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

Ladan Goshayeshi,1,2 Kamran Ghaffarzadegan,3 Alireza Khooei,4 Abbas Esmaeilzadeh,1,5 Mahla Rahmani Khorram,6 Hooman Mosannen Mozaffari,1,6 Behzad Kiani,7 Benyamin Hoseini8

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 (72.7%) 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 can manage considering 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. The studies performed in Iran reported up to 25%–37.8% of early onset CRC. The increased incidence of early onset CRC together with its aggressive nature, although LS CRC is considered to be less aggressive with better overall survival than sporadic CRC, 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. Perea reported 14.8% prevalence of early onset MSI cases had germline MMR mutation. Additionally, some studies compared early onset CRCs with late-onset ones (≥50 years) and revealed that both prevalence of MMR deficiency (dMMR) and 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. Microsatellite instability (MSI) for testing tumour and immunohistochemistry (IHC) staining to identify the absence of MMR protein expression are acceptable methods to screen LS and have a similar sensitivity that is >80%. Notably, the IHC-based method is more cost-effective. 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. Using MSI testing, a study performed in Saudi Arabia revealed 11.6% prevalence of early onset MSI while only one showed BRAF mutation. 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. A recent study conducted in central Iran reported the high prevalence of FCCTX (77.4%) in early onset CRCs using a selective strategy. 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. 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. 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.

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.
Goshayeshi L, et al. BMJ Open 2018;8:e023102. doi:10.1136/bmjopen-2018-023102

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).

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.
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±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 (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±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 (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±14.68 years; p=0.35), and it was the same for early onset and late-onset when performed separately (table 5).

**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) | 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.
Baiocchi et al reported an extremely high prevalence of 50%, the prevalence ranged from 9.2% to 21.3%, 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.

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 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, 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 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  | 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          |
| 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.

| 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 |
|---------|-------------------------------------------------------------------------------------|
| Gold standard | Amsterdam II criteria | 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 | |
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. Moreover, Hampel et al suggested tumour sequencing approach as a replacement for current multitest LS screening, 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, other studies performed in Middle-East countries confirmed the current research findings. 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.

Previous studies indicated that an indicator of a hereditary component is early onset CRC. However, LSs represent 9.2%–21.3% of cases in this subgroup and this research like other current studies 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.

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 North-eastern 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

| 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 |
| --- | --- | --- |
| 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 |

| Table 5 | Mean difference of age between cases screened as negative for Lynch syndrome (LS) vs those at high risk of LS |
| --- | --- | --- |
| Age group | LS status | Negative LS Mean (SD) | At high risk of 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 |
diagnose LS. The family history of CRC among early onset LS CRCs was much 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.

**Author affiliations**

1. Gastroenterology and Hepatology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran  
2. Oncology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran  
3. Pathology Department, Education and Research Department, Razavi Hospital, Mashhad, Iran  
4. Department of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran  
5. Department of Health Information Technology, Neyshabur University of Medical Sciences, Neyshabur, Iran

**Acknowledgements** The authors would like to thank all study participants of Imam Reza hospital laboratory center, Moayed pathology laboratory, Mashhad pathology laboratory and staff for their contributions to this project, in particular, F. Salamati, F. Ahmad, A. Mokhtarifar and S. Jangjoo. The authors would also like to thank Dr Reza Malekzadeh and Dr Faraz Bisheshari for their contributions.

**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.

**Funding** The study was funded by the research committee at Mashhad University of Medical Sciences and the Tehran Digestive Disease Research Institute (DDRI).

**Competing interests** None declared.

**Patient consent** Not required.

**Ethics approval** Ethics approval was obtained from the ethics committee of Mashhad University of Medical Sciences.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**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.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

**REFERENCES**

1. Dirijena WN, Dubbink HJ, Wagner A. Guidelines on genetic evaluation and management of Lynch syndrome. *Am J Gastroenterol* 2015;110:192–3.

2. Moller P, Seppälä T, Bernstein I, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. *Gut* 2017;66:464–72.

3. Moller P, Seppälä TT, Bernstein I, et al. Incidence of and survival after subsequent cancers in carriers of pathogenic MMR variants with previous cancer: a report from the prospective Lynch syndrome database. *Gut* 2017;66:1657–64.

4. Moller P, Seppälä TT, Bernstein I, et al. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the prospective Lynch syndrome database. *Gut* 2018;67:1306–16.

5. Aaltonen LA, Salovaara R, Kristo P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998;338:1481–7.

6. Barnetson RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. *N Engl J Med* 2006;354:2751–63.

7. Berginc G, Bracko M, Ravnik-Glavac M, et al. Screening for germline mutations of MLH1, MSH2, MSH6 and PMS2 genes in Slovenian colorectal cancer patients: implications for a population specific detection strategy of Lynch syndrome. *Fam Cancer* 2009;8:421–9.

8. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* 2005;352:1851–60.

9. Jin HY, Liu X, Li VK, et al. Detection of mismatch repair gene germline mutation carrier among Chinese population with colorectal cancer. *BMC Cancer* 2008;8:44.

10. Moreira L, Balaguer F, Lindor N, et al. Identification of Lynch syndrome among young patients with colorectal cancer. *JAMA* 2012;308:1555–65.

11. Salovaara R, Loukola A, Kristo P, et al. Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J Clin Oncol* 2000;18:2193–200.

12. Ward RL, Hicks S, Hawkins NJ. Population-based molecular screening for Lynch syndrome: implications for personalized medicine. *J Clin Oncol* 2013;31:2551–62.

13. Goshayeshi L, Khooee A, Ghaffarzadehgan K, et al. Screening for Lynch syndrome in cases with colorectal carcinoma from Mashhad. *Arch Iran Med* 2017;20:332–7.

14. Goel A, Nagasaka T, Spiegel J, et al. Low frequency of Lynch syndrome among young patients with non-familial colorectal cancer. *Clin Gastroenterol Hepatol* 2010;8:966–71.

15. Giráldez MD, Balaguer F, Bujanda L, et al. MSH6 and MUTYH deficiency is a frequent event in early-onset colorectal cancer. *Clin Cancer Res* 2010;16:5402–13.

16. Lee-Kong SA, Markowitz AJ, Giogiosi E, et al. Prospective immunohistochemical analysis of primary colorectal cancers for loss of mismatch repair protein expression. *Clin Colorectal Cancer* 2010;9:255–9.

17. Limburg PJ, Harmsen WS, Chen HH, et al. Prevalence of alterations in DNA mismatch repair genes in patients with young-onset colorectal cancer. *Clin Gastroenterol Hepatol* 2013;11:947–52.

18. Wright DM, Arnold JL, Parry B, et al. Immunohistochemistry to detect hereditary nonpolyposis colorectal cancer in young patients: the 7-year Auckland experience. *Dis Colon Rectum* 2011;54:552–8.

19. Chew MH, Koh PK, Tan M, et al. Mismatch repair deficiency screening via immunohistochemical staining in young Asians with colorectal cancers. *World J Surg* 2013;37:2468–75.

20. Steinhagen E, Shia J, Markowitz AJ, et al. Systematic immunohistochemical screening for Lynch syndrome in early age-of-onset colorectal cancer patients undergoing surgical resection. *J Am Coll Surg* 2012;214:214–21.

21. Stigliano V, Sanchez-Mete L, Martayan A, et al. Early-onset colorectal cancer patients without family history are ‘at very low risk’ for Lynch syndrome. *J Exp Clin Cancer Res* 2014;33:1.

22. Boyle P, Ferlay J. Cancer incidence and mortality in Europe, 2004. *Ann Oncol* 2005;16:1481–9.

23. Siegel RL, Jemal A, Ward EM. Incidence of colorectal cancer among young men and women in the United States. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research. *Cancer Epidemiol Biomarkers Prev* 2009;18:1695–9.

24. Samowitz WS, Curtin K, Lin HH, et al. The colon cancer burden of genetically defined hereditary nonpolyposis colon cancer. *Gastroenterology* 2001;121:830–8.

25. Meyer JE, Narang T, Schnoll-Sussman FH, et al. Increasing incidence of rectal cancer in patients aged younger than 40 years: an analysis of the surveillance, epidemiology, and end results database. *Cancer* 2010;116:4354–9.
26. Siegel RL, Miller KD, Fedewa SA, et al. Colorectal cancer statistics, 2017. CA Cancer J Clin 2017;67:177–93.

27. Mahdaviani M, Bishehsari F, Ansari R, et al. Family history of colorectal cancer in Iran. BMC Cancer 2005;5:112.

28. Kocián P, Whitley A, Blaha M, et al. Colorectal cancer in patients under the age of 40 years: experience from a tertiary care centre in the Czech Republic. Acta Chir Belg 2017;117:356–62.

29. Silla IO, Rueda D, Rodríguez Y, et al. Early-onset colorectal cancer: a separate subset of colorectal cancer. World J Gastroenterol 2017;23:10131–9.

30. Shia J. Evolving approach and clinical significance of detecting DNA mismatch repair deficiency in colorectal carcinoma. Semin Diagn Pathol 2015;32:352–61.

31. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol 2008;26:5783–8.

32. Terdiman JP, Gurn JR, Conrad PG, et al. Efficient detection of hereditary nonpolyposis colorectal cancer gene carriers by screening for tumor microsatellite instability before germline genetic testing. Gastroenterology 2001;120:21–30.

33. Snowsill T, Coelho H, Huxley N, et al. Molecular testing for Lynch syndrome in people with colorectal cancer: systematic reviews and economic evaluation. Health Technol Assess 2017;21:1–238.

34. Amira AT, Mouna T, Ahliem B, et al. Immunohistochemical expression pattern of MMR protein can specifically identify patients with colorectal cancer microsatellite instability. Tumour Biol 2014;35:6289–91.

35. Palomaki GE, McClain MR, Mellilo S, et al. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med 2009;11:42–65.

36. Kidambi TD, Blanco A, Myers M, et al. Selective versus universal screening for Lynch syndrome: a six-year clinical experience. Dig Dis Sci 2015;60:2483–9.

37. Mvundura M, Grosse SD, Hampel H, et al. The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer. Genet Med 2010;12:93–104.

38. Chaves P, Cruz C, Lage P, et al. Immunohistochemical detection of mismatch repair gene proteins as a useful tool for the identification of colorectal carcinoma with the mutator phenotype. J Pathol 2000;191:355–60.

39. Suzuki O, Eguchi H, Chika N, et al. Prevalence and clinicopathologic/molecular characteristics of mismatch repair-deficient colorectal cancer in the under-50-year-old Japanese population. Surg Today 2017;47:1135–46.

40. Alqahtani M, Griev F, Carrello A, et al. Screening for Lynch syndrome in young colorectal cancer patients from Saudi Arabia using microsatellite instability as the initial test. Asian Pac J Cancer Prev 2016;17:1917–23.

41. Zeinalian M, Hadian M, Hashemzadeh-Chaleshtori M, et al. Familial colorectal cancer type X in central Iran: a new clinicopathologic description. Int J Hematol Oncol Stem Cell Res 2017;11:240–5.

42. Perea J, Rueda D, Canal A, et al. Age at onset should be a major criterion for subclassification of colorectal cancer. J Mol Diagn 2014;16:116–26.

43. Baldocchi GL, Portalini N, Verbi W, et al. Lynch syndrome from a surgeon’s perspective: retrospective study of clinical impact of mismatch repair protein expression analysis in colorectal cancer patients less than 50 years old. BMC Surg 2014;14:9.

44. Carethers JM. Differentiating Lynch–like from Lynch syndrome. Gastroenterology 2014;146:602–4.

45. Carethers JM, Stoffel EM. Lynch syndrome and Lynch syndrome mimics: the growing complex landscape of hereditary colon cancer. World J Gastroenterol 2015;21:9253–61.

46. Pearlman R, Frankel WL, Swanson B, et al. Prevalence and spectrum of germline cancer susceptibility gene mutations among patients with early-onset colorectal cancer. JAMA Oncol 2017;3:464–71.

47. Shia J, Tang LH, Vakiani E, et al. Immunohistochemistry as first-line screening for detecting colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: a 2-antibody panel may be as predictive as a 4-antibody panel. Am J Surg Pathol 2009;33:1639–45.

48. Zeinalian M, Hashemzadeh-Chaleshtori M, Akbarpour MJ, et al. Epidemiologic feature of early-onset colorectal cancer at-risk for Lynch syndrome in Central Iran. Asian Pac J Cancer Prev 2015;16:4647–52.

49. Stoffel EM, Koepe E, Everett J, et al. Germline genetic features of young individuals with colorectal cancer. Gastroenterology 2018;154.

50. Hampel H, Pearlman R, Beightol M, et al. Assessment of tumor sequencing as a replacement for Lynch syndrome screening and current molecular tests for patients with colorectal cancer. JAMA Oncol 2018;4:806.

51. Schofield L, Watson N, Griev F, et al. Population-based detection of Lynch syndrome in young colorectal cancer patients using microsatellite instability as the initial test. Int J Cancer 2008;124:1097–102.

52. Siraj AK, Prabhakaran S, Bavi P, et al. Prevalence of Lynch syndrome in a Middle Eastern population with colorectal cancer. Cancer 2015;121:1762–71.

53. Liang JT, Huang KC, Cheng AL, et al. Clinicopathological and molecular biological features of colorectal cancer in patients less than 40 years of age. Br J Surg 2003;90:205–14.

54. Losi L, Di Gregorio C, Pedroni M, et al. Molecular genetic alterations and clinical features in early-onset colorectal carcinomas and their role for the recognition of hereditary cancer syndromes. Am J Gastroenterol 2005;100:2280–7.

55. Campos FG. Colorectal cancer in young adults: A difficult challenge. World J Gastroenterol 2017;23:5041–4.

56. Ballester V, Rastkat S, Boardman L. Clinical and molecular features of young-onset colorectal cancer. World J Gastroenterol 2016;22:1736–44.

57. Kohtani N, Teer JK, Abbott AM, et al. Increased incidence of FBXW7 and POLE proofreading domain mutations in young adult colorectal cancers. 2016;122:2828–35.