

## RESEARCH ARTICLE

# Ability of Biochemical Parameters to Distinguish between Bile Duct Cancer and Gall Bladder Stones - A Case Control Study in a Tertiary Care Hospital of Pokhara Valley

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### Abstract

**Background:** The present study was designed to comparatively assess alteration of biochemical parameters in bile duct cancer and gall stone disease. **Materials and Methods:** A hospital based case-control study was carried out in the Department of Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal between 1<sup>st</sup> January 2010 and 31<sup>st</sup> December 2012. The variables collected were age, gender, serum total cholesterol, total bilirubin, AST, ALT, serum alkaline phosphatase, albumin and hemoglobin. One way ANOVA was used to examine the statistical significance of differences between groups. A post-hoc LSD test was applied for the comparison of means of control versus case groups. A p-value of <0.05 (two-tailed) was considered significant. **Results:** The mean age of cases and controls was 53.2±21.2 years. The levels of serum cholesterol were higher in cases of cancer 192.5±21.5 mg/dl in comparison to stone cases 168.7±16.1 mg/dl (p value: 0.0001). The total bilirubin showed the marked difference in cases of cancer 7.6±3.2 mg/dl in comparison to stone cases 2.5±0.8 mg/dl of bile duct. There was discernible divergence in values of alkaline phosphatase in cases of cancer 251.5±20.1 IU/l when compared to stone cases 173.2±12.6 IU/l of bile duct. In contrast, there was no apparent deviation in values of aspartate transaminases and alanine transaminases in cases of cancer 59.1±8.9 IU/l and 105.5±26.5 IU/l when compared to stone cases 56.9±7.9 IU/l and 84.5±13.5 IU/l respectively. **Conclusions:** LFT analysis for pre-operative assessment was a good predictive marker in setting apart bile duct cancer and gall bladder stone.

**Keywords:** Biochemical parameters - bile duct cancer - gall bladder stone - Pokhara - Nepal

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### Introduction

Bile duct cancer instigates from the bile duct epithelium; it is further classified into intrahepatic cholangiocarcinoma, extrahepatic bile duct cancer, and gallbladder cancer (Luke et al., 2010). Large gallstones (>3 cm) are one of the predominant risk factors for gallbladder cancer. (Kumar et al., 2009). The other risk factors for bile duct cancer include bile stasis, chronic inflammation, chronic hepatitis, carcinogenic exposures, choledochal cyst, liver fluke (especially in Asia), genetic factors, diet, parity, obesity, bacterial infection, poverty, benign neoplasm of gallbladder, congenital abnormalities and porcelain gallbladder and, in Western countries, primary sclerosing cholangitis (Nishino, 2006). Sick cell disease is associated with the development of pigment gallstones (Jeong et al., 2006). Incidence of gall stones is higher for various non modified and modified factors such as family history, genetic predilection, age, ethnic background

and obesity, metabolic syndrome, diabetes mellitus, dyslipidemia, thiazide diuretics, female sex hormones, reduced physical activity, TPN, Crohn's disease, cirrhosis, rapid weight loss respectively (Premkumar et al., 2012). The rate of occurrence of gallstones becomes 4-10 times in older individuals particularly after the age of 40. The stone types also alter with age, to begin with predominantly of cholesterol but in late life tending to be black pigment stones (Schirmer et al., 2005).

Various metabolic changes such as hepatic insulin resistance, low HDL, high cholesterol level, hypertriglyceridemia have been implicated in gall stone formation and bile duct cancer (Xu et al., 2011). Although, clinical history and blood variables such as total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, haemoglobin (Hb), erythrocyte sedimentation rate (ESR), total leukocyte count (TLC), urea and sugar are routinely measured for every gallstone patient, (Habib

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et al., 2009), the variation of these parameters to make a distinction between bile duct cancer and gall bladder stone has not been appraised. Thus, the objective of present study was designed to assess the alteration of biochemical parameters in bile duct cancer and gall stone disease.

## Materials and Methods

It was a hospital based case control study carried out in the Department of Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal between 1<sup>st</sup> January 2010 and 31<sup>st</sup> December 2012. The variables collected were age, gender, serum total cholesterol, total bilirubin, AST and ALT serum alkaline phosphatase, albumin and hemoglobin. Approval for the study was obtained from the institutional research ethical committee. Estimation of total cholesterol was done by CHOD-PAP method respectively (Trinder, 1969). The total bilirubin was estimated by Jendrassik/Grof method (Garber, 1981). The transaminases (AST and ALT) were estimated by liquid UV test (Henley et al., 1955). Estimation of serum alkaline phosphatases was done by standard enzymatic method (German, 1972). The albumin was measured by BCG method (Doumas et al., 1971). Estimation of hemoglobin was done by a portable hemoglobinometer (Gong et al., 1999). All these laboratory parameters were analyzed using Human reagent kits and with the help of semi autoanalyser (Human, Germany). Analysis was done using descriptive statistics and testing of hypothesis. The data was analyzed using Excel 2003, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version. The One way ANOVA was used to examine the statistical significant difference between groups. Post Hoc test LSD used for the comparison of means of control versus case groups. A p-value of <0.05 (two-tailed) was used to establish statistical significance.

## Results

Out of 90 cases selected, 30 were of cancer, 30 were of stone and rest were controls.

Table 1 illustrates that mean age of cases and controls were 53.3±21.24 yrs. There was total 60 cases and out of that 30 were of cancer origin and rest were of stone. Number of females were more in comparison to males in cases of bile duct cancer and gall stone. Out of 30 cases of cancer origin, 16 were of bile duct cancer, 2 were of intrahepatic cancer, 8 were of perihilar cancer, 2 were of extrahepatic cancer and 2 were of gall bladder cancer. Out of 30 cases of stone, 20 were of gall bladder stone, 7 were

of common bile duct stone, and 3 were of intrahepatic duct stone.

Table 2 depicts that levels of serum cholesterol was more in cases of cancer 192.45±21.50 mg/dl in comparison to stone cases 168.65± 16.05 mg/dl of bile duct (p value: 0.0001\*). The total bilirubin showed the marked difference in cases of cancer 7.6±3.2 mg/dl in comparison to stone cases 2.5±0.8 mg/dl of bile duct. There was discernible divergence in values of alkaline phosphatase in cases of cancer 251.5±20.1 IU/l when compared to stone cases 173.2±12.6 IU/l of bile duct. In contrast to that, there was no apparent deviation in values of aspartate transaminases and alanine transaminases in cases of cancer 59.1±8.9 IU/L and 105.5±26.5 IU/l when compared to stone cases 56.88±7.9 IU/l and 84.5±13.5 IU/l of bile duct respectively. Furthermore, serum albumin was less in cases of cancer 3.1±0.6 g/dl in comparison to stone cases 4.1±0.8 g/dl of bile duct. The hemoglobin was mildly reduced in cancer cases 10.3±2.6 g/dl in comparison to stone cases 12.8±1.2 g/dl.

## Discussion

Cholelithiasis is a major health problem world wide, particularly in adult population. There is a considerable geographical and regional variation in its prevalence. Intra operative ultrasonography is not widely available in our health care system especially in Nepal for accurate differentiating between bile duct cancer and stones. Furthermore, the long list of investigations makes the diagnostic pathway complex and expensive in developing countries. The components of LFT's appears to be a easily available, cheap and better indicator for differentiating between bile duct cancer and gall stones. In our present

**Table 1. Elaboartion of Cases of Cancer and Stone in Accordance to Their Numbers**

Age (years)	53.25±21.24
Number of cases of cancer origin	30
Males/Females	13/17
Bile duct cancer	16
Intrahepatic cancer	2
Perihilar cancer	8
Extrahepatic cancer	2
GB cancer	2
Number of cases of stone=30	
Males/Females	10/20
Intrahepatic duct stone	3
Common bile duct stone	7
Gall Bladder stone	20
Normal controls	30
Males/Females	15/15

**Table 2. Variation of Biochemical Characteristics in Cases of Bile Duct Cancer and Gall Bladder Stone**

Variables	Bile duct cancer cases	Gall bladder stone cases	Controls	p value
Total Cholesterol (mg/dL) (normal range: 100-220)	192.5±21.5 (182.9,203.1)	168.7±16.1 (160.9, 176.5)	138.1±12.7(131.8,144.4)	0.0001*
Total Bilirubin (mg/dL) (normal range: 0.2-1.2)	7.6±3.20 (6.00, 9.2)	2.5±0.8 (2.10,2.9)	0.5±0.2 (0.4,0.6)	0.0001**
Alkaline phosphatase (IU/L) (normal range: 38-115)	251.5±20.1 (241.8, 261.1)	173.2±12.6 (167.5,176.9)	84.5±13.5 (78.1, 90.1)	0.0001**
Aspartate transaminase (IU/L) (normal range: 13-34)	59.1±8.90 (56.10, 64.1)	56.8±7.9 (51.2,61.4)	26.4±3.8 (24.1, 28.9)	0.525
Alanine transaminase (IU/L) (normal range: 5-46)	105.5±26.5 (92.8,118.2)	84.5±13.5 (78.1,90.1)	31.3±5.2 (28.7, 33.9)	0.0132
Albumin (g/dL) (normal range: 3.3-5.3)	3.1±0.60 (2.80,3.4)	4.1±0.8 (3.7,4.5)	4.8±0.6 (4.5,5.1)	0.0001**
Hemoglobin (g/dL) (normal range: 13.0-17.0)	10.3±2.60 (9.00,11.6)	12.8±1.2 (12.2,13.4)	14.6±1.2 (14.0,15.2)	0.0001**

study, number of females were more in cases of bile duct cancer and gall stone as mutagenic toxins secreted reside longer in the gallbladder due to stasis from impaired contractility associated with the female hormone, progesterone. The cholesterol levels were more in cases of bile duct cancer  $192.45 \pm 21.50$  mg/dl (182.92, 203.06) and gall stone  $168.65 \pm 16.05$  mg/dl (160.85, 176.54) when compared to controls  $138.1 \pm 12.7$  mg/dl (131.8, 144.4) ( $p: 0.0001^*$ ). Our results concurred with Andreotti (Andreotti et al., 2008). Hepatic insulin resistance which is common metabolic dearrangement in both the gall stone formation and bile duct cancer may act by enhancing hepatic cholesterol secretion, depressing bile salt synthesis and/or impairing gallbladder motility (Biddinger et al., 2008). The serum total bilirubin showed a marked difference for bile duct cancer ( $7.6 \pm 3.2$  mg/dl) when compared to gall stone formation ( $2.5 \pm 0.8$  mg/dl) as there was severe obstruction in cancer cases ( $p: 0.0001^*$ ). The study done by park also showed similar results (Park et al., 2006). In both cancer and stone cases, mildly raised AST and ALT suggested the very less injury to hepatocytes. The serum alkaline phosphatase was markedly raised in cancer cases  $251.5 \pm 20.1$  IU/L (241.8, 261.1) when compared to stone cases  $173.2 \pm 12.6$  IU/L (169.5, 176.9) and controls  $84.5 \pm 13.5$  IU/L (78.1, 90.1) due to more severe injury to bile duct epithelial cells in cancer cases ( $p: 0.0001^*$ ) (Bhudhisawasdi et al., 2004). Furthermore, it was more raised in females owing to increased bone turn over or simultaneous formation of osteoid. Reduced hepatic function in cancer cases  $3.1 \pm 0.6$  g/dl (2.8, 3.4) lead to decreased synthesis of albumin when compared to stone  $4.1 \pm 0.8$  g/dl (3.7, 4.5) and controls  $4.8 \pm 0.6$  g/dl (4.5, 5.1) ( $p: 0.0001^*$ ) (Zare et al., 2011). Thus, this study found statistically significance positive correlation of biochemical parameters to make a distinction between bile duct cancer and gall bladder stones.

In conclusion, LFT's analysis in pre-operative assessment was a good predictive marker in setting apart bile duct cancer and gall bladder stone.

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