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Research Article

Serum estrogen level and lipid profile in Gall stone and Gallbladder cancer: a case control study

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Abstract

Background: Carcinoma of the gallbladder is a highly fatal disease with late diagnosis, limited treatment options and deprived prognosis. It is the fifth most common cancer of gastrointestinal tract and the most common cause of death from biliary malignancies. Gallstones and gallbladder cancer predominate in females and are associated with obesity and multiple pregnancies.In postmenopausal women, hormone replacement therapy significantly increases the risk of gallbladder diseases.In the present study, we have estimated the Level of Estradiol and Lipid profile in different study groups and healthy controls from northern India.

Results: The estradiol level was significantly higher ($p<0.001$) among patients with both of gall bladder cancer & stone, gallbladder stone, gall bladder cancer compared with controls. The lipid profile and estradiol levels were almost similar among males in both study and control group while significantly different among females.

Conclusions: These results indicate that the female gender is strongly associated with the disease and the possible reason is increased level of total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein and decreased level of high density lipoprotein, and also the higher level of estrogen.

Keywords: Gallbladder stone, Gallbladder cancer, Estrogen, Lipid Profile

1. Introduction

Carcinoma of the gallbladder is a highly fatal disease with late diagnosis, limited treatment options and deprived prognosis.[1] Gallbladder cancer is the fifth most common cancer of gastrointestinal tract and the most common cause of death from biliary malignancies.[2] The Indian Council of Medical Research Cancer Registry has recorded an incidence of 4.5 and 10.1 per 100,000 males and females respectively in the northern parts of India, and 1.2 per 100,000 population in females in southern parts of India.[3]

Moreover, a plethora of epidemiological studies has shown strong association of gallbladder cancer with cholesterol gallstone disease. [4] Gallstone formation is noticeably multifactorial and for any individual, some risk factors are irreversible, such as advancing age, being female, genetic factors and ethnicity. Other factors can be modified, such as obesity, rapid weight loss, diet and drugs.[5]

Development of cholelithiasis has been associated with the use of oral contraceptive drugs. Furthermore, gallstones are more frequently observed in women and particularly more common in multiparous females[6] Cholesterol crystals are glued together by bile proteins to make gallstones[7-10]. Cholesterol precipitation results from an imbalance of these three components in bile; cholesterol, bile salts and phospholipids. These changes in bile composition are closely related to the disorders of lipid metabolism in liver.[11].

In postmenopausal women, hormone replacement therapy significantly increases the risk of gallbladder diseases[12,13] suggesting a noteworthy role of sex hormones in the etiology of gallbladder cancer[14-18]. The sex hormone estrogen is a collective term for the naturally occurring female hormones estradiol, estriol, and estrone. In females, estrogen is important in the development of secondary sexual characteristics, in the regulation of the menstrual cycle, and in pregnancy[19]. Estrogen derivatives of estrone (E1), estradiol (E2), and estriol (E3), the C18 steroids are derived from cholesterol. Cholesterol is taken up by steroidogenic cells, stored, and moved in to the site of steroid synthesis[20]. The different steroids are formed by reduction in the number of carbon atoms from 27 to 18 [21].

Gallstones and gallbladder cancer predominate in females and are associated with obesity and multiple pregnancies. Conditions related to higher levels of estrogens, suggesting that endogenous estrogens are involved in the pathogenesis of these conditions by altering bile acid composition and gallbladder motility.Although the mechanism underlying this association is still unclear.[22-24].

In the present study, we have estimated the level of estradiol and Lipid profile in different study groups and healthy controls from northern India, where the incidence of carcinoma gallbladder is one of the highest in the world.

2. Material and Method

This is a population based case control study, conducted in the department of Physiology with the collaboration of department of General Surgery, Gastroenterology and Pathology at King George's Medical University, Uttar Pradesh, Lucknow, India. Total 300 subjects were enrolled in the study on the basis of well-defined inclusion and exclusion criteria from the Outdoor Patients Department (O.P.D) of General Surgery and Gastroenterology, King George's Medical University, Uttar Pradesh, Lucknow, India. Out of 300 subjects, 150 subjects were in study group and 150 subjects were in control group. The confirmed diagnosed cases of Gallbladder cancer as well as stone were taken from ward (admitted) of general surgery and gastroenterology department. The subjects were excluded from the study with condition which may affect the level of estrogen and lipid such as metabolic syndrome, polycystic ovarian syndrome, coronary artery disease and negative history of gall bladder cancer as well as stone. Furthermore the study group was again divided in 3 sub-groups, subjects with cancer (62), subjects with Stone (80) and subjects with cancer and stones both (8).

2.1 Biochemical Analysis

After taking the ethical approval from institutional ethical committee of King George's Medical University, U.P., Lucknow, India and obtaining informed consent, total 3 ml. venous blood sample was drawn from each participant. Serum was separated, aliquoted and stored at -80° C. Estimation of Total cholesterol, Triglycerides and High density lipoprotein was done by using Merk kit with the help of semi-automated analyzer *(Microlab 300, Merck)* on the same day of sample collection. Low density lipoprotein and Very low density lipoprotein was calculated by the *Friedewald Formula* [Low Density Lipoprotein-Cholesterol = Total Cholesterol - (High Density Lipoprotein-Cholesterol + Very Low Density Lipoprotein-Cholesterol) & Very Low Density Lipoprotein-Cholesterol = Triglycerides/5][40]. Estimation of Serum Estradiol level was done by using commercially available ELISA Kit *(DRG Instruments, GmbH Germany)* with the help of Bio-Rad ELISA reader.

2.2 Statistical Analysis

The data collected was entered in Microsoft Excel computer program and checked for any inconsistency. The results were presented as mean (±) SD and percentages. The chi-square test was used to compare dichotomous/categorical variables among the groups. The one way analysis of variance (ANOVA) was used to compare the means among the groups with Tukey's pairwise comparison test for normally distributed variables. The Kruskall-Walis test was used to compare the non-normal variables among the groups. The p-value <0.05 was considered as significant. All the analysis was carried out by using SPSS 16.0 version.

3. Results

The age was similar among the patients of gall bladder cancer, stone, gall bladder cancer & stones both and controls groups. Majority were females in all the groups (Table-1). The Total Cholesterol was significantly ($p<0.001$) higher among gall bladder cancer patients as compared to controls. Total Cholesterol was significantly ($p<0.0001$) higher among patients with gall bladder cancer & stone both compared with gall bladder cancer and stone patients. Similar observation was found for Triglycerides, Low Density Lipoprotein and Very Low Density Lipoprotein. However, High Density Lipoprotein was significantly ($p<0.001$) lower among patients with gall bladder cancer & stone both than patients with gall bladder cancer, stone and healthy controls (Table-2). The estradiol level was significantly ($p<0.001$) higher among patients with gall bladder cancer & stone both, gallbladder stone, gall bladder cancer compared with controls (Fig.1). The lipid profile and estradiol levels were almost similar among males in both study and control group while significantly different among females (Fig.2).

Table-1: Age and sex distribution of the patients and controls

Age and gender	Gall Bladder Cancer (n=62)		Stone (n=80)		Gall Bladder Cancer + Stone (n=8)		Control (n=150)	
	No.	%	No.	%	No.	%	No.	%
Age in years								
<30	1	1.6	15	18.8	0	0.0	6	4.0
30-40	16	25.8	32	40.0	2	25.0	48	32.0
41-50	28	45.2	16	20.0	4	50.0	55	36.7
51-60	12	19.4	11	13.8	2	25.0	30	20.0
>60	5	8.1	6	7.5	0	0.0	11	7.3
Mean±SD*	47.32±10.19		44.33±11.92		46.50±5.42		45.53±10.12	
Gender**								
Male	2	3.2	4	5.0	1	12.5	20	13.3
Female	60	96.8	76	95.0	7	87.5	130	86.7

*p=0.08, **p=0.05

Table-2: Comparison of lipid profile among cases and controls

Lipid profile	Gall Bladder Cancer (n=62)	Stone (n=80)	Gall Bladder Cancer + Stone (n=8)	Control (n=150)	p-value
Total Cholesterol (TC)	187.55 ±11.02[1,2]	172.00 ±7.99[1,2]	202.72 ±40.80[1,2]	162.10 ±3.48[1,2]	0.03*
Triglycerides (TG)	186.07 ±14.82[1]	142.53 ±6.09[1]	241.99 ±59.37[1]	133.37 ±2.63[1]	0.04*
High Density Lipoprotein	17.67 ±1.19[1]	24.39 ±0.89[1,2]	15.34 ±3.57[2]	38.98 ±0.86[1,2]	<0.0001*
Low Density Lipoprotein	132.67 ±10.54[1]	119.10 ±7.83[1]	138.98 ±36.81[1]	96.45 ±3.47[1]	0.01*
Very Low Density Lipoprotein	37.21 ±2.96[1]	28.51 ±1.21[1]	48.40 ±11.87[1]	26.67 ±0.52[1]	0.04*
Estradiol (E2)	49.58 ±6.44[1,2]	61.06 ±5.80[1]	63.52 ±10.30[2]	39.85 ±2.99[1,2]	0.01*

[1] p=0.001, [2] p<0.0001 (Multiple comparison test), *Significant (Kruskall Walis test), values are in mean±SE

Fig.1: Comparison of estradiol (E2) among cases and controls

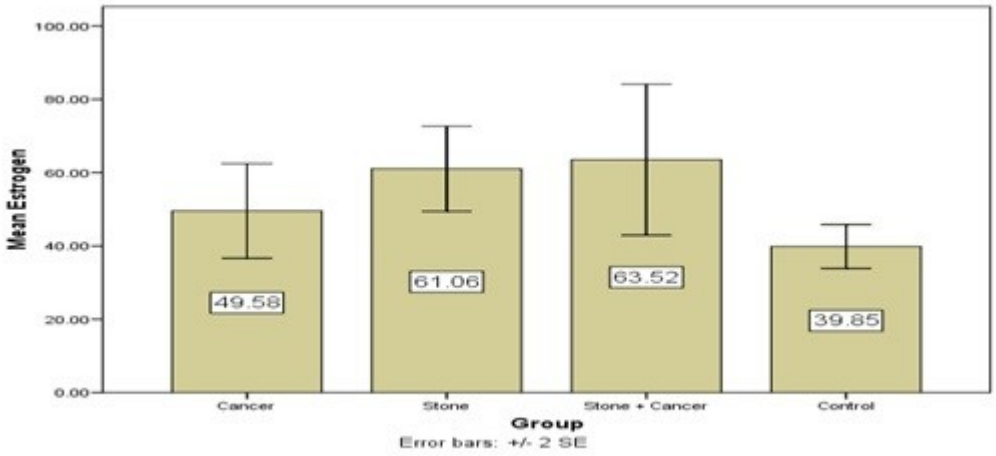
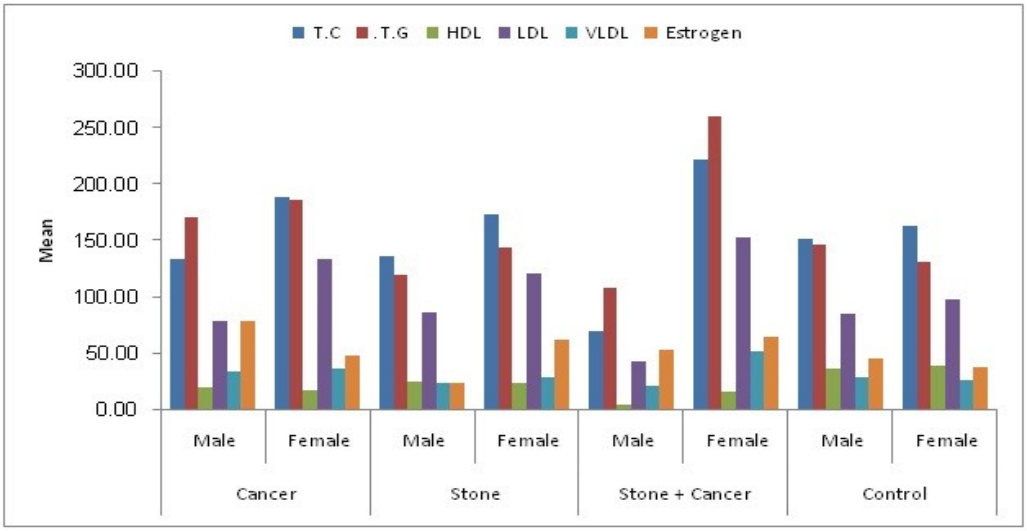


Fig.2: Comparison of lipid and estradiol level by gender



4. Discussion

There are several lines of evidence suggesting that the incidence of gallstones is related to female hormones and the disease is more frequent in women than in men[25], and supports our findings as most of the subjects were female with the age group of 41-50 years in present study.

Our findings suggest that the high serum level of triglyceride, total cholesterol, low density lipoprotein, very low density lipoprotein was strongly associated with gall bladder cancer and stone. The serum level of total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein was found significantly higher in all study group compared with control subjects. However high density lipoprotein was significantly lower in study group compared with control subjects. This finding was consistent with previous reports from different countries[26-30] while some could not find such relationship with gallbladder cancer[31,32]. The exact reason for this controversy was still unknown, but possibly it may be due to the differences in study populations, different ethnic group or region and different environmental factor. Interestingly, between the groups analysis the serum level of total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein was found significantly higher in subject with gall bladder cancer and stone both compared with cancer and stone patients. The high density lipoprotein was significantly lower in subjects with gall bladder cancer and stone both compared with cancer and stone patients. This suggests that altered serum lipid profile do play a significant role in the pathogenesis of gall bladder cancer with the development of gall bladder stone.

Although there was very few studies have been done with direct association of lipid profile and gall bladder cancer. As in our study, it was found that the increased level of total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein and decreased level of high density lipoprotein, significantly associated with gall bladder cancer. These associations were independent of other lipids examined in the study and were not confounded or modified by other demographic risk factors, including gallstones. Higher levels of triglycerides and lower levels of high density lipoprotein, has been reported in several other malignancies, including tumors of the colon, breast and prostate,[33-35] and has been implicated in gallbladder cancer etiology, due to its close relationships with gallstones, obesity, high-fat diet, and diabetes[36,37] which are also linked to gallbladder cancer[38, 39].

In present study, the serum estradiol level was significantly higher in all study groups compared to the controls. As per our literature survey there was very limited studies have been done in this direction to reveal the exact association of estrogen and gall bladder cancer[22-24]. We also found that the lipid profile and estradiol levels were almost similar among males and significantly different among females.

This study elucidates the impact of serum estrogen level and lipid profile in gallbladder cancer and gallstone for the first time in North India. Although it was multi factorial cause but as per our study findings, it may be concluded that the female gender is strongly associated with disease and the possible reason is increased level of total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein and decreased level of high density lipoprotein, and also the higher level of estrogen.

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