

CIRCULATING CONCENTRATIONS OF IL-2, IL-6, TNF-ALPHA AND
ENDOTOXIN IN PATIENTS OF CARCINOMA GALL BLADDER

Induja Awasthi* and Lav Kumar Kacker

*Department of Surgical Gastroenterology, CSMMU, India

*Corresponding Author: induja.awasthi@gmail.com

This article is available online at www.ss-journals.com

ABSTRACT

Introduction: Gallbladder Cancer represents the most common and aggressive type among the biliary tree cancers (BTCs). Carcinoma Gallbladder has an unusual geographic distribution. The highest incidence of carcinoma of gallbladder is in Chileans, American Indians, and in parts of North India.

Objective: The present study was designed to characterise the circulating concentrations of endotoxin and different inflammatory mediators in patients with Carcinoma Gall bladder.

Methods and Material: One hundred twenty eight Carcinoma Gall bladder patients were included in the study. Enzyme-linked immunosorbent assay was used for determining the circulating concentrations of Interleukin-2 (IL-2), Interleukin -6 (IL-6), Tumor Necrosis Factor- α (TNF- α) and Endotoxins in human serum by LAL Method. A cross sectional trial was performed

Results: The mean age of the patients was 50.86 ± 12.6 years. The mean duration of jaundice was since last 74.91 ± 50.0 days. Fifty five percent patients had cholelithiasis association with Carcinoma Gall bladder. The mean Body Mass Index (BMI) of Carcinoma Gall bladder patients were 18.33 ± 2.8 . The mean circulating concentrations of IL-2 were 33.31 ± 56.6 pg/ml, IL-6 were 167.16 ± 216.4 pg/ml, TNF- α were 56.00 ± 56.7 pg/ml and endotoxin were 0.66 ± 0.2 EU/ml.

Conclusions: Mean circulating concentrations of IL-2 and TNF- α were lower and mean circulating concentrations of IL-6 were higher than the previous studies. The mean circulating concentrations of endotoxin were within the normal limits. Which concludes that cytokines may participate in gall bladder carcinogenesis and the study provides evidence for a strong link between chronic inflammation and Carcinoma Gall bladder.

Keywords: Obstructive jaundice, Cholelithiasis, Karnofsky score

1. Introduction:

Carcinoma of the gallbladder is the most common malignant tumour of the biliary tract and high incidence is observed in Chile, Japan, Latin America and Northern India. The prevalence of carcinoma of the gallbladder is highly variable across the world.^{1,2} The Risk factors of carcinoma of gall bladder are: Gender, approximately twice more common in women than men, mostly between the ages of 50 and 60. Early diagnosis is not generally possible.³ The prognosis of Carcinoma Gall bladder is poor due to unsatisfactory treatment.⁴ Obstructive jaundice causes increased exposure to endotoxin by lack of bile in the gut lumen and diminished clearance of endotoxin by Kupffer cells.⁵ The present study was designed to characterize the circulating concentrations of endotoxin and different inflammatory mediators in patients with CAGB.

2. Materials and Methods:

Five millilitre blood samples were obtained from the patients. Serum was centrifuged and then

stored at -20°C until testing. Biochemical tests were performed and a questionnaire was used to obtain the general information and biochemical test results.

2.1 Selection and Description of Participants:

One hundred and twenty eight Carcinoma Gall bladder patients were included in the study. The patients were taken from the outpatient department and were between 20-80 years of age. Patients were eligible for inclusion in the study when they presented with obstructive jaundice with bilirubin concentrations less than five mg%. Patients were excluded from the study if bilirubin concentrations greater than five mg%. Malignancy was confirmed by Fine Needle Aspiration Cytology/Biopsy/Brush cytology. Prior consent was obtained by the patients. The study was designed so that all patients were cases of carcinoma gall bladder.

2.2 Technical Information:

Serum concentration of Cytokines were assessed by the highly sensitive, AviBion Human Interleukin-two, Interleukin-six and Tumor Necrosis Factor- α Enzyme-linked immunosorbent assay

(ELISA) kits according to the manufacturer's instructions (Orgenium Laboratories Business Unit, Vantaa Finland). Nutritional status was determined by Body Mass Index and functional status was determined by Karnofsky Score. The study was ethically approved by the institutional ethics committee. (Letter no. 5029/R.Cell-10, Dated: 11/01/10, Ref. code-XLEM/A-P4)

2.3 Statistics: A cross sectional trial was performed. Results are expressed as mean \pm SD and proportion, as appropriate. Stata version 11.1 was used to analyze the data.

3. Results:

During the three year period, 128 cases of Carcinoma Gall bladder were recorded. The mean age of was 50.86 ± 12.6 years and 73 patients i.e. 57.03% were female. The presence of jaundice in patients was since 74.91 ± 50.0 days and majority of patients had cholelithiasis associated with GBC (70 patients i.e. 54.69% (Table 1). The major signs and symptoms present were Loss of appetite, Weight loss, Pruritus, Weakness and fatigue (Fig. 2). Main USG findings were GB wall thickening, Dilated CBD, Intra Hepatic Biliary Radical Dilatation, Contiguous liver spread and Non- Contiguous liver spread. Mass in the gallbladder was present in 71 patients (55.47%) (Table 2, Fig.1). The mean body mass index of patients in the present study was (18.33 ± 2.8) and majority of the patients were malnourished (Table 3). The mean Functional status of the patients was above 60% (Table 3), which suggests that the patients required some help with daily work, were caring for self, could take care of most personal requirements but were not capable of normal activity or work. The mean hemoglobin level in the study was 10.69 ± 1.8 g/dl i.e. the patients were anaemic and the mean leukocyte count was found to be 7880.08 ± 2559.7 (Table 3). The mean S. Bil. (Serum Bilirubin) level (total) was 15.93 ± 7.0 mmol/L, mean S. Bil. level (direct) was 11.60 ± 4.9 mmol/L, mean SGPT (Serum glutamic pyruvic transaminase), SGOT (Serum glutamic oxaloacetic transaminase) levels were 103.86 ± 80.9 IU/L and 110.53 ± 67.6 IU/L and the mean S.Alp. (Serum Alkaline Phosphatase) level was 1586.97 ± 1103.6 IU/L (Table 3). The levels of IL-2 in the present study were 33.31 ± 56.6 pg/ml (Table 3). IL-6 levels were 167.16 ± 216.4 pg/ml. The endotoxin level in the observed 128 Carcinoma Gall bladder patients was found to be 0.66 ± 0.2 EU/ml and the TNF- α level was 56.00 ± 56.7 pg/ml (Table 3). None of the patients were positive for HIV, HbSAg or HCV.

4. Discussion:

The aim of this study was to determine the circulating concentrations of IL-2, IL-6, TNF- α and Endotoxin in Patients of Carcinoma Gall bladder. Early carcinoma gallbladder has no specific clinical presentation and preoperative diagnosis is rarely possible. Most of these patients are asymptomatic while a few present with clinical features suggestive of benign disease such as right upper abdominal pain interspersed with occasional attack of nausea and vomiting.⁶

Cancer of the gallbladder is an aggressive malignancy that occurs predominantly in elderly people. More than 75% of patients with this malignancy are older than 65 years.⁷ Studies from Pakistan have shown that the age of occurrence is younger than this.⁴ The mean duration of jaundice in the present study was 74.91 ± 50.0 days prior to presentation, whereas in previous studies the average duration of jaundice was 1.15 months i.e. 45-46 days.⁸ In another study, patients had symptoms for an average duration of 3.8 months prior to presentation.⁹ The female to male ratio in the study was 11:3 (73 female: 55 male), in previous studies it was found to be 2:1 (57 female: 29 male).² A high incidence among females suggests a role of female hormones in the aetiology of the disease and the majority of females were in 5th and 6th decade of life.^{1,4} GBC usually arises in a background of cholelithiasis cholecystitis.¹⁰ In a study by Kelvin *et al* fifty patients (58.1%) had comorbidities, including hypertension, diabetes mellitus and other signs and symptoms present were: 93% of the patients presented with abdominal pain and 34% had jaundice at presentation.² In the present study, 69.53% patients presented with abdominal pain and 93% patients had jaundice. In previous studies, presentation of patients was; jaundice with mass right hypochondrium 16 patients (66.6%) and pain right hypochondrium 8 patients (33.3%). Among clinical signs, jaundice was seen in 96.99% of the patients and 35.34% of the patients experienced tenderness in the abdomen.¹¹

The symptoms of carcinoma of the gall bladder mimic benign gall bladder disease until persistent pain due to invasion of the surrounding structure gives a clue to the correct diagnosis. Anorexia, weight loss and jaundice are common but occur late in the disease. Hepatomegaly (often nodular) and a palpable

gall bladder lump indicate an advanced stage of the disease,¹² which in the present study was found in 67(52.34%) patients (Fig.-2). In a study by Burgess *et al*, out of 29 patients eighteen patients (62%) presented with jaundice, 11 patients (38%) had a palpable mass, only eight (28%) had abdominal pain on diagnosis.¹³ The most common symptoms in the study by Pandey *et al* was pain in the abdomen (82.8%), followed by anorexia (41.4%) and significant weight loss (39.4%). An abdominal mass was present in about three-fourths of the cases.⁹ Nausea/vomiting were present in 69(53.91%) patients in the present study (Fig. 2), whereas in a study by Ballinger *et al* five of 19 (26.3%) patients complained of mild nausea and constipation was present in two patients.¹⁴ In the present study, Constipation was present in 96 patients (Fig. 2).

Ultrasonography examination of the abdomen was carried out in all the patients and it showed the presence of gallstones in 69 (69.7%) cases. A mass in the gallbladder was evident in 93 patients (93.9%); it was associated with a calculus in 63.6% patients. Sixty-seven patients (67.7%) had a sonographic evidence of spread of disease beyond the gallbladder. The diagnosis was confirmed preoperatively in 73 patients who presented with a palpable abdominal lump; intraoperatively hepatic involvement was seen in 53 (76.8%) patients.⁹ In the present study, mass in gall bladder was present in 71 (55.47%) cases (Fig. 3).

Short periods of biliary obstruction rarely induce malnutrition but prolonged obstruction of the bile duct with low bile acid secretion may affect fat absorption. It has also been suggested that the presence of endotoxins and liver dysfunction may lead to anorexia. The high incidence of malnutrition in obstructive jaundice-related neoplasia may be caused by the tumor per se and by the anorexic properties of tumor necrosis factor- α (TNF α).¹⁵ Present study supports the study by Rai *et al*. in which, 43% of GBC patients were malnourished with low body mass index.¹⁶

Majority of the patients in present study were anemic (Table 3). In previous studies, Anemia was present in 86 (86.9%) patients.⁹ The hemoglobin levels in case and control groups in the study by Rai *et al* were 10.87 g/dl (+/-1.81 SD) and 11.62 g/dl (+/-1.89 SD), respectively (P < 0.001).¹⁶ The bilirubin levels both total and direct, SGPT, SGOT and alkaline phosphatase were quite high in the present study (Table 3). Total bilirubin concentration was less than 5

mg/dl only in 3.1% of the patients and the level increased to above 16 mg/dl in 31.4% of them. Increased serum SGOT and SGPT levels were found in 72.2% and 71.8% of the patients and a high level of serum alkaline phosphatase was found in 74.8%.¹¹

IL-2 is a cytokine normally produced by the body during an immune response and IL-6 is a pro-inflammatory cytokine secreted by T-cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage leading to inflammation. Aggarwal *et al*. suggested that IL-2, IL-6, TNF are responsible for Autocrine growth.¹⁷ In the present study, the IL-2 and IL-6 levels were 33.31 \pm 56 and 167.16 \pm 216.4 pg/ml (Table 3). In a study by Awasthi *et al* the IL-2 and IL-6 levels were 13.93+ 26.80 and 79.43+ 96.83 pg/ml which increased significantly if no intervention was done.¹⁸ In the study by Tang *et al* the IL-2 levels were 256.21 \pm 55.66 pg/ml and TNF- α levels were 285.01 \pm 42.25 pg/ml.¹⁹ In other study by Kimmings *et al* the IL-6 levels were 4.2 (0.9) pg/ml and TNF - α levels were 21.7 (3.1) pg/ml.⁵ TNF, a kind of cytotoxic protein, is produced by monocytes and macro phagocytes because of production of bacterial endotoxins, tissue injury or tumor cells. Endotoxin is a strong substance to stimulate the production of TNF. Obstructive jaundice causes increased exposure to endotoxin by two different mechanisms. Firstly, the lack of bile in the gut lumen is thought to be responsible for an increase in translocation of endotoxins through the intestinal mucosa. Secondly, biliary obstruction causes a reduction in liver reticuloendothelial system function leading to a diminished clearance of endotoxin by Kupffer cells.⁵ In a study by Yang *et al*. the endotoxin levels in Carcinoma Gall bladder patients was 0.70 \pm 0.25 EU/ml and the TNF- α levels were 2.43 \pm 0.57 pg/ml.²⁰ The endotoxin levels were lower in the present study but the TNF- α levels comparatively were very high (Table 3).

Inflammatory interleukins have been linked with tumorigenesis, which suggests that inflammation is associated with cancer development. Interleukins mediate different steps in the pathway leading to tumorigenesis. The mean circulating concentrations of IL-2 and TNF- α were lower than previous studies. The mean circulating concentrations of IL-6 were higher than the previous studies and the mean circulating concentrations of endotoxin were within the normal limits. Since the TNF- α is dysregulated, it can mediate cancer and also can act as an endogenous tumor promoter. This

concludes that cytokines may participate in gallbladder carcinogenesis and the study provides evidence for a strong link between chronic inflammation and Carcinoma Gall bladder.

References:

1. Sheih CJ, Dunn E, Standard JE. Primary carcinoma of the gallbladder: a review of the 16 year experience at the Waterbury Hospital Health Center. *Cancer*. 1981; 47: 996-1004.
2. Kelvin KNG, Chan SY, Poon RTP, Sheung TF. Management of Carcinoma of the Gallbladder: A Single-Institution Experience in 16 Years. *J of Surg Oncology*. 2008; 97:156–164.
3. Misra S, Chaturvedi A, Misra NC, Sharma ID. Carcinoma of the gallbladder. *Lancet Oncology*. 2003; 4:167–176.
4. Parvez T, Parvez B, Alharbi TM. Advanced carcinoma gallbladder. *J Coll Physicians Surg Pak*. 2007; 17:175-179.
5. Kimmings AN, Deventer SJH, Obertop H, Rauws EAJ, Huibregtse K, Gouma DJ. Endotoxin, cytokines, and endotoxin binding proteins in obstructive jaundice and after preoperative biliary drainage. *Gut*. 2000; 46:725-73.
6. Gupta SK, Shukla VK. Gall Bladder Cancer etiopathology And Treatment. *Health Administrator*. 2003; 17: 134-42.
7. Townsend CM, Beauchamp DR, Evers BM, Mattox KL. Malignant Biliary Disease, Sabiston Textbook of Surgery. 18th Edition. 2008. Available from: <http://www.mdconsult.com>. (Accessed October 29, 2011).
8. Han X, Li J, Han G. Surgical mortality in patients with Carcinoma Gall bladder: a multivariate discriminant analysis. *Hepatobiliary & Pancreatic Diseases International*. 2003; 2 : 435-440.
9. Pandey M, Pathak AK, Gautam A, Aryya NC, Shukla VK. Carcinoma of the Gallbladder- A Retrospective Review of 99 Cases. *Digestive Diseases and Sciences*. 2001; 46:1145–1151.
10. Wistuba II, Gazdar AF. Gallbladder cancer: Lessons from a rare tumor. *Nature Reviews*. 2004; 695-706.
11. Moghimi M, Marashi SA, Salehian MT, Sheikhvatan M. Obstructive jaundice in Iran: factors affecting early outcome. *Hepatobiliary Pancreat Dis Int*. 2008; 7: 515-519.
12. Chattopadhyay TK, Kumar A, Kapoor VK, Sharma LK, Kapur MM, Kapur BML, et al. Carcinoma of the gall bladder - can we do anything? *Postgraduate Med J* .1988; 64: 593-595.
13. Burgess P, Murphy PD, Clague MB. Adenocarcinoma of the gallbladder- a 5 year review of outcome in Newcastle upon Tyne. *Journal of the Royal Society of Medicine*. 1991; 84: 84-86.
14. Ballinger AB, McHugh M, Catnach SM, Alstead EM, Clark ML. Symptom relief and quality of life after stenting for malignant bile duct obstruction. *Gut*. 1994; 35:467-470.
15. Padillo FJ, Andicoberry B, Muntane J, Lozano JM, Min˜o G, Sitges-Serra A, et al. Factors Predicting Nutritional Derangements in Patients with Obstructive Jaundice: Multivariate Analysis. *World J Surg*. 2001; 25: 413–418.
16. Rai A, Tewari M, Mohapatra SC, Shukla HS. Correlation of nutritional parameters of gallbladder cancer patients. *J Surg Oncol*. 2006; 93:705-708.
17. Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G. Inflammation and cancer: How hot is the link? *Biochem Pharmacol*. 2006; 72:1605– 1621.
18. Awasthi I, Chandra A, Kacker LK, Wahal AK, Natu SM, Kumar P. Role of glutamine supplementation on improving the nutritional, functional and immunological status of Carcinoma Gall bladder patients at a tertiary care centre. *Int J Biomed Res* .2011; 2:213-221.
19. Tang ZF, Ling YB, Lin N, Hao Z, Xu RY. Glutamine and recombinant human growth hormone protect intestinal barrier function following portal hypertension surgery. *World J Gastroenterol*. 2007; 13: 2223-2228.
20. Yang YJ, Shi JS, Xie SM, Zhang DT, Cui BS. Effects of different drainage procedures on levels of serum endotoxin and tumor necrosis factor in patients with malignant obstructive jaundice. *Hepatobiliary and Pancreatic Diseases International*. 2003; 2: 426-430.

Table 1: Baseline Patients' General Characteristics:

Variables	n	Mean ± SD	
Age (years)	128	50.86±12.6	
Duration of jaundice (days)	128	74.91±50.0	
Female sex	128	73 (57.03)	
Presence of Cholelithiasis	128	70 (54.69)	
Co-morbidities	None	128	
	Diabetes	128	102 (79.69)
	Hypertension	128	13 (10.16)
	Others	128	4 (3.13)

Figures in parenthesis are %

Table 2: Ultrasonographic Findings of the Patients:

Variables	n	N (%)
GB wall thickening	128	64 (50.00)
GB mass	128	71 (55.47)
Dilated CBD	128	98 (76.56)
IHBRD	128	116 (90.63)
Contiguous liver spread	128	53 (43.44)
Non- Contiguous liver spread	128	71 (58.20)

Figures in parenthesis are %

Table 3: Clinical Characteristic of Patients:

Variables	n	Mean ± SD
BMI	122*	18.33±02.80
Karnofsky score	128	66.02±17.20
Hemoglobin (g/dl)	128	10.69±01.80
Total Leukocyte count (/mm ³)	128	7880.08±2559.7
Serum creatinine (mg/dl)	128	0.71±0.3
S. Bil. (total) (mmol/L)	128	15.93±7.0
S. Bil. (direct) (mmol/L)	128	11.60±4.9
SGPT (IU/L)	128	103.86±80.9
SGOT (IU/L)	128	110.53±67.6
S. Alp. (IU/L)	128	1586.97±1103.6
Cytokines: IL-2 (pg/ml)	128	33.31±56.6
IL-6 (pg/ml)	128	167.16±216.4
TNF-α (pg/ml)	128	56.00±56.7
Endotoxins (EU/ml)	128	0.66±0.2

*3patients had ascites and 3 had edema.

Fig.1: Ultrasonographic Findings Of The Patients (% of patients).

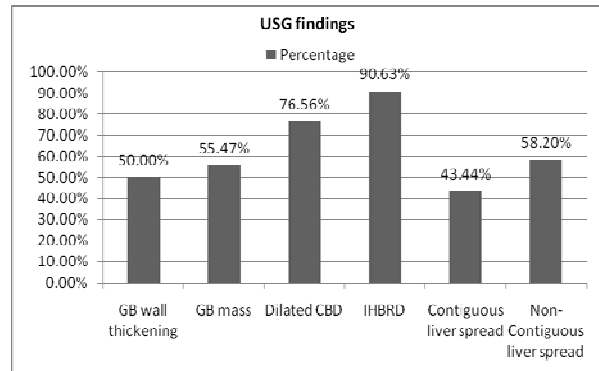


Fig.2: Signs and Symptoms of Carcinoma Gall bladder (% of patients).

