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Prevalence and clinicopathological characteristics of mismatch repair-deficient colorectal carcinoma in early onset cases as compared with late-onset cases: a retrospective cross-sectional study in Northeastern Iran

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ABSTRACT

Objectives Lynch syndrome (LS), a genetically inherited autosomal disorder, increases the incidence of colorectal carcinoma (CRC). We aimed to perform a universal strategy to assess the prevalence and clinicopathological characteristics of early onset CRCs at high risk of LS versus late-onset ones in the Iranian population.

Setting A local population-based study from Northeastern Iran.

Participants 321 consecutive CRCs and pathology specimen screened between 2013 and 2016.

Primary and secondary outcome measures Retrospectively, information regarding the clinical criteria was obtained by interviewing the patients with CRC or, their families. Pathologists tested tumours with immunohistochemistry (IHC) staining of four mismatch repair (MMR) proteins (*MLH1, MSH2, MSH6* and *PMS2*). Tumours with absent IHC staining of *MLH1* were tested for BRAF mutations to exclude sporadic CRCs. Prevalence of early onset CRCs at high risk of LS and familial CRC type X were assessed as primary and secondary outcome measures, respectively.

Results Of 321 CRCs (13/123 (10.57%), early onset vs 21/198 (10.6%) late-onset) were detected to be MMR-deficient (dMMR). Nine early onset cases and 14 late-onset ones with a loss of *MLH1* underwent testing for the BRAF mutation, none of the early onset and four (2.02%) late-onset were recognised as sporadic. The difference in the outcome of IHC-analysis between early and late-onset CRCs at high risk of LS was not statistically significant (p=0.34). Majority of the suspected LS tumours from early onset patients had arisen in distal part (8/11 (72.72%) vs 8/14 (57.14%)), all of which were occurred in the rectum or sigmoid.

Conclusion Clinically, these findings suggest that in case of limitation for BRAF testing, the practitioner in Iran may consider managing early onset dMMR cases like LS until access to BRAF testing becomes available to them, before germline testing to accurately diagnose LS.

Strengths and limitations of this study

- The first comprehensive study to evaluate the prevalence and clinicopathological characteristics of colorectal carcinomas (CRCs) at high risk of Lynch syndrome (LS) in the early onset Iranian CRCs using a universal strategy.
- Conducted using participants from one province in Northeastern Iran.
- Unable to contact all CRCs; therefore, all consecutive CRCs were included.
- Lack of germline mutation testing in clinical practice made the differentiation between true lynch and lynch-like early onset syndrome to be difficult.

INTRODUCTION

A genetically inherited autosomal disorder that increases the risk of many types of cancer is known as Lynch syndrome (LS). The disorder is diagnosed due to molecular testing in patients with mutations in one of the four mismatch repair (MMR) genes, including *MLH1, PMS2, MSH6* and *MSH2*.[1] The lifetime risk of colorectal carcinoma (CRC) in LS pathogenic variant carriers under endoscopic surveillance is usually up to 45%–50% at 75 years for *MLH1* and *MSH2*, and less for *MSH6* and *PMS2*.[2–4] Moreover, these patients are at the high risk of endometrial, ovarian, renal, gastric, pancreatic, skin and brain extracolonic cancers.[1]

According to some studies, LS might cause up to 2%–9% of all CRCs,[5–13] and accounts for 9.2%–21.3% of patients with early onset (≤50 years) CRC.[14–21] Approximately, 2%–8% of all CRCs are early onset[22 23] and mean age of CRC development in LS is about 45 years.[5 24]

Recent studies have revealed an increased incidence of CRC in early onset patients.[23 25 26] The studies performed in Iran reported up to 25%–37.8% of early onset CRC.[13 27] The increased incidence of early onset CRC together with its aggressive nature,[28] although LS CRC is considered to be less aggressive with better overall survival than sporadic CRC,[2] highlights the importance of early evaluation in young individuals with symptoms. Early onset CRC is one of the 'hallmarks' for hereditary CRC syndromes which represent 15%–20% of cases in this group.[23 29] Identification of hereditary carcinoma syndromes has significant implications for patients and families, as it facilitates risk assessment, directs proper colon cancer screening, the most common type inherited in this group.

LS is often underdiagnosed; selective strategies such as Amsterdam II criteria and the revised Bethesda guidelines detect cases at high risk of LS but with low sensitivity or specificity.[13 30] Microsatellite instability (MSI) for testing tumour and immunohistochemistry (IHC) staining to identify the absence of MMR protein expression are acceptable methods to screen LS[31–33] and have a similar sensitivity that is >80%.[1 34 35] Notably, the IHC-based method is more cost-effective.[36–38] Suzuki *et al* performed IHC screening of the early onset CRCs in Japan and reported 8.4% and 5.9% prevalence of MMR-deficient (dMMR) and LS, respectively.[39] Using MSI testing, a study performed in Saudi Arabia revealed 11.6% prevalence of early onset MSI while only one showed BRAF mutation.[40] In addition to LS, familial colorectal cancer type X (FCCTX) refers to subjects with CRC who met the Amsterdam II criteria but show no MMR deficiency.[1] A recent study conducted in central Iran reported the high prevalence of FCCTX (77.4%) in early onset CRCs using a selective strategy.[41]

Some studies compared early onset CRCs with late-onset ones (>50 years) and revealed that both prevalence and clinicopathological characteristics of LS CRC among these patients are different.[42 43] Perea *et al* reported 14.8% MSI for early onset and 9.3% for late-onset CRCs, in which 83% of early onset MSI cases had germline MMR mutations. Whereas late-onset CRCs showed frequent BRAF mutations, early onset MSI cases showed different tumour locations and more family history of cancer (FHC) than late-onset ones in their study.[42]

Although IHC screening of dMMR among early onset CRCs has become routine in many countries, no comprehensive study has been attempted previously in the Iranian early onset CRCs, to identify cases with dMMR and/or LS. Accordingly, the present study conducted in Iran aimed to assess the prevalence and clinicopathological characteristics of early onset CRCs at high risk of LS versus late-onset ones with the universal strategy using IHC for MMR protein findings of dMMR CRCs.

MATERIALS AND METHODS

Setting and participants

This retrospective cross-sectional study of a local population-based cohort of consecutive CRCs was performed in Mashhad, Northeastern Iran between January 2013 and February 2016. Initially, 841 patients with CRC registered in the databases of three referral centres were included. Of these 841 cases, 170 were unavailable due to changes in address and/or phone number, and 126 refused to be interviewed. Of the remaining 545 CRCs, 222 cases were not eligible for IHC screening of the MMR proteins due to lack of access to the pathology block or clinical features. Finally, 323 (~38%) cases (123 early onset, 198 late-onset and 2 unreported age cases) underwent IHC screening of the MMR proteins. The flow chart of including and excluding cases in the study and detecting CRCs at high risk of LS is outlined in figure 1.

Family history of cancer

Information regarding the history of cancer in relatives of at least the second degree and beyond was obtained by interviewing the patients or, in the circumstance of their death, their siblings and/or parents. The cancer characteristics of each patient were documented via information obtained through archives, pathology reports and interviews. Such information included sex, age at diagnosis, tumour site, history of CRC or non-CRC in first-degree and second-degree relatives and histological features for the revised Bethesda criteria reported by two expert pathologists in gastroenterology. However, some variables remained with missing value owing to lacking CRC registry in the study setting and/or lacking access to some colonoscopy reports (tables 1 and 2). Informed consent was obtained from all participants before interviewing and/or testing.

Patient and public involvement

No patients and the public were involved in the study design, the outcome measures, data analysis or interpretation of the results. There are no plans to disseminate the results of the research to study participants or the relevant patient community. The study participants are thanked in the acknowledgements.

Clinical criteria and IHC investigation of CRCs at high risk of LS

Patients that fulfilled the Amsterdam II and revised Bethesda criteria were also documented. The revised Bethesda guidelines, a third set of clinicopathological criteria, identify patients fit for further investigation of LS with microsatellite instability and/or IHC.[36]

An IHC screen was considered abnormal if IHC staining was absent for any of the four MMR proteins (*MLH1, MSH2, MSH6* and *PMS2*). Tumours without IHC staining of *MLH1* were tested for BRAF V600E mutations to exclude sporadic CRCs with acquired promoter hypermethylation. Patients without MMR proteins and normal BRAF status (if *MLH1* was absent) were considered 'at high risk of LS'. The germline mutations of MMR genes were not assessed in these cases; therefore, true lynch from lynch-like syndrome was not distinguished in the current study.[44 45]

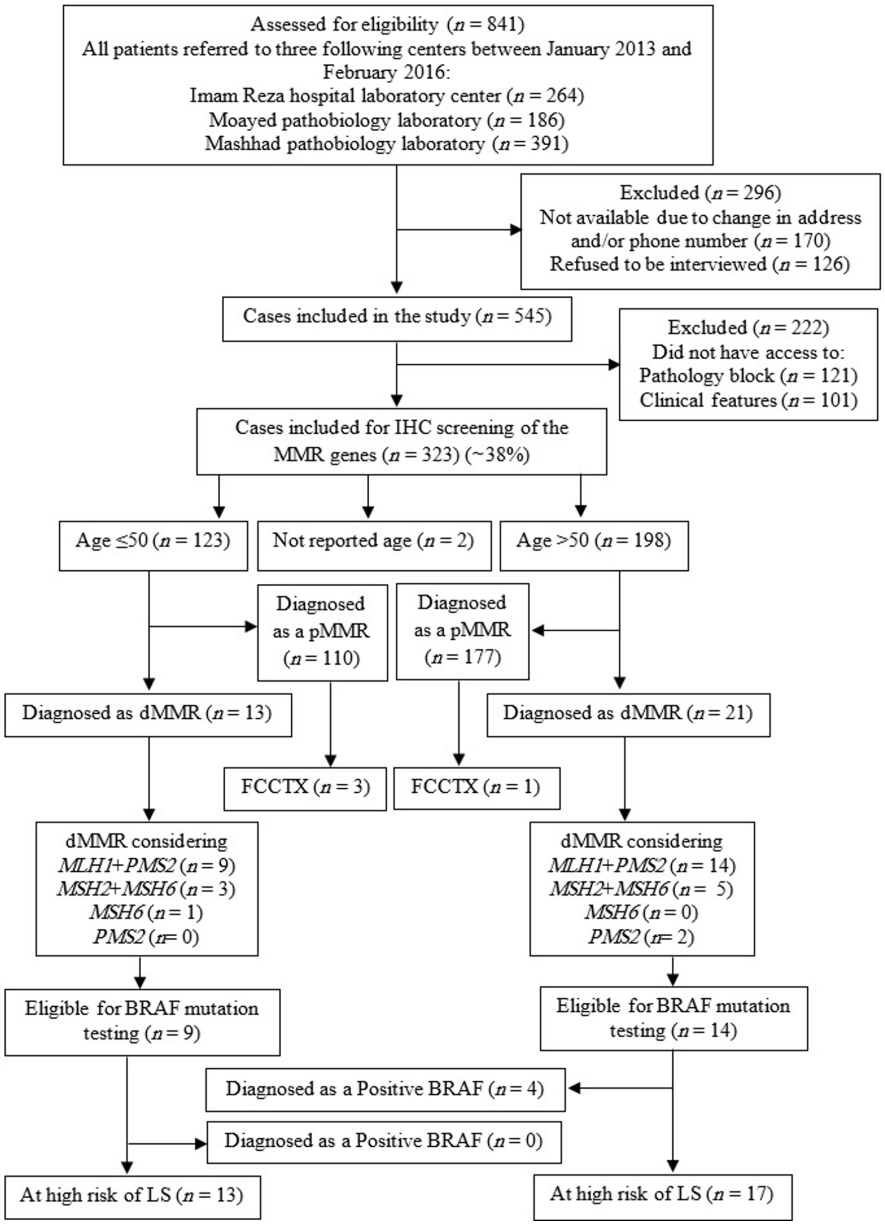

Figure 1 Flow chart of detecting early onset colorectal carcinomas (CRCs) at high risk of Lynch syndrome (LS) as compared with late-onset ones. FCCTX, familial colorectal cancer type X; IHC, immunohistochemistry; dMMR, MMR-deficient; pMMR, MMR-proficient; MMR, mismatch repair.

STATISTICAL ANALYSIS

Chi-square test, Fisher's exact test and Student's t-test were used for statistical evaluation. Reported p values of <0.05 were considered statistically significant. Continuous variables were expressed as mean±SD SPSS software V.16 (SPSS, Chicago, Illinois, USA) was used to analyse the data.

RESULTS

A total of 123 early onset CRCs with a mean age of 40.33±6.848 years and 198 late-onset ones with a mean age of 65.11±9.326 were screened for LS. Thirteen (10.57%) early onset and 21 (10.6%) late-onset cases were detected to be dMMR. All *MLH1* and *PMS2* proteins in early onset and all *MSH2* and *MSH6* in late-onset were abnormal simultaneously. In early onset CRCs, all *MSH2*-abnormal cases were abnormal for *MSH6* and in late-onset ones all *MLH1*-abnormal cases were abnormal for *PMS2* (figure 1). Nine early onset cases and 14 late-onset ones with a loss of *MLH1* underwent testing for the BRAF mutation, none of the early onset and four (2.02%) late-onset were recognised as positive BRAF mutation. Finally, 13 (10.57%) early onset with the mean age of 40.69±6.62 years vs 17 (8.59%) late-onset with mean age of 62.71±9.732 were detected as 'at high risk of LS' (figure 1) and the difference in outcome of IHC analysis between them was not statistically significant (p=0.34).

Table 1 Association of LS status in early onset cases (n=123) screened negative for LS vs those at high risk of LS with gender (n=123) and location of CRC (n=84), Amsterdam II (n=78), revised Bethesda (n=123), history of CRC in FDR (n=81), history of CRC in SDR (n=83) and FHC (n=77)

	Negative LS (n=110)	At high risk of LS (n=13)	
	No. of cases (%)	No. of cases (%)	P values
Gender (n=123)			
Female (n=70)	65 (59.1)	5 (38.5)	0.13
Male (n=53)	45 (40.9)	8 (61.5)	
Location of CRC (n=84)*			
Proximal (n=11)	8 (12.70)	3 (18.18)	0.15
Distal (n=73)	65 (87.30)	8 (81.81)	
Amsterdam II (n=78)*			
Absent (n=71)	62 (95.4)	9 (69.2)	0.01
Present (n=7)	3 (4.6)	4 (30.8)	
Revised Bethesda (n=123)			
Absent (n=0)	0 (0)	0 (0)	0.35
Present (n=123)	110 (100)	13 (100)	
History of CRC in FDR (n=81)*			
No (n=75)	68 (97.1)	7 (63.6)	0.003
Yes (n=6)	2 (2.9)	4 (36.4)	
History of CRC in SDR (n=83)*			
No (n=72)	64 (91.4)	8 (61.5)	0.012
Yes (n=11)	6 (8.6)	5 (38.5)	
FHC (n=77)*			
Absent (n=55)	49 (75.4)	6 (50)	0.09
Present (n=22)	16 (24.6)	6 (50)	

*Indicating variables with missing value.
CRC, colorectal cancer; FDR, first-degree relatives; FHC, family history of cancer; LS, Lynch syndrome; SDR, second-degree relatives.

All variables related to 321 cases underwent IHC screening of dMMR had missing value except age and gender. Missing value status of these variables is outlined in tables 1 and 2. Of the 78 early onset and the 112 late-onset CRCs that had sufficient information for evaluation of the Amsterdam II criteria, 7 early onset cases and 2 late onset ones met the Amsterdam II criteria (8.97% vs 1.78%), 3 early onset and 1 late-onset were FCCTX (figure 1). The predictivity of the Amsterdam II criteria for early onset CRCs at high risk of LS and each dMMR complex (*MLH1* vs *MSH2*) is outlined in table 3. The sensitivity of the Amsterdam II criteria was 30.76%, which increased to 50% for the *MSH2* complex.

Table 1 compares demographic and clinicopathological variables between early onset cases screened as negative LS and those that were at high risk of LS. The same analysis was performed for late-onset ones, but there was no significant positive association between LS status and any demographic or clinicopathological variables. Among the 13 early onset and 17 late-onset CRCs that were at high risk of LS, 25 had information on the location of the CRCs (11 early onset and 14 late-onset), and 8 of the early onset CRCs were distal (table 2).

Age distribution of early onset CRCs underwent IHC screening was between 21 and 50 years, but it was between 30 and 50 years in cases at high risk of LS. Most prevalence of CRCs at high risk of LS (10.71%) occurred in the age interval of under 40 years (table 4).

Mean age of 30 cases at high risk of LS was not less than that of 289 cases screened as negative for LS (53.17±13.91 vs 55.71±14.68 years; p=0.35), and it was the same for early onset and late-onset when performed separately (table 5).

DISCUSSION

To the best of the authors' knowledge, this is the first research in Iran on the assessment of prevalence and characteristics of LS in the early onset CRCs when compared with late-onset ones. The results of the study revealed that the prevalence of both dMMR and CRCs at high risk of LS was 10.57% (13/123) among early onset in Northeastern Iran, while it was 10.6% and 8.59% among late-onset ones, respectively. Previous studies performed in Japan[39] and the USA[46] reported dMMRs of 8.4% and 10.7% for early onset CRCs, respectively. Although

Table 2 Profile of CRCs at high risk of LS considering age vs other factors (n=30)

	≤50 years (n=13)	>50 years (n=17)
Gender (n=30)		
Female (n=10)	5	5
Male (n=20)	8	12
Location of CRC (n=25)*		
Proximal (n=8)	2	6
Distal (n=17)	9	8
Amsterdam II (n=24)*		
Absent (n=19)	9	10
Present (n=5)	4	1
Revised Bethesda (n=24)*		
Absent (n=7)	0	7
Present (n=17)	13	4
History of CRC in FDR (n=22)*		
No (n=17)	7	10
Yes (n=5)	4	1
History of CRC in FDR (n=24)*		
No (n=18)	8	10
Yes (n=6)	5	1
Family history of cancer (n=23)*		
Absent (n=13)	6	7
Present (n=10)	6	4
Location (n=25)*		
Caecum (n=3)	0	3
Sigmoid (n=5)	4	1
Rectum (n=9)	4	5
Rectosigmoid (n=2)	0	2
Transverse (n=5)	3	2
Ascending (n=1)	0	1

*Indicating variables with the missing value.
CRC, colorectal cancer; FDR, first-degree relatives; FHC, family history of cancer; LS, Lynch syndrome; SDR, second-degree relatives.

Baiocchi *et al* reported an extremely high prevalence of 50%,[43] the prevalence ranged from 9.2% to 21.3%,[14–21] which demonstrate that the present study is in line with other evidence.

An interesting finding of the research is that there was no BRAF mutation in dMMR early onset CRCs, while late-onset ones showed 19% (4/21) BRAF mutation. These findings suggest that *MLH1* methylation, suggested by BRAF mutation, responsible for positive dMMR is more common in late-onset versus early onset CRCs and extends the involvement of epigenetic-driven mechanisms for late-onset dMMR+ CRCs more commonly compared with early onset cases. Clinically, these findings suggest that in case of limitation for BRAF testing, the practitioner in Iran may consider managing early onset dMMR+ cases like LS until access to BRAF testing becomes available to them, before germline testing to accurately diagnose LS. These findings are in line with those reported by Perea *et al*, which found no BRAF mutation in early onset MSI CRCs while BRAF mutation in late-onset CRCs were frequent.[42]

We found that two-antibody panels were efficient as four-antibody panels to diagnose dMMRs. Two-antibody panel testing, composing of *PMS2* and *MSH6*, was previously reported by Shia *et al*[47] to be as efficient as the current four-antibody panel for detecting dMMR. In the current study, it was possible to detect all dMMR by considering two-antibody panel (*PMS2* and *MSH6*) instead of the four-antibody panel (*MLH1, MSH2, MSH6* and *PMS2*) in the early onset cases. We suggest that *PMS2* and *MSH6* staining in the early onset CRCs can be an acceptable approach if the four-panel testing is not available.

Amsterdam II criteria, with the relatively low sensitivity of 30.76% and predictivity of 57.14%, was able to diagnose seven early onset CRCs, among which four were dMMR+, and three were negative, and likely belong to FCCTX. In contrast with recent studies performed in central Iran,[41 48] the current research did not show that the prevalence of FCCTX among Iranian CRCs is higher than that of Western countries.

Also, a significant correlation between the history of CRC in FDR\SDR and LS status in early onset cases was

Table 3 Predictivity of Amsterdam II criteria for early onset colorectal cancers (CRCs) at high risk of Lynch syndrome in the study considering four-panel/two-panel mismatch repair (MMR) and BRAF mutation testing as the gold standard in 78 early onset CRCs

		Amsterdam II criteria		
Gold standard		**Positive**	**Negative**	**Sensitivity–specificity (positive predictive value)**
Four-panel MMR and BRAF mutation testing	Positive	4	9	30.76%–95.39% (57.14%)
	Negative	3	62	
MLH1 complex and BRAF mutation testing	Positive	2	7	22.22%–92.75% (28.57%)
	Negative	5	64	
MSH2 complex and BRAF mutation testing	Positive	2	2	50%–93.24% (28.57%)
	Negative	5	69	

Table 4 Age distribution of colorectal cancers (CRCs) underwent immunohistochemistry screening of mismatch repair mutation testing (n=321) and cases at high risk of Lynch syndrome (LS) (n=30) in the study

	Participants		
Age interval	No. of CRCs (%)	No. of cases at high risk of LS (%)	Percentage (%) of cases at high risk of LS at each age interval
Age≤40	56 (17.44)	6 (20)	10.71
40<age ≤ 50	67 (20.88)	7 (23.33)	10.44
50<age ≤ 60	82 (25.54)	8 (26.67)	9.76
60<age ≤ 70	53 (16.51)	4 (13.33)	7.54
Age>70	63 (19.63)	5 (16.67)	7.93

observed (table 1), while there was no significant positive association between LS status and any demographic or clinicopathological variable in late-onset ones. It seems that in areas where IHC screening of all CRCs is not possible, at least early onset patients with a history of CRC in FDR\SDR should be referred to tertiary centres for IHC of their MMR. But more than half of our early onset cases did not have any family history of CRC in FDR\SDR, so we cannot rely only on clinical criteria to find LSs and IHC for MMR recommended for all early onset CRCs. These findings are in line with those reported by other studies, which found Germline testing with a multigene panel should be addressed for all early onset CRCs.[46 49] Moreover, Hampel *et al* suggested tumour sequencing approach as a replacement for current multitest LS screening,[50] but these new strategies are not widely available in Iran and are too expensive.

We observed that the majority of early onset CRCs at high risk of LS have arisen in the distal part (8/11 (72.72%) vs 8/14 (57.14%) in late-onset ones) (table 2). Although studies reported that majority of the positive LS tumours from early onset CRCs occurred in the proximal part in Western countries,[42 51] other studies performed in Middle-East countries confirmed the current research findings.[40 48 52] These findings suggest that there may be differences in the pathogenesis and aetiology of dMMRs between Middle-East CRCs and Western ones. Further studies are needed to investigate this subject. Also, the study reported that all distal tumours in dMMR had appeared in the rectum or sigmoid, so wide-scope studies in this subgroup of early onset cases seem necessary.

Table 5 Mean difference of age between cases screened as negative for Lynch syndrome (LS) vs those at high risk of LS

	LS status		
Age group	At high risk of LS Mean (SD)	Negative LS Mean (SD)	P values
Age≤50	40.69±6.62	40.28±6.87	0.83
Age>50	62.71±9.73	65.08±9.21	0.31
Total	53.17±13.91	55.71±14.68	0.35

Previous studies indicated that an indicator of a hereditary component is early onset CRC.[53 54] However, LSs represent 9.2% –21.3% of cases in this subgroup[14–21] and this research like other current studies[55–57] revealed it is a heterogeneous disease, which includes cases with a high familial component other than LS as well as a substantial proportion of sporadic cases with distal location.

The highest prevalence of CRCs at high risk of LS (10.71%) occurred among early onset CRCs under 40 years. However, to recommend this age interval to steer the IHC screening, the number of cases in this group was too small.[33]

To the authors' knowledge, this study was the first to comprehensively evaluate the prevalence of early onset CRCs at high risk of LS in Iran using universal strategy. However, the research had some limitations; first, it was conducted using participants from one province in Northeastern Iran, and the results need to be confirmed in more extensive studies. The authors were not able to contact all CRCs; therefore, all consecutive CRCs were included. The research suggests a comprehensive registry of all CRCs, which will enable researchers to perform more extensive multicentre studies to investigate the prevalence of LS in Iran. Lack of germline mutation testing in clinical practice made the differentiation between true lynch syndrome and lynch-like syndrome early onset CRCs to be difficult, but it is ongoing and will be reported in future studies. These limitations include a lack of generalisability of results and the strategy used to identify CRCs at high risk of LS, but we still think it will be useful for low-income and middle-income countries especially in Middle-East region where there is a restriction of the resources available like Iran.

CONCLUSION

This informative study estimated the prevalence of early onset CRCs at high risk of LS to be 10.57% and 8.58% for late-onset ones in Northeastern Iran. There was no BRAF mutation in early onset dMMR CRCs, while BRAF mutation in late-onset ones was frequent. Clinically, these findings suggest that in case of limitation for BRAF testing, the practitioner in Iran may consider managing early onset dMMR cases like LS until access to BRAF testing becomes available to them, before germline testing to accurately

diagnose LS. The family history of CRC among early onset LS CRCs was more common versus late-onset ones; however, clinical criteria and family history of CRC have low sensitivity to detect LS and IHC screening for MMR with at least a two-antibody panel (*PMS2, MSH6*) should be performed for both newly diagnosed early onset and late-onset cases. Majority of the positive LS tumours from early onset patients occurred in the rectum or sigmoid in the study area that opens up room for future studies. The next step of the ongoing research is to follow-up surviving dMMR early onset patients and perform germline mutation analysis of MMR genes in these patients.

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Contributors Designing the study: LG, AE, BH. Performing the experiments: KG, AK. Conducting the sample collection: MRKh, LG, HMM. Analysing and interpreting the data: BH, LG, BK. Drafting the manuscript and final approval of the version to be published: BH, LG. All authors read and approved the final manuscript.

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Patient consent Not required.

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Data sharing statement All relevant data are within the paper. The data underlying this study are available to all interested researchers. To gain access to these data, please submit a proposal the Mashhad University of Medical Sciences (MUMS) at http://research.mums.ac.ir. Ethical approval by MUMS Research Ethics Committee is needed before any data release.

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