A Study of Red Cell Distribution Width as a Biomarker to Differentiate Between Benign and Malignant Diseases of Gall Bladder

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Abstract

Introduction: Gall bladder malignancies have got poor prognosis primarily due to its delayed initial presentation and dissemination of the disease at the time of diagnosis. Recent studies have reported an increased mortality in subjects with elevated red cell distribution width (RDW). This study proposes to see whether RDW can be used to differentiate benign from malignant gall bladder (GB) pathology preoperatively.

Materials and methods: This single-center prospective cross-sectional study was conducted over a period of 2 years. A total of 110 patients of which 27 subjects having GB malignancy and 83 subjects having benign gall stone disease were enrolled into the study after getting informed consent. Data were recorded and the patients were treated as per indication. Statistical analysis was done.

Results: The mean RDW in the carcinoma GB group was 16.6±1.67%, and in the benign gall stone disease group was 13.6±1.36%. The variation of RDW between the two groups was statistically significant. Receiver Operating Characteristic (ROC) curve plotted with the data showed sensitivity and specificity for a cut off value of 15.05% at 77.8% and 84.3% respectively. Statistically significant association was there between the stages of presentation of GB malignancy and mean RDW value. Higher levels of RDW were seen in GB malignancy with advanced disease.

Conclusion: Red cell distribution width (RDW) is an effective inflammatory biomarker to predict carcinoma GB in the pre-operative period and can be used to differentiate between the benign and malignant diseases of gall bladder.

Keywords: Biomarker, Carcinoma GB, Gall Bladder, Red Cell Distribution Width

INTRODUCTION

Gallstone disease is one of the commonest benign disorders of the gall bladder. The prevalence of gall stones among the adult population in India is around 7-8% [1]. Female gender, obesity and advancing age are also considered as risk factors for gall stone disease [2,3]. Among the entire population who are affected by the gall stones, 80% remains asymptomatic. Only the remaining 20% of the population will show any symptoms of the disease during their lifetime. Around 2% of this population will develop symptoms per year [4,5].

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The association of gall stones with gall bladder carcinoma is well studied. It is implicated that the stone size of more than 3cm is a potential risk factor for gall bladder malignancy [6-9]. Gall bladder malignancies have got poor prognosis primarily due to its delayed initial presentation, dissemination of the disease at the time of diagnosis. The 5-year survival rate of gall bladder malignancies is around 4-5% and median survival period in advanced gall bladder malignances is less than 6 months [10,11]. It is one of the most lethal malignancies and in advanced disease states, the long-term survival rates are very low.

The incidence of gall bladder malignancy in the northern part of India is more than that in the southern part. Indian Council of Medical Research (ICMR) database shows that the majority of cases of gall bladder malignancy are from the northern states. It is more common in females, and it is the most common cancer of the digestive system in females. The highest incidence is seen in females of age above 65 years.

Early stages of gall bladder carcinoma remain purely asymptomatic. Even the symptomatic cases may mimic as biliary colic or chronic cholecystitis. Advanced stages may present as jaundice, anaemia, deranged liver function profile, oedema etc. The tumour markers commonly used in gall bladder carcinoma are carcinoembryonic antigen (CEA) and Carbohydrate Antigen 19-9 (CA 19-9). Elevated CEA has a specificity of 90% in gall bladder malignancy but has only a low sensitivity of 50% [12]. At levels higher than 20 U/mL, the sensitivity and specificity of CA 19-9 is around 75% in gall bladder malignancies [13].

Ultrasonography, Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) scan, Fluorodeoxyglucose positron emission tomography (FDG-PET) scan are the imaging studies useful in evaluation of gall bladder malignancy for assessing extension of the malignancy [14]. In the operable cases of gall bladder malignancy, preoperative biopsies are contraindicated as it may cause tumour seeding in biopsy tracts, peritoneum and surgical incisions [15-17]. If diagnosis is suspected and the imaging features are suggestive of an operable lesion, we must proceed for a definitive surgical procedure.

A higher and sustained level of inflammatory response is seen in malignancies. The increased production of cytokines like interlukin-6, C-reactive protein (CRP) and tumour necrosis factor alpha (TNF-α) have been implicated to this sustained inflammatory response [18].

The normal red blood cells are non-nucleated biconcave disc shaped cells. The average diameter of a red blood cell ranges between 6-8 μm and is 2 μm thick. Normal mean corpuscular volume (MCV) is 85±9 fL. Red cell distribution width (RDW) measures the variation in the volume of erythrocytes which is termed as anisocytosis. The Coefficient of Variation (C_v) is presented as a volume curve. The distribution width of the volume curve is the red cell distribution width.

Red cell distribution width (RDW) measures the heterogenicity of RBC sizes, which is calculated by dividing the standard deviation (SD) of mean corpuscular volume (MCV) of erythrocytes by the MCV.

$$RDW = \frac{SD \ of \ MCV}{MCV} \times 100$$

Automated blood cell counting machines are widely available all over the world and it gives the report of RDW. It is easily available, reproducible and is also cheap.

Recent studies have been reporting an increased mortality in subjects with elevated red cell distribution width [19,20]. The mechanisms for this association have not been elucidated yet. But elevated RDW values can be due to multifactorial etiologies such as malnutrition (folic acid, cyanocobalamin, and iron deficiency), defective erythropoiesis related to ageing and chronic inflammation [19-22]. In malignancies and malnutrition, chronic inflammatory changes are seen which may be responsible for the increase in red cell distribution width [23]. Hence red cell distribution width reflects the nutritional status and chronic inflammation, and therefore can be used as a predictor of malignancy and marker of cancer progression. Red cell Distribution Width is correlated with malnutrition, chronic inflammation, deranged renal parameters and abnormal erythropoietin synthesis function [24,25]. Correlation of red cell distribution width with malignancies have been studied and, in some studies, it is used as a parameter to differentiate between malignant and benign lesions [26-31]. However, data on gall bladder malignancy is lacking.

Hence, we conducted a study to see the correlation of RDW to differentiate benign from malignant GB pathology at RIMS hospital, Imphal.

MATERIALS AND METHODS

This prospective cross-sectional study was conducted in the Department of General Surgery and Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur (RIMS) from August 2018 to July 2020 after obtaining clearance from the Research Ethics Board of the institute. A total of 110 patients diagnosed of gall bladder diseases admitted in surgery wards during the study period in RIMS, Imphal were enrolled into the study after getting informed consent, of which 27 subjects were having GB malignancy and 83 subjects were having benign gall stone disease.

Inclusion criteria

1. Age >18 years and <90 years
2. Both sexes
3. Benign and malignant gallbladder diseases

Exclusion criteria

1. Known cases of hematologic malignancy or disorder
2. Patients on immunotherapy
3. Presence of active infection
4. Known cases of Autoimmune diseases
5. Presence of any other solid organ malignancies
6. History of splenectomy
7. Pregnancy
8. Patients not willing to participate in the study

Study variables

    Independent variables:

1. Red Cell Distribution Width (RDW)
2. Hemoglobin
3. Platelet
4. Mean corpuscular volume (MCV)
5. Mean corpuscular Hemoglobin (MCH)
6. Mean corpuscular Hemoglobin Concentration (MCHC)
7. Total Leucocyte Count

8. Neutrophil count and lymphocyte count
9. Liver Function Test (LFT)
10. Kidney Function Test (KFT)

Outcome variables:

Benign or malignant disease of gall bladder.

Working definition

Red cell Distribution Width (RDW) is a Complete Blood Count parameter with normal value ranging from 12% to 16%.

Study tools

1. SYSMEX XS-800i- automated cell counter used to measure the red cell distribution width and other CBC parameters.

2. Case proforma for recording details like:

   - Demographic details
   - Brief history of the patient
   - Complete blood count parameters
   - Liver function test parameters
   - Kidney function test parameters
   - Imaging study reports etc.

Procedure

All patients with gall bladder diseases who were enrolled into the study according to the inclusion and exclusion criteria were evaluated with a complete blood count, liver function tests and kidney function tests as a part of routine investigations done for the patient before undergoing treatment. Patients were treated as per indication. Benign gall stone disease patients underwent laparoscopic or open cholecystectomy. Operable cases of carcinoma gall bladder underwent radical surgery. Inoperable cases of carcinoma gall bladder underwent palliative therapy. Post-operative histopathological analysis was done to confirm the diagnosis of benign or malignant diseases.

Statistical analysis

Data analysis was done using Statistical Package for the Social Sciences (SPSS) software version 22 (IBM Corp., Armonk, NY, United States). Continuous data obtained were expressed as mean ± standard deviation, median and range, whichever is appropriate. Chi-square test (for categorical variables), Independent t-

test and ANOVA test (for continuous variables) were used for inferential statistics. A p-value <0.05 was considered as significant.

RESULTS

A total of 110 subjects ranging from 18 years to 84 years were included in the study. Among them 27 subjects were in the carcinoma GB group (malignant group) and the remaining 83 were in the benign gall stone disease group (benign group).

Among the total 110 patients who were included in the study, 67 were female and 43 were male. The male: female ratio in the carcinoma GB group was 1:4.4 whereas the male: female ratio in the benign gall stone disease group was 1:1.18 (Table 1). An overall female preponderance was noted which was more in the carcinoma GB group.

Table 1: Sex distribution among the study population (N=110)

Groups	N	Males	Females	p-value
Carcinoma GB	27	5	22	0.012
Benign gall stone disease	83	38	45	
Total	110	43	67	

Table value = 6.360

AGE:

The mean age of patients in the carcinoma GB group was 62.96±9.84 years whereas in the benign gall stone disease group was 41.10±13.67 years (Table 2).

Table 2: Age distribution among the study population (N=110)

Groups	N	Mean (in years)	Range (in years)	Median	Mode	p-value
Carcinoma GB	27	62.96±9.84	44-84	61	60	0.000
Benign gall stone disease	83	41.10±13.67	18-71	40	30	

Table value = 4.951

COMORBIDITY:

Among the total 110 subjects included in the study, 13 patients had diabetes mellitus and 14 patients had systemic hypertension (Table 3). The remaining 83 subjects had no other significant comorbidities at the time of presentation.

Table 3: Comorbidity distribution among the study population (N=110)

Groups	N	Diabetes	Hypertension	Nil
Carcinoma GB	27	5	4	18
Benign gall stone disease	83	8	10	65
Total	110	13	14	83

HEMOGLOBIN:

The mean hemoglobin of patients in the carcinoma GB group was 10.30±1.80 g/dL whereas in the benign gall stone disease group was 12.75±1.52 g/dL (Table 4). The variation in hemoglobin was statistically significant (p = 0.000).

Table 4: Mean hemoglobin comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (g/dL)	Range (g/dL)	Median	Mode	p-value
Carcinoma GB	27	10.30±1.80	6.7-14.2	10.8	11.4	0.000
Benign gall stone disease	83	12.75±1.52	8.4-15.6	12.8	12.8	

Table value = 1.410

MEAN CORPUSCULAR VOLUME (MCV):

The mean MCV of patients in the carcinoma GB group was 83.98±6.63 fL whereas in the benign gall stone disease group was 87.4±5.94 fL (Table 5). The variation in MCV was statistically significant (p = 0.012).

Table 5: Mean MCV comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (in fL)	Range (in fL)	Median	Mode	p-value
Carcinoma GB	27	83.98±6.63	73.2-98.1	82.0	79.2	0.012
Benign gall stone disease	83	87.4±5.94	72.0-102.1	88.0	85.0	

Table value = 0.942

MEAN CORPUSCULAR HEMOGLOBIN (MCH):

The mean MCH of patients in the carcinoma GB group was 28.85±3.38 pg whereas in the benign gall stone disease group was 29.49±2.56 pg (Table 6). The variation in MCH was not statistically significant (p = 0.302).

Table 6: Mean MCH comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (in pg)	Range (in pg)	Median	Mode	p-value
Carcinoma GB	27	28.85±3.38	23.1-42.2	28.1	26.9	0.302
Benign gall stone disease	83	29.49±2.56	23.7-36.2	29.1	28.4	

Table value = 0.230

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC):

The mean MCHC of patients in the carcinoma GB group was 32.89±2.96 g/dL whereas in the benign gall stone disease group was 33.4±2.11 g/dL (Table 7). The variation in MCHC was not statistically significant (p = 0.311).

MCV (in fL)

MCV (in fL)

Table 7: Mean MCHC comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (g/dL)	Range (g/dL)	Median	Mode	p-value
Carcinoma GB	27	32.89±2.96	26.2-38.9	33.3	30.1	
Benign gall stone disease	83	33.4±2.11	25.3-38.7	33.5	34.7	0.311

Table value = 7.483

RED CELL DISTRIBUTION WIDTH (RDW):

The mean RDW of patients in the carcinoma GB group was 16.6±1.67% whereas in the benign gall stone disease group was 13.6±1.36% (Table 8). The variation in RDW was statistically significant (p =0.000).

Table 8: Mean RDW comparison between carcinoma GB group and benign gall stone disease group (N=110) Table value = 2.432

Groups	N	Mean (in %)	Range (in %)	Median	Mode	p-value
Carcinoma GB	27	16.6±1.67	12.9-19.6	16.2	16.1	
Benign gall stone disease	83	13.6±1.36	11.2-18.2	13.6	14.1	0.000

A Receiver Operating Characteristic (ROC) curve made from the above data showed area under curve of 0.914. A cut off value of 15.05% showed sensitivity and specificity of 77.8% and 84.3% respectively (Figure 1).
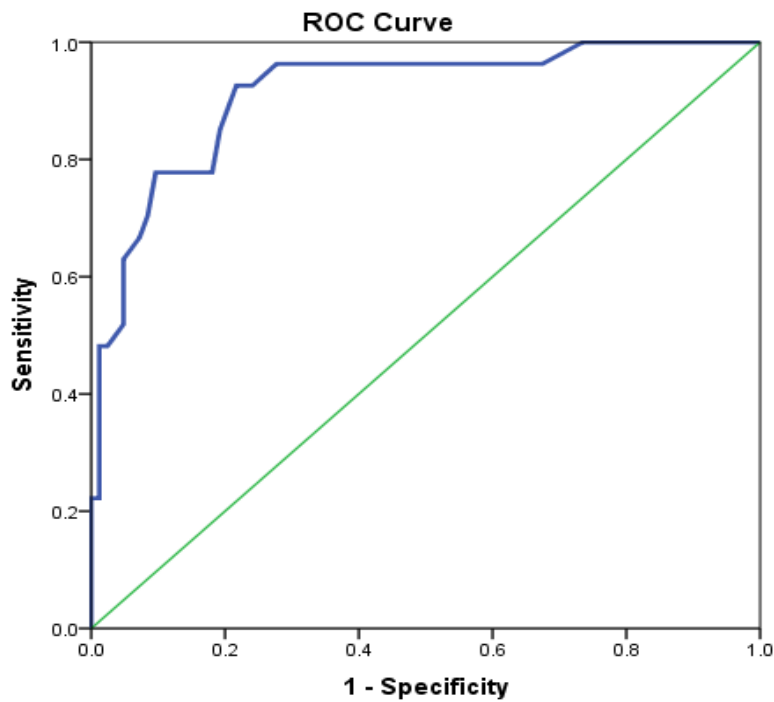
Figure 1: ROC Curve for RDW (N=110)

STAGE OF GB MALIGNANCY AND RDW:

Among the 27 patients in the carcinoma GB group, 12 patients were found to have stage IV disease (44.4%), 8 patients had stage III disease (29.6%), and 6 patients had stage II disease (22.2%). Only 1 patient presented with stage I disease (3.7%). Association between the stage of presentation of GB malignancy and mean RDW value was checked using one-way Anova test, and it showed statistically significant association with a p value of 0.02. Higher levels of RDW was seen in patients with advanced disease (Table 9).

Table 9: Mean RDW values in different stages of presentation in carcinoma GB group (N=110)

Stage of GB malignancy	N	RDW Mean ± SD	p-value
Stage I	1	14.90 ± 0.00	0.02
Stage II	6	15.35 ± 0.65	
Stage III	8	16.20 ± 2.16	
Stage IV	12	17.59 ±1.09	

Table value = 3.49

TOTAL LEUCOCYTE COUNT:

The mean total leucocyte count of patients in the carcinoma GB group was 9037.04±3223.7/µL whereas in the benign gall stone disease group was 7309.40±1883.1/µL (Table 10). The variation in total leucocyte count was statistically significant (p = 0.001).

Table 10: Mean Total Leucocyte Count comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (per µL)	Range (per µL)	Median	Mode	p-value
Carcinoma GB	27	9037.04±3223.7	3000-17000	8300	6800	0.001
Benign gall stone disease	83	7309.40±1883.1	3100-12500	7100	5600	

Table value = 9.255

PLATELET:

The mean platelet count of patients in the carcinoma GB group was $242\pm135\times10^3$/µL whereas in the benign gall stone disease group was $220\pm89\times10^3$/µL (Table 11). The variation in platelet count was not statistically significant (p =0.351).

Table 11: Mean Platelet count comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (per µL)	Range (per µL)	Median	Mode	p-value
Carcinoma GB	27	$242\pm135\times10^3$	$90\text{-}510\times10^3$	211×10^3	111×10^3	0.351

Benign gall stone disease	83	$220\pm89\times10^3$	$28\text{-}571\times10^3$	212×10^3	210×10^3	

Table value = 7.489

MEAN PLATELET VOLUME (MPV):

The mean MPV of patients in the carcinoma GB group was 11.56 ± 1.67fL whereas in the benign gall stone disease group was 11.63 ± 2.20fL (Table 12). The variation in MPV was not statistically significant (p = 0.885).

Table 12: Mean MPV comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (in fL)	Range (in fL)	Median	Mode	p-value
Carcinoma GB	27	11.56 ± 1.67	8.7-15.5	11.3	10.2	0.885
Benign gall stone disease	83	11.63 ± 2.20	8.0-15.6	11.0	10.9	

Table value = 5.904

     A Receiver Operating Characteristic (ROC) curve made from the above data showed area under curve 0.510. A cut off value of 10.95 showed sensitivity and specificity of 59.3% and 49.4% respectively (Figure 2).
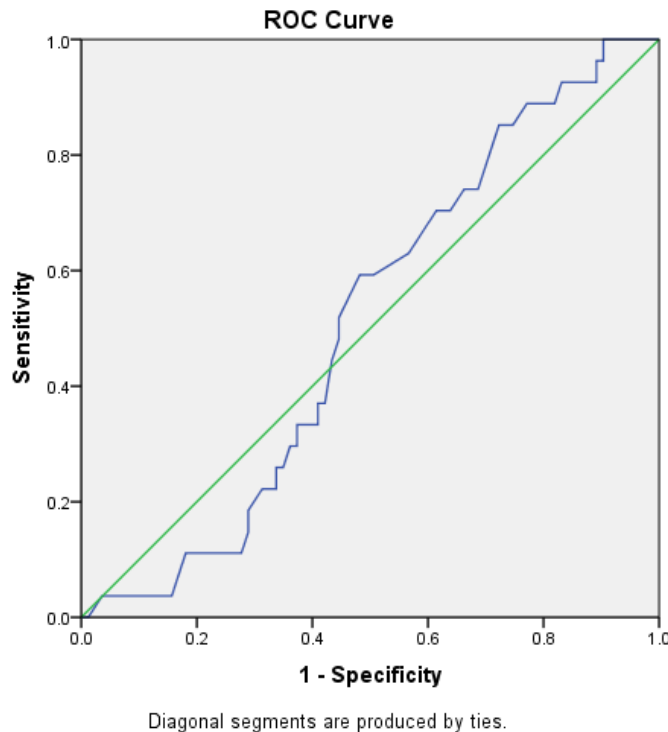

Diagonal segments are produced by ties.

Figure 2: ROC Curve for MPV (N=110)

NEUTROPHIL LYMPHOCYTE RATIO (NLR):

The formula to calculate NLR is

NLR = (Total Neutrophil Count) ÷ (Total Lymphocyte Count)

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The mean NLR of patients in the carcinoma GB group was 3.22±2.04 whereas in the benign gall stone disease group was 2.36±1.10 (Table 13). The variation in NLR was statistically significant (p = 0.007).

Table 13: Mean NLR comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean	Range	Median	Mode	p-value
Carcinoma GB	27	3.22±2.04	1.61-11.27	2.26	1.61	0.007
Benign gall stone disease	83	2.36±1.10	1.10-7.44	2.07	1.10	

Table value = 10.047

A Receiver Operating Characteristic (ROC) curve made from the above data showed area under curve of 0.643. A cut off value of 2.02 showed sensitivity and specificity of 63% and 48.2% respectively (Figure 3).
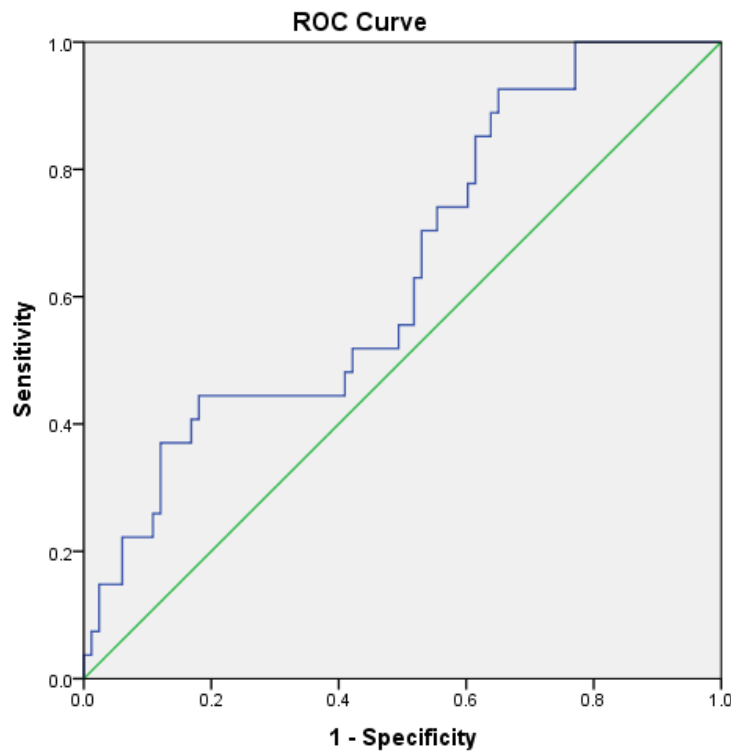

Figure 3: ROC Curve for NLR (N=110)

PLATELET LYMPHOCYTE RATIO (PLR):

PLR is calculated as PLR = (Platelet Count) ÷ (Total Lymphocyte Count)

The mean PLR of patients in the carcinoma GB group was 123.92±92.57 whereas in the benign gall stone disease group was 113.15±61.41 (Table 14). The variation in PLR was not statistically significant (p = 0.490).

Table 14: Mean PLR comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean	Range	Median	Mode	p-value

| Carcinoma GB | 27 | 123.92±92.57 | 23.49-388.54 | 101.76 | 23.49 | |
| Benign gall stone disease | 83 | 113.15±61.41 | 11.84-237.60 | 96.06 | 218.75 | 0.490 |

Table value = 3.452

A Receiver Operating Characteristic (ROC) curve made from the above data showed area under curve of 0.490. A cut off value of 96.77 showed sensitivity and specificity of 51.9% and 50.6% respectively (Figure 4).
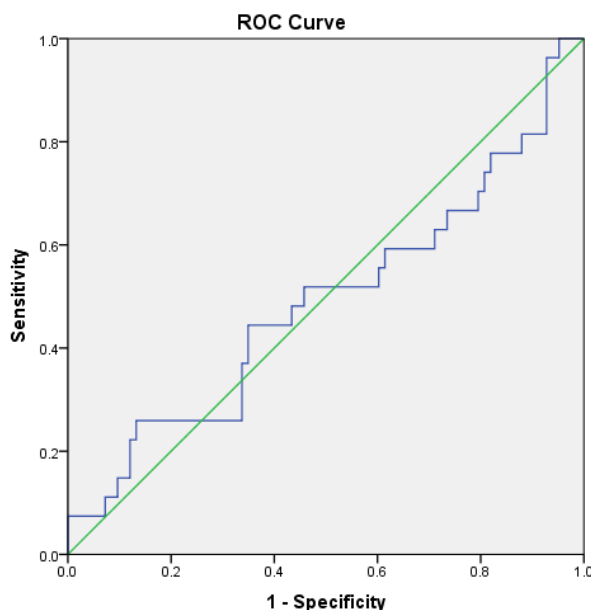

Figure 4: ROC Curve for PLR (N=110)

TOTAL BILIRUBIN:

The mean total bilirubin levels of patients in the carcinoma GB group was 1.3±1.9mg/dL whereas in the benign gall stone disease group was 0.6±0.2mg/dL (Table 15). The variation in the total bilirubin levels was statistically significant (p =0.002).

Table 15: Mean total bilirubin comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (mg/dL)	Range (mg/dL)	Median	Mode	p-value
Carcinoma GB	27	1.3±1.9	0.2-10.9	0.9	0.5	
Benign gall stone disease	83	0.6±0.2	0.1-1.2	0.6	0.4	0.002

Table value = 9.566

TOTAL PROTEIN:

The mean total protein of patients in the carcinoma GB group was 6.6±0.6 g/dL whereas in the benign gall stone disease group was 7.4±0.4g/dL (Table 16). The variation in the total protein levels was statistically significant (p =0.000).

Table 16: Mean total protein comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (g/dL)	Range (g/dL)	Median	Mode	p-value
Carcinoma GB	27	6.6±0.6	5.4-7.6	6.8	5.9	0.000
Benign gall stone disease	83	7.4±0.4	6.4-8.6	7.5	7.5	

Table value = 10.871

ALBUMIN:

The mean albumin levels of patients in the carcinoma GB group was 3.3±0.6g/dL whereas in the benign gall stone disease group was 4.1±0.3g/dL (Table 17). The variation in the albumin levels was statistically significant (p =0.000).

Table 17: Mean albumin comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (g/dL)	Range (g/dL)	Median	Mode	p-value
Carcinoma GB	27	3.3±0.6	2.2-4.5	3.4	2.9	0.000
Benign gall stone disease	83	4.1±0.3	2.8-5.2	4.1	3.9	

Table value = 24.720

ALANINE AMINOTRANSFERASE:

The mean ALT levels of patients in the carcinoma GB group was 52.33±30.7 IU/L whereas in the benign gall stone disease group was 38.65±21.9 IU/L (Table 18). The variation in the Alanine Aminotransferase was statistically significant (p=0.013).

Table 18: Mean Alanine Aminotransferase levels comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (IU/L)	Range (IU/L)	Median	Mode	p-value
Carcinoma GB	27	52.33±30.7	16-114	39	30	0.013
Benign gall stone disease	83	38.65±21.9	13-112	31	23	

Table value = 11.971

ASPARTATE AMINOTRANSFERASE:

The mean AST levels of patients in the carcinoma GB group was 48.85±25.0 IU/L whereas in the benign gall stone disease group was 33.89±18.4 IU/L (Table 19). The variation in the Aspartate Aminotransferase was statistically significant (p=0.001).

Table 19: Mean Aspartate Aminotransferase levels comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (IU/L)	Range (IU/L)	Median	Mode	p-value

Carcinoma GB	27	48.85±25.0	16-110	40	30		0.001
Benign gall stone disease	83	33.89±18.4	12-118	29	28		

Table value = 5.168

ALKALINE PHOSPHATASE:

The mean Alkaline Phosphatase levels of patients in the carcinoma GB group was 259.67±211.2 IU/L whereas in the benign gall stone disease group was 186.48±118.2 IU/L (Table 20). The variation in the Alkaline Phosphatase levels was statistically significant (p=0.026).

Table 20: Mean Alkaline Phosphatase levels comparison between carcinoma GB group and benign gall stone disease group (N=110)

Groups	N	Mean (IU/L)	Range (IU/L)	Median	Mode	p-value
Carcinoma GB	27	259.67±211.2	80-1250	210	180	
Benign gall stone disease	83	186.48±118.2	83-880	146	110	0.026

Table value = 0.821

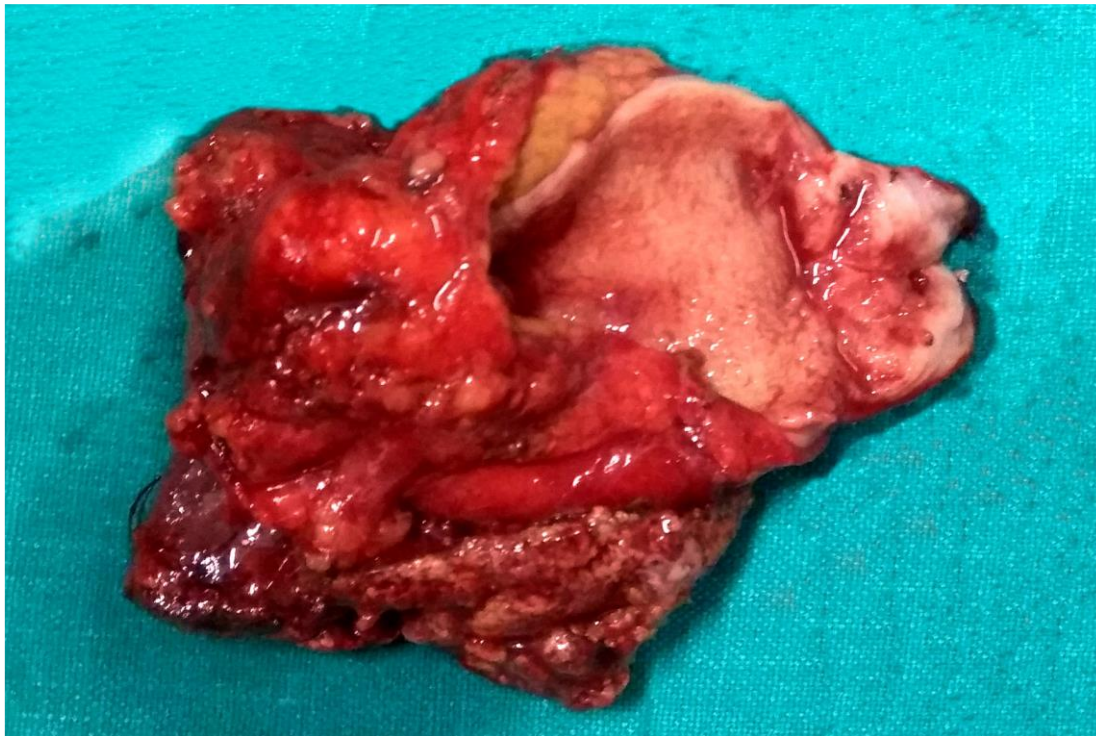**Figure 5: Laparoscopic view of Gallbladder and Calots triangle**

Figure 6: Specimen showing growth at fundus of gall bladder

DISCUSSION

Carcinoma of gall bladder is the most common malignancy of the biliary tract. It is also the fifth most common malignancy of the gastrointestinal tract [32]. The prevalence of gall bladder malignancy is more among the Asian race. It can be attributed to the high prevalence of the risk factors like cholelithiasis and chronic Salmonella infections [32]. The mortality and morbidity associated with the carcinoma of GB is high due to it being asymptomatic in the early stages and hence at the time of diagnosis, dissemination would have already occurred.

Chronic inflammation can damage cellular DNA, leading to repeated proliferation and repair of tissues. This process involves the release of cytokines, which makes cells susceptible to malignant transformation and ultimately to cancer. Tumour cytokine-mediated inflammation can promote tumour growth, invasion and metastasis. As one of the inflammation markers, RDW can reflect the progress of chronic diseases and is related to the results of chronic diseases and malnutrition. It is increased in many malignancies [26-31].

In this study, we tried to compare between the benign and malignant diseases of gall bladder and to analyse whether there is any variation in the blood parameters among these two groups.

In this study, the mean age of patients in the carcinoma GB group was 62.96 ± 9.84 years, whereas in benign gall stone disease group was 41.10 ± 13.67 years. Mean age for carcinoma GB patients in the study done by Dutta et al [33] in the Indian population was 55 ± 11.7 years. In the study conducted by Johansson et al [34] the mean age of patients with benign gall stone disease was 46 years.

The male : female ratio in the carcinoma GB group in our study was 1:4.4 whereas the male : female ratio in the benign gall stone disease group was 1:1.18. An overall female preponderance was noted which was more in the carcinoma GB group which is in consistent with the study conducted by Bush N et al [35] which states that women are having 2-6 times higher risk for developing GB malignancy.

The mean hemoglobin concentration of patients in the carcinoma GB group was 10.30 ± 1.80 g/dL whereas in the benign gall stone disease group was 12.75 ± 1.52 g/dL. The variation in hemoglobin levels between the two groups was statistically significant with a p value < 0.001. Ong et al [36] in 73 patients with carcinoma GB showed that mean hemoglobin was 12.26 g/dL.

Beliaev et al [37] in one another study on a cohort of 45 subjects with cholecystitis stated that the mean hemoglobin for the subjects was 13.3g/dL.

The mean MCV of patients in the carcinoma GB group was 83.98 ± 6.63 fL whereas in the benign gall stone disease group was 87.4 ± 5.94 fL. The variation in MCV was statistically significant with a p value 0.012. This can be attributed to the increased incidence of microcytic anaemia associated with malignancies.

In the carcinoma GB group, the mean RDW was $16.6 \pm 1.67\%$ with values ranging from 12.9%-19.6%. In the benign gall stone disease group, it was $13.6 \pm 1.36\%$ with values ranging from 11.2%-18.2%. The variation of RDW between the two groups was statistically significant with a p value <0.001. ROC curve plotted with the data showed sensitivity and specificity for a cut off value of 15.05% at 77.8% and 84.3% respectively (Figure 1). The area under the curve (AUC) was 0.914. Beyazit et al [27], in their study to distinguish benign and malignant biliary strictures showed that RDW cut-off level of 14.8% was best to predict a malignant biliary stricture (sensitivity and specificity of 72% and 69% respectively, with AUC 0.755). The study by Yazici et al [38], in acute cholecystitis patients showed that the mean RDW in a group 72 patients was $14.3 \pm 1.3\%$. The study conducted by Xie Y et al [39] among 108 GB malignancy patients and 119 age and gender matched individuals also showed that GB malignancy patients had significantly higher RDW values than the control group which can be compared to our study.

In our study, among the 27 patients in the carcinoma GB group, 12 patients were found to have stage IV disease (44.4%), 8 patients had stage III disease (29.6%), 6 patients had stage II disease (22.2%) and only 1 patient presented with stage I disease (3.7%). The association between stage of presentation of GB malignancy and mean RDW value was statistically significant with a p value of 0.02. Higher levels of RDW was seen in patients with advanced disease. This correlates with the study conducted by Amit G et al [40] which also demonstrated higher levels of RDW with advanced stages of GB malignancy.

Ong et al [36] in their study among carcinoma GB patients showed mean TLC as 9600/µL. Beliaev et al [37] in another study among a cohort of cholecystitis patients states that the mean TLC was 7400/µL. In our study the mean total leucocyte count of patients in the carcinoma GB group was 9037.04 ± 3223.7/µL whereas in the benign gall stone disease group was 7309.40 ± 1883.1/µL. The variation in total leucocyte count was statistically significant (p = 0.001).

The study by Yazici et al [38] showed that the mean platelet counts in a group of carcinoma GB was 331×10^3/µL. But in our study the mean platelet count of patients in the carcinoma GB group was $242 \pm 135 \times 10^3$/µL whereas in the benign gall stone disease group was $220 \pm 89 \times 10^3$/µL. The variation in platelet count was not statistically significant (p =0.351).

The mean Neutrophil Lymphocyte Ratio (NLR) of patients in the carcinoma GB group was 3.22 ± 2.04 whereas in the benign gall stone disease group was 2.36 ± 1.10. The variation in NLR was statistically significant with a p value of 0.007. Receiver Operating Characteristic (ROC) curve made from the above data showed area under the curve (AUC) of 0.643. A cut off value of 2.02 showed sensitivity and specificity of 63% and 48.2% respectively (Figure 2). The area under the curve (AUC) was 0.643. NLR value more than 5 is a poor prognostic marker [38]. Increased NLR leads to a decreased overall survival and relapse free survival for malignancies of biliary tract [41]. Beal et al [42] in his study included 187 patients with GB malignancy and among them 145 had a NLR < 5 and 42 had NLR > 5. Patients with NLR < 5 had fewer complications, higher overall survival and shorter duration of stay. The mean NLR of 73 subjects included in the study by Ong et al [36] was 4.12. Zhang et al [43] in their study of 145 subjects with GB malignancy devised a cut off value of NLR at 1.94, with sensitivity and specificity of 64.2% and 76% respectively. Subjects having high NLR had a lower 5-year survival rate than subjects having low NLR. NLR was also an independent prognostic factor in carcinoma GB subjects. NLR, CRP and total bilirubin in the high NLR group was higher than the low NLR group. This was significant because CRP and total bilirubin were correlated with the median survival period in this study. The study done by Beliaev et al [37] among a cohort of 45 patients with cholecystitis showed the mean NLR as 1.73.

The mean Platelet Lymphocyte Ratio (PLR) of patients in the carcinoma GB group was 123.92 ± 92.57 whereas in the benign gall stone disease group was 113.15 ± 61.41. But the variation in PLR was not

statistically significant (p = 0.490). A Receiver Operating Characteristic (ROC) curve made from the above data showed area under curve of 0.490. A cut off value of 96.77 showed sensitivity and specificity of 51.9% and 50.6% respectively (Figure 3). The mechanism for increased PLR and poor prognosis is not clear. The associations with inflammation might be the probable reason. Platelets could accelerate tumor growth and invasion by releasing several platelet derived mediators [44,45]. Zhang et al [43] in their study of 145 subjects with carcinoma GB devised a cut off value of PLR at 113.34 with a sensitivity and specificity of 62.5% and 76% respectively. Pang et al [46] in his study of 316 subjects with GB malignancy devised a cut off value of PLR at 117.7 with a sensitivity and specificity of 73.6% and 53.2% respectively.

In the case- control study conducted by Pedrazzani et al [47] in colorectal cancer patients, the preoperative values of NLR was higher than that in controls (NLR 3.1±1.8 versus 1.8±1, with a p value <0.001). Similarly, the PLR among cases were 194 ± 98 and in controls 126 ± 38 with p value < 0.001.

The mean total bilirubin levels of patients in the carcinoma GB group was 1.3 ± 1.9mg/dL whereas in the benign gall stone disease group was 0.6 ± 0.2mg/dL. The variation in the total bilirubin levels was statistically significant with a p value of 0.002. Beliaev et al [37] in their study showed that the mean bilirubin among benign gall stone disease patients was 0.7mg/dL which is comparable to our study.

The mean total protein of patients in the carcinoma GB group was 6.6±0.6 g/dL whereas in the benign gall stone disease group was 7.4 ± 0.4 g/dL. The variation in the total protein levels was statistically significant (p <0.001). The mean albumin levels of patients in the carcinoma GB group was 3.3 ± 0.6g/dL whereas in the benign gall stone disease group was 4.1 ± 0.3g/dL. The variation in the albumin levels was statistically significant (p <0.001). The mean albumin levels in the study conducted by Ong et al [36] among 73 subjects having carcinoma GB was 3.5 mg/dL.

The mean Alanine Aminotransferase (ALT) levels of patients in the carcinoma GB group was 52.33 ± 30.7 IU/L whereas in the benign gall stone disease group was 38.65 ± 21.9 IU/L. The variation in the Alanine Aminotransferase was statistically significant with a p value of 0.013. The mean ALT levels of 73 subjects having carcinoma GB by Ong et al [36] was 38 IU/L. Beliaev et al [37] in their study showed that the mean ALT levels among benign gall stone disease patients was 38 IU/L.

The mean Aspartate Aminotransferase (AST) levels of patients in the carcinoma GB group was 48.85 ± 25.0 IU/L whereas in the benign gall stone disease group was 33.89 ± 18.4 IU/L. The variation in the Aspartate Aminotransferase was statistically significant with a p value of 0.001. Beliaev et al [37] in their study showed that the mean AST levels among benign gall stone disease patients was 27 IU/L.

The mean Alkaline Phosphatase (ALP) levels of patients in the carcinoma GB group was 259.67±211.2 IU/L whereas in the benign gall stone disease group was 186.48±118.2 IU/L. The variation in the Alkaline Phosphatase levels was statistically significant with a p value of 0.026. The mean ALP levels among the subjects having carcinoma GB in the study by Ong et al [36] was 213 IU/L. This study using ROC curve was analysed and predicted that age >68 years, platelet count >$345×10^6$, total count >$7.6×10^6$/L, ALP >124 IU/L, bilirubin >0.94 mg/dL, will most likely be unresectable during surgery. Beliaev et al [37] in their study showed that the mean ALP levels among benign gall stone disease patients was 79 IU/L.

CONCLUSION

The study was conducted in 110 subjects, which consisted of 43 males and 67 females. Among the total 110 subjects, 27 subjects had carcinoma GB and the remaining 83 subjects had benign gall stone disease. The mean RDW in the carcinoma GB group was 16.6±1.67%, and in the benign gall stone disease group, it was 13.6±1.36%. The variation of RDW between the two groups was statistically significant with a p value <0.001. ROC curve plotted with the data showed sensitivity and specificity for a cut off value of 15.05% at77.8% and 84.3% respectively. Statistically significant association was there between the stages of presentation of GB malignancy and mean RDW value with a p value of 0.02. Higher levels of RDW were seen in GB malignancy with advanced disease.

We concluded that Red cell distribution width (RDW) is an effective inflammatory biomarker to predict carcinoma GB in the pre-operative period and can be used to differentiate between the benign and malignant

diseases of gall bladder. RDW being a simple, cost-effective, and readily available blood parameter, can support the diagnosis of carcinoma gall bladder before undergoing surgery.

However, further study with a larger sample size and longer follow-up is required to enable us to comment regarding the prognostic value of RDW in GB malignancies.

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