

## RESEARCH ARTICLE

# Association of Cytochrome-17 (MspA1) Gene Polymorphism with Risk of Gall Bladder Stones and Cancer in North India

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### Abstract

**Background:** Cholelithiasis is associated in 54%-98% of patients with carcinoma of the gallbladder, and a high incidence among females suggests a role of female hormones in the etiology of the disease. Cytochrome P450C17 $\alpha$  (CYP-17) is a key enzyme involved in estrogen metabolism and polymorphisms in CYP-17 are associated with altered serum levels of estrogens. Thus, we investigated whether the CYP-17 MspA1 gene polymorphism might impact on risk of gall bladder cancers or gallstones, as well as to determine if this gene polymorphism might be linked with estrogen serum levels and lipid profile among the North Indian gall bladder cancer or gallstone patients. **Materials and Methods:** CYP-17 gene polymorphisms (MspA1) were genotyped with PCR-RFLP in cancer patients (n=96), stone patients (n=102), cancer + stone patients (n=52) and age/sex matched control subjects (n= 256). Lipid profile was estimated using a commercial kit and serum estrogen was measured using ELISA. **Results:** The majority of the patients in all groups were females. The lipid profile and estrogen level were significantly higher among the study as compared to control groups. The frequency of mutant allele A2 of CYP17 MspA1 gene polymorphism was higher among cancer (OR=5.13, 95% CI=3.10-8.51, p=0.0001), stone (OR=5.69, 95% CI=3.46-9.37, p=0.0001) and cancer + stone (OR=3.54, 95% CI=1.90-6.60, p=0.0001) when compared with the control group. However there was no significant association between genotypes of CYP17 MspA1 gene polymorphism and circulating serum level of estrogen and lipid profile. **Conclusions:** A higher frequency of mutant genotype A1A2 as well as mutant allele A2 of CYP-17 gene polymorphism is significantly associated with risk of gallbladder cancer and stones. Elevated levels of estrogen and an altered lipid profile can be used as predictors of gall bladder stones and cancer in post menopausal females in India.

**Keywords:** Gallbladder cancer - gallbladder stone - CYP-17 gene polymorphism - estrogen - lipid profile

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### Introduction

Gall bladder cancer (GBC) is a rare neoplasm with varying demographic distribution in different parts of the world and moreover, it is common in the population of Chile, Japan, Peru and Korea (Curado et al., 2007). Carcinoma of gall bladder is the fifth most common cancer of gastrointestinal tract and the most common cause of death from biliary malignancies (Khan et al., 2010; Pavlidis et al., 2012). In Indian population the incidence of gall bladder cancer shows the varying geographic distribution, as the incidence of gall bladder cancer is much higher in Delhi population (North India) in comparison of South Indian population. 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 (National Cancer Registry Programme, 2001).

Various epidemiological studies conducted in different countries suggesting that the gallstone formation is closely related to the carcinoma of gall bladder (Hart et al., 1971; Diel, 1980; 1983; Zatonski et al., 1992). Cholelithiasis is associated in 54%-98% of patients with carcinoma of the gallbladder, (Kumar et al., 2009; Cha et al., 2014) and a high incidence among females suggest a role of female hormones in the etiology of the disease (Castro et al., 2013). The highest frequency of the disease is found among females over the age of 65 years (Tada et al., 1990). The higher rate of gallbladder cancer in women has been attributed to such hormonal factors as high parity, early age at first full-term birth, oral contraceptive use, hormone replacement therapy and obesity via increased estrogen levels (Hsing et al., 2006). Service et al. (1998) has been suggested that estrogen might be a complete carcinogen and capable of causing genetic alterations as well as tumor initiation (Service et al., 1998). The estrogen acts on its target tissue by binding itself to its receptor

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ESR1 and ESR2, which has been detected in different tissue including biliary tract (Nakamura et al., 1989; Hewitt et al., 2000). In females, estrogen is important in the development of secondary sexual characteristics in the regulation of the menstrual cycle, and in pregnancy (Report on Carcinogens, 2011). 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 (Scallen et al., 1985).

Zuber et al. (1986) has been reported that the cytochrome P450C17 $\alpha$  (CYP-17) is a key enzyme involved in estrogen metabolism by mediating both steroid 17  $\alpha$ -hydroxylase and 17, 20-lyase activity and encoded by P450c17  $\alpha$  (CYP-17) gene (Zuber et al., 1986). The human CYP-17 gene located on chromosome 10 q 24.3, spans 6,569 bp, and is divided into eight exons (Picado et al., 1987). A single nucleotide polymorphism (T to C substitution) has been first time identified by Carey et al. (1994) at 34 base pair upstream of the initiation of translation but downstream from the transcription start site in the 5' promoter region of CYP-17 gene. The T to C substitution (CCACT-CCACC) polymorphism may create a new additional Sp-1 promoter site (CCACC box) and introduces a restriction site for MspA1 restriction enzyme (Carey et al., 1994). The T allele and C allele were reported as A1 and A2 alleles in the literature respectively. The A2 allele carrying women were associated with higher Serum levels of androgens and estrogens (Feigelson et al., 1998; Haiman et al., 1999). However some studies have reported contradictory findings, they suggested that the promoter activities and mRNA expression of CYP-17 do not differ significantly between A1 and A2 allele carriers, and no additional Sp1 transcription factor binding activity is created by carrying the A2 allele (Nedelcheva et al., 1999; Ambrosone et al., 2003; Miyoshi et al., 2003; Yilmaz et al., 2011). Previous epidemiologic studies suggested that the CYP-17 gene polymorphism in some, but not all, has been associated with hormonally related cancer including the breast, endometrium and prostate (Feigelson et al., 1997; Haiman et al., 2001; Ambrosone et al., 2003; El-Ezzi et al., 2014). The specific role of CYP-17 gene polymorphism in estrogen synthesis and metabolism is still unclear. Therefore we planned the study, to see the association of CYP-17 MspA1 gene polymorphism with risk of gall bladder cancers or gallstones, as well as, to see whether this gene polymorphism associated with estrogen serum level and lipid profile among the North Indian gall bladder cancers or gallstone patients.

## Materials and Methods

This is a population based case control study conducted in the department of Physiology and Biochemistry at King George's Medical University, Uttar Pradesh, Lucknow, India with the collaboration of department of Human Genetics at Sanjay Gandhi Post Graduate Institute, Uttar Pradesh, Lucknow, India. Prior ethical approval was taken from institute. Subjects were recruited with written informed consent from department of General Surgery, Gastroenterology, King George's Medical University,

Uttar Pradesh, Lucknow, India. Total 506 subjects (n=250 cases and n=256 control subjects) between the age group of 18 to 70 years were enrolled in study on the basis of well defined inclusion and exclusion criteria. The sample size was statistically calculated with 90% of power. Subjects having gall bladder cancer (histopathologically proven), gall bladder stone (confirmed by USG) and subjects having both cancer and stone were recruited as study group and controls were recruited from teaching/non teaching staff of the institute as well as from other outdoor patient department of institutes coming for minor medical and surgical problem. Subjects with conditions which may affect the level of estrogen and lipid such as polycystic ovary syndrome, thyroid dysfunctions, metabolic syndrome, pregnancy, chronic diseases, infection, and coronary artery disease were excluded. Further the study group was divided in 3 sub-groups, subjects with cancer (n=96), subjects with Stone (n=102) and subjects with cancer and stones both (n=52). Total 5 ml venous blood sample was collected from each subject and out of which 3 ml blood was separated in plain vial and rest 2 ml in EDTA vial. Serum was separated immediately, aliquot prepared and stored at -80°C till further analysis.

**Biochemical Analysis:** Estimation of total cholesterol (TC), triglycerides (TG) and High density lipoprotein (HDL) was carried out by using commercially available kit (Merk Specialities Pvt. Ltd.) with the help of semi automated analyzer (Microlab 300, Merck) on the same day of sample collection. Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) was calculated using the Friedewald Formula [LDL= TC-(HDL+VLDL) & VLDL= TG/5] (Friedewald et al., 1972). 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.

**Genotyping:** Genomic DNA was prepared from peripheral blood collected in EDTA vial using Quick-gDNA™ Blood MiniPrep kits (Zymo Research Corp) according to manufacturer's instructions. The CYP-17 MspA1 (rs.743572) polymorphism at nucleotide 27 (T27C) was detected by using polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP). The 209-bp DNA fragment in the 5' region of the CYP-17 gene was amplified from genomic DNA (100ng) with forward primer 5'-GGC TCC AGG AGA ATC TTT C-3' and reverse primer 5'-GGG CCA AAA CAA ATA AGC TA-3' (Hou et al., 2006) by using a thermal cyclar (Applied Biosciences) and Dream Taq™ Green PCR Master Mix (Fermentas). DNA was amplified with cycling conditions of 94°C for 5 min, 94°C for 1 min, 57°C for 1 min 45 sec, and 72°C for 45 sec for 30 cycles with final extension of 7 min at 72°C. The 209bp amplicon was digested with MspA1 restriction enzyme (Fermentas) to identify the T&C (A1 & A2) allele. The digested PCR product was analyzed by gel electrophoresis using 3% agarose gel. A1/A2 heterozygote mutant genotype shows three bands of 209 bp, 123 bp & 86 bp and A1/A1 homozygote wild genotype shows one band of 209 bp. (Figure 1). About 20% samples were randomly re-genotyped by other worker for validation of genotyping accuracy.

### Statistical analysis

The data collected was entered in Microsoft Excel computer program and checked for any inconsistency. The results are presented as mean±SD and percentages. The chi-square test was used to compare dichotomous/categorical variables among the groups and Hardy-Weinberg equilibrium testing. The one way analysis of variance (ANOVA) was used to compare the means among the groups with Tukey's pair wise comparison test for normally distributed variables. Kruskal-Wallis test was used to compare non-normal data. The multivariate binary logistic regression analysis was carried out to find the risk factors for cancer, stone, and cancer+stone patients compared with controls. All the statistical test were two tailed and p-value<0.05 was considered as significant. All the analysis was carried out by using SPSS 16.0 version

## Results

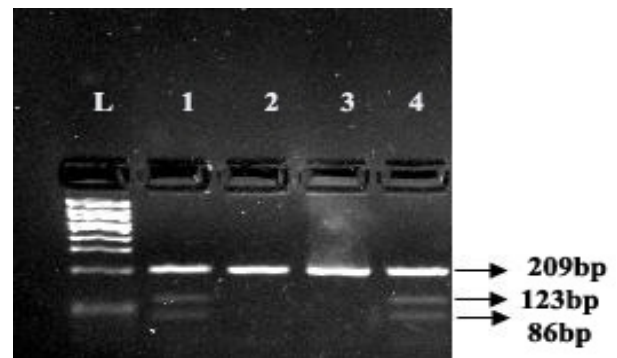
*In the present study, majority of the patients in all groups were females and between 31-50 years*

Lipid profile and estrogen level among the study and control group: The lipid profile and estrogen level were significantly different among the groups. The serum level of total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein, estrogen were significantly higher and the serum level of high density lipoprotein were significantly lower among all the study groups as compared with controls. (Table 1)

After the post-hoc comparison test of lipid and estrogen levels between the groups, there was no significant (p>0.05) difference between the Cancer and Stone group as well as Cancer and Cancer + Stone groups. The comparison between stone and cancer + stone group shows that the level of TC, TG, LDL, VLDL, estrogen was significantly (p=0.0001) higher and HDL was significantly lower among cancer+stone group. Similarly the comparison of cancer, stone and cancer+stone (all

study group) with the control group shows that the level of TC, TG, LDL, VLDL, estrogen was significantly higher and HDL was significantly lower among the all study groups. (Table 1)

Allele and genotype frequency of CYP-17 MspA1 gene polymorphism among the study and control group: Hardy-Weinberg expectations were fulfilled in control subjects, as we could not find any significant deviation (p=0.064). The frequency of heterozygous mutant genotype A1A2 was significantly higher among cancer (OR=5.13, 95%CI=3.10-8.51, p=0.0001), stone (OR=5.69, 95%CI=3.46-9.37, p=0.0001) and cancer + stone (OR=3.54, 95%CI=1.90-6.60, p=0.0001) as compared with controls. In present study we could not find the homozygous mutant genotype A2A2 in our population. The frequency of wild allele A1 was higher among cancer, stone, cancer + stone and control group as compared with the frequency of mutant allele A2 of groups. However the frequency of mutant allele A2 was higher among cancer, stone, cancer+stone patients group when compared with the control group. (Table 2)



**Figure 1. Restriction Fragment Length Polymorphism Analysis of CYP-17 MspA1 Gene: M: 100 bp DNA molecular weight marker, Lane 1 and 4: A1/A2 Heterozygous mutant, Lane 2 and 3: A1/A1 Homozygous Wild**

**Table 1. Comparison of Lipid and Estrogen Levels among Cases and Controls**

Parameters	Cancer (n=96)	Stone (n=102)	Cancer + Stone (n=52)	Control (n=256)	p-value <sup>1</sup>	
	mean±SD	mean±SD	mean±SD	mean±SD		
TC	186.67±77.69	175.42±67.36	209.76±89.98	156.68±38.00	0.0001*	
TG	170.26±85.29	165.51±63.25	201.50±102.26	125.75±59.60	0.0001*	
HDL	26.54±14.72	29.00±13.29	23.66±14.35	41.09±12.19	0.0001*	
LDL	126.08±78.37	113.32±68.87	145.80±92.27	90.44±39.67	0.0001*	
VLDL	34.05±17.06	33.10±12.65	40.30±20.25	25.15±11.92	0.0001*	
Estrogen	51.02±47.38	54.64±48.40	51.69±45.28	32.88±20.32	0.002**	
Parameters	p- value <sup>2</sup> (Tukey's Post-hoc test)					
	Cancer vs Stone	Cancer vs Cancer+Stone	Stone vs Cancer+stone	Cancer vs Control	Stone vs Control	Cancer+ Stone vs Control
TC	0.55	0.11	0.005 <sup>#</sup>	0.0001 <sup>#</sup>	0.04 <sup>#</sup>	0.0001 <sup>#</sup>
TG	0.96	0.06	0.01 <sup>#</sup>	0.0001 <sup>#</sup>	0.0001 <sup>#</sup>	0.0001 <sup>#</sup>
HDL	0.55	0.58	0.08	0.0001 <sup>#</sup>	0.0001 <sup>#</sup>	0.0001 <sup>#</sup>
LDL	0.46	0.24	0.01 <sup>#</sup>	0.0001 <sup>#</sup>	0.009 <sup>#</sup>	0.0001 <sup>#</sup>
VLDL	0.96	0.06	0.01 <sup>#</sup>	0.0001 <sup>#</sup>	0.0001 <sup>#</sup>	0.0001 <sup>#</sup>
Estrogen	0.89	1	0.96	0.0001 <sup>#</sup>	0.0001 <sup>#</sup>	0.004 <sup>#</sup>

\*p-value<sup>1</sup> Comparison of cases with controls, \*Significant (ANOVA); \*\*Significant (Kruskal Wallis test), p-value<sup>2</sup> Post-hoc multiple comparisons test between the groups (Tukey's test); <sup>#</sup>Significant (Tukey's test)

**Table 2. Distribution of Genotypes and Allele Frequency of CYP 17 MspA1 Gene Polymorphism among the Cases and Controls**

Genotype	Cancer (n=96)		Stone (n=102)		Cancer+Stone (n=52)		Control (n=256)	
	No.	%	No.	%	No.	%	No.	%
A1A1 <sup>a</sup>	41	42.7	41	40.2	27	51.9	203	79.3
A1A2	55	57.3	61	59.8	25	48.1	53	20.7
OR	5.13		5.69		3.54		1.00	
(95%CI),	(3.10-8.51),		(3.46-9.37),		(1.90-6.60)			
p-value	0.0001*		0.0001*		0.0001*			
Allele								
A1 <sup>b</sup>	137	71.4	143	70.1	79	76	459	89.6
A2	55	28.6	61	29.9	25	24	53	10.4
OR	3.48		3.69		2.74		1.00	
(95%CI),	(2.28 - 5.30)		(2.44 - 5.59)		(1.61 - 4.67)			
p-value	0.0001*		0.0001*		0.0002*			

<sup>a</sup> considered as reference wild genotype for comparison; <sup>b</sup> considered as reference wild allele for comparison, OR-Odds ratio, CI-Confidence interval, \*Significant p-value<0.05

**Table 3. Comparison of Lipid and Estrogen Levels According to Genotypes of CYP 17 MspA1 Gene Polymorphism among the Cases and Controls**

Parameters	Cancer	Stone	Cancer+stone	Controls
TC				
Wild (A1A1)	184.21±76.98	165.57±58.09	208.64±87.73	156.86±38.64
Heterozygous (A1A2)	188.50±78.88	182.04±72.65	210.98±94.15	155.98±35.80
p-value	0.79	0.22	0.92	0.88
TG				
Wild (A1A1)	184.60±88.43	159.31±60.47	208.70±96.15	129.46±60.70
Heterozygous (A1A2)	159.56±82.05	169.67±65.21	193.73±109.92	111.57±53.32
p-value	0.15	0.42	0.6	0.05*
HDL				
Wild (A1A1)	27.06±15.04	29.65±14.40	23.46±13.57	41.18±12.30
Heterozygous (A1A2)	26.15±14.59	28.56±12.60	23.88±15.43	40.71±11.84
p-value	0.76	0.68	0.91	0.8
LDL				
Wild (A1A1)	120.23±81.00	104.06±61.04	143.43±90.27	89.79±39.96
Heterozygous (A1A2)	130.44±76.81	119.54±73.50	148.35±96.18	92.95±38.80
p-value	0.53	0.26	0.85	0.61
VLDL				
Wild (A1A1)	36.92±17.69	31.86±12.09	41.74±19.23	25.89±12.14
Heterozygous (A1A2)	31.91±16.41	33.93±13.04	38.75±21.98	22.31±10.66
p-value	0.15	0.42	0.6	0.05*
Estrogen				
Wild (A1A1)	53.26±47.65	58.34±52.87	48.89±35.00	32.36±20.00
Heterozygous (A1A2)	49.35±47.55	52.15±45.42	54.70±54.88	34.91±21.55
p-value	0.33	0.64	0.86	0.42

\* Significant p-value<0.05

**Table 4. Multivariate Logistic Regression Analysis Evaluating Significant Risk Factors for Cancer, Stone and Cancer+Stone Patients Compared with Controls**

Risk Factors	OR (95.0% C.I.), p-value		
	Cancer vs Control	Stone vs control	Cancer+stone vs Control
Age	0.97 (0.96-0.99), 0.03*	0.96 (0.93-0.99), 0.005*	-
TC	1.01 (1.00-1.01), 0.03*	-	1.01 (1.00-1.02), 0.001*
TG	1.01 (1.00-1.01), 0.0001*	1.01 (1.00-1.02), 0.005*	1.01 (1.00-1.01), 0.002*
HDL	0.93 (0.91-0.95), 0.0001*	0.93 (0.90-0.95), 0.0001*	0.89 (0.86-0.93), 0.0001*
CYP 17 MspA1 Genotype			
Wild (A1A1)	1.00 (Ref.)	1.00 (Ref)	1.00 (Ref)
Heterozygous (A1A2)	1.75 (1.15-2.67), 0.008*	1.16 (1.09-2.33), 0.0001*	1.18 (1.12-2.12), 0.0001*

SE-Standard deviation, OR-Odds ratio, CI-Confidence, Ref: Reference, \*Significant p-value<0.05

Genotypes of CYP-17 MspA1 gene polymorphism versus serum level of lipid profile and estrogen level among the study and control group: There was no significant ( $p>0.05$ ) difference in the lipid and estrogen levels between the wild and heterozygous genotype of CYP-17 gene polymorphism among the all groups. However, among the controls only TG and VLDL were significantly different between the wild and heterozygous genotype of CYP-17 gene polymorphism (Table 3).

Risk factors for cancer, stone and cancer + stone patients: In the multivariate logistic regression analysis, age, TC, TG, HDL and increased frequency of heterozygous genotype of CYP-17 gene polymorphism were the significant factors for cancer patients. Among the stone patients age, TG, HDL and increased frequency of heterozygous genotype of CYP-17 gene polymorphism were found to be the significant factors. Similarly among the cancer+stone patients TC, TG, HDL and increased frequency of heterozygous genotype of CYP-17 gene polymorphism were found to be the significant factors (Table 4).

## Discussion

Carcinoma of the gallbladder is a highly fatal disease because late diagnosis, limited treatment options (Khan et al., 2010; Le et al., 2011; Pavlidis et al., 2012). In the present study, we are reporting the association of CYP-17 MspA1 gene polymorphism, lipid profile and serum estrogen level with gall bladder cancer and gallstone among the North Indian population. In our study we observed that the maximum number of cases were female between the age group of 41-50 years (cancer and cancer+stone) and 31-40 years (stone). The findings were consistent with previous reports, as investigated that the carcinoma of the gallbladder occurs with a high frequency among the females and explaining the significant role of female hormones in the etiology of the disease (Castro et al., 2013). Furthermore, there was no significant difference in gender and age between the study and control groups, so the present study is age and sex matched.

It was reported that the cholelithiasis is strongly associated with carcinoma gallbladder and is the most common associated factor independent of age or sex by many authors (Hart et al., 1971; Diel, 1983). There are three stages of gallstone formation, super saturation, nucleation and aggregation (Bennion et al., 1978). Cholesterol crystals form on the surfaces of these vesicles and grow within the mucin gel and glued together by bile proteins to make gallstones (Ho, 1977; Ahlberg, 1979; Carey, 1992; Juvonen, 1994). The cholesterol solubility in bile depends on the imbalanced relative concentrations of cholesterol, bile salts and phospholipids. These changes in bile composition are closely related to the disorders of lipid metabolism in liver (Small, 1967; Admirand et al., 1968). Several studies have reported that gallbladder cancer and gallstone patients had hyperlipidemia (Mohr et al., 1991; Channa et al., 2010; Yadav et al., 2013; Singh et al., 2013; Dwivedi et al., 2013). The findings of our study were agreement with these reports, as in our study the serum level of total cholesterol, triglyceride,

low density lipoprotein and very low density lipoprotein was found significantly higher in all study group, and high density lipoprotein was significantly lower in study group compared with control subjects. However, some investigators could not found such an association with gallbladder cancer and gallstone patients (Pettiti et al., 1981; Cavallini et al., 1987) and possibly it may be due to the differences in study populations, sample size and different ethnic group. The exact reason for this inconsistency is still unknown.

Interestingly, between the groups analysis, there was no significant difference of lipid level between the Cancer and Stone group as well as Cancer and Cancer + Stone groups. The comparison between stone and cancer + stone group shows that the level of TC, TG, LDL, VLDL, estrogen was significantly higher and HDL was significantly lower among cancer+stone group. Similarly, the comparison between the all study group (cancer, stone and cancer+stone) and control group shows that the level of TC, TG, LDL, VLDL, estrogen was significantly higher and HDL was significantly lower among the all study groups. Thus, in our study the significant higher level of TC, TG, LDL, VLDL, estrogen and lower HDL level among the patients of gallbladder cancer and gallstone indicating a significant association of lipid as well as estrogen metabolism in the etiology of disease and the findings were in agreement with previous report (Mohr et al., 1991; Channa et al., 2010; Dwivedi et al., 2013; Singh et al., 2013; Yadav et al., 2013). In postmenopausal women, hormone replacement therapy was significantly associated with the gallbladder diseases (Gallus et al., 2002; Cirillo et al., 2005) and suggesting that a noteworthy role of sex hormones in the etiology of gallbladder cancer (Nakamura et al., 1989; Khan et al., 1999; Zhang et al., 2013). It was clearly mentioned by investigators that the higher level of estrogen increases the cholesterol saturation in the bile, and thereby enhance the gallstone formation (Moerman et al., 1994; Feigelson et al., 1998; Dowling, 2000).

To the best of our knowledge this is the first study conducted to see the association of CYP-17 MspA1 gene polymorphism with estrogen serum level and lipid profile in the gall bladder cancer and stone among the North Indian patients. In this population based case control study, the heterozygous mutant genotype A1A2 and mutant allele A2 of CYP-17 MspA1 gene polymorphism was significantly associated with the gall bladder cancer and stone. As we have observed the higher frequency of A1A2 genotype and A2 allele of CYP-17 MspA1 gene polymorphism among the study group in comparison of control group. As per the various report concerning with the association of this gene polymorphism and hormone related cancer (e.g., breast, endometrium, prostate) yet not been clearly outlined, because many epidemiological studies dealing with such association have reported inconsistent results, with reports showing either the A1 or A2 allele being associated with an increased risk (Feigelson et al., 1997; Haiman et al., 1999; Haiman et al., 2001; Ambrosone et al., 2003; El-Ezzi et al., 2014). The reason for the inconsistency of results might be due to the population difference, ethnicity, life style or some

environmental factors. There was an only study which explores the association of MspA1 polymorphism with gall bladder cancer and stone, conducted by Lifang Hou et al. (2006) among Chinese population. Our findings are inconsistent with this study as, they have observed that the A1 allele carrier females were at higher risk of gall bladder cancer (Hou et al., 2006). This inconsistency may be due to the differences in genetic makeup of population, life style and absence of A2A2 homozygous mutant genotype in our study population. The absence of A2A2 homozygous mutant genotype in our study population might be due to the very infrequent frequency of A2A2 genotype, which leads to a small sample size of the compared groups (Nedelcheva et al., 1999).

In our study, we could not find any association of CYP-17 MspA1 gene polymorphism with the serum level of lipid and estrogen. Our result support the finding of Weber et al., (2000) who reported that the pathway of estrogen metabolism is mediated by the activities of multiple genes, such as CYP-17, CYP1A1, COMT, and HSD17B1 (Weber et al., 2000) not only by CYP-17 gene. However some reports were suggested that the A2 allele carrying women were associated with higher Serum levels of androgens and estrogens (Feigelson et al., 1998; Haiman et al., 1999). Insignificant association between estrogen level and CYP-17 gene polymorphism might be due to the limited impact of such gene polymorphism on estrogen metabolism and the current sample size. Furthermore, the multivariate logistic regression analysis indicated that the age, TC, TG, HDL and increased frequency of heterozygous genotype A1A2 of CYP-17 gene polymorphism were the significant risk factors for cancer patients. Among the stone patients age, TG, HDL and increased frequency of heterozygous genotype of CYP-17 gene polymorphism were found to be the significant risk factors. Similarly among the cancer + stone patients TC, TG, HDL and increased frequency of heterozygous genotype of CYP-17 gene polymorphism were found to be the significant risk factors.

On the basis of our findings, we may concluded that the higher frequency of heterozygous mutant genotype A1A2 and mutant allele A2 of CYP-17 MspA1 gene polymorphism is significantly associated with the risk of gall bladder cancer and stone among the North Indian patients. The female gender is also an important risk factor for the disease. The elevated level of estrogen as well as altered level of lipid is also a significant risk factor for the disease among the post menopausal females. As we could not find the association of CYP-17 MspA1 gene polymorphism with the serum level of lipid and estrogen, therefore, it is very necessary to conduct the study with multiple gene polymorphism involved in estrogen metabolism in larger population to reduce the inconsistency of results.

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