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The effect of *Entamoeba histolytica* lectin antigen and microRNA-643 on the development of microsatellite instability (MSI) in colorectal adenocarcinoma

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Abstract

Entamoeba histolytica remains a significant cause of global mortality. The involvement of protozoa in microsatellite instability (MSI) and the potential of miRNA-643 as biomarkers for amoebic and colorectal diseases have not been extensively researched. The relationship between the antigenic structure of *Entamoeba histolytica* Lectin (*Eh-lectin*) and the altered expression of miRNA-643 and the X-Linked Inhibitor of Apoptosis (XIAP) is still unclear. This study aimed to identify *Eh-lectin*, miRNA-643, XIAP, and MSI in 150 colorectal cancer biopsy samples using a comprehensive approach that included immunohistochemistry (IHC), Multiplex PCR, RT-qPCR, and Real-Time PCR. To enhance the accuracy of MSI diagnosis, PCR-Multiplex was performed alongside IHC. Among the 150 colorectal cancer biopsy samples analyzed, 39 (comprising 28 MSI-H and 11 MSI-L) showed MSI, while the remaining 111 were MSI-negative. Notably, 11 samples demonstrated a co-occurrence of MSI and *Eh-lectin*, with increased expression of miRNA-643 relative to XIAP. The presence of MSI in conjunction with *Eh-lectin* positivity and elevated miRNA-643 expression, along with reduced levels of the XIAP inhibitor gene in colorectal adenocarcinoma biopsy samples, strongly indicates that this protozoan parasite may play a role in the development of MSI by affecting apoptosis.

Keywords *Entamoeba histolytica* lectin, miRNA-643, MSI, XIAP, Immunohistochemistry (IHC), PCR

Introduction

Despite extensive research on *Entamoeba histolytica* infections, significant gaps remain in our understanding of its natural history [1]. While the majority of infected individuals remain asymptomatic carriers in the large intestine, the protective mechanisms the host employs to prevent invasive disease are still not fully understood. A new overview could reignite interest and help fill this crucial knowledge void [2]. Current estimates indicate that around 480 million people are infected globally, with 36 million suffering from severe colitis or extra-intestinal abscesses [3].

In Iran, the prevalence of *E. histolytica* and *E. dispar* ranges from 2 to 30%, likely reflecting the varied climatic and environmental conditions across different regions [4]. Lectin, a crucial adherence factor for *E. histolytica*, helps differentiate it from its subtypes by promoting attachment to intestinal cells. Gal-lectin, a type of lectin inhibited by galactose/N-acetylgalactosamine, is essential for binding to mucin, adhering to host cells, and

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contributing to disease development [5]. Most intestinal cells express MUC-2, which has a strong affinity for the carbohydrate recognition domain (CRD Hgl) of Gal-lectin. The heavy and light chains of Gal-lectin are linked by disulfide bonds on a lipid raft, facilitating the activation of β2 and β7 integrins, important for intracellular signaling in *E. histolytica* [6]. Thus, Gal-lectin plays dual roles in both identifying and binding to the mucus and mucin found in the colon's mucosa and submucosa., while also granting *E. histolytica* resistance to the complement system [7]. Interestingly, elevated cholesterol levels may enhance the adhesion of *E. histolytica* to galactose on the cells' surfaces [8].

In *E. histolytica*, the voracious protozoan responsible for human amoebiasis, phagocytosis is a key factor in its virulence [9]. It has the inherent capacity to ingest other cells through phagocytosis and trogocytosis, a processes crucial for both reproduction and pathogenicity [10]. The activation of cytolytic and proteolytic enzymes leads to cleaving caspases, ultimately leading to cell death - apoptosis [11]. The role of host non-coding RNAs, particularly microRNAs, in this precise regulation is still unclear. These small, single-stranded RNA molecules are involved in various infectious diseases [12]. Recent studies have identified additional structural sequences crucial for efficient miRNA production. These include the length of the UGU motif in the apical loop, a GHG motif in the stalk, and a UG and CNNC motif in the basal region of the pri-miRNA [13].

This parasite, which causes human amoebiasis, relies heavily on phagocytosis for its virulence [9], enabling it to ingest other cells through processes essential for reproduction and pathogenicity [10]. The activation of cytolytic and proteolytic enzymes results in the cleavage of caspases, ultimately leading to cell death, or apoptosis [11]. However, the role of host non-coding RNAs, such as microRNAs, in regulating this process remains unclear. These small, single-stranded RNA molecules guide post-transcriptional gene silencing, impacting mRNA degradation and translation inhibition, and have implications in various infectious diseases [12]. Recent studies have highlighted additional structural sequences important for effective miRNA production. These include the length of the UGU motif in the apical loop, a GHG motif in the stalk, and a UG and CNNC motif in the basal region of the pri-miRNA [13].

Moreover, RNA-binding proteins are crucial regulators of miRNA production and function, with interactions between RNA-RNA and protein-RNA being vital for post-transcriptional regulation during normal development; however, these processes become dysregulated in disease. MicroRNAs (miRNAs) often show atypical expression in various cancers, including gastrointestinal cancers [14]. Distinct miRNA expression profiles can differentiate various digestive cancers, such as those of the esophagus, stomach, liver, pancreas, colon, and rectum [15]. Due to their stability outside cells and presence in cancer-associated genomic regions, microRNAs are being explored as potential cancer biomarkers [16]. Apoptosis is linked to colon cancer progression through changes in chromosomal structure and instability of microsatellites, known as microsatellite instability (MSI) [17, 18]. It has been reported that about 15% of hereditary colorectal cancers are MSI positive, with microsatellite stability evaluated through five indices: NR27, BAT-26, BAT-25, NR21, and NR24, categorizing MSI into high (H-MSI), stable (MSS), and low (L-MSI) groups [19], with H-MSI comprising more than 90% of these mutations [20].

Building on earlier findings that *E. histolytica* can induce apoptosis in colon cancer cell lines via increased miRNA-643 expression [21], it is important to note the lack of clinical research examining miRNA-643 expression levels in tumoral adenocarcinoma cells, particularly in relation to MSI and its connection with the antigenic presence of *Eh-lectin* and the anti-apoptotic gene XLAP. This study aims to achieve two main objectives: (1) To identify a specific *E. histolytica* surface protein, such as Gal-lectin, on Colorectal adenocarcinoma (CRA) tumor cells, and (2) To explore the association between miRNA-643 expression and MSI in affected patients. Through correlation analysis and functional assays, this research seeks to uncover potential diagnostic, prognostic, or therapeutic implications that could lead to more effective treatment strategies for colorectal cancer [22].

Materials and methods

Sample collection

To investigate *E. histolytica* antigen (*Eh-lectin*) and MSI in 150 colorectal adenocarcinoma biopsy samples, 3–5 µm sections were prepared on Poly-L-lysine-coated slides from paraffin blocks for immunohistochemical staining. Additionally, DNA was extracted for MSI detection using Multiplex PCR. For miRNA assessment via RT-qPCR and Real-Time PCR, 5 micro tubes, each containing 3 sections of 15 µm, were collected from separate samples.

DNA extraction

Microtubes collected after deparaffinization with xylene were used for the extraction of genomic DNA from colorectal biopsy specimens with the GeneAll® Exgene™ FFPE Tissue DNA kit (South Korea) according to the manufacturer's instructions.

Multiplex PCR

Multiplex PCR was conducted using various markers and specific primers (Table 1). Each 25 µL reaction mixture

Table 1 Primers sequences and amplicon size of PCR program for MSI detection

Marker	Primer sequences(5′ to3′)	Amplicon size (bp)	Gene
BAT-25	F: TACCAGGTGGCAAAGGGCA R: TCTGCATTTTAACTATGGCTC	153	c-Kit
BAT-26	F: CTGCGGTAATCAAGTTTTTTAG R: AACCATTCAACATTTTTAACCC	183	HMSH2
NR-27	F: AACCATGCTTGCAAACCACT R: CGATAATACTAGCAATGACC	87	Inhibitor of apoptosis protein-1
NR-21	F: GAGTCGCTGGCACAGTTCTA R: CTGGTCACTCGCGTTTACAA	109	SLC7A8
NR-24	F: GCTGAATTTTACCTCCTGAC R: ATTGTGCCATTGCATTCCAA	131	Zinc finger 2

comprised 5.5 µL of Taq Master Mix, 10 pmol of each primer (1 µM for both forward and reverse), 4 µL of template DNA, and nuclease-free water to reach the final volume. The PCR protocol included an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 40 s, annealing at 54 °C for 33 s, and extension at 72 °C for 1 min. A final extension at 72 °C was carried out for 30 min. Amplified products were visualized via electrophoresis on a 2% agarose gel containing red gel dye.

Immunohistochemical staining

To investigate *Eh*-lectin and MSI, immunohistochemical staining was performed on tissue sections. Following deparaffinization, dehydration, and peroxidase inhibition with 3% hydrogen peroxide in methanol, antigen retrieval was achieved in citrate buffer (pH 6) at 121 °C for 15 min under 15 psi pressure in an autoclave. After autoclaving and cooling, the slides were placed in PBS buffer.

After cooling, the slides were incubated with an anti-Gal-lectin monoclonal antibody (HK-9 strain) from GeneTex to detect the specific *Eh-lectin*, followed by a blocking protein step. To investigate MSI, antibodies against PMS2, MSH6, MSH2, and MLH1 from Master Company (Master diagnostica, ready to use) were subsequently applied. Incubation with specific antibodies lasted 1.5 h, followed by a master secondary antibody. Diaminobenzene (DAB) substrate and Mayer's hematoxylin were employed for detection and analysis of the antibody reactions, with hematoxylin providing counterstaining.

Detecting miRNA-643 and XIAP in biopsy samples

RNA was extracted from deparaffinized colorectal biopsy samples using the QIAGEN RNeasy® FFPE kit (Cat No. 73504) according to the manufacturer's instructions. Subsequently, cDNA was synthesized with primers designed by Bon Yakhte Company (mir-643-F: GAACTTGTATGG TATCTCAGGT and RNU6-F: AAGGATGACACGCAA ATTC) using a temperature program of 37 °C for 10 min, 55 °C for 60 min, and 70 °C for 10 min.

Table 2 Sequence of miRNA-643, XIAP, GAPDH primer

Marker	Primers Sequence(5′to3′)
has-46 F:	GTG CTC GTC TCG GCA GCA CAT ATA C
has-46 R:	AAA AAT ATG GAA CGC TTC ACG AAT TTG
has-XIAP F:	TTC ACT TGA CGA GTG TCT GGT
has-XIAP R:	TGT CCT TGA AAC TGA ACC CCA
has-GAPDH F:	TTG ACC TCA ACT ACA TGG TTT ACA
has-GAPDH R:	GCT CCT GGA AGA TGG TGA TG
has-miRNA 643 F:	GCA GAC TTG TAT GCT AGC TCA
has-miRNA 643 R:	GTC CAG TTT TTT TTT TTT TTT CTA CCT

To assess the expression levels of miRNA-643 and X-Linked Inhibitor of Apoptosis (XIAP), an anti-apoptotic gene, qRT-PCR was conducted using SYBR-Green on an ABI7500 system (Applied Bio systems, USA). Three replicates of each experiment were performed. Real-time reactions followed a temperature program of 95 °C for 2 min, then 40 cycles of 95 °C for 5 s, and 60 °C for 30 s.

The relative expression of miRNAs was quantified using the comparative Ct method (2^−ΔΔCT) and the Delta-Delta-Ct (ddCt) Algorithm, with GAPDH serving as an internal control for data normalization (Table 2).

Statistical analysis

Data were presented as Mean ± SD and analyzed using SPSS version 23.0, with a *P*-value of less than 0.05 deemed statistically significant. The associations between miR-643 expression levels and colorectal cancer (CRC) parameters were assessed using the Pearson χ^2 test. The kappa test evaluated the concordance between miR-643 expression levels and MSI status. Differences in miR-643 levels between MSI-positive and *E. histolytica* -positive cases were analyzed with the McNemar test, with $P < 0.05$ indicating significance.

Results

General results

Of the 150 colorectal adenocarcinoma samples examined, 19 cases tested positive for the *Eh-lectin* light chain antigen by immunohistochemistry. Meanwhile, 39 cases were positive for MSI using PCR (Fig. 1), and 37 cases with IHC (Fig. 2). The degree of concordance between PCR and IHC methods in detecting MSI positivity was 95%.

Out of a total of 39 MSI + cases, 28 cases had more than 2 positive indices, placing them in the MSI-H group, and 11 cases had a positive index, indicating MSI-L, which signifies a higher percentage of MSI-H (71.7%) in MSI-positive samples (Table 3).

Out of 39 MSI + cases, 11 cases were positive for *E. histolytica* light chain antigen. Out of 131 cases of MSI-, 8

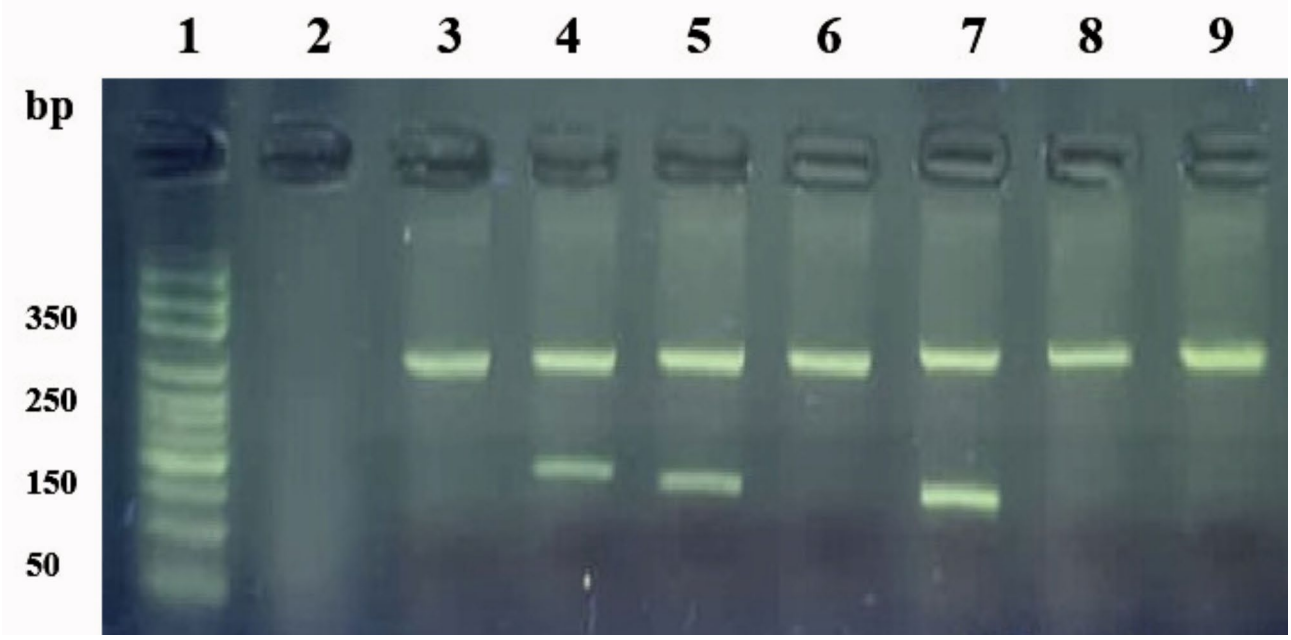

Fig. 1 MSI electrophoresis gel: with three positive indicators (H-MSI). Number 1: Ladder (50 bp); 2: Blank; 3: negative control; 4: BAT-26 positive; 5: BAT-25 positive; 6: NR-21 negative; 7: NR-24 positive; 8: NR-27 negative; 9: Negative control

cases were confirmed by light chain antigen of this protozoan (Table 4; Fig. 3).

The expression results of miRNA-643 and XIAP gene

The measurement of miRNA-643 and XIAP gene expression levels classified the samples into four groups: The first group: MSI positive but *Eh-lectin* negative. The second group: MSI negative but *Eh-lectin* antigen positive. The third group: Both *Eh-lectin* antigen and MSI positive and the fourth group: Compared the expression of miRNA-643 and the anti-apoptotic gene XIAP, where *Eh-lectin* was positive. The miRNA-643 and XIAP expression results in the four investigated groups is shown in Table 5. miRNA-643 and XIAP were most highly expressed in groups 3 and 2, respectively.

According to Table 5, the first group exhibited a threefold increase in XIAP gene expression compared to miRNA-643, and the second group showed a fourfold increase in anti-apoptotic gene expression. The third group revealed a threefold increase in miRNA-643 expression compared to the anti-apoptotic gene. In the fourth group, related to *E. histolytica* positive antigen samples and their relationship with miRNA-643, the co-expression of miRNA-643 and XIAP was indicated, which was not statistically significant with a p-value > 0.5. All groups were measured based on the GAPDH gene with an expression level of 1.

The samples positive for MSI and *Eh-lectin* antigen, demonstrated an increase in miRNA-643 expression compared to the XIAP anti-apoptotic gene, based on the 2^−ΔΔCT results from data analysis ($P < 0.019$) (Fig. 4).

Discussion

Entamoeba histolytica is recognized for its ability to induce apoptosis in host cells, with ongoing research focused on how D-galactose and N-acetyl-D-galactosamine (Gal/GalNAc) lectin may initiate this process, the function of amoeba pores in activating host caspases, and the capacity of amoebic proteases to directly stimulate effector caspases upon entry into the cytoplasm of host cells [23]. Colorectal cancer is one of the most common cancers in Iran, ranking third in incidence [24]. The role of microsatellite instability (MSI) as a diagnostic biomarker is gaining traction, particularly for enhancing treatment strategies for colorectal cancer patients, with roughly 15% of colorectal cancers classified as MSI-positive [25]. However, the environmental and acquired factors that contribute to MSI development remain largely unexplored. In contrast, some studies have established links between specific bacteria, such as *Fusobacterium nucleatum* and *Epstein-Barr* virus, and colon cancer tissue, along with their correlation with MSI at the tumor site [26].

Consequently, alongside molecular techniques, immunohistochemical methods have been utilized due to improvements in antigen detection in tissue samples [27]. Immunohistochemistry is a key technique widely utilized in both diagnostic and research settings across numerous laboratories. It is essential for tumor immunophenotyping and assessing tissue-specific genes, which are vital for tumor classification at various diagnostic stages [28]. Haghighi et al. (2017) employed this technique to identify the specific antigen of this protozoan in biopsy samples

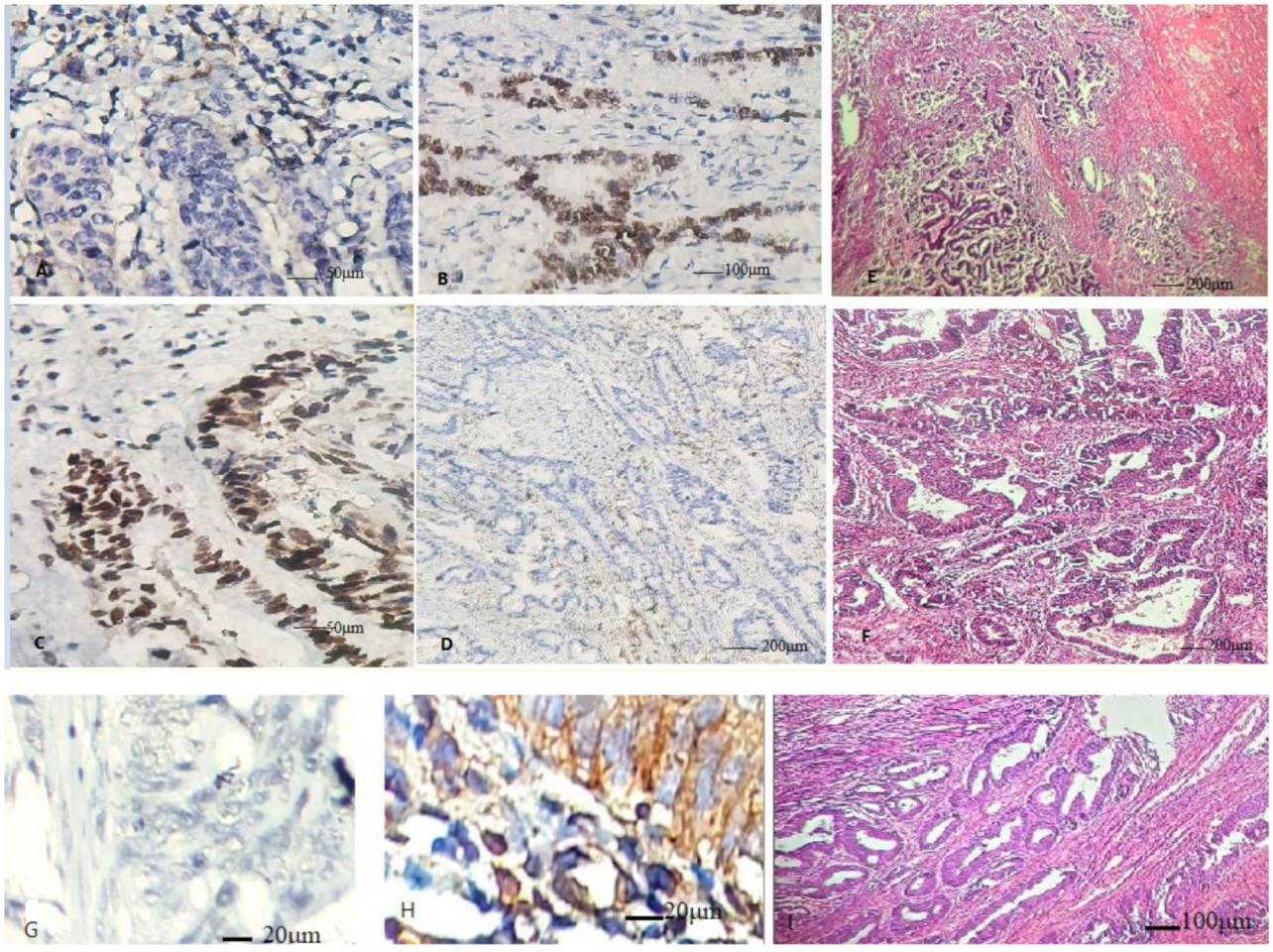

Fig. 2 Immunohistochemical staining pictures related to microsatellite instability are presented as follows: **A** depict positive staining for MutL homolog 1 (MLH1) and **B&C** depict positive staining for MutS Homolog 2 (MSH2) and MutS Homolog 6 (MSH6) mutations, while **D** show case negative staining for mutations of Postmeiotic segregation increased 2 (PMS2). Edge-enhanced and bright-field imaging (**E&F** Imaging) of **H&E** staining. It is worth mentioning that in the analysis of immunohistochemical slides of microsatellite samples, the color ability of tumoral cell nuclei with chromogen indicates the absence of mutation in the investigated marker and is considered negative from the point of view of the specific antibody. In other words, the absovation of cells not stained with chromogen indicates a disruption in the DNA mismatch repair (MMR) system. MMR is a system for recognizing and repairing erroneous insertion, deletion, and mis-incorporation of bases that can arise during DNA replication and recombination, as well as repairing some forms of DNA damage G: Negative and H: Positive cytoplasmic staining image for *Eh-lectin* light chain antigen. I: H&E slide of adenocarcinoma of colorectal

Table 3 Comparison of MSI-H and MSI-L percentages in 39 MSI + cases

	MSI High *N* (%)	MSI Low *N* (%)	Chi-square Statistics	*P*-value
MSI+ (N Total = 39)	28 (0.72)	11 (0.28)	7.41	0.009

Table 4 The relationship between Adenocarcinoma, *E. Histolytica - lectin and MSI*

	No.	MSI+ No. (%)	MSI- No. (%)	Sig *P*-value
E. histolytica (*Eh-L*)+	19	11 (58%)	8 (42%)	< 0.05
E. histolytica (*Eh-L*)-	131	28 (21.37%)	103 (78.62%)	< 0.009
Adenocarcinoma	150	39 (26%)	111 (74%)	< 0.001

from colorectal lesions, discovering the presence of lectin antigen on the surface of tumor cells in 8 MSI-positive samples [29]. The absence of *E. histolytica* DNA in adenocarcinoma biopsy samples, as determined by PCR, indicated that patients with adenocarcinoma were not simultaneously infected with this protozoan [29]. However, the presence of this protozoan's antigen on colorectal tumor cell surfaces still requires further investigation for confirmation [30].

Additionally, alterations in the intestinal microbiome have been associated with colorectal cancer and may facilitate the invasion of intestinal parasitic diseases. The invasion process of *E. histolytica* trophozoites leads to significant modifications in the host's genetic programs, a

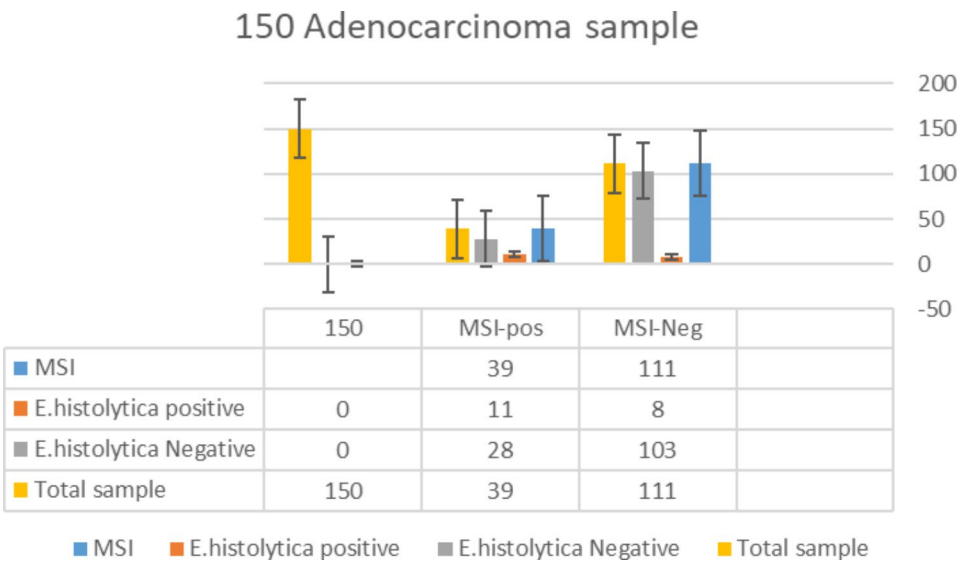

Fig. 3 Comparison of *Eh-lectin* antigen positive percentage in MSI positive and MSI negative group. The difference in *Eh-lectin* antigen positivity between the MSI + and MSI − groups was not statistically significant based on Fisher's exact test ($P > 0.05$). However, a significant difference was observed when comparing *Eh-lectin* antigen negative samples with positive and negative MSI status ($P < 0.009$). Additionally, a significant difference was noted in the concurrent examination of MSI and *Eh-lectin* antigen ($P < 0.05$). Out of the 39 MSI-positive samples, MSI + was observed in 3 samples, and there was a significant difference between these two groups ($P < 0.05$)

Table 5 miRNA-643 and XIAP expression results in the four investigated groups

	mir643			XIAP		
	Expression	***P* value**	**Regulated**	**Expression**	***P* value**	**Regulated**
Group 1	0.146	0.001	DOWN	3.769	0.088	UP
Group 2	3.9	0.014	UP	12.036	0.005	UP
Group 3	1.437	0.033	UP	0.425	0.019	DOWN
Group 4	0.555	0.595	UP	0.342	0.653	UP

Group 1: MSI positive, *Eh-lectin* antigen negative; Group 2: MSI negative, *Eh-lectin* antigen positive; Group 3: MSI and *Eh-lectin* antigen positive; Group 4: Relationship of miRNA643 with *Eh-lectin* antigen positive

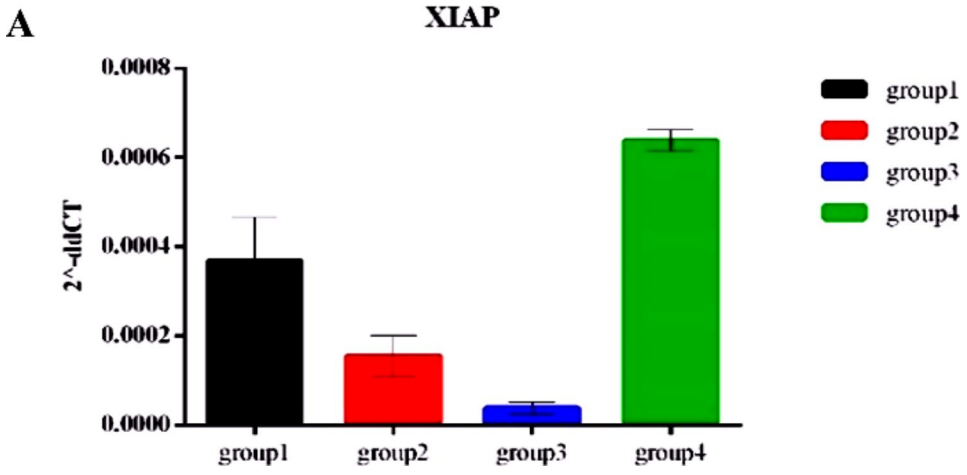

Fig. 4 (**A**) The 2^−ΔΔCT values related to the XIAP anti-apoptotic gene in four groups were as follows: Group 1 - MSI negative, *E. histolytica* negative; Group 2 - MSI positive, *E. histolytica* negative; Group 3 - MSI positive and *E. histolytica positive*; Group 4 - *E. histolytica* positive and related to the expression of miRNA643 and XIAP gene; (**B**) The results for the miRNA-643 gene in these groups showed that group 3 exhibited a threefold increase in miRNA-643 expression compared to the anti-apoptotic gene

phenomenon largely explained by protozoan trogocytosis [31].

Moreover, the role of microRNAs in the apoptosis of colorectal tumor cells has been investigated using various detection methods [32]. The biogenesis of miRNAs begins in the cell nucleus, where they are transcribed from specific genes by RNA polymerase II or III as primary transcripts (pri-mRNA). MiRNAs can interact with their target genes in two primary ways: if a miRNA binds to an mRNA with a highly complementary sequence, it triggers mRNA degradation; if the binding is less complete, it leads to translation repression of the mRNA [33].

In recent years, miRNAs have emerged as potential diagnostic and prognostic biomarkers, as well as modulators of chemo-resistance and novel therapeutic options [34]. The tumor-suppressive abilities of miR-643 have attracted attention, with research indicating that miR-643 can inhibit cell proliferation, invasion, and metastasis by down regulating rapidly accelerated fibrosarcoma (RAF1) expression in lung cancer cells while enhancing radiation sensitivity during radiotherapy [35]. Furthermore, miR-643 has been shown to reduce cell proliferation mediated by TPC-1 (Two pore segment channel 1) and increase the expression of the KI67 antigen along with apoptosis-related proteins like Bax and caspase-3 in papillary thyroid carcinoma cells [36].

Conversely, cells possess genes that inhibit cell death or apoptosis. One such gene, XIAP, encodes a protein that prevents cell death by inhibiting caspases, which are essential for cellular functions. This gene is significantly up regulated in cancer cells [37]. Mutations in the XIAP gene are associated with various diseases, including Crohn's disease [38].

A study found that miRNA-643, which was up regulated in SW-480 tumor cells exposed to *E. histolytica* trophozoites, may counteract the effects of the XIAP gene that inhibits apoptosis. However, the role and mechanisms of miR-643 in colorectal cancer are not well understood, although some studies suggest that it can inhibit cell viability and promote apoptosis in gastric cancer [39]. Analysis of colon adenocarcinoma biopsy specimens, particularly those that were positive for MSI and *E. histolytica* antigen, showed a threefold increase in miRNA-643 expression, consistent with observations from SW480 cell line cultures [40].

Given that PCR results ruled out active amoebic infections and that immunohistochemistry and Western blotting confirmed the presence of *Eh-lectin* light chain on tumor cell surfaces, two hypotheses arose: first, patients may have had a prior infection with this protozoan, leaving the lectin antigenic markers in their cells; second, tumor cells might mimic antigens similar to those of *E. histolytica*, which could be useful as a treatment marker for colorectal adenocarcinoma using recombinant protozoan lectin. The increase in miRNA-643 expression in adenocarcinoma cells suggests its potential role as an apoptosis-inducing factor in these patients, indicating the need for further research.

Conclusion

Current cancer treatment guidelines advocate for targeted strategies based on the results of clinical trials. The results of this study indicate that MSI-positive samples, which also tested positive for *Eh-lectin* light chain antigen and exhibited increased expression of miRNA-643, support the involvement of this antigen in the development of MSI. This suggests that *E.h*-Lectin may serve as an auxiliary biomarker for detecting mutations such as NRAS, BRAF, and KRAS in patients with colorectal adenocarcinoma. Furthermore, the study proposes the potential use of the protozoan's lectin antigenic index as a means to enhance drug delivery to tumor cells and promote apoptosis. This idea highlights the necessity for further research in this area to investigate its practical applications and therapeutic advantage for colorectal cancer.

The broader significance of our study lies in identifying *E. histolytica antigen* as a potential biomarker for colorectal cancer associated with specific mutations, opening new pathways for understanding cancer progression. It presents an innovative approach for improved diagnostics and treatments.

While the findings are promising, additional validation and clinical studies are crucial to establish the reliability of *Eh*-lectin as a biomarker. Ongoing research will reinforce these findings and facilitate the development of personalized cancer therapies and diagnostic tools.

Abbreviations

MSI	Microsatellite instability
E. histolytica	*Entamoeba histolytica*
Entamoeba histolytica Lectin	*Eh-lectin*
CRA	Colorectal adenocarcinoma
IHC	Immunohistochemistry
CRD	Carbohydrate recognition domain
miRNAs	MicroRNAs
H-MSI	High MSI
MSS	Stable MSI
L-MSI	Low MSI
XIAP	X-Linked Inhibitor of Apoptosis
DAB	Diaminobenzene
ddCt	Delta-Delta-Ct
CRC	Colorectal cancer
H&E	Hematoxylin and eosin
PMS2	Postmeiotic segregation increased 2
E&F Imaging	Edge-enhanced and bright-field imaging
MLH1	MutL homolog 1
MSH2	MutS Homolog 2
MMR	Mismatch repair
Gal/GalNAc	D-galactose and N-acetyl-D-galactosamine
RAF1	Rapidly accelerated fibrosarcoma

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Author contributions
L.H.: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. A.D.: Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization. M.P.: Writing – original draft, Validation, Software, Formal analysis. F.G.: Writing – original draft, Visualization, Validation, Methodology.

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Data availability
All data generated or analyzed during this study are included in this published article. The raw data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The Ethical Committee of Tarbiat Modares University (Tehran, Iran), approved this study with Code No.: IR.MODARES.REC.1402.004. Informed written consent was obtained from all patients for the inclusion of their colorectal biopsy samples. Our study complied with the Helsinki Declaration.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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