

Research Article

Consumption of heme iron: A major factor in pigment gallstone formation

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Abstract

Objective: To study the various risk factors responsible for pigment gallstone formation. As there is not any reported medicine which can dissolve pigmented gallstone.

Design: Study of 526 cases of cholelithiasis and 526 control cases was done. Query forms were filled with various risk factors like age, gender, BMI, diabetes, genetics, diet, smoking, etc. Collected data and hypothesis were statistically analysed. ICP-OES was done to know the elementary composition of gallstone.

Statistical Analysis: Univariate and multivariate were done using SPSS to conclude the risk factors for gallstone disease. Chi-square was applied for the relation between types of stone and age while ANOVA and post hoc ANNOVA, Least significant difference (LSD) were use for the analysis of variance between prevalence of different types of stone with risk factors.

Results: In our study, non-veg diet and ground water rich in iron beyond the permissible limit, as a drinking source were at higher risk factor for the gallstone disease ($p < 0.0001$). Non-veg diet as a rich source of heme iron and supplementation of iron from drinking water source may led to pigmented gallstone formation.

Conclusion: Controlled dietary habit and use of supply water may reduce the chance for pigmented gallstones.

Keywords: Heme iron, pigmented gallstone, bilirubin, gallstone disease

1. Introduction

Gallstone is the most common digestive disease after gastro-oesophageal reflux disease¹. It occurs mainly due to imbalance between biliary pronucleating and antinucleating proteins². Gallstones can be asymptomatic or symptomatic. Asymptomatic remains silent^{3,4} but in symptomatic condition, stones block the passage of bile into the intestine, which developed various symptoms in different cases, like right hypochondrium pain or epigastric pain⁵, fat intolerance^{6,7}, nausea or vomiting⁸ and jaundice⁹. Symptomatic gallstone necessitates surgical removal of gallbladder^{10,11} known as cholecystectomy. In US, seven lakhs of cholecystectomies were performed annually and this incurs medical expenses of \$ 6.5 million¹².

Epidemiology of different types of gallstone formation may depend on demographic characteristics and social customs¹³, also the incidence and composition of gallstone changes with time¹⁴. So it is necessary to revisit the types of gallstone from time to time in different geographical regions. Diet^{7,15,16}, age^{17,18}, gender^{19,20}, rapid weight loss and obesity²¹, genetics^{22,23} are the various risk factors for gallstone formation. Pigmented gallstone is funnelled by diseases which effect the bile composition like Crohn disease²⁴, liver cirrhosis²⁵, Gilbert's syndrome^{26,27}.

Dietary factors are seen to be more fluctuating with the social customs and geographical areas. For example in South India, use of Malabar tamarind (*Garcinia camborgrinia*) is associated with prevalence of pigmented gallstone^{13,28}, whereas fat rich diet with predominance of cholesterol gallstone in North India^{29,30}. Even the elementary composition of gallstones changes with demographical characteristics. A study in Erbil (Iraq) reflects a strong relationship between the Calcium content in drinking water in Erbil and that in gallstones of the patients from the same location³¹. This indicates that the main source of Ca in gallstones comes from the water that people drink³².

Effect of regional factors on the types of gallstone rooted the epidemiological study of gallstone formation in each geographical region. In Jharkhand region (Eastern India) we are the first to deal with the epidemiology of different types of gallstones. Here, in Jharkhand, cases of gallstone constitute 67.2% of different types of stones like renal stone, ureteric stone and bladder stone³³. This high prevalence rate of gallstone intrigued us to study its epidemiology which is not yet conducted. In our study we found non-veg diet (rich in heme iron) as well as drinking water containing iron beyond the permissible limit (0.3ppm-1ppm) as a risk factor for pigmented gallstone. Pigmented gallstone predominant over cholesterol and mixed gallstones in the Jharkhand region of India, another concerned serious matter. In case of cholesterol gallstone patients bile acid as oral treatment can be given but it is limited to the cholesterol gallstone that too effective in 8-10% of the population.

2. Materials and Methods

2.1 Sample Collection

Case control studies of 1052 cases were done. Of these 526 cases were of cholelithiasis and 526 (age- 25-55yrs) non-gallstones, who were taken as control. Gallstones of the patients were collected, washed with normal saline and air dried. Gallstones were classified on the basis of their cross section and morphology³⁴ into three categories: Cholesterol stones, pigmented stones and mixed stones.

2.2 Measures for probing risk factors for gallstone formation:

Query form of the gallstone patients were filled which included age, gender, weight, height, source of drinking water, dietary habit, genetic relation, sugar level or diabetic status and smoking habit etc. BMI (Body Mass Index) were calculated as weight and height ratio.

2.3 Collection of data of Drinking Water

Data of elementary constituents of ground water of 21 blocks of Jharkhand, India were collected from State Level Water Laboratory, Drinking Water and Sanitation Department, Ranchi, Jharkhand.

2.4 Elementary analysis of Gallstone

Elementary analysis of human gallstones was determined by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy). This is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the samples. For the elementary analysis, 0.2 gm of gallstones were digested with a mixture of HNO₃ (5 ml) and H₂O₂ (3 ml) in teflon stub³⁵. After digestion the samples were diluted to 100 ml with deionised water. Then the samples were subjected to ICP-OES.

2.5 Statistical analysis

Univariate analysis as well as multivariate analysis was used to correlate between independent variants with the prevalence of gallstone disease. Chi-square applied for the relation between types of stone and age while ANOVA and post hoc ANNOVA Least significant difference (LSD) is use for the analysis of variance between prevalence of different types of stone with non-veg diet and source of drinking water. For all the analysis SPSS 16 software was used.

3. Results

The study included 526 gallstone patients and 526 non-gallstone persons. Out of 526 patients 384 were female and 142 were male. Females were 2.7 times more prone to gallstone. Out of 526 cases dealt, only 459 gallstones were collected. Rest of the 67 stone were either sucked along the suction pressure during surgery or fully crushed. Out of these, pigmented gallstones (238/459) were predominant followed by Cholesterol (104/459) and Mixed gallstones (117/459). Though pure cholesterol gallstones were not seen, as some pigmentation were observed in their inner surface as shown in Figure: 1. Like others study dietary factors were found dominating one in Jharkhand region also. Individuals having non-veg diet (O.R=2.3204-3.8717; $p<0.0001$) and ground water (O.R=2.1345; 1.6677-2.7320; $p<0.0001$) as a drinking water source were at higher risk. The Sources of drinking water were also seen to be related with the chemical constituent of gallstones. According to the data from Level Water Laboratory, Drinking Water and Sanitation Department, Ranchi, Jharkhand of 21 blocks of Jharkhand, shows that the iron content of ground water of Jharkhand is very high beyond the permissible limit (0.3ppm-1ppm). Mean \pm SD iron content of ground water of these 21 blocks were found to be 2.831 \pm 0.5 ppm and the ICP-OES (Perkin Elmer, USA; Optical 2100DV) results of pigmented and Cholesterol gallstones shows the mean \pm SD iron content of 0.83 \pm 0.12 ppm and 0.514 \pm 0.09 respectively. Pigmentations in cholesterol (Figure: 1) gallstone may be due to presence of iron which may get absorbed from drinking water source ($F=22.29$; $p<0.0001$; Chol vs Pigmented and mixed; $p<0.0001$). Average BMI for diseased and control were 22.34 and 21.57 respectively, which is a normal range as per WHO (World Health Organization) Expert Committee. Thus BMI was not an influencing factor for gallstone formation in our study. Consumption of beverages like tea, coffee or combination of tea and coffee by gallstone patient and control cases were also compared. 293, 121 and 147 patients were having tea, coffee or combination of both respectively in their routine life while 302, 115 and 153 control cases were having tea, coffee or combination of both respectively.

Figure 1: Pigmentation in the inner surface of gallstone.



4. Discussions

Previous studies stated BMI³⁶, ageing¹⁷, diet⁷, Gender¹⁹ are the major risk factors for gallstone formation. Whereas diabetes, genetics, smoking, beverages like tea and coffee are still controversial³⁷. Of these factors, diets have major effect in influencing the types of gallstones as well as renal gallstone³⁸ formation. Additionally the composition of gallstones is greatly affected by the elementary constituents of the sources of

drinking water. Shareef et al, 2008 found higher concentration of calcium in gallstone as well as in the source of drinking water in Erbil (Iraq)³⁹. In our case based study also, non-vegetarian taking ground water as drinking water sources are at higher risk factor with types of gallstones on univariate (Table: 1) as well as multivariate analysis (Table: 2). From our study we found that 72% of the cases were regularly having non-vegetarian diet. Non-vegetarian food (meat, poultry and fish) are the good source of heme form of iron^{40,41} which is greatly associated with the gallstone disease in comparison to nonheme iron⁴². Heme iron breakdown to form bilirubin⁴³ which precipitates to initiate pigmented gallstone formation. The gallstone sample collected by us also shows the variance of pigmented gallstone with relation to non-veg diet as shown in Table 3.

Table: 1 Incidence of gallstone cases with variable risk factors (Univariate analysis).

Factors affecting gallstone formation	No. of cases out of 526	Control out of 526	Odd ratio (95% CI)	Chi-square	p-value
Non-vegetarian	378	242	2.9973 (2.3204- 3.8717)	72.65	<0.0001
Ground water	331	233	2.1345(1.6677- 2.7320)	36.71	<0.0001
Fat rich diet	225	297	0.5789 (0.4536 to 0.7388)	19.42	<0.0001
Diabetes	25	209	1.0355 (0.7681 to 1.3959)	0.57	0.8192
Genetic	48	63	0.7380 (0.4963- 1.0973)	2.27	0.1333
Smoking (male)*	116	141	0.7725 (0.5825-1.0245)	15.01	0.0732
Tea	293	302	0.9327 (0.7309-1.1903)	1.84	0.5756
Coffee	121	115	1.0678 (0.7991-1.4267)	5.09	0.6575
Combination of coffee and tea	147	153	0.4600 (0.3555-0.5953)	0.6	0.0001

*None of the females either experimental or control were found to be addicted of smoking.

Table: 2 Incidence of gallstone cases with variable risk factors (Multivariate Logistic Analysis).

Variable Risk Factors	Coefficient	Std. Error	Adjusted Odd Ratio at 95% CI	p-value
Non-vegetarian	0.896	0.220	2.45 (1.593-3.767)	<0.0001
Ground water as a drinking source	0.758	0.126	2.135(1.668-2.732)	<0.0001
Fat rich diet	-0.551	0.125	0.576 (0.452-0.736)	<0.0001
Diabetes	0.35	0.152	1.035 (0.768-1.396)	0.819
Genetic	-0.304	0.202	0.738 (0.496-1.097)	0.133
Smoking	-0.258	0.144	0.772 (0.583-1.025)	0.073
Tea	-0.046	0.124	0.955 (0.748-1.218)	0.709
Coffee	0.066	0.148	1.068 (0.799-1.427)	0.657
Combination of coffee and tea	0.056	0.137	0.946 (0.723-1.236)	0.682

Table: 3- Prevalence of different types of gallstone with non-vegetarians and ground water users and mean age.

Types of gallstone	No. of cases	Cases in non-vegetarian	ANOVA	Cases in Ground water user	ANOVA	Mean age±SE	Chi-square
Cholesterol stone	104	63	F=16.573; LSD- Pigmented vs Cholesterol & mixed; p<0.0001	28	F=22.288; LSD- Cholesterol vs Pigmented & Mixed; p<0.0001	43.5±1.2	$\chi^2=6.00$; p=0.199
Pigmented stone	238	195		153		31.14±0.5	
Mixed stone	117	66		58		45.14±1.7	

Though patients were also taking non-heme iron in their diet but supplementation of iron abate the absorption of non-heme iron⁴⁴. The source of drinking water was supplementing the iron in those patients. According to the data from Level Water Laboratory, Drinking Water and Sanitation Department, Ranchi, Jharkhand, mean iron content of ground water was 2.831 ± 0.5 ppm which is far higher than the permissible limit (.3ppm- 1ppm). Such a high content of body iron is regulated primarily by absorption, since human have no physiological mechanism by which excess iron is excreted. Excess of iron remains within shed intestinal cells, in bile, urine and even in small amount in sweat, nails and hairs⁴⁵. Accumulation of iron in bile may expedite the iron content in gallstones. ICP-OES result also show the presence of iron in gallstones. The mean iron content was found to be 0.514 ± 0.09 ppm, 0.72 ± 0.1 and 0.83 ± 0.12 ppm in cholesterol, mixed and pigmented stones respectively. This proportion of iron in cholesterol stone is because of some pigmentation on their inner surface as shown in Figure: 1 which may be from the drinking water source (p<0.0001).

With increasing age absorption of iron decreases, the increased iron is accumulate in the bile, may expedite the pigmented gallstone in higher age group but no such relation with the types of stone were seen biostatistically in our study ($\chi^2=6$; p=0.199). This study is contradicted by Diehl et al, 1995, who found cholesterol stone in the patients with age below 40yrs and pigmented stone in the age of more than 40yrs⁴⁶. Also Kurtin et al, 2000 stated that the cholesterol content of stone steadily decreases after the age of 50⁴⁷. Genetic is related with gallstone disease, as its frequency varies between ethnicities. But in this study we have not found such an association with gallstone formation (p=0.1333). Likewise, cigarette smokers have lower level of HDL in comparison to non-smokers, a risk factor for gallstone formation in some studied^{48,49} but not in all^{18,37} which is supporting our analysis (p=0.0732).

5. Conclusions

Use of heme iron rich diet and high concentration of iron in drinking water may predispose pigmented stone formation.

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