyceride and body fat (Kritchevsky and Klurfeld, 1983, Parker *et al.*, 2002, Zhang *et al.*, 2005). This effect of proteins has been suggested by many animal studies. Ozban *et al.* suggested that the proteins in soya bean decrease the cholesterol level and have a negative effect on biliary stones formation (Ozben, 1989); moreover, Kritchevsky et al. demonstrated similar effect of vegetable protein on biliary stone formation (Kritchevsky and Klurfeld, 1983). The effect of plant protein and animal protein are different in the formation of biliary stones (Tsai *et al.*, 2004). The possible reason for this difference may be related to the significant effect of the plant protein on the serum concentration of triglyceride (Anderson *et al.*, 1995). The current study results suggest that the vegetable proteins in men are associated with lower risk of biliary stone compared to animal proteins. These results are aligned with previous studies. Lander et al. suggested that plant proteins are significantly related to lower risk of gallstone disease in post-menopausal female (Lander *et al.*, 2016); moreover, in the study of Tsai et al. the risk of cholecystectomy due to biliary stone was inversely associated with vegetable proteins intake (Tsai *et al.*, 2004).

This study has some limitations. Firstly, we designed a case-control approach which led to the inability to figure out the causal relationship between plant and animal protein consumption and biliary stone disease. Moreover, although family history is an obvious risk factor for biliary stone diseases, we did not collect any information of family history of biliary stone diseases. Moreover, the protein content of the food might be affected by some other factors like food preservation, agriculture conditions, etc. The other limitation of this study is the geographical issue; we evaluated the patients who were in Tehran, the capital of Iran. It will be better to conduct feature study as an epidemiologic evaluation with larger samples.

Despite these limitations, the present study has several strengths; this study was among the few studies to assess the association of protein intake with the risk of biliary stone among Iranian individuals. Previous related studies evaluated just gallstone patients, but this was the first evaluation of biliary stone (gallstone and CBD stone and history of last 6 months cholecystectomy). Furthermore, interview bias was declined, as the data was collected by the same interviewer. To decrease the recall bias that might have occurred regarding the frequency of food consumption and quantification of this questionnaire, all case subjects were recently diagnosed (within 6 months).

Conclusion

These results suggest that dietary habit is associated with biliary stone formation. The consumed type of proteins has a positive or negative effect on the formation of biliary stones. In this study, it was shown that the vegetable protein intake in male can act as a healthy contributor in biliary stone diseases, while the animal protein consumption positively affects biliary stone formation and this can be one of the major risk factors among men compared to women.

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whom this study was impossible.

Authors' contributions

K. Naseri, F. Pashayee-Khamene, and H. Asadzadeh-Aghdaei designed the research; S.R Sobhani, M.A Shahrbaf, and K. Esmaeilinejad conducted the research; F. Hosseini-Oskuiee and S. Hadavi analyzed the data; A. Hekmatdoost, A. Sadeghi, and M. Nazari wrote the paper. S. Saadati had primary responsibility for final content. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest to declare.

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