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A PROSPECTIVE OBSERVATIONAL STUDY TO EVALUATE THE ROLE OF RED CELL DISTRIBUTION WIDTH AS A MARKER TO PREDICT TUMOR BURDEN IN GALLBLADDER CANCER.

General Surgery

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KEYWORDS

INTRODUCTION:

Approximately 80-90% of the biliary tract cancers are Gall Bladder cancers, making it one the most common malignancy of the biliary tract. It is a rare malignancy that has silent course, initially asymptomatic and when later detected usually is fatal. An early diagnosis is the key to curative treatment.[1] The prognosis of gallbladder malignancy is grave and mainly depends on the histological subtype, grade and stage of the tumor at the time of presentation. The overall survival is usually 6 months with 5- year survival rate being less than 5%.[2]

In India, there are marked geographical variations in the prevalence of gallbladder cancer i.e. more in the northern part of India compared to the southern part. The mean age of presentation of GBC in India (51±11 years) is younger as compared to the western population (71.2±12.5 years) but is similar to the Latin American countries.Usually, it manifests as diffuse thickening of the GB wall or as a GB mass arising from the fundus, neck or body of the GB .[3] Metaplasia proceeds to dysplasia, carcinoma in situ, and then invasive cancer, according to the postulated carcinogenesis of GBC in 5 to 15 years.[4,5] The frequent rapid and silent course of gall bladder malignancy is responsible for its poor prognosis and a satisfactory outcome usually depends on an early diagnosis and surgical resection. Tumor markers such as CEA, cancer antigens (CA) CA125, CA242, and CA199 have been widely used for the diagnosis of different types of cancer (*e.g.* liver, gastric, colorectal, and pancreatic) & combined detection of these markers could increase the sensitivity for diagnosis and prognosis of GBC. Other more readily available and cheap blood markers are being searched.

The red blood cell distribution width (RDW) is a measure of the heterogeneity of the tumor distribution of red blood cell size. The RDW is calculated from the coefficient of variation of the red cell volume distribution histogram.[6] It is one of the routine whole blood investigations performed in hospital settings. RDW is also linked with chronic inflammation and shows a positive correlation with other markers like ESR and C-reactive protein (CRP) and is important for tumor progression and prognosis.[7,8]

The preoperative diagnosis of gallbladder malignancy is the field of concern in oncologic studies. The relative prognostic factors for GBC have not yet been identified, and previous research has suggested that age, sex, CA19.9 levels and tumour-node-metastasis (TNM) stage might affect the survival of these patients. Taking into account, the relationship between chronic inflammation and gallbladder malignancy, RDW is suggested as a good indicator of the inflammatory state and is subsequently looked at as a prognostic indicator in GBC.[9] Studies have found that preoperative Neutrophil lymphocyte ratio , Platelet lymphocyte ratio and serum carbohydrate antigen 19–9 (CA19–9) levels are associated with poor prognosis of GBC.[10,11]

CA19-9 is a tumor-associated antigen, initially reported by Koprowski et al.[12]It has been shown that CA19-9 is a prognostic indicator in GBC.[13] However, the optimal cutoff value has remained controversial. In our analysis, we used 250.90U/mL as the cutoff point of CA19-9. The use of CA19-9 might provide an opportunity for guiding personalized neoadjuvant therapy in patients with GBC.

It is also a tumor marker used primarily in the management of pancreatic cancer.[14]CA19-9 can be elevated in many types of malignant and benign conditions. It can also be elevated in people with obstruction of the bile ducts.In patients who lack the Lewis antigen (a blood type antigen on red blood cells), CA19-9 is not produced by any cells, even in those with large tumors.

The aim of this study is to establish the correlation between levels of Red cell distribution width with tumor burden of gallbladder cancer. Also to establish a relationship between Red cell distribution width with tumor markers like CA 19.9 in gallbladder cancer**.**

METHODS AND MATERIALS:

The study was an hospital based observational study conducted in the department of General surgery, S.M.S. hospital, Jaipur from July 2020 to June 2021. Study population comprises all patients admitted in surgical OPD, Oncosurgery OPD, Surgical Gastroenterology OPD and Emergency department of S.M.S. Hospital with Gallbladder cancer, who went through inclusion and exclusion criteria. Inclusion criteria includes patients 20 to 70 years of age presented with gallbladder cancer, confirmed by radiological and pathological investigations and waiting for further management with given written and informed consent. Patients without consent, with previously treated malignancy, with hematological disorders, severe anemia & iron supplementation therapy, with infectious or inflammatory disease, recent deep venous thrombosis (past 6 months), recent blood transfusion (past 3 months), chronic obstructive pulmonary disease, hepatitis B or C, severe liver and renal insufficiency & kidney transplantation, pregnancy, cardiac failure, arrhythmia, untreated thyroid disease were excluded.

The sample size of 114 patients of gallbladder cancer was calculated at 95% confidence interval at 80% of study power and alpha (α) error 0.05 assuming PSM correlation coefficient of 0.26 between CA 19.9 & RDW as found in the seed article . It is further rounded off to 120 patients of gallbladder cancer for study purposes considering 5% attrition. Qualitative data was expressed in the form of proportions and percentages. Quantitative data was expressed in Mean +/- Standard Deviation. Qualitative data was compared using the chi square test. Unpaired t-test was used to infer the difference in means. After the patient selection using ultrasonography and ct imaging, red cell width was measured and staging of gallbladder carcinoma was done using AJCC TNM staging (eight edition). Palliative or operative strategy was adopted for treatment.

RESULTS:

In our study, 25% of patients were in stage T2A, 21.67% in stage T2B, 19.17% in stage T4A while 17.5% in stage T3A. Gall bladder carcinoma was found 41.6% more common in females than males out of which 63.3 % of patients were more than 50 years old. In our study, 18.3% of patients were smokers and 81.7 % of patients were non-smokers.In our study, 22.50% patients presented with jaundice, 12.50% patients presented with pain abdomen, 23.3% patients presented with fever, 39.1% patients presented with loss of appetite. So, the most common presentation of GBCs in our study was loss of appetite.

Our study reveals that the Mean RDW was 15.79± 2.13 while Mean Serum CA 19.9 level was 145.6±285.6. 28 patients had serum RDW <14mg/dl and 92 patients had between >14mg/dl. Also, 64 patients had CA 19.9 <37 u/ml , 20 patients had between 38-100 u/ml and 36 patients had >100u/ml. The stage of gall bladder carcinoma and CA

19.9 level had higher trends in stage IV and the p value is significant.P value of relationship between stage IV GBC and RDW value did come out as significant in this study.However the higher RDW levels in advanced stages of GBC indicates that RDW levels can be correlated with advancement of disease.

Our study also shows that RDW and CA 19.9 levels are correlated and this relationship is statistically significant (0.00132). Mean CA19.9 value is 573 in 20-30 yrs, 158.03 in 31-40 yrs, 163.77 in 41-50 yrs,136.13 in 51-60 yrs and 113.37 in 61-70 yrs and p value of mean CA19.9 is <0.05 which is significant . Mean RDW levels are 16.5 ±4.73 in 20-30 yrs, 16.1±2.33 in 31-40 yrs, 15.7±1.69 in 41- 50 yrs, 15.5±2.19 in 51-60 yrs, 15.5±2.26 in 61-70 yrs group.

Correlation between RDW levels and total bilirubin, with direct bilirubin levels and ESR is significant while it is insignificant with hemoglobin , platelet and TLC levels.

DISCUSSION:

The thesis was conducted in the Department of General surgery, SMS Medical College, Jaipur during July 2020 to June 2021. The incidence of gallbladder carcinoma has increased in recent years worldwide. In this study we analyzed the correlation between tumor markers and different clinicopathological features of gallbladder carcinoma and compared the mean level of these markers in gallbladder carcinoma cases.

Gallbladder Carcinoma (GBC) is the most common malignancy of the biliary tract and the sixth most common malignancy of the gastrointestinal tract worldwide.It is an aggressive and a late symptomatic disease and most of the patients are treated at advanced stages.

The prognosis is usually dismal and the 5 year survival rates have been reported to be less than 5% for the more advanced stages. The countries with a high incidence of gallbladder cancer include Chile, Poland, India and Japan.A very high incidence of this cancer has been reported among women in northern India (21.5/100,000) and among female Native American Indians (14.5/1000,000).

In our study maximum number of patients presented with stage 2 (n=56)(46.67%), rest were stage 1(n=1)(0.83%),stage 3(n=26)(21.67%) and stage 4 (n=37) (40.83%). **Henson DE, et al**[15] found 26.4% stage 1, 5% stage 2 , 28.8% stage 3 and 39.8% stage 4 disease. Maximum number of patients in their study were in stage 4 at the time of presentation.The changing trend of early presentation of GBC can be attributed to the use of increasing imaging modalities in evaluation of symptomatology related to GBC.

In our study, 120 gall bladder carcinoma patients were included with a mean age of 53.53±10.5 years. Average age of gallbladder cancer was 50- 55 years in various other studies in India and other Asian countries. In a study done by **Globocan 2018**[16] ,out of the 120 cases about 1.6%, 10.8%, 24.1%, 33.33% & 30% were in the age group 20-30 years, 31-40 years,41-50 years,51-60 years & 61-70 years respectively. It shows that the age of presentation is according to the current trends of gallbladder in various age groups.**Dutta U et al (2019)**[3] found that GBC in India usually affects younger patients in contrast to Western population which can be linked to multiple risk factors like gallstones and lower socioeconomic status.

In the present study, 70.8% cases were female, 41.6% higher than males.**A Duffy MD et al(2008)**[17] found that among the 435 gallbladder cancer cases, 285 were (65.5%) females and 150 (34.5%) were males. **Rawla P et al(2019)**[18] found that women are two to six times more affected with gallbladder cancer probably due to effect of hormone estrogen to increase the saturation of cholesterol in bile, thus increasing the risk of gallstone formation.[19] **Konstantinidis IT et al (2009)**[20] found 72.3% patients were females out of 332 patients of gall bladder malignancy.

In our study, 18.3% of patients were smokers and 81.7% of patients were non-smoker. **S Kono et al(2002)**[21] found that cigarette smoking is unrelated to gallbladder malignancy and other gallbladder disorders. However, latest studies by **Ding Wenbin et al** (2013)[22] concluded that cigarette smoking is significantly associated with increased risk of gallbladder carcinoma. Thus further studies are required to show an association between GBC and smoking. In our study, 22.5% patients presented with jaundice, 23.30% patients presented with fever, 12.50% patients presented with abdominal pain and lump, 39.1% patients presented with loss of appetite.**Kridis WB et al(2018)**[23] found that the common presenting complaints in non-emergency cases were usually nonspecific i.e. abdominal pain (72–77%) ,jaundice (58%) (secondary to either tumor invasion or extrinsic compression of the bile ducts by lymphadenopathy or tumor or by the presence of liver metastases),nausea and vomiting (20–49%) and mass of the right hypochondrium(15–50%) along with loss of appetite, fever, chronic abdominal discomfort, weight loss and itching (pruritus)in GBC patients.

RDW in GBC: In our study of 120 GBC patients, mean RDW was 15.79±2.13. **Xie Youjun et al (2019)**[24] found in his study that GBC patients with stage III+IV had higher levels of RDW(%) than stage I+II (16.1±2.5 vs 14.9±2.0, *P*=.011). Correlation analysis showed that RDW had positive correlations with TNM stage (correlation coefficient=0.302, *P*=.002). The cut-off value of RDW was observed to be 14.5% in patients with GBC (area under the curve=0.757, 95% confidence interval =0.677–0.838, *P*=.000). The role of RDW as a potential biomarker in malignant diseases is well documented in literature .Most of these studies in the literature are mostly related to the inflammatory aspect of malignancies and since RDW is a marker of chronic inflammation it can be used as prognostic marker, however few studies have mirrored the relationship between RDW and gallbladder cancer and relationship between raised RDW and advancement of the disease.[25] **John Aaron et al (2018)**[26] showed that RDW and other inflammatory markers (Neutrophil- lymphocyte ratio, platelet lymphocyte ratio) have significant role in predicting gallbladder cancer.

Various studies have utilized RDW as a marker of malignancy. **Fang-Ming Wang et al** (2014)[27] in his study found that higher RDW is associated with increased risk of Renal cell carcinoma along with positive association with cancer stage. **Junji Ichinose et al** (2016)[28] showed that higher RDW values were significantly associated with higher morbidity and reduced survival in elderly patients who underwent resection for NSCLC.Similarly **Beyazit Y et al (2012)**[29] showed that higher RDW is useful as a predictor to differentiate between benign and malignant causes of biliary stricture.

Serum CA 19.9 in GBC : In our study, Mean Serum CA 19.9 level was 145.6 which is consistent with higher CA 19.9 values in biliary malignancies. **Sergio Renato Pais-Costa et al (2012)**[30] showed that CA19.9 is a significant prognostic factor for gallbladder carcinoma. **Yu Tunan et al (2014)**[31] found that by a combined utilization of CA 19-9 and CEA, individualized prediction of survival can be done in resectable GBC before operation .He also showed that extended radical operation brings the most prognostic benefits in patients with non elevations of either CA 19-9 or CEA. **W Steinberg et al (1990)**[32] showed that CA 19.9 with an upper limit of normal of 37 U/ml, has overall sensitivity of approximately 80% and its specificity is 90%. If higher cutoffs are used, the specificity rises so that, at levels greater than 1000 U/ml, the marker's specificity approaches 100%. Acute cholangitis and cirrhosis are two benign conditions that might raise this assay significantly. This tumor-associated marker is also helpful in predicting unresectability of pancreatic adenocarcinoma, as 96% of tumors that result in blood levels greater than 1000 U/ml have been found to be unresectable.After potentially curative surgery, the CA 19-9 can help prognosticate survival. **K S Goonetilleke et al** (2007)[14] showed that CA19.9 should be used in contemporary algorithms for diagnosis of pancreatic carcinoma and it is superior to other biomarkers available. **Zhu, Ji-Qiao et al** (2015)33 showed that elevated CA19-9 combined with CEA and/or CA125, a polyp greater than or equal to 1.2 cm, and focal gallbladder wall thickening of more than or equal to 5 mm indicated a higher risk of developing incidental gallbladder cancer in elderly patients.

In our study, mean CA 19.9 level was 573 in 20-30 yrs, 158.03 in 31-40 yrs, 163.77 in 41-50 yrs, 136.13 in 51-60 yrs and 113.37 in 61-70 yrs and p value of mean CA19.9 is <0.05 which is significant . Mean RDW levels was 16.5 ±4.73 in 20-30 yrs, 16.1±2.33 in 31-40 yrs, 15.7±1.69 in 41- 50 yrs, 15.5±2.19 in 51-60 yrs, and 15.5±2.26 in 61-70 yrs. The level of CA19.9 varies according to the age group of patients of GBC with the p value of <0.05 ,which is statistically significant. It shows age group has significant impact on Mean CA19.9 levels in GBC's.

CONCLUSION:

Association between Gallbladder cancer cases and serum RDW levels

was found statistically significant with p value <0.001. Higher RDW levels in advanced stages of GBC indicates that RDW levels can be correlated with advancement of disease. Stage of gallbladder carcinoma and CA19.9 levels had higher trends in stage IV and the p value is significant.RDW and CA 19.9 levels are related and this relationship is statistically significant with p value 0.00132. Serum RDW levels and CA19.9 levels in different age groups of patients with GBC do not have much difference. So age group has no impact on Serum RDW and CA19.9 levels in GBC's. The Serum RDW was found significant with direct bilirubin , ESR and total bilirubin with p-value <0.001.

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