Original Article

Estimation of Preoperative Serum Lactate Dehydrogenase and Alkaline Phosphatase Enzymes and their Correlation with Histological Grade and Stage of Colorectal Cancer

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Abstract

Background: *Colorectal cancer (CRC) remains one of the main public health problem throughout the world. Prognosis of the patient is largely dependent on its histological grade and stage. It has recently been observed that lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) enzymes play critical role in cancer expansion. Current study was enrolled for investigating serum levels of LDH and ALP before surgery, as well as to assess association of these enzymes with the histological grade and stage of CRC.*

Methods: *This cross-sectional study was carried out in the Pathology department of Mymensingh Medical College, Mymensingh, during February 2018 to December 2019. Preoperative blood samples were collected and evaluated for LDH and ALP enzymes from patients diagnosed as colorectal carcinoma by colonoscopy in surgery department of MMCH who underwent colectomy. All the resected specimens were sectioned, stained and studied. Tumors were graded as well, moderately and poorly differentiated as per WHO criteria. Staging was done according to TNM classification as per AJCC Cancer Staging Manual.*

Results: *Histologically most of them were adenocarcinoma (93.3%). The mean LDH was 160.33±27.02 in grade I, 199.26±31.1 in grade II and 246.71±36.32 in grade III tumor. On the other hand, mean LDH was 179.71±41.74 in stage I, 166±33.94 in stage II, 218.1±40.12 in stage III disease. The mean LDH was significantly (p<0.05) associated with both grade and stage. Mean ALP was 63.67±8.62 U/L in grade I, 75±10.15 U/L in grade II and 109.14±31.89 U/L in grade III tumor; 13.6% patients had high ALP in stage III and in other stages it was within normal limit. The mean ALP was significantly (p<0.05) elevated with increased grade and stage, which indicates that ALP was significantly increased with advance grade and stage. LDH had a positive significant correlation with both grade (r=0.423; p=0.004) and stage (r=0.498; p=0.001) of CRC. Similarly, ALP had also a positive significant correlation with both grade (r=0.477; p=0.001) and stage (r=0.489; p=0.001).*

Conclusion: *These finding suggest that serum levels of LDH and ALP appears to be of some prognostic value in CRC.*

Key words: *Colorectal cancer, LDH, ALP, tumor grade, tumor stage.*

Introduction

Colorectal cancer is among the most common malignancies and leading causes of cancer related deaths in developed countries. It is also increasingly being reported in the developing countries. CRC is evaluated as the third most common cancer overall and accounts for nearly 10% of all cancers[1]. Early detection of cancer can be potentially cured through surgery especially, when the tumor is very small and has not metastasized. In view of this, there is need for simple biochemical investigations, for early detection such as the use of tumor markers which include some enzymes[2]. Despite recent advances in treatments available for patients with CRC, 5-year survival rate ranges between 44% and 93%, decreasing to 12% in patients with advanced stages of the disease[3]. Therefore, it is necessary to discover biomarkers that can identify patients that are at-risk for advanced disease and reduced survival.

Traditional prognostic factors of CRC include the tumor size, histologic type, Tumor Node Metastasis (TNM) and 'potential' residual disease after initial surgery[4] .TNM staging system has been regarded as a standard staging system for colorectal cancer, but there are still variations between patients who have the same stage; a set of patients will have minimal response and rapid disease progression that culminates in death within a year of diagnosis[5] .Commonly used biomarkers such as fecal occult blood test; carcinoembryonic antigen (CEA); and carbohydrate antigen (CA-19.9) have low sensitivity for CRC and are not specific to the organ[6]. Subsequently, there is a real need for more efficient biomarkers that are low-cost, highly efficient, sensitive in the preoperative period, beneficial in clinical usage and with both prognostic and predictive features.

LDH was closely associated with analysis of glucose and mitochondrial shortage that is demanded for tumor conservation. ALP could be considered as a tumor marker for cancer in ovary, testis, lung and colorectal tract. Increase in ALP or LDH could suggest a heavy tumor burden and tumor spread. Increase ALP level was suggested to be associated with poor prognosis in various kinds of tumors[7].

Cancer cells rely heavily on aerobic glycolysis to support their growth, a process that is known as the Warburg effect[8]. Lactate dehydrogenase plays an important role in this process by mediating the conversion of pyruvate and lactate, and this enzyme is an emerging anticancer target. In addition, elevated lactate dehydrogenase levels are consistently reported as a prognostic factor for poor survival among several cancer groups. Although a large number of studies have been performed among patients with CRC, the prognostic value of lactate dehydrogenase levels among them remains controversial[9].

Methods

It was a cross-sectional observational study which was carried out from February 2018 to December 2019 at the Department of Pathology, Mymensingh Medical College, Mymensingh. The study population was 45 admitted patients diagnosed as CRC by colonoscopy in Surgery department of MMCH who underwent colectomy. Preoperative blood sample was collected from those patients and was evaluated in Department of Pathology in MMC.

Inclusion criteria: (i) Patients histopathologically diagnosed as colon and/or rectum carcinoma from all ages and both sexes (ii) Patients who gave written consent.

Exclusion criteria: (i) Patients with any form of malignancy other than colon and rectum. (ii) Patients with non-epithelial colorectal tumour. (iii) Diagnosed case of colorectal carcinoma patients treated with neoadjuvent chemo and/or radiotherapy (iv) Patient who had a history of conditions that may have influenced blood cell lines (connective tissue disorder, lymphoma and leukemia).

Blood was collected from each patient and was sent to biochemistry laboratory for estimation of serum LDH and ALP. Fresh unfixed specimens were obtained after surgical resection and were transferred to 10% formalin for overnight fixation. In the next morning the

specimens were examined during gross cut up and tissue blocks were taken. All the tissue blocks were submitted for routine processing and paraffin embedding in the Pathology laboratory of MMC following standard protocol. Then H&E stained slides were examined thoroughly to confirm the diagnosis and to evaluate the histopathologic characteristics, including histologic type of tumor, histologic grade of tumor, presence of lymphovascular invasion, perineural invasion, necrosis etc. Tumour was graded as well, moderately and poorly differentiated carcinoma according to the WHO grading criteria. Staging was done according to TNM classification by using the AJCC Cancer Staging Manual. Prior to commencement of this study the protocol was approved by Institutional Review Board (IRB) of Mymensingh Medical College, Mymensingh. The statistical analysis of data was carried out using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

A total number of 45 patients were included in this study. Qualitative variables were expressed as frequencies and percentages and test of significance was performed by Chi-square (χ^2) test. Quantitative variables were expressed as mean±standard deviation and unpaired t-test was performed for test of significance. ANOVA test was done to find out the association of LDH and ALP with grade and stage of CRC. Bonferroni test was done for intergroup comparison. Correlation of above mentioned biochemical parameters with grade and stage of CRC were analyzed by Spearman's rank correlation coefficient. P value <0.05 was considered as statistically significant.

Table I: **Distribution of the study patients by demographic profile**

Age (years)	Frequency (n=45)	Male (n=27)		Female (n=18)		P value
		n	%	n	%	
16-25	2	1	3.7	1	5.6	
26-35	7	6	22.2	1	5.6	
36-45	8	3	11.1	5	27.8	
46-55	13	7	25.9	6	33.3	
56-65	12	8	29.6	4	22.2	
>65	3	2	7.4	1	5.6	
Mean±SD		50.33±14.66		49.78±11.74		0.894
Range (Min-Max)		18-72		15-70		

In this present study, out of 45 cases more than one fourth (13 cases, 28.9%) patients had tumor in sigmoid colon, followed by 12(26.7%) had tumor in ascending colon, 10(22.2%) in ceacum, 5(11.1%) in rectum, 4(8.9%) in descending colon and 1(2.2%) was situated in transverse colon (Fig-1).
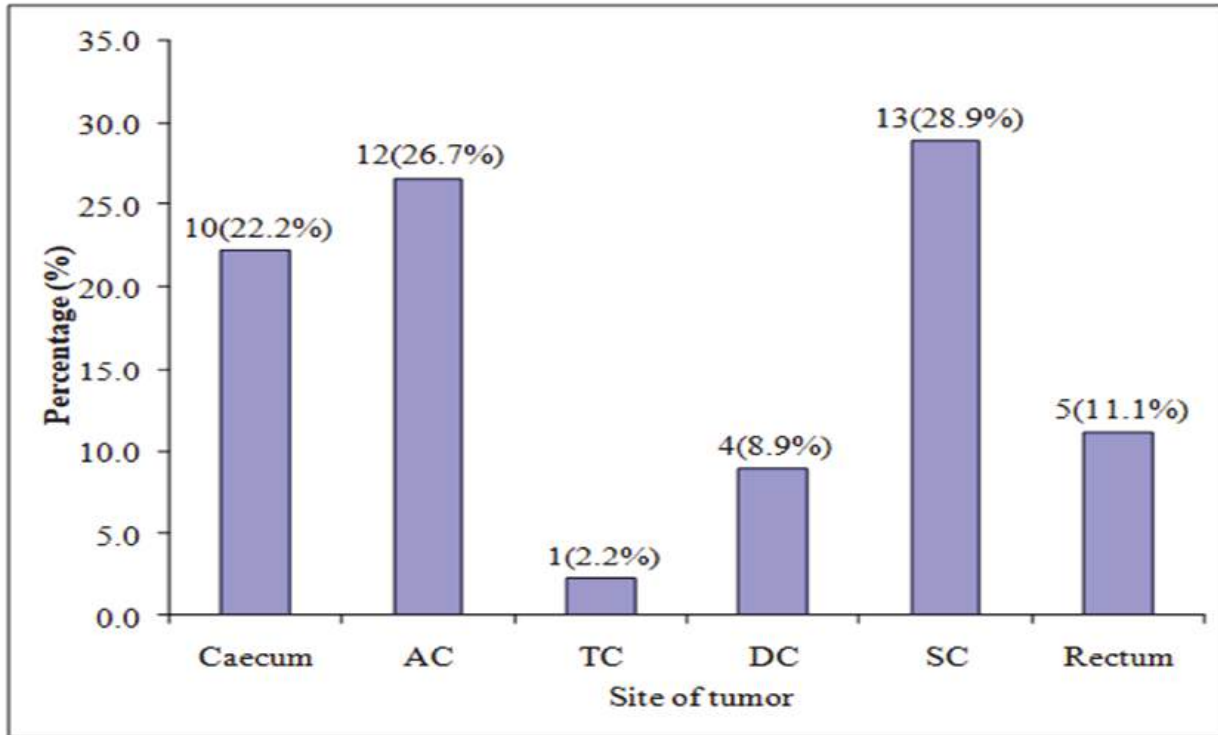

Figure 1: Bar diagram showing distribution of patients according to location of tumor (AC: Ascending Colon, TC: Transverse Colon, DC: Descending Colon, SC: Sigmoid Colon)
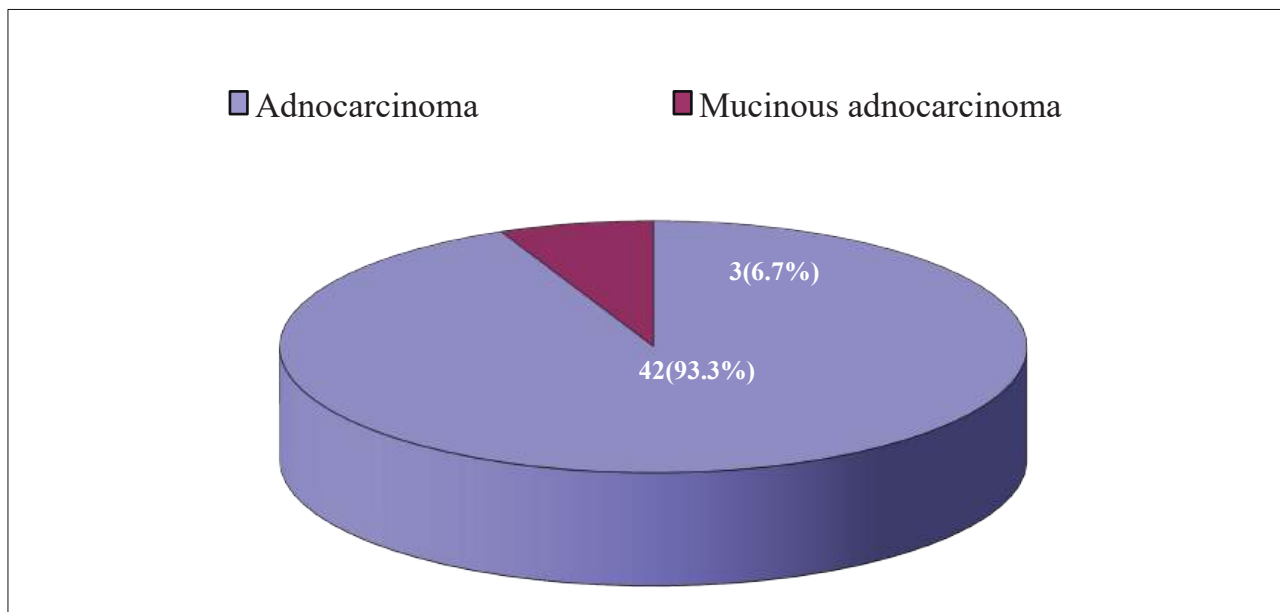

Figure 2: Pie chart showing histological types of colorectal carcinoma

Out of 45 cases, the study shows more than three fourth (34 cases, 75.6%) of the patients had Grade-II (Moderately differentiated), 7(15.6%) had Grade-III (Poorly differentiated) and 4(8.9%) had Grade-I (Well differentiated) carcinoma. It was observed that 21(46.7%) cases belonged to stage I, 2(4.4%) stage II and 22(48.9%) stage III carcinoma.

In this study we found that all the patients had anaemia, 19(42.2%) patients had leukocytosis, 11(24.4%) had thrombocytosis, 14(31.1%) had high serum LDH and 3(6.7%) had high serum ALP (Table-II).

Table II: Distribution of the study patients according to haematological findings

Haematological parameters	Number of patients	Percentage
Hb - Low	45	100
WBC		
High	19	42.2
Normal	26	57.8
Platelet		
High	11	24.4
Normal	34	75.6
LDH		
High	14	31.1
Normal	31	68.9
ALP		
High	3	6.7
Normal	42	93.3

Table-III: Association of LDH with histological grading of the study patients (n=45)

Histopathological Grading	LDH (U/L)		P value
	n	(Mean ±SD)	
Well differentiated	4	160.33±27.02	
Moderately differentiated	34	199.26±31.1	
Poorly differentiated	7	246.71±36.32	
Statistical analysis			
G-I vs G-II vs G-III			0.001[s]
G-I vs G-II			0.022[s]
G-I vs G-III			0.001[s]
G-II vs G-III			0.017[s]

ANOVA followed by Bonferroni test was performed to measure the level of significance and compare between groups.s= significant

Table III shows association of LDH with histological grade of the study patients. It was observed that the mean LDH value was 160.33 in well differentiated (G-I), 199.26 in moderately differentiated (G-II) and 246.7 in poorly differentiated (G-III) carcinoma. The difference was statistically significant ($p<0.05$).

Table III: Association of serum LDH with pathological stage of the study patients (n=45)

LDH	Stage						p value
	I (n=21)		II (n=2)		III (n=22)		
	n	%	n	%	n	%	
High	3	14.3	0	0.0	11	50.0	[a]0.025[s]
Normal	18	85.7	2	100.0	11	50.0	
Mean±SD	179.71±41.74		166±33.94		218.1±40.12		[b]0.008[s]
Range (min-max)	136-308		142-190		145-326		

s= significant; [a]p value reached from Chi-square test; [b]p value reached from ANOVA test

Table IV: Association of ALP with histological grade of the study patients (n=45)

Histopathological Grading	ALP (U/L)		P value
	n	Mean±SD	
Well differentiated (G-I)	4	63.67±8.62	
Moderately differentiated (G-II)	34	75.0±14.15	0.001[S]
Poorly differentiated (G-III)	7	109.14±31.89	
Statistical analysis			
G-I vs G-II vs G-III			0.001[S]
G-I vs G-II			0.039[S]
G-I vs G-III			0.023[S]
G-II vs G-III			0.017[S]

s =significant

ANOVA followed by Bonferroni test was performed to compare between groups and measure the level of significance.

Table V: Association of serum ALP with pathological stage of CRC in the study patients (n=45)

ALP	Stage						P value
	I (n=21)		II (n=2)		III (n=22)		
	n	%	n	%	n	%	
High	0	0.0	0	0.0	3	13.6	
Normal	21	100.0	2	100.0	19	86.4	
Mean±SD	68.81±10.53		76±14.14		89.19±21.84		0.001[S]
Range (min-max)	(52-90)		(66-86)		(58-145)		

s= significant

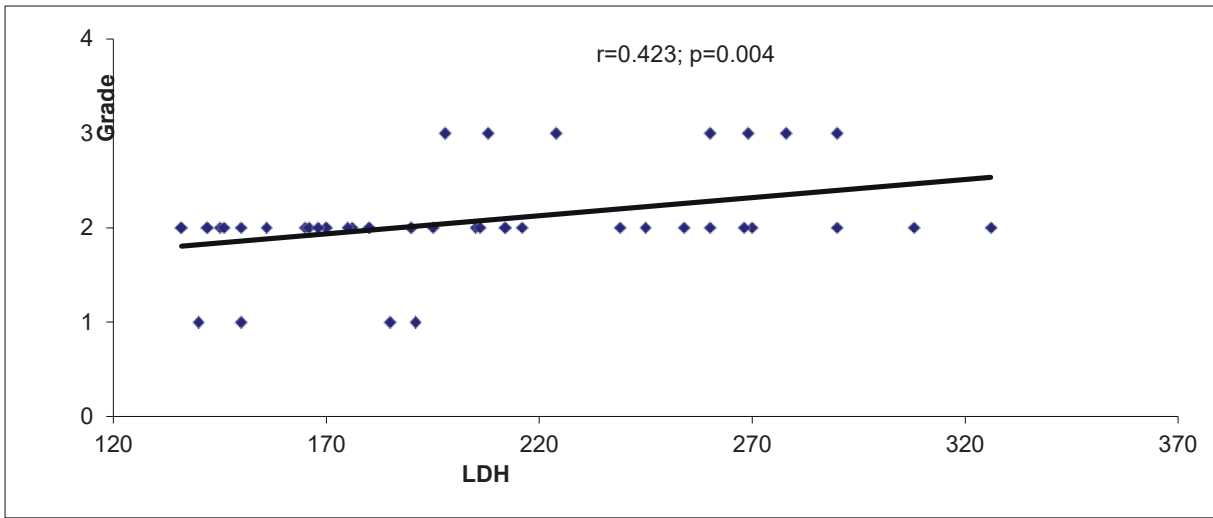p value reached from ANOVA test

Figure 3: Scatter diagram showing positive significant spearmen rank correlation (r=0.423; p=0.004) between LDH and grade.
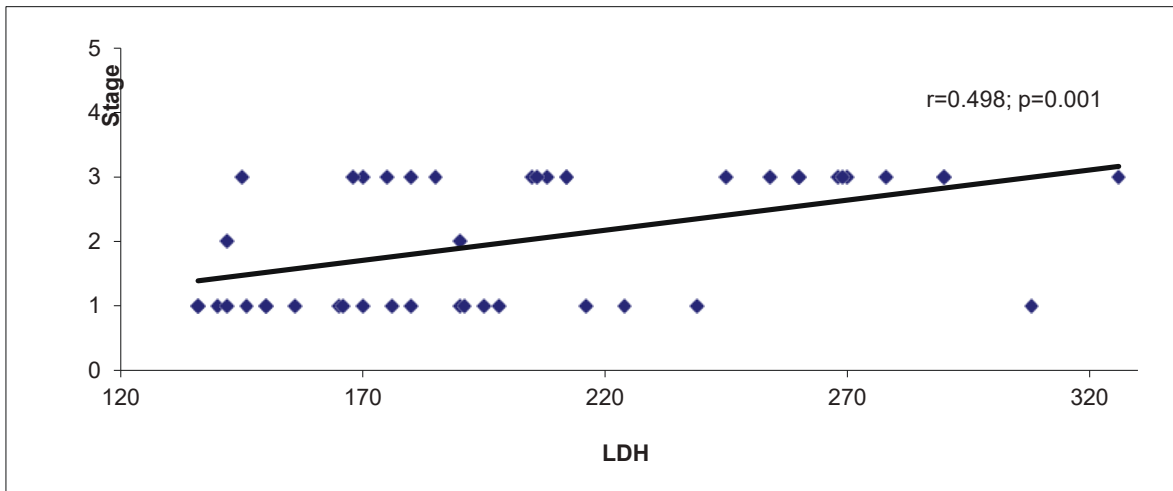

Figure 4: Scatter diagram showing positive significant spearmen rank correlation between LDH with stage.
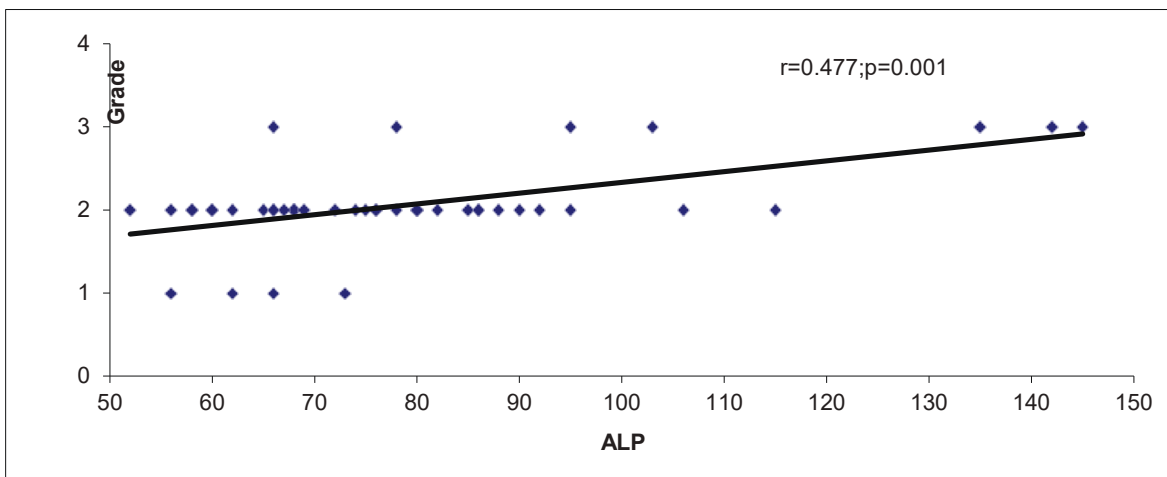

Figure 5: Scatter diagrams showing positive significant spearmen rank correlation (r=0.477; p=0.001) between ALP with grade.
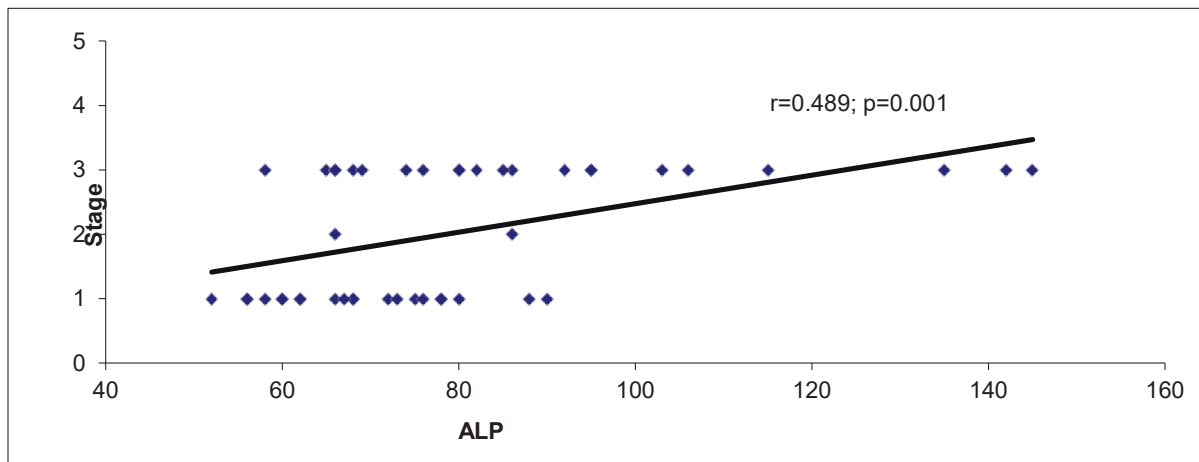
Figure 6: Scatter diagrams showing positive significant spearmen rank correlation (r=0.489; p=0.001) between ALP with stage.
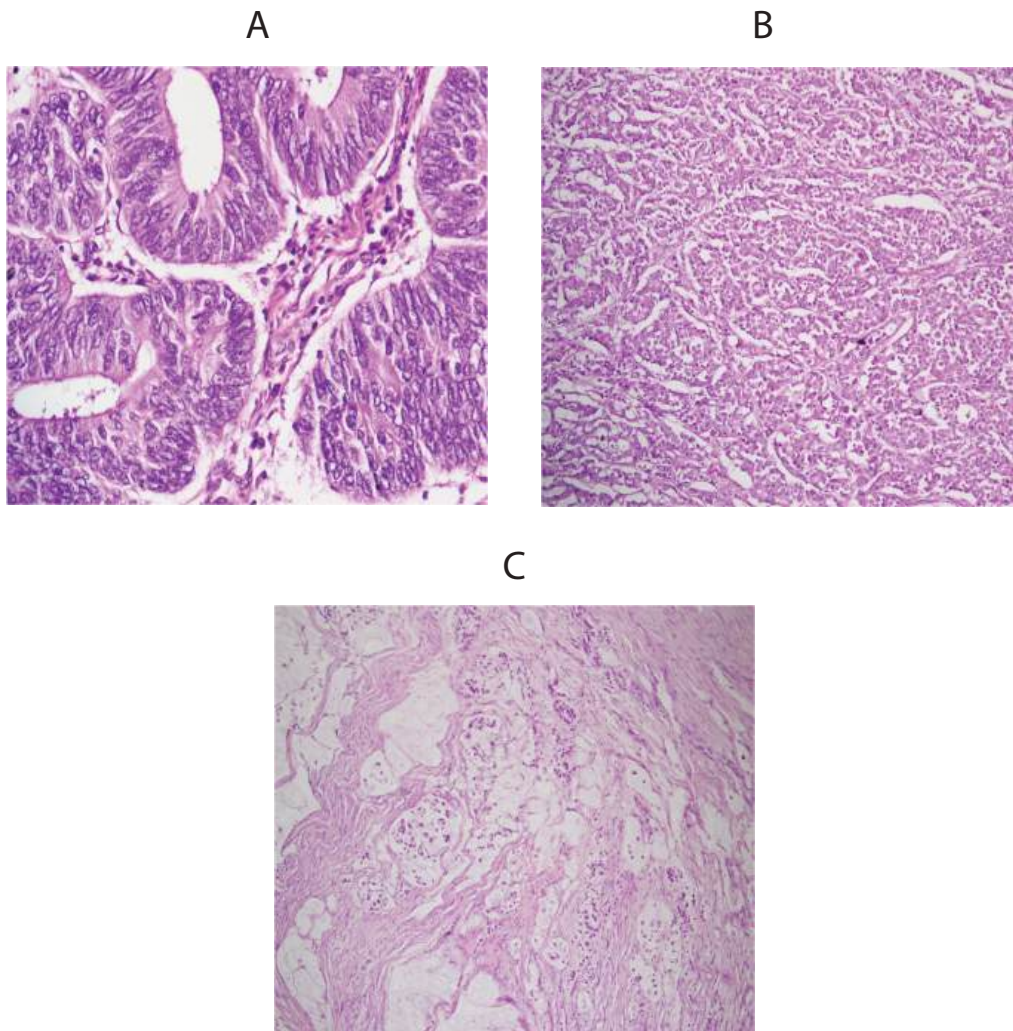

Figure 7: Photomicrograph showing moderately differentiated adenocarcinoma (A) poorly differentiated adenocarcinoma (B) mucinous adenocarcinoma (C)

Discussion

Colorectal cancer is increasingly being reported in the developing countries, where screening is unusual and the diagnosis is made after the onset of symptoms[10]. Over the last few years there has been an increased interest in clinical and molecular prognostic factors of CRC due to recent advances in treatment options which have extended patient survival. The role of immune system on disease progression has been investigated previously and the prognostic importance of biochemical parameters has been shown in various malignancies. This cross-sectional study was carried out with an aim to evaluate the selected preoperative biochemical parameters of patient with colorectal cancer and to assess their relationship with cancer grade and stage.

This study involved 45 patients with age range from 16-72 years. The mean age was 50.33±14.66 years in male and 49.78±11.74 years in female with no statistically significant (p>0.05) difference (Table-1). In a similar study Vayrynen found the mean age was 66.7±11.1 years and Stojkovic Lalosevic found higher mean age (61.63±10.94 years)[11,12]; may be due to geographical, genetic, racial and ethnic differences. Our study showed male to female ratio was 1.5:1 that were in accordance with another Bangladeshi study done by Raza[13] This increased vulnerability of man may be due to a number of biological and gender-related (behavioral) factors. On histological examination of 45 cases, 42(93.3%) were usual adenocarcinoma and 3(6.7%) were mucin secreting adenocarcinoma (Fig-2). Raza found 12% mucinous adenocarcinoma in their study. Sample size may be the cause of this variation[13].

According to WHO grading criteria 75.6% patients had Grade II cancer. Similarly, Vayrynen and Al-Saeed also found predominantly Grade II Tumor in their study.[11,14] On TNM staging it was found that 46.7% patients belonged to stage I, 4.4% stage II and 48.9% stage III disease. Stojkovic Lalosevic[12] study found the distribution of patients in TNM stage I was 27.0%, stage II 25.0%, stage III 31.0%, and stage IV 17.0% and Watt[15] showed the majority had TNM stage II (50.0%)

and stage III disease (39.0%). Their result differ with the present study may be due to single centered study as well as variation in sample size.

LDH plays necessary part in the transformation of pyruvate to lactate; each of them has been noticed to have prognostic value in many tumors. Based on prior researches with various types of malignancies, increase in ALP or LDH could suggest a heavy tumor burden and tumor spread.[16] About the association of LDH with grade in this current study, it was observed that the mean LDH was 160.33±27.02 in grade I, 199.26±31.1 in grade II and 246.71±36.32 in grade III tumor. On the other hand, mean LDH was 179.71±41.74 in stage I, 166±33.94 in stage II, 218.1±40.12 in stage III disease. The mean LDH was significantly (p<0.05) associated with both grade and stage. Other important results in Al-Ghurabi study are that the mean value of LDH and ALP in patients increased with advance stage as well as the significant reduction in the levels of serum enzymes postoperatively as compared to their levels at baseline[7].

Regarding the association of ALP with grade and stage, the study found that the mean ALP was 63.67±8.62 U/L in grade I, 75±10.15 U/L in grade II and 109.14±31.89 U/L in grade III tumor, 13.6% patients had high ALP in stage III and in other stages it was within normal limit. The mean ALP was significantly (p<0.05) elevated with increased grade and stage, which indicates that ALP was significantly increased with advance grade and stage, which is similar with Al-Ghurabi[7] study. Hung[17] observed that the elevated preoperative ALP levels were not only associated with liver metastasis, but it was also related with advanced tumor status and indicated a poor survival in colon and rectal cancer patients. Faloppi[18] and Wei[16] obtained in their respective studies that both LDH and ALP are indicators of tumor spread and evolution, and they have been assured as prognostic biomarkers in several types of tumors. LDH had a positive significant correlation with both grade (r=0.423; p=0.004) and stage (r=0.498; p=0.001) of CRC. Similarly, ALP had also a positive significant correlation with both grade (r=0.477; p=0.001) and stage (r=0.489; p=0.001).

Conclusion

This cross sectional study was performed to assess the relationship between selected preoperative biochemical parameters and histological grade and stage of disease in patients with cancer of the colon and rectum. We examined the predictive values of various blood parameters for colorectal cancer grade and stage. Our results confirmed that serum LDH and ALP level are significantly associated with both histological grade and stage. We also found positive significant correlation between serum LDH and ALP with both grade and stage colorectal cancer.

Histological grade and stage of CRC has been shown to have enormous importance in disease course, overall prognosis and treatment. Regular use of some costly investigations like molecular techniques and immunohistochemistry are not quite feasible in our country and there is also constrains of infrastructural set up. So, some commonly done biochemical parameters like LDH, ALP etc. are both easily available and cost-effective, may play a role in predicting grade and stage of CRC and may be used as adjunct to clinical, radiological and pathological findings in disease evaluation.

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