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RESEARCH ARTICLE

Lactate Dehydrogenase in Patients with Metastatic Colorectal Cancer: Retrospective Study to Explore a Target Subgroup for Utilization as a Tumor Marker

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ABSTRACT

Aim: Serum lactate dehydrogenase (LDH) may be a prognostic marker in metastatic colorectal cancer (mCRC). However, mCRC is a heterogeneous disease, and data on LDH-related subgroups are limited. This study aimed to investigate clinical and molecular features associated with LDH in mCRC.

Method: Demographic, clinical, treatment response, and survival data from a retrospective cohort of patients diagnosed with synchronous mCRC between 2019 and 2023 were analyzed according to serum LDH levels. Lactate dehydrogenase A (LDHA) gene expression and molecular features were assessed in an independent cohort.

Results: The clinical cohort included 135 patients. The median LDH level was 231 U/L (range: 106-5,655), and 55.1% (n=75) of patients had high LDH. The presence of liver metastases (p=0.037), the number of liver metastases (≥5 vs. <5, p=0.035), carcinoembryonic antigen (p=0.000), carbohydrate antigen 19-9 (p=0.042), and C-reactive protein (p=0.002) levels were significantly associated with high LDH. Among patients with liver-only metastases, high LDH was significantly associated with worse overall survival (OS) (19.7 months [95% confidence interval (CI): 13.8-28.1] vs. 39.0 months (95% CI: 19.8-59.2), p=0.017). Non-responders to 5-fluorouracil, leucovorin, and oxaliplatin had higher LDH levels (p=0.016) and worse OS [11.4 months (95% CI: 6.2-12.7) vs. not reached, p=0.002]. Among 84 patients in the independent mCRC cohort, 16.7% (n=14) had high LDHA expression in tumor tissue. High LDHA expression was associated with lower microsatellite instability scores (p=0.048) and higher hypoxia scores (p for Buffa=0.001, Winter=0.003), but not with tumor mutational burden or aneuploidy score. Expression of metabolic–epithelial–mesenchymal transition pathway genes was correlated with LDHA expression.

Conclusion: LDH may be a potential marker in microsatellite-stable (MSS), nonimmunogenic, liver-dominant mCRC. Whether LDH could serve as a biomarker for immunotherapy studies in MSS colorectal cancer warrants investigation in future studies.

Keywords: Lactate dehydrogenase, metastatic colorectal cancer, liver metastasis, tumor marker, microsatellite stability, microsatellite stable, hypoxia

Introduction

Colorectal cancer (CRC) ranks as the third leading cause of cancer-related deaths globally, with more than 1.85 million new cases and 850,000 deaths each year. Approximately 20% of patients present with synchronous metastatic disease at diagnosis. Despite advances in management, the 3-year survival rate of metastatic colorectal cancer (mCRC) is nearly 30%.[1]

Well-established predictive and prognostic biomarkers, such as rat sarcoma (RAS) and B-Raf proto-oncogene (BRAF) mutations and microsatellite instability (MSI), are already incorporated into routine clinical practice for the treatment of mCRC.[2] However, mCRC is a clinically and molecularly heterogeneous disease, and novel treatment options, such as immunotherapy, are under investigation.[2] Therefore, there is a need for additional biomarkers and for further characterization

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of existing biomarkers according to disease presentation, clinical setting, patient subgroups, disease biology, and molecular subtypes.[3]

Most cancer cells rely on aerobic glycolysis, also known as the Warburg effect, to sustain growth even in the presence of oxygen, resulting in increased lactate production and secretion. Lactate dehydrogenase (LDH) plays a pivotal role in glucose metabolism by regulating the interconversion of pyruvate and lactate. The lactate dehydrogenase A (LDHA) subunit catalyzes the conversion of pyruvate to lactate.[4] Serum LDH levels have been reported to have predictive and prognostic value in mCRC, and serum LDH has also been considered an indirect marker of hypoxia and angiogenesis.[5] However, LDH-related clinical and molecular characteristics in mCRC remain poorly defined. Characterization of these features is required to implement LDH as a biomarker in mCRC, a highly heterogeneous disease. The objective of this study is to investigate circulating serum LDH levels and tumor LDHA gene expression in mCRC to define a framework for its potential use as a tumor marker, based on the hypothesis that LDH is associated with specific clinical and molecular features.

Materials and Methods

Patients and Clinical Assessments

Retrospective cohort data from patients newly diagnosed with mCRC between 2019 and 2023 were analyzed. Patients of any gender aged ≥18 years who were newly diagnosed with synchronous (*de novo*) mCRC, with resectable, potentially resectable, or unresectable disease, were included. Patients who developed metastatic disease following recurrence of localized disease (that is, not de novo metastatic disease) and patients without LDH data were excluded.

Age, gender, comorbidities (diabetes, hypertension, or coronary artery disease), smoking history, primary tumor location, metastatic sites (liver, peritoneum, lung, or bone), number of liver metastases, serum LDH, plasma carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), C-reactive protein (CRP) levels, neutrophil-to-lymphocyte ratio, serum uric acid levels at diagnosis, presence of urgent surgery, MSI, RAS and BRAF mutational status, systemic and local treatments and treatment response, disease progression, and survival data were recorded. Patients were divided into two groups, high LDH and normal LDH, according to serum LDH levels at diagnosis (LDH greater than the upper limit of normal (ULN) of 214 U/L and LDH less than or equal to the ULN). Demographic and clinical parameters were compared between the high and normal LDH groups.

Response to first-line treatment was evaluated according to Response Evaluation Criteria in Solid Tumors version 1.1. Progression-free survival (PFS) was defined as the time from initiation of treatment to first documented disease progression or death. Overall survival (OS) was defined as the time from initiation of treatment to death or the patient's last hospital visit.

Ethical approval was obtained from the Clinical Research Ethics Committee of Ankara University Faculty of Medicine (decision no.: İ11-694-22, date: 10.01.2023) in compliance with the Declaration of Helsinki. The study analyzed retrospective, anonymized clinical data. Therefore, informed consent was not required, and the ethics committee granted a waiver for this purpose.

Molecular Assessments

Data from The Cancer Genome Atlas (TCGA) Program PanCancer Atlas for colorectal adenocarcinoma were utilized, and cBioPortal was used for metadata collection and analyses.[6] Only patients with metastatic disease within TCGA cohort were included. Messenger RNA expression z scores of the LDHA gene in tumor samples relative to normal samples [log RNA sequencing version 2 RSEM (z score threshold ±2] were analyzed. Patients were divided into two groups, LDHA high expression and LDHA normal expression, according to the z score (LDHA high >2 and LDHA normal ≤2).

MSI Microsatellite Analysis for Normal-Tumor InStability scores[7], Buffa and Winter hypoxia scores[8,9], tumor mutational burden (TMB), and aneuploidy scores[10] were compared between the LDHA high and LDHA normal expression groups. Genes whose expression correlated with LDHA expression and showed the highest correlation coefficients were identified. The correlation between LDHA expression and gene sets corresponding to consensus molecular subtype (CMS) 3 (metabolic) was analyzed.[11] In addition, the correlation between LDHA expression and the expression of epithelial–mesenchymal transition genes, representing CMS4 (mesenchymal), was evaluated.[11] Gene sets for metabolic pathways were obtained from the Kyoto Encyclopedia of Genes and Genomes as referenced in the original study.[11,12] The gene set for epithelial-mesenchymal transition was obtained from Gene Set Enrichment Analysis.[13,14]

Statistical Analyses

Continuous variables were reported as median (minimum-maximum), and categorical variables were presented as percentages. Missing data were reported in the tables and included in the statistical analyses. Univariable comparisons were performed using the chi-square test, Fisher's exact test, Student's t-test, the Mann-Whitney U test, and Cox regression, as appropriate. All p-values were based on two-

tailed tests of significance, with a significance threshold of p=0.05. All statistical analyses were conducted using MedCalc® Statistical Software version 22.026 (MedCalc Software Ltd, Ostend, Belgium). Calculated metadata from cBioPortal were used where applicable.[6]

Results

Serum LDH Levels and Clinical Characteristics

A total of 135 patients with synchronous mCRC were included (Figure 1). The demographic and clinical characteristics of the overall study population and the LDH subgroups are presented in Table 1. The median age was 60 years (range: 30-80), and 61.5% (n=83) of patients were men. The primary tumor location was rectal in 44.5% (n=60) of patients. Liver metastases were present in 88.1% (n=119) of patients. RAS mutations were detected in 42.2% (n=57). The median serum LDH level was 231 U/L (range: 106–5,655), and 55.1% (n=75) of patients had LDH levels above the ULN. Among these patients, 16.0% (n=12) had LDH levels exceeding 1,000 U/L. Liver metastases were significantly more frequent in the high-LDH group than in the normal-LDH group (93.3% vs. 81.6%, p=0.037). When the number of liver metastases was categorized as ≥5 versus <5, the proportion of patients with ≥5 liver metastases was significantly elevated in the high-LDH group (69.3% vs. 43.3%, p=0.035). Median CEA levels [134 (0.93-17,796)] vs. 14.7 [0.94–949] ng/mL, p=0.00), median CA19-9 levels [149.80 (0.80-12,443) vs. 80.75 (0.80-19,300)] U/mL, p=0.042), and median CRP levels [19.75 (0.30-239.60) vs. 9.00 (0.80-163.10) mg/L, p=0.002] were significantly elevated in the high-LDH group. When LDH was analyzed as a continuous variable, the strength of the associations increased, with improved significance levels (p<0.001 for the presence of liver metastases, p=0.002 for the number of liver metastases, p=0.017 for CEA, and p<0.001 for CRP). These findings indicate that serum LDH is associated with both the presence and burden of liver metastases in synchronous mCRC.

Serum LDH Levels and Survival

As serum LDH was found to be associated with liver metastases, survival outcomes were evaluated in patients with liver-only mCRC. PFS did not differ between the high-LDH and normal-LDH groups (11.2 months (95% CI: 6.9-14.2) vs. 11.4 months [(95% CI: 7.0-13.7), p=0.276)] (Figure 2a). In contrast, high LDH was significantly associated with worse OS [19.7 months (95% CI: 13.8-28.1) vs. 39.0 months [95% CI: 19.8-59.2), p=0.017] (Figure 2b).

Treatment and response characteristics according to LDH groups are presented in Table 2. Despite differences in the

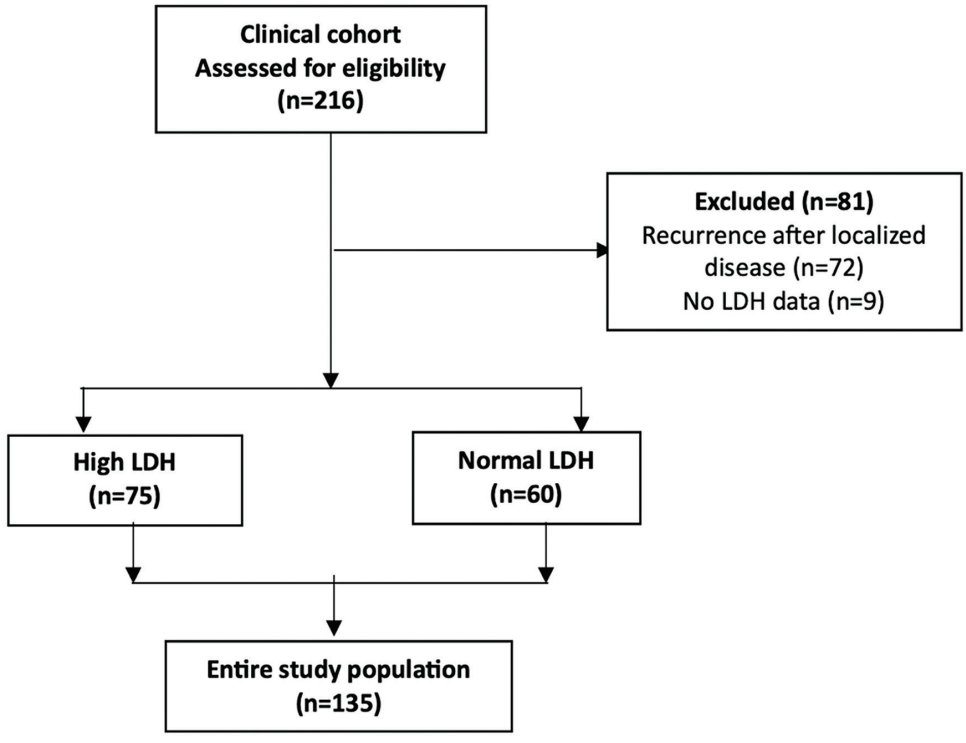

Figure 1. Patient flow diagram of the study
LDH: Lactate dehydrogenase

presence and burden of liver metastases, first-line systemic treatments, local treatments, and best response rates did not differ between the LDH groups. Patients with liver-only metastatic disease who received first-line 5-fluorouracil (5-FU), leucovorin, and oxaliplatin chemotherapy were further evaluated for treatment response in relation to serum LDH levels. Non-responders (stable disease or progressive disease) had a median LDH level of 250 U/L (range: 167-1,898), which was significantly higher than that observed in responders (complete or partial response) [median LDH

Table 1. Characteristics of the patients and LDH groups

	Study population (n=135)	**LDH>ULN (n=75, 55.5%)**	**LDH<ULN (n=60, 44.5%)**	**p**
Age, median (min-max)	60 (30-80)	59 (30-76)	60 (31-80)	0.447
Gender, n (%)				
Male	83 (61.5)	45 (60)	38 (63.3)	
Female	52 (38.5)	30 (40)	22 (36.7)	0.692
Comorbidities, n (%)				
Diabetes	25 (18.5)	5 (6.6)	10 (16.7)	0.620
Hypertension	43 (31.9)	20 (26.6)	23 (38.3)	0.148
Coronary disease	13 (9.7)	5 (6.6)	8 (13.3)	0.192
Hypothyroidism	9 (6.6)	6 (8)	3 (5)	0.487
Smoking history, n (%)	70 (51.9)	41 (54.7)	29 (48.3)	0.464
Primary site, n (%)				
Colon	75 (55.5)	46 (61.3)	29 (48.3)	0.307
Rectum	60 (44.5)	32 (38.7)	28 (51.7)	
Metastatic site, n (%)				
Liver	119 (88.1)	70 (93.3)	49 (81.6)	**0.037**
Lung	35 (25.9)	17 (22.7)	18 (30)	0.334
Bone	6 (4.4)	4 (5.3)	2 (3.3)	0.693
Peritoneal	18 (13.3)	11 (14.7)	7 (11.6)	0.610
Number of liver metastasis, n (%)				
≥5	78 (57.7)	52 (69.3)	26 (43.3)	**0.035**
<5	35 (25.9)	16 (21.3)	19 (31.6)	
MSI-H, n (%)	3 (2.2)	2 (1.4)	1 (<1)	-
RAS mutant, n (%)	57 (42.2)	31(41.3)	26 (43.3)	0.482
RAF mutant, n (%)	1 (<1)	1 (<1)	0	-
ABO group, n (%)				
AB	9 (6.5)	7 (9.7)	4 (5.9)	
A	60 (44.9)	38 (50)	26 (44)	
B	19 (14.1)	7 (9.7)	9 (14.9)	0.393
O	42 (31)	21 (27.4)	19 (31.6)	
UK	5 (3.5)	2 (3.2)	2 (3.6)	
CEA, ng/mL, median (min-max)	38.75 (0.93-17,796)	134 (0.93-17,796)	14.7 (0.94-949)	**<0.001**
Ca19-9, U/mL, median (min-max)	93.50 (0.80-19,300)	149.80 (0.80-12,443)	80.75 (0.80-19,300)	**0.042**
CRP, mg/L, median (min-max)	14.15 (0.30-239.60)	19.75 (0.30-239.60)	9 (0.80-163.10)	**0.002**
NLR, median (min-max)	3.14 (1-12.56)	3.28 (1.13-12.56)	2.95 (1-8.57)	0.109
Albumin, g/dL, median (min-max)	3.99 (2.52-4.91)	3.96 (2.52-4.85)	4.09 (2.98-4.91)	0.142
Uric acid, mg/dL median (min-max)	5 (2-9.5)	5 (2.2-9.5)	4.95 (2-7.4)	0.746

LDH: Lactate dehydrogenase, ULN: Upper limit of normal, CEA: Carcinoembryonic antigen, Ca19-9: Carbohydrate antigen 19-9, CRP: C-reactive protein, NLR: Neutrophile/lymphocyte ratio, RAS: Rat sarcoma, MSI-H: Microsatellite instability-high

198 U/L (range: 137-1,554), p=0.016] (Figure 3a). Among these patients, high LDH was significantly associated with worse OS [11.4 months (95% CI: 6.2-12.7)] vs. not reached, p=0.002) (Figure 3b).

Tumor LDHA Expression and Molecular Characteristics

Among 594 patients with colorectal adenocarcinoma in TCGA cohort, 14.1% (n=84) had metastatic disease. Of these, 16.7% (n=14) exhibited high LDHA expression in tumor tissue.

MSI MANTIS scores, Winter and Buffa hypoxia scores, TMB, and aneuploidy scores were compared between LDHA high-expression and LDHA normal-expression groups among patients with available data (Figure 4). The median MSI MANTIS score was significantly lower in the LDHA high-expression group [2,817 (33-3,807) vs. 3,325 (66-8,168), p=0.048]. Median Winter and Buffa hypoxia scores were significantly increased in the LDHA high-expression group [Winter: 32 (14-52) vs. 15 (-20-32), p=0.003; Buffa: 35 (13-39) vs. 17 (-17-29), p=0.001]. Median TMB was

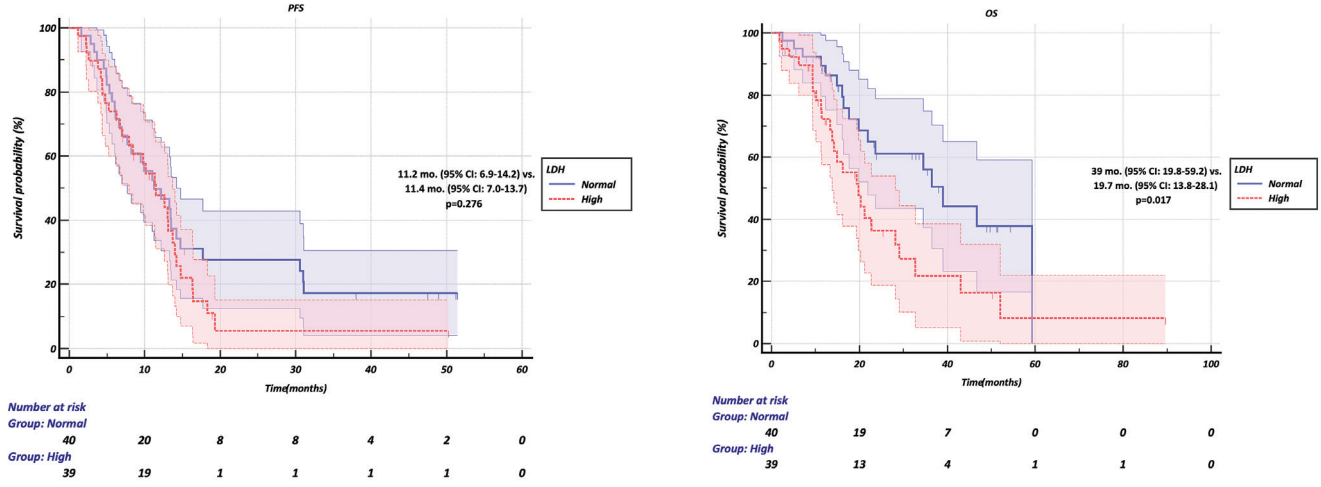

Figure 2. (a) Progression-free survival (PFS) and (b) overall survival (OS) among patients with liver-only metastatic colorectal cancer according to serum LDH levels.
LDH: Lactate dehydrogenase, CI: Confidence interval

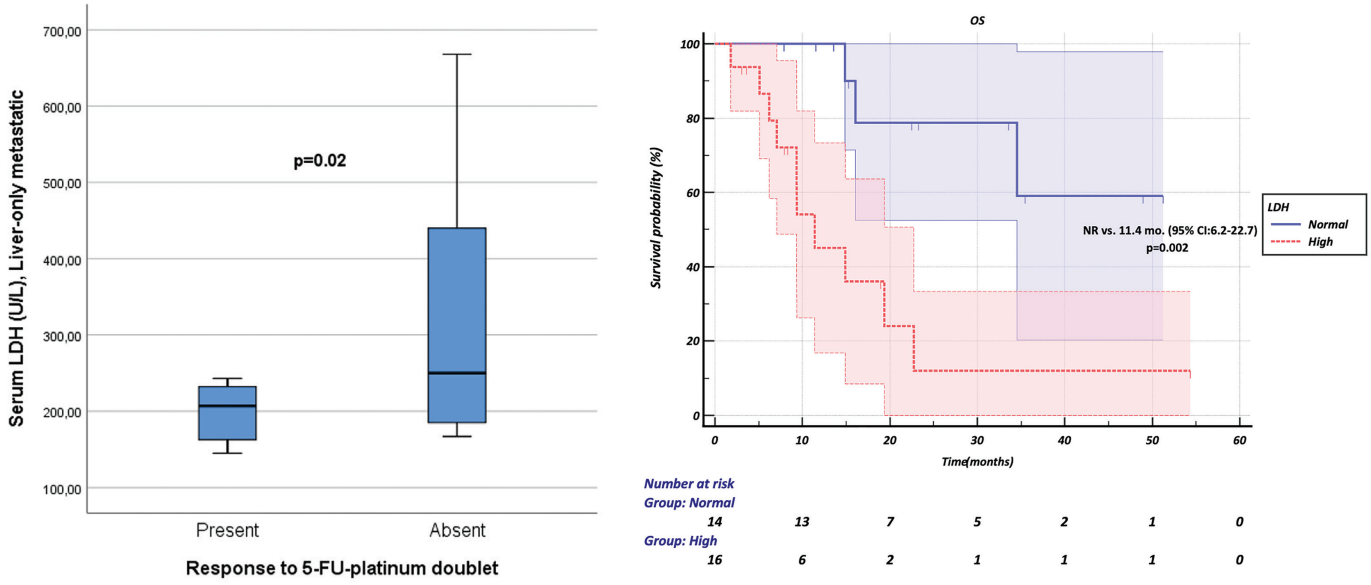

Figure 3. (a) Comparison of serum LDH levels between responders and non-responders to first-line FOLFOX chemotherapy among patients with liver-only metastatic colorectal cancer and (b) overall survival (OS) according to LDH level in this patient group.
LDH: Lactate dehydrogenase, CI: Confidence interval

3.13 mutations/Mb (range: 1.73-5.83) in the LDHA high-expression group and 3.01 mutations/Mb (range: 0.00-7.67) in the LDHA normal-expression group (p=0.320). Median aneuploidy scores did not differ between groups [14.5 (4-25) vs. 16 (2-29), p=0.270]. These findings suggest that LDH is associated with hypoxia and metabolic pathways in CRC cells rather than with genomic instability or immunogenicity.

Gene expression correlation analyses further supported a role for LDHA in nonimmunogenic, metabolic, and epithelial–mesenchymal transition pathways in mCRC. Genes most strongly correlated with LDHA expression included phosphoglycerate kinase 1, PSMA1, ELP4, SAAL1, RRM1, ADM, AKIP1, COQ2, MTCH2, SAP30, MELK, ALDOA, and ANKRD37 (Table S1). Phosphoglycerate kinase 1, a key enzyme in the glycolytic pathway, showed the strongest correlation with LDHA expression (Spearman's rho=0.58, p<0.001, q<0.001). Among the gene sets related to metabolic pathways (14 pathways in total), the glucose–pentose pathway showed the strongest association with LDHA expression, which was significantly positively correlated with 48.1% (17 of 23) of genes within the glucose–pentose pathway. Within the epithelial-mesenchymal transition gene set, LDHA expression was significantly correlated with CD44 (p<0.001, q=0.007, a cell-surface glycoprotein involved in cell adhesion and migration), CD59 (p<0.001, q=0.021, a cell-surface glycoprotein), *PLOD2* (p=0.001, q=0.052, a catalyst of the hydroxylation of lysyl residues in collagen-like peptides), *SAT1* (p<0.001, q=0.017, an acetyltransferase involved in polyamine metabolism), and *TPM4* (p<0.001, q=0.033, a member of the tropomyosin family).

Discussion

This study explored LDH-associated clinical and molecular features to define specific patient subgroups in mCRC to facilitate further investigation and support the use of LDH as a tumor marker. Serum LDH was associated with both

Table 2. Treatment characteristics of patients and LDH groups

	Study population (n=135)	LDH>ULN (n=75, 55.5%)	LDH<ULN (n=60, 44.5%)	p
First-line treatment, n (%)				
5-FU-OX doublet	72 (52.9)	32 (42.7)	40 (66.7)	
5-FU-OX doublet + bevacizumab	31 (22.8)	18 (24)	13 (21.7)	
5-FU-OX doublet + anti-EGFR	23 (16.9)	16 (21.3)	7 (11.6)	
Only 5-FU	1 (0.7)	1 (1.3)	0 (0)	
5-FU-IRI doublet	1 (0.7)	1 (1.3)	0 (0)	
5-FU-IRI doublet + bevacizumab	1 (0.7)	1 (1.3)	0 (0)	0.138
5-FU-IRI doublet + anti-EGFR	2 (1.5)	2 (2.7)	0 (0)	
Triplet	1 (0.7)	1 (1.3)	0 (0)	
Triplet + bevacizumab	3 (2.2)	2 (2.7)	0 (0)	
Triplet + anti-EGFR	1 (0.7)	1 (1.3)	0 (0)	
Local treatment, n (%)				
Surgery	24 (17.6)	12 (16)	12 (20)	0.601
TARE, TACE, or RFA	33 (24.3)	21 (28)	11 (18.3)	0.160
Best response to first-line treatment, n (%)				
Complete	14 (10.3)	4 (5.3)	10 (16.7)	
Partial	70 (51.5)	40 (53.3)	29 (48.3)	
Stable	36 (26.5)	20 (26.7)	16 (26.7)	0.133
Progression	11 (8.1)	8 (10.7)	3 (5)	
Unknown	5 (3.7)	3 (4)	2 (3.3)	

LDH: Lactate dehydrogenase, ULN: Upper limit of normal, 5-FU: 5-fluorouracil, OX: Oxaliplatin, EGFR: Epidermal growth factor receptor, IRI: Irinotecan, TARE: Transarterial radioembolization, TACE: Transarterial chemoembolization, RFA: Radiofrequency ablation

the presence and burden of liver metastases in synchronous mCRC and correlated with serum CEA levels. High serum LDH levels were associated with worse OS and poorer response to 5-FU-platinum chemotherapy in liver mCRC. Tumor gene expression profiles were associated with microsatellite stability, hypoxia, metabolic pathways, and mesenchymal features, but not with TMB or aneuploidy scores.

The prognostic role of serum LDH in mCRC has been evaluated in several studies. In a meta-analysis, high LDH levels were associated with poor OS [hazard ratio (HR)=1.75 (95% CI: 1.52-2.02)].[15] The prognostic significance was independent of metastatic status and the use of antiangiogenic chemotherapy. No prognostic value was observed for PFS. However, the studies included in this meta-analysis were heterogeneous, reinforcing the rationale for subgroup specification, as addressed in the present study. Additionally, dynamic changes in serum LDH levels have been reported to be prognostic in mCRC.[16] Another meta-analysis demonstrated that high serum LDH levels were associated with shorter PFS [HR=1.43 (95% CI: 1.05-1.94), p=0.023] and OS [HR=1.667 (95% CI: 1.230-2.259), p=0.001] in patients with mCRC treated with bevacizumab-based first-line chemotherapy.[17] High serum LDH levels were also identified as a prognostic factor for worse survival in patients with mCRC receiving irinotecan-based second-line chemotherapy.[18] Our findings confirm the prognostic value of LDH in mCRC and further support its potential utility in microsatellite-stable (MSS), liver-metastatic disease.

Several previous studies have reported molecular features associated with LDH in CRC, largely consistent with our findings. Tumor gene expression of LDHA, vascular endothelial growth factor receptor 1, and vascular endothelial growth factor A has been shown to correlate with serum LDH levels in mCRC.[19] Expression profiling of invasion margins in colorectal tumors has revealed increased lactate metabolism and expression in aggressive phenotypes, supporting a role in epithelial-mesenchymal transition.[20,21] Cetuximab-resistant CRC cells have been reported to produce significantly elevated levels of lactate, suggesting that enhanced anaerobic metabolism is a

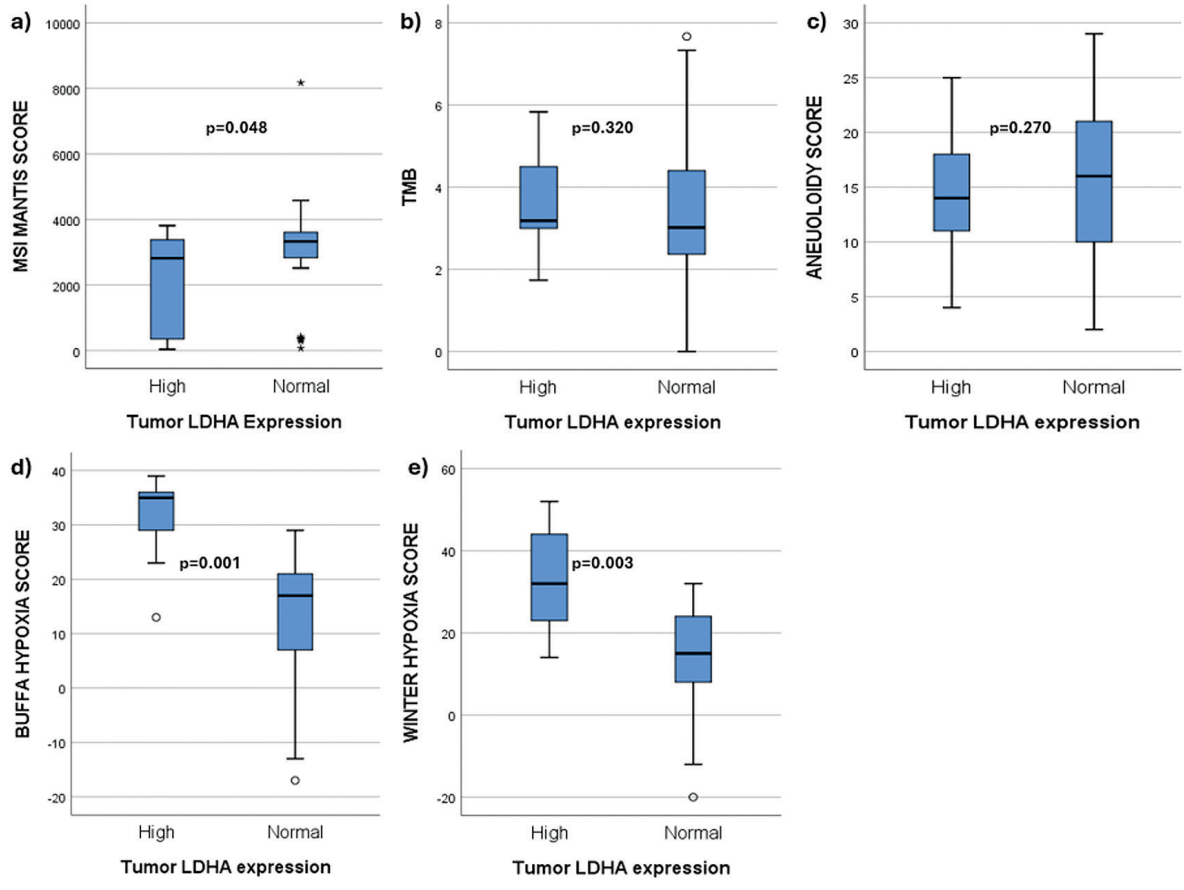

Figure 4. Molecular comparisons between LDHA high- and normal-expression groups: (a) MSI MANTIS score, (b) tumor mutational burden (TMB), (c) aneuploidy score, (d) Buffa hypoxia score, and (e) Winter hypoxia score.

LDHA: Lactate dehydrogenase A, MSI: Microsatellite instability, MANTIS: Microsatellite Analysis for Normal-Tumor InStability

prominent feature of resistance to anti-epidermal growth factor receptor therapy.[22] Notably, inhibition of LDHA by microRNA-34a has been shown to resensitize colon cancer cells to 5-FU.[23] Consistent with these findings, we observed that patients who did not respond to 5-FU–platinum doublet chemotherapy had increased serum LDH levels. Together, these results suggest that elevated LDH levels may indicate a need for more aggressive treatment strategies and the addition of biologic agents to chemotherapy, highlighting the link between LDH and molecular pathogenesis.

In a study comparing the expression of aerobic glycolysis-related genes between primary tumors and liver metastases in CRC, LDHA was the only gene expressed at an elevated level in liver metastases.[24] In line with this observation, our study demonstrated an association between elevated serum LDH levels and both the presence and burden of liver metastases.

The Colorectal Cancer Subtyping Consortium evaluated high-throughput transcriptomic data to define intrinsic molecular subtypes of CRC.[11] Four CMSs were identified, each with distinct characteristics: CMS1 (MSI immune, 14%), characterized by hypermutation, MSI, and strong immune activation; CMS2 (canonical, 37%), epithelial tumors with marked activation of Wingless-related integration site signaling and myelocytomatosis oncogene signaling; CMS3 (metabolic, 13%), epithelial tumors with evident metabolic dysregulation; and CMS4 (mesenchymal, 23%), characterized by prominent transforming growth factor-β activation, stromal invasion, and angiogenesis. Based on our clinical findings and initial molecular analyses, LDH appeared to be more closely associated with CMS3 (metabolic) and CMS4 (mesenchymal) than with CMS1 (MSI immune) or CMS2 (canonical). Accordingly, we explored associations between LDHA gene expression and metabolic and epithelial-mesenchymal transition pathways. The observed correlations support a link between LDH and CMS3-CMS4 subtypes. Furthermore, correlations with epithelial-mesenchymal transition pathway genes suggest that LDH may contribute to epithelial-mesenchymal transition in CRC, thereby facilitating metastatic spread, consistent with its clinical association with liver metastasis and metastatic burden in our cohort.

Although the efficacy of immunotherapy in MSI-high CRC has been well established and has substantially altered treatment paradigms, ongoing research is focused on extending immunotherapy to MSS CRC. A major challenge in this setting is the identification of biomarkers that can select patients with MSS CRC who may benefit from immunotherapy.[25] Our hypothesis-generating findings suggest that LDH may warrant further investigation as a potential biomarker in immunotherapy research for this patient population.

Study Limitations

This study has several limitations. First, the clinical analyses are subject to the inherent limitations of a single-center retrospective design. As all eligible patients during the study period were included, a formal sample size calculation was not performed; however, studies with larger cohorts would provide more robust conclusions. Survival analyses comparing high and normal LDH groups were restricted to patients with liver metastases, as this was the primary difference in baseline characteristics and treatment patterns. Nonetheless, the single-center design and exclusion of certain patients introduce a potential risk of selection bias. Future studies incorporating multicenter cohorts, multivariable-adjusted analyses, and a broader set of clinical variables may strengthen the proposed associations. In addition, matched clinical and molecular analyses were not available. Molecular assessments were primarily based on correlative gene expression analyses. Mechanistic studies and prospective validation cohorts are needed to confirm these findings and establish definitive conclusions.

Conclusion

This study adds to the existing literature on LDH in mCRC by identifying a clinically and molecularly relevant patient subgroup. LDH may serve as a potential tumor marker in MSS, non-immunogenic, liver-dominant mCRC. Based on the hypothesis-generating results of this study, the potential role of LDH as a biomarker in immunotherapy research for MSS CRC warrants evaluation in future studies.

Acknowledgment

The data of this study was partly presented as a poster at ESMO Gastrointestinal Cancers Congress 2024, in Munich, Germany, from 26 to 29 June 2024.

The results published here are in part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Clinical Research Ethics Committee of Ankara University Faculty of Medicine (decision no.: İ11-694-22, date: 10.01.2023) in compliance with the Declaration of Helsinki.

Informed Consent: The study analysed retrospective, anonymous clinical data of the patients. Therefore, informed consent of the patients was not required, and waiver/exempt was granted by the Ethics Committee for this purpose.

Footnotes

Authorship Contributions

Concept: E.A., B.B.K., U.T., G.U., Design: E.A., B.B.K., U.T., G.U., Data Collection or Processing: E.A., B.B.K., U.T., Analysis or Interpretation: E.A., B.B.K., G.U., Literature Search: E.A., Writing: E.A., B.B.K., U.T., G.U.

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Table 1S. Genes with the highest correlation coefficient with LDHA expression

Gene	Spearman's rho	p	Function-pathway
PGK1	0.58	5.95e-9	Phosphoglycerate kinase 1, glycolysis and gluconeogenesis
PSMA1	0.57	1.45e-8	Proteasome 20S Subunit Alpha 1
ELP4	0.56	3.05e-8	Elongator Acetyltransferase Complex Subunit 4, chromatin organization and mesodermal commitment
SAAL1	0.55	6.11e-8	Serum Amyloid A Like 1
RRM1	0.55	6.55e-8	Ribonucleotide Reductase Catalytic Subunit M1, pyrimidine deoxyribonucleotides biosynthesis from CTP and purine nucleotides de novo biosynthesis
ADM	0.54	1.12e-7	Adrenomedullin, GPCR downstream signaling and Presynaptic function of Kainate receptors
AKIP1	0.51	4.94e-7	A-Kinase Interacting Protein 1
COQ2	0.51	8.51e-7	Coenzyme Q2, Polyprenyltransferase, Peroxisomal lipid metabolism and Metabolism of water-soluble vitamins and cofactors
MTCH2	0.50	1.081e-6	Mitochondrial Carrier 2
SAP30	0.50	1.086e-6	Sin3A Associated Protein 30, RNA Polymerase I Promoter Opening and infectious disease
MELK	0.50	1.092e-6	Maternal Embryonic Leucine Zipper Kinase
ALDOA	0.50	1.115e-6	Aldolase, Fructose-Bisphosphate A, glycolysis (BioCyc) and response to elevated platelet cytosolic Ca2+
ANKRD37	0.50	1.266e-6	Ankyrin Repeat Domain 37

LDHA: Lactate dehydrogenase A