EPIDEMIOLOGY OF INFECTIOUS GALLBLADDER DISEASES IN PATIENTS AND ASSOCIATED RISK FACTORS OF GALLBLADDER DISEASES

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ABSTRACT

Gallbladder diseases occur frequently in developed countries. Gallbladder disease is a condition that affects mainly women, although men can suffer too **(1).** GB disease is one of the most common surgical problems in the world today with over 500,000 GB operations performed annually in the India **(2).** Considerable evidences are available, but man in the various areas of the world has suffered from the biliary tract diseases. The present study was undertaken to investigate epidemiology of bacterial gall bladder diseases and also associated risk factors. The survey of patients will be done in government hospital and also some private clinics. The study comprised investigation of frequent infection in GB diseases. In present study, 165 patients suffering from cholecystitis, GBC and cholangitis were included. We have also investigated factors, which are responsible for infectious GB diseases and the effect of risk factors on patients

Keywords: Gallbladder,cholecystitis,cholangitis,biliary tract diseses,gallstone.

INTRODUCTION:

Gallbladder disease is an exceptionally common source of morbidity in the India, where an estimated 20 million people either have gallstones or have had surgery for gallstones.The most common manifestation of GBD is biliary pain, but serious complications including gallbladder inflammation, pancreatitis and bile duct obstruction may result. Gallstones may also contribute to the development of gallbladder cancer. A major risk factor for GBDs is bacterial infection in bile, biood, gallstone and tissue of GB. The GB probably does not harbour bacteria at most times in normal individuals .The conditions necessary for the development of infection of the biliary tract or for the production of infected bile are not in general known. It is agreed that infection regularly follows certain septicaemic illnesses, notably typhoid fever, and that infection so initiated may become chronic is indicated by the occurrence of the carrier state.

A risk factor is something that increases chance of getting a disease or condition. Bacterial infection is one of the most important factor for GB diseases. Other risk factors for gallbladder diseases include age, gender, obesity, race, use of cholesterol-lowering drugs, diabetes, rapid weight loss and fasting, previous gallstones, diseases of the gallbladder and ducts, blood diseases, including sickle cell anemia. The relative risk of gallbladder cancer is 2.4-10 times higher in patients with gallstones than in those without. Due to long term biliary drainage in biliary malignancy increases the risk of bacterial culture rate. (**3**).

MATERIALS AND METHODS:

The study comprised the pathological group of patients having GB diseases (Cholecystitis, Cholangitis, GBC). These patients were admitted for surgical treatment in Jaya Arogya hospital and private clinics, Gwalior. No therapy prior to surgery has been given to the patients of this group. The study comprised investigation of frequent infection in GB diseases. In present study, 165 patients suffering from cholecystitis, GBC and cholangitis were included. Study was performed on 165 patients, including 115 females and 50 males suffering from GB diseases/biliary tract diseases. The ratio of men to women was 1:2.3. In our study, we have identified a pathological group of patients with infection in GB.

(1.) COLLECTION OF SAMPLES FROM GALLBLADDER PATIENTS

The clinical specimen i.e. bile, blood, stone and tissue samples will be collected from gallbladder patients

(i) Bile: 1 ml bile sample was collected from each patient of GB diseases (cholecystitis, cholangitis and GBC).

(ii) Blood: One ml venous blood was collected from each GB patient with the help of syringe.

(iii) Stone: Some patients have only one stone; others develop hundreds. GB and CBD stones were obtained from all patients undergoing cholecystectomy and/or CBD exploration. Gallstones specimens taken from the GB were washed by sterile saline repeatedly to remove surface contaminants. They were crushed and ground to powder afterwards. Under sterile condition, the powder was put into a sterile culture tubes. Powder of gallstone mixed in nutrient broth then incubates for 24 hours.

(iv) Tissue: Tissue specimens were taken during surgery from GB suspected of being infected, by aspirating pus or by swabbing infected areas, taking care to avoid contact with adjacent mucous membranes. The specimens were transported to the laboratory in the syringe used to collect them, capping the needle with a rubber stopper. Small piece of tissue were placed in nutrient broth for 24 hours incubation then plated on appropriate media.

(2.) ISOLATION OF BACTERIA FROM GALLBLADDER PATIENTS

After the collection of samples from patients, we turn on next process that is isolation of test bacteria.Glasswares,instruments and culture media are used for isolation of test bacteria

(3)GLASSWARE PREPARATION **:**

The glasswares were immersed overnight or atleast 1 hour in a container of 2500-ppm chlorine disinfected. Make sure that all the glasswares were fully immersed with air bubble expelled. The container should not be overloaded. The glasswares were washed with detergent using a test tube brush then rinsed well with water and dried. Afterward they were wrapped with paper and kept for sterilization in a hot air oven at 160^0c for ½ an hour.

(4) CULTURE OF BACTERIA:

Samples was inoculated on nutrient agar, Mac-conkey agar and blood agar media. Then it was incubated for 24 hours at 37^0c.

(5)COLONY MORPHOLOGY:

After incubation of 24-48 hours the bacterial colonies was observed by its appearance on nutrient agar, Mac-Conkey agar and blood agar. It includes size, texture, optical nature and hemolysis etc. Colony counts were performed for each isolated organism. The isolated microorganisms were further purified and maintained on nutrient agar slants at 4^0c for the characterization.

RESULTS

The total no. Of patients registered at J.A. hospital and private clinics, gwalior (M.P.) from March 2005 to march 2008 was 200. Present study revealed that cholecystitis and GB cancer are placed in group (1) because their infection initially start in GB, after a period of time it is seen that the infection spread to the biliary tract but the main pathosis lies in the GB. While in case of cholangitis the infection starts in common bile duct (CBD) and the GB gets secondarily infected (due to spread of infection from CBD), so it is categorized in group (2).

The patients were divided into following two groups-

Group (1) - This group comprised of 120 patients (72.72%) with major infective GB complication. Out of 120 patients, 35 were male (29.16%) and 85 female patients (70.83%). The ratio of men to women was 1:2.4. This group was further subdivided into group 1A, which comprised of 77 patients cholecystitis (64.13%). Group1B, which comprised of 43 patients with tumor in gallbladder (35.86%).

Group (2) - This group includes 45 patients (27.27%), which were suffering from associated infective gallbladder complication, which is cholangitis (100%). Out of 45 patients, 15 were male (33.33%) and 30 female patients (66.66%). The ratio of men to women was 1:2.

During this study we tried to analyze the number of persons affected with GB diseases in different gender, age, and dietary habits among the patients studied. We also tried to analyze the most frequent type of GB disease in the patients studied. Incidence study found the occurence and factors responsible for GB diseases.

Table-1. Occurrence of GB diseases in different genders:

Sr.no.	Number of patients	
	Males	Females
1.	50 (30.30%)	115 (69.69%)

Table-2. Occurrence of GB diseases in different age groups:

Sr.no	**Age group**	**Number of cases**	**Percentage**
1.	< 20	5	3%
2.	21-30	12	7.27%
3.	31-40	21	12.72%
4.	41-50	38	23.03%
5.	51-60	48	29.09%
6.	> 60	41	24.84%
Total		**165**	

Table-3. Occurrence of GB diseases:

Sr.no.	Number of patients		
	Cholecystitis	GBC	Cholangitis

1.	77 (46.66%)	43 (26.06%)	45 (27.27%)

Table-4.Occurrence of different types of GB diseases in males and females:

Sr. no.	Number of patients					
	Males			Females		
	Cholecystitis	Cholangitis	GBC	Cholecystitis	Cholangitis	GBC
1.	25 (32.46%)	15 (33.33%)	12 (27.90%)	52 (67.53%)	30 (66.66%)	31 (72.09%)

Table-5.Number of GB patients studied from different location during present study:

Sr.no.	Number of patients					
	Gwalior	Morena	Dabra	Shivpuri	Datia	Bhind
1.	132 (80%)	6 (3.6%)	5 (3%)	7 (4.2%)	8 (4.8%)	7 (4.2%)

Table-6. Occurrence of GB cases in different age group:

Sr.no.	**Age group**	**Number of cases**	**Value of $\chi 2$ (At 5% level of significance)**	**Percent**
1.	< 20	5	18.409	3%
2.	21-30	12	8.736	7.27%
3.	31-40	21	1.536	12.72%
4.	41-50	38	4.009	23.03%

5	51-60	48	15.281	29.09%
6.	> 60	41	6.627	24.84%
Total		**165**		**100%**
		Mean ± SD **49.42±4.33**		

Tabular value of $\chi 2$ =11.070 (At 5% level of significant)

Tabular value of $\chi 2$ > Calculated value of $\chi 2$

(2), (3), (4), (6) values are significant and (1), (5) are not significant.

Table-7. Occurrence of GB diseases in patients who have family history of GB diseases:

Sr.no.	**Age group**	**Genetic factor**	**Value o $\chi 2$ (At 95% level of significance)**	**Percent**
1.	< 20	1	6.775	1.92%
2.	21-30	6	0.817	11.53%
3.	31-40	9	0.00017	17.30%
4.	41-50	11	0.632	21.15%
5	51-60	16	6.221	30.76%
6.	> 60	9	0.013	17.30%
Total		**52**		**31.51%**
		Mean ± SD **46.92 ± 4.35**		

Tabular value of $\chi 2$ =1.145 (At 95% level of significant)

Tabular value of $\chi 2$ > Calculated value of $\chi 2$

(1), (5), values are significant and (2), (3), (4), (6) are not significant.

Table-8. Occurrence of GB diseases in diabetic patients:

Sr.no.	Age group	Diabetes	Value of $\chi 2$ (At 95% level of significance)	Percent
1.	< 20	2	5.602	3.63%
2.	21-30	5	1.893	9.09%
3.	31-40	11	0.366	20%
4.	41-50	12	0.876	21.81%
5	51-60	15	3.713	27.27%
6.	> 60	10	0.075	18.18%
Total		**55**		**33.33%**
		Mean ± SD **46.45 ± 4.35**		

Tabular value of $\chi 2$ =1.145 (At 95% level of significant)

Tabular value of $\chi 2$ > Calculated value of $\chi 2$

(1), (2), (5) values are significant and (3), (4), (6) are not significant.

Table-9. Occurrence of GB diseases in the people who have over weight:

Sr.no.	Age group	Obesity (Weight > 90 to 110)	Value of $\chi 2$ (At 1% level of significance)	Percent
1.	< 20	0	11.66	0%
2.	21-30	0	11.66	0%
3.	31-40	0	11.66	0%
4.	41-50	20	69.55	28.57%
5	51-60	35	544.75	50%
6.	> 60	15	11.15	21.42%
Total		**70**		**42.42%**
		Mean ± SD **52.14 ± 4.29**		

Tabular value of $\chi 2$ =15.086 (At 1% level of significant)

Tabular value of $\chi 2$ > Calculated value of $\chi 2$

(4), (5), values are significant and (1), (2), (3), (6) are not significant.

Table-10.Effect of weight loss or cycling on GB diseases:

Sr.no.	Age group	Weight loss (1.5 kg with in one week)	Value of $\chi 2$ (At 95% level of significance)	Percent
1.	< 20	13	0.789	21.31%

2.	21-30	15	2.298	24.59%
3.	31-40	13	0.789	21.31%
4.	41-50	10	0.0027	16.39%
5	51-60	10	0.0027	16.39%
6.	> 60	10	0.0027	16.39%
Total		**61**		**36.36%**
		Mean ± SD **36.47 ± 4.44**		

Tabular value of $\chi 2$ =1.145 (At 95% level of significant)

Tabular value of $\chi 2$ > Calculated value of $\chi 2$

Only (2) value are significant and (1), (3), (4), (5), (6) values are not significant.

Table-11. Occurrence of GB diseases in the people with different dietary habit among studied group:

Sr.no.	**Age group**	**Dietary habit (Eat rich fat content diet)**	**Value of $\chi 2$ (At 95% level of significance)**	**Percent**
1.	< 20	1	11.404	1.25%
2.	21-30	12	0.132	15%
3.	31-40	15	0.209	18.75%
4.	41-50	20	3.337	25%
5	51-60	21	4.413	26.25%
6.	> 60	11	0.407	13.75%

Total		**80**		**48.48%**
		Mean ± SD **43.75± 4.36**		

Tabular value of $\chi 2$ =1.145 (At 95% level of significant)

Tabular value of $\chi 2$ > Calculated value of $\chi 2$

Only (1), (4), (5) values are significant and (2), (3), (6) values are not significant.

Table-12. Effect of alcohol consumtion on GB patients:

Sr.no.	**Age group**	**Alcoholic persons**	**Value of $\chi 2$ (At 95% level of significance)**	**Percent**
1.	< 20	1	1.035	6.25%
2.	21-30	3	0.035	18.75%
3.	31-40	5	2.058	31.25%
4.	41-50	2	0.163	12.5%
5	51-60	2	0.163	12.5%
6.	> 60	3	0.043	18.75%
Total		**16**		**9.6%**
		Mean ± SD **41.25± 4.37**		

Tabular value of $\chi 2$ =1.145 (At 95% level of significant)

Tabular value of $\chi 2$ > Calculated value of $\chi 2$

Only (1), (3), values are significant and (2), (4), (5), (6) values are not significant.

Statistical analysis: The mean and standard deviation for each quantitative variable and each patient group were calculated. Statistical analysis of the results was performed using $\chi 2$ test. In all cases, some are significant and some are not.

Table-13. Number of positive and negative samples:

Total patients		Number of samples from each patient	Total bacteria isolated from 165 patients	Total gram positive bacteria isolated from 165 patients	Total gram negative bacteria isolated from 165 patients
+Ve Sample	–Ve Sample				
165	35	4 (Bile, blood, stone, tissue)	550	220 (40%)	330 (60%)

Table-14.Number of gram positive and gram-negative bacteria isolated from each sample:

Samples	Number of bacteria	
	Gram positive (out of 220)	Gram negative (out of 330)
Bile	75 (34.09%)	96 (29.09%)
Blood	23 (10.45%)	60 (18.18%)
Stone	51 (23.18%)	79 (23.93%)
Tissue	71 (32.27%)	94 (28.48%)

Table-15. Number of bacteria isolated from group G1 and G2

	Group G1 (120 patients)		Group G2 (45 patients)
	Cholecystitis	GBC	Cholangitis
Number of patients	77 (64.13%)	43 (35.86%)	45 (100%)
Number of bacteria isolated from group G1 and G2	380		170

Table-16. Number of gram positive and gram-negative bacteria isolated from patients of cholecystitis, GBC and cholangitis:

Bacteria	Cholecystitis	GBC	Cholangitis
Gram positive	80/380=21.5%	60/380=15.78%	80/170=47.05%
Gram negative	140/380=36.84%	100/380=26.31	90/170=52.94%

Table-17. Number of gram positive and gram-negative bacteria isolated from samples of cholecystitis patients, GBC patients and cholangitis patients:

Samples	Cholecystitis		GBC		Cholangitis	
	Gram +ve	Gram–ve	Gram+ve	Gram–ve	Gram+ve	Gram–ve
Bile	40/80=50%	50/140=35.71%	35/60=58.33%	41/100=41%	25/80=31.25%	13/90=14.44%
Blood	5/80=6.25%	17/140=12.14%	4/60=6.6%	7/100=7%	20/80=25%	35/90=38.88%
Stone	15/80=18.75%	27/140=19.28%	4/60=6.6%	15/100=15%	20/80=25%	32/90=35.55%
Tissue	20/80=25%	46/140=32.85%	17/60=28.33%	37/100=37%	15/80=18.75%	10/90=11.11%

DISCUSSION:

Gallstone formation is seen in twice as many women as men, particular those between the ages of 20 and 60 (**4**). Females are at least twice as "lithogenic" as males **(5).** Women between 20 and 60 years of age are

twice as likely to develop gallstones as men **(6)**. In present study, out of 165 patients 115 were females and 50 males which suffered with gallbladder diseases.

The previous study showed the infection of the bactibilia in the bile sample were common in the age > 65 **(7)**. The infection in bile is more common after 40 year of age (>40) **(8)**. Severity of bactibilia in GB patients is most common between age group of 40 to 60 **(9).** While in our study the incidences of infection in bile sample were observed in the age group of 51 to 60 years (About 67% significant) (49.42±4.33).

Obesity significantly associated with increased risk of gallbladder disease **(10).** Obesity is a major risk factor for gallstones. A large clinical study showed that being even moderately overweight increases one's risk for developing gallstones **(11).** GB diseases are associated with obesity **(12).** In present study some cases are significant (34%) and some are not significant by the use of χ**2** test (52.15±4.29).

Those genetic factors are responsible for at least 30% of symptomatic gallstone disease **(13).** Family member or close relative with infective gallbladder disease may increase the risk of infective gallbladder disease **(14).** By the use of statistical analysis, some results of present study favour these studies (34% significant, 46.92±4.35).

People with diabetes generally have high levels of fatty acids called triglycerides. These fatty acids increase the risk of GB diseases **(15)**. People with diabetes are at higher risk for gallstone and have a higher risk than average of acalculous infective gallbladder disease **(16)**. Present study shows 51% significant (46.45±4.35).

As the body metabolizes fat during rapid weight loss, it causes the liver to secrete extra cholesterol into bile, which can cause gallstone **(17).** The rapid weight loss or cycling further increases cholesterol production in the liver, with resulting supersaturation and risk of gallstone **(18).** Fasting decreases gallbladder movement, causing the bile to become over concentrated with cholesterol, which can lead to gallstones **(19)**. Results of present study are 17% significant (36.47±4.44).

High calorie diet may also be a causative factor **(20)**. Dietary habit of infective gallbladder patients takes rich fat content diet. It concludes that fats have been associated with infective gallbladder diseases. High intake of fibres, vegetable protein, lecithin, coffee, vitamin C can reduce the risk of infective gallbladder diseases **(21).** Significance of present study is 51% (43.75±4.36).

Consume alcohol in small amounts (occasionally) shows offcourse gallbladder complication but severity of infective gallbladder disease is very low than non-alcoholic patients **(22).** Small intake of alcohol reduces the risk of infective gallbladder diseases **(23).** Present study shows 34% of significance (41.25±4.37).

Reported for gallstone diseases, the most common organisms cultured were gram-negative bacteria (74%) followed by gram positive (15%). In the case of gallstone disease, organisms cultured gram positive (50%) and gram negative (50%) both. **Manzanilla *et al,* 2004** in the case of prolonged gallstone, *Enterococcus sp* found in maximum number from patient's sample. In our study, the gram-negative bacteria were more frequent that is 60% than gram-positive bacteria (40%). Various studies reported, the positive infection rate was higher in gram-negative bacteria than gram-positive bacteria **(24).** Most of the gram-negative bacteria shows higher rate of infection than gram positive in the case of cholangitis and cholecystitis **(25).** Maximum number of infection in GB can occur due to gram-negative bacteria than gram positive in the case of GB cancer **(26)**. Our study was also in accordance with these studies

CONCLUSION: Cholecystitis is the most common of all the three: cholecystitis, GBC and cholangitis.Epidemiological distribution of diseases state that it is most common in chambal region that too in Gwalior.Diabetes and dietary habits (rich fat contents in diet) are found to be one of the most important risk factors associated with GB diseases. Genetic factor, alcohol consumption and overweight are also found to be associated with it. Though over weight loss may also related to such diseases but its role is not very significant.Out of samples taken from GB patients, most of the bacteria are detected in bile.

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