COVER PAGE:

I am submitting a manuscript for consideration of registration in Clinical Trials. The manuscript is entitled "**Does timing of completion radical cholecystectomy determine the survival outcome in incidental carcinoma gallbladder – a single center retrospective analysis**".

NCT number – not available

Date – 05.11.2021 (Results were last updated on 01.01.2019)

STUDY PLAN:

Surgical Gastroenterology department at our institute is a tertiary referral center for hepatobiliary malignancies; covers a large part of North and North-east India. The details and progress report of patients of Gallbladder Cancer (GBC) being referred to and treated at the institute is maintained in a prospective manner on hospital data base. This included demographic data, presenting complains, details of relevant investigations done outside and in the hospital, previous surgery, pre-hospital course, operative findings, post operative course and final histopathology. During the study period (Jan 2009 to Dec 2018), 1049 participants with the diagnosis of GBC (including 91 IGBC patients) were admitted; 239/958 participants with the diagnosis of GBC and 48/91 participants with the diagnosis of IGBC could undergo resection with curative intent. Information regarding adjuvant therapy, recurrence, and survival were retrieved through data maintained on follow up out-patient cards till December 2019. Few participants who stopped visiting the hospital were contacted telephonically or through letters.

Tumors were staged as per 8th edition of AJCC-TNM staging. Preoperative workup included contrast enhanced axial imaging (CECT – contrast enhanced computed tomography). In few cases with high risk features (residual disease on CT scan or late presentation), Positron Emission Tomography (PET) scan was added. Participants found fit for surgery and those with resectable disease on imaging were considered for the definite procedure. Laparoscopy was done preferably in those presenting beyond 4 weeks of index surgery or residual disease (local or nodal) on imaging to rule out metastasis. In absence of obvious distant metastasis (ascites, peritoneal nodules, mesenteric nodules, serosal nodules), inter-aortocaval (IAC) tissue was sent for evaluation of nodal metastasis. Once IAC node was found negative for deposits on frozen-section examination, radical re-resection was attempted. It included wedge resection of liver or Segment 4b/5 excision with regional lymphadenectomy. Extrahepatic bile duct excision was added in presence of positive cystic duct margin or direct invasion into the duct.

All available histopathology blocks and slides following the index cholecystectomy were re-reviewed by the pathologists at our institute. Tumor type, differentiation and primary T stage were documented for majority of cases. Interval between index cholecystectomy and date of re-operation was calculated for all the participants and divided into 3 groups: Early (<4weeks),

Intermediate (4-12 weeks) and Late (>12 weeks). Primary objective was to assess the effect of tumor characteristics and the time gap (between index surgery and re-resection) on overall survival following curative (R0/R1) resection.

Statistical Analysis:

Continuous variables were presented in median (range) while categorical data was expressed in frequency (%). A variable was considered as normally distributed when the Z-score was within ±3.29 (n≥50). To compare the means/medians between two groups, independent samples t-test/Mann Whitney U test was used. To test the association between categorical variables, Chi square tests or Fisher exact test was used. Event free survival was calculated for the patients using the Kaplan-Meier survival plots using Log rank test and Breslow test. Univariate and multivariate Cox regression analysis were used to identify the predictors of survival. Variables found significant in Univariate analysis were included for multivariate analysis. A two tailed p-value of <0.05 was considered statistically significant. Statistical package for social sciences version-23 (SPSS-23, IBM, Chicago, USA) and MedCals software was used for data analysis.

RESULTS:

During the study period, 48 participants {11 (22.9%) in Group 'E', 31 (64.5%) in Group 'I' and 6 (12.5%) in Group 'L'} underwent re-resection with curative intent. The demographic profile (age and gender), co-morbidities, tumor characteristics, extent of resection, resection margin and nodal clearance were similar in the three groups (each p>0.05). Median age of participants with IGBC was 55 years and 2.7 times more common among females.

Similarly, presence of residual disease, grades of the tumor, primary stage of the tumor following index cholecystectomy, final stage after curative resection, adjuvant therapy and associated morbidity were statistically equal among the groups (each p>0.05). No post operative mortality was recorded. The most common complication in the postoperative period was surgical site infection (SSI), recorded in 8 patients. Three patients required postoperative percutaneous drainage for intra-abdominal collection. No patient required prolonged hospital stay or reoperation.

Overall Survival (OS) and Disease-free survival (DFS):

Mean and median follow-up was 51.6 and 40.6 months, respectively (range: 1.2-130.6). Total 9(18.7%) patients were reported lost to follow up during the study period. Mean overall survival (OS) in study patients was 91.75(95% CI: 8.32-75.44) months. As OS percentage did not reach <50%, resultant median survival could not be computed. Similarly the mean DFS following curative resection was 85.63 (95% CI: 69.48-101.79) months (As the patient recurrence free survival percentage did not reach below 50%, resultant median recurrence free survival time could not be computed).

In present study, 29(60.4%) of those undergoing definitive surgery received chemotherapy. Around 58% received >1 cycle and 44% could complete their therapy. Radiotherapy was given to 42% participants. The most important indications for adjuvant therapy included good performance status with advanced stage and margin positive and/or node positive disease. OS among participants who received adjuvant therapy was similar to those who were not offered chemotherapy [88.10 (95% CI: 67.18-109.03) Vs 87.56 (95% CI: 61.66-113.45) months (p=0.916) (Fig 1). Similarly DFS among participants who received adjuvant therapy was similar to those who did not receive any post operative treatment. [86.18 (95% CI: 65.73-106.64) Vs 85.37 (95% CI: 59.82-110.91) months (p=0.915).
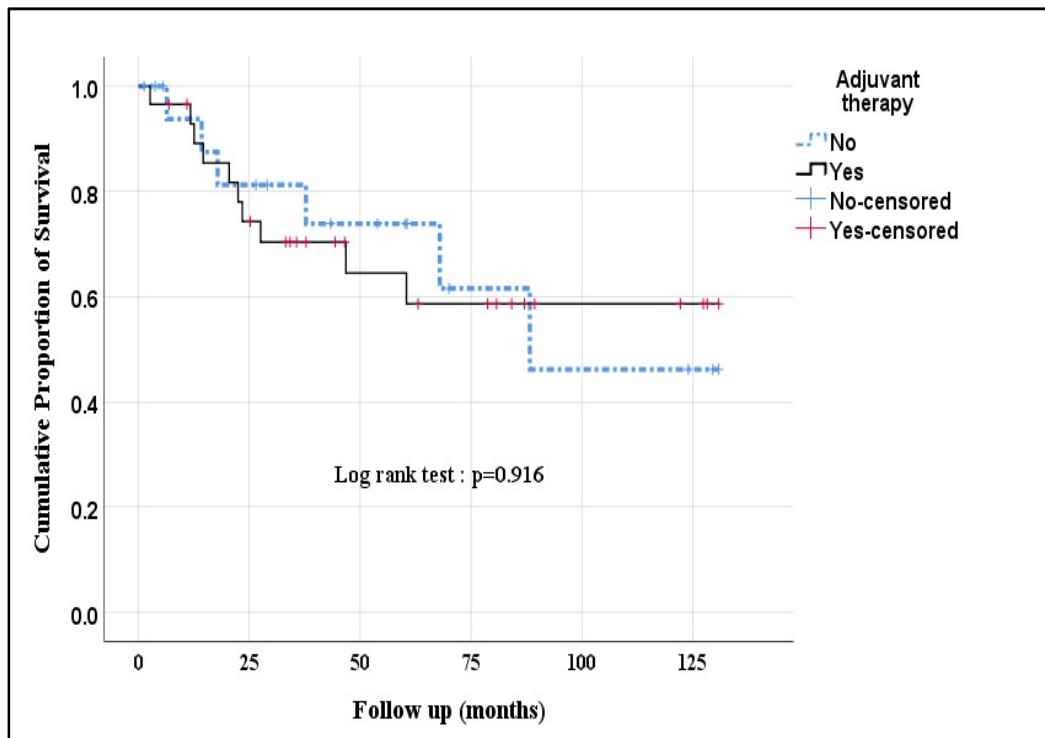
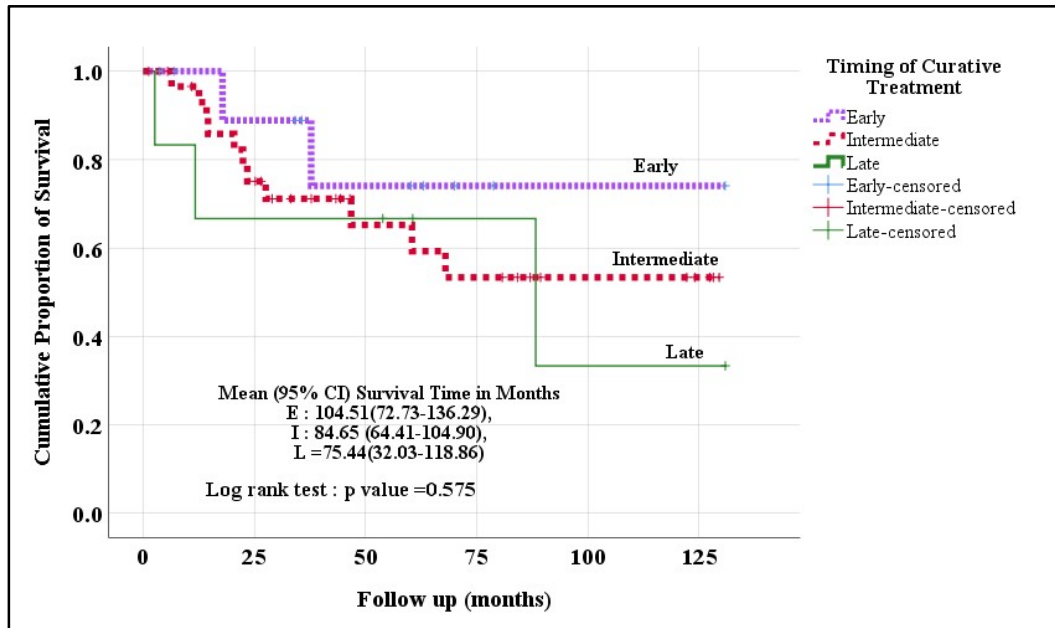Figure 1: Overall impact of adjuvant therapy on event free survival.

Figure 2 : Overall event free survival in study patients as per timing of treatment.

Kaplan Meier method (log rank test) was used to compare the event free and disease-free survival time of the patients among the three groups. Though the participants operated within 4 weeks and between 4-12 weeks fared a better OS and DFS than those operated beyond 12 weeks, the difference could not achieve statistical significance. (Event free survival time (p=0.575) and
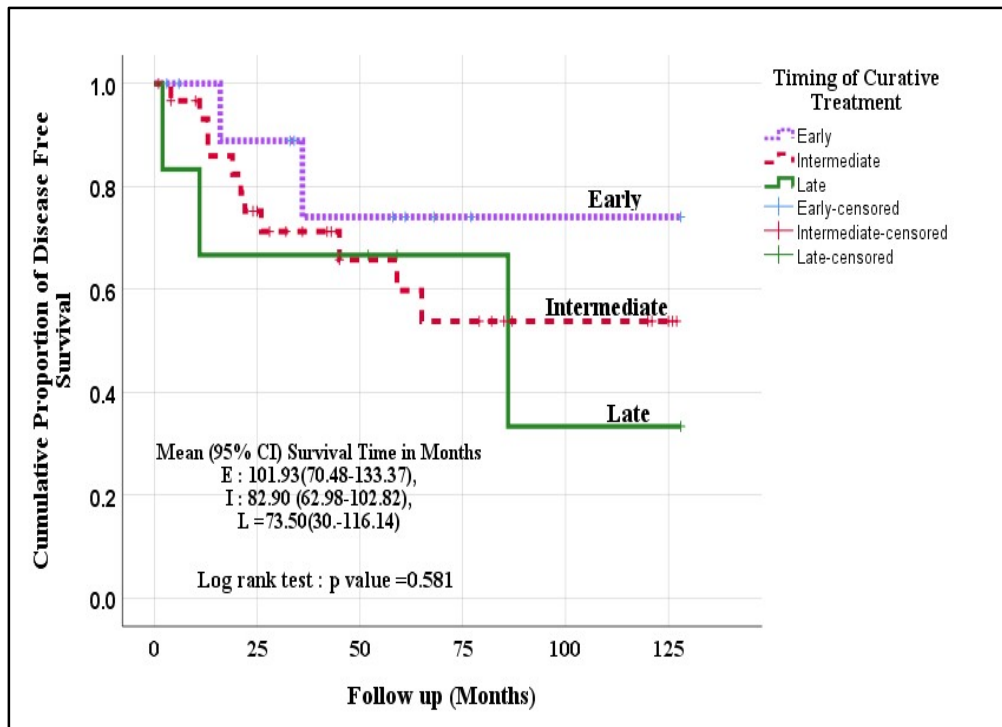
disease-free survival time (p=0.581))(Figures 2&3)

Figure 3: Overall disease free survival in study patients as per timing of treatment.

Survival Analysis:

On Univariate analysis of factors associated with significantly poor survival following curative resection were: presence of nodal metastasis, poor differentiation and lesions necessitating CBD excision. Other factors portending an unfavorable outcome included presence of residual disease, lympho-vascular invasion and delay in re-resection beyond 12 weeks, though the difference was not statistically significant. Primary tumor stage, resection margin and use of adjuvant therapy did not affect the overall survival (each p>0.05). Residual disease was present in 20 out of 48 (41.6%) participants. The distribution of residual disease following re-resection, according to the index T-stage was (16% in T1, 29% in T2 and 72% in T3 tumors).

To further assess the predictors of survival among the participants, multivariate Cox regression analysis was used. Three variables (Node status, Grades and Extent of resection) which were significant on Univariate analysis were included in multivariate analysis. Results showed that disease grade was the only significant independent predictor of survival. Presence of positive

nodes (p=0.052) and the need for CBD excision (0.133) was associated with increased relative risk of disease related mortality, although the difference was not statistically significant.