Original Article

Screening for Lynch Syndrome in Cases with Colorectal Carcinoma from Mashhad

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Abstract

Introduction: Lynch Syndrome (LS) is a genetically inherited autosomal disorder that increases the risk of many types of cancer, especially colorectal cancer (CRC). Identifying these subjects improves morbidity and mortality. We aimed to assess the prevalence of LS with both clinical criteria and universal strategy in Mashhad, Iran.

Methods: In this retrospective study, we screened 322 patients with CRC between 2013 and 2016 in Mashhad, Iran. CRCs were screened based on Amsterdam II criteria, revised Bethesda guideline, and universal strategy. Information regarding the clinical criteria was obtained by interviewing the patients or, their families. Tumors were screened by pathologists with IHC staining of four Mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, and PMS2). Tumors with absent IHC staining of MLH1 were tested for BRAF mutations to exclude sporadic CRCs.

Results: Of 322 CRCs, 33 cases were found to be deficient-MMR; 22 of these had concurrent loss of MLH1 and PMS2, followed by concurrent loss of MSH2 and MSH6 in 8 CRCs. Twenty-two cases with a loss of MLH1 underwent testing for the BRAF mutation, 4 of which were recognized as a positive BRAF mutation. Finally, 29 CRCs were found as being positive screen for LS. Poor sensitivity (21.74%) was found for the Amsterdam II criteria and a poor positive predictive value (15.39%) for the revised Bethesda.

Conclusion: Application of clinical criteria may not be effective enough to identify LS and at least 2-antibody panel (PMS2, MSH6) should be conducted for newly diagnosed CRCs.

Keywords: Cancer screening, colorectal carcinoma, immunohistochemistry, Lynch syndrome, mismatch repair

Introduction

Lynch Syndrome (LS), often called hereditary nonpolyposis colorectal cancer (HNPCC) is a genetically inherited autosomal disorder that increases the risk of many types of cancer. This phenomenon is diagnosed by molecular testing in patients with mutations in one of four mismatch repair (MMR) genes, including MLH1, PMS2, MSH6 and MSH2.[1] There is a high lifetime risk between approximately 70–80% for colorectal

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cancer (CRC) in predisposed individuals with LS.[2] It should be noted that the mean age for CRC development is approximately 45 years.[3,4] Moreover, patients with CRC are at a high risk of endometrial, ovarian, renal, gastric, pancreatic, skin and brain extra-colonic cancers.[1] According to some studies, LS might account for 2–7% of all CRCs.[3,5-11] Remarkably, informing patients and their family members who are at risk for such cancers to adherence to specialized endoscopic screening programs[12,13] has improved clinical outcomes, thus resulting in studies on LS screening[14] and the evolution of diagnostic tools and strategies.[15]

Former approaches such as Amsterdam II criteria and the revised Bethesda guidelines detect high-risk patients based on family history of cancer (FHC), age at diagnosis of CRC, and tumor histology. However, these approaches have shown low sensitivity or specificity.[15] Microsatellite instability (MSI) for testing tumor and immunohistochemistry (IHC) staining to identify absent MMR protein expression are acceptable methods to screen for Lynch patients[16,17] and have a similar sensitivity that is upwards of 90%.[1,12] Notably, the IHC-based method is less expensive, easy to access, and can identify potential target genes for confirmatory, germline genetic testing and reduce the unnecessary analysis of other genes.[14,18] More recently, in order to decrease the chance of missing LS, universal IHC testing has been suggested for the MMR protein in every patient diagnosed with CRC.[1]

In addition to LS, familial colorectal cancer type X (FCCTX) refers to subjects with CRC who meet the Amsterdam II criteria

but show no MMR deficiency.[1]

Various studies have reported that the incidence rate of LS varies globally between 2–7%[3,5-11]; for example, the rate is 5.5% in China,[8] 7.3% in Sloviansk had MSI-H tumors of whom 1.3% had germline defects,[6] and 3.1% in the USA.[14]

Data on the outcome of LS screening by IHC in Iranian CRCs are scanty. Using the Amsterdam II criteria, Mahdavinia *et al.* found 21 (4.7%) probands clinically diagnosed as HNPCC.[19] In a study by Molaei *et al.*, 14% of the 343 CRC study units showed abnormal outcomes of IHC staining for MMR proteins. The sporadic and germline MSI were not differentiated as BRAF mutation status was not assessed (20). In another study from Isfahan, patients at high risk for LS were selected based on age and family history and then MMR protein expression was assessed only in these 25 CRCs, considered to be the high risk group. Based on the results obtained in the study, 2% and 2.9% HNPCC and FCC were observed, respectively, with the help of a selective strategy and only in those who met the Amsterdam II criteria and had early onset CRC (21).

Despite the AGA guidelines on universal strategy, there was no difference between the selective and universal strategies in a recent investigation conducted in the USA (14).

Therefore, the objectives of the present study conducted in Iran were to determine the prevalence of FCCTX and assess the outcomes of universal strategy using IHC screening for LS and clinicopathological findings of deficient-MMR (dMMR) CRCs.

Material and Methods

Initially, we included 840 patients with CRC registered in the databases of three referral centers between January 2013 and February 2016 in Mashhad, Northeastern Iran. Of these 840 cases, 170 were unavailable due to changes in address and/or phone number, and 126 refused to be interviewed. Of the remaining 544 cases, IHC screening for the MMR protein was performed for only 322 cases because we did not have access to the pathology block or clinical features. Figure 1 shows the process of including and excluding cases in the study and detecting dMMR cases by considering their characteristics.

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
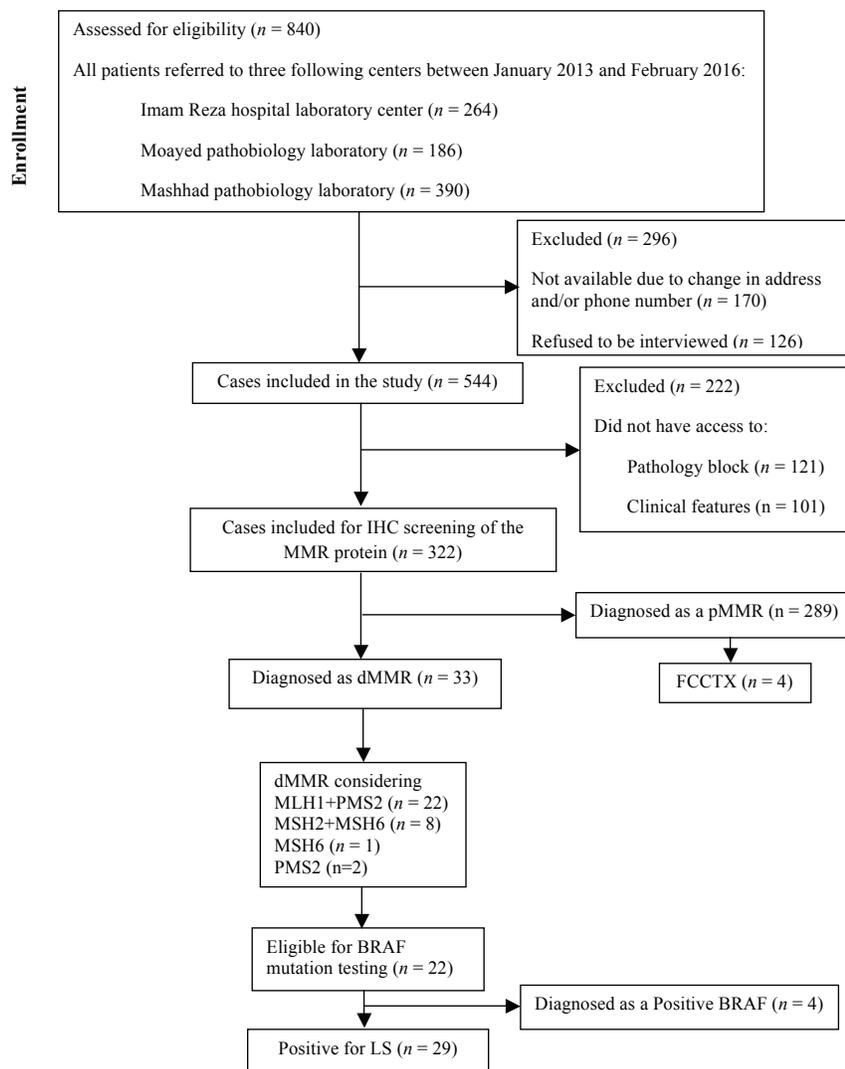

Figure 1. Trend of detecting positive LS.

documented using information gathered through archives, pathology reports and interviews. Such information included sex, age at diagnosis, tumor site, history of CRC or non-CRC in first- and second-degree relatives and histological features for the revised Bethesda criteria reported by 2 expert pathologists in gastroenterology. Early-onset CRC was regarded as onset < 50 years.

Patients fulfilling the Amsterdam II and revised Bethesda criteria were also documented. The revised Bethesda guidelines, a third set of clinicopathological criteria, identify patients for whom it is justified to further investigate for LS with microsatellite instability and/or IHC.[14]

An IHC screen was considered abnormal if IHC staining was absent for any of the four MMR proteins (MLH1, MSH2, MSH6 and PMS2). Tumors with absent IHC staining of MLH1 were tested for BRAF V600E mutations to exclude sporadic CRCs with acquired promoter hypermethylation. Patients with absent MMR proteins, and normal BRAF status (if MLH1 was absent) were considered "screened positive for LS". The germline mutations of MMR genes were not assessed in these cases; therefore, true Lynch and Lynch-like patients are not distinguished in our study.[22,23]

The ethics committee of Mashhad University of Medical Sciences approved this study.

Statistical Analysis

We used chi-square, Fisher's exact, spearman, and Student's t tests for statistical evaluation. Reported p-values less than 0.05 were considered statistically significant. SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used to analyze the data.

Results

A total of 322 CRCs with a mean age of 55.63 ± 14.802 years were screened for LS. Thirty-three cases were detected to be dMMR; 22 of these had concurrent loss of MLH1 and PMS2, followed by concurrent loss of MSH2 and MSH6 in 8, isolated loss of MSH6 in 1, and isolated loss of PMS2 in 2 CRCs (Figure 1). It was possible to detect all dMMR through considering 2-antibody panel (PMS2 and MSH6) instead of 4-antibody panel (MLH1, MSH2, MSH6 and PMS2). Twenty-two cases with loss of MLH1 underwent testing for the BRAF mutation, 4 of whom were recognized as a positive BRAF mutation. Finally, 29 CRCs with mean age of 53.72 ± 13.812 years were detected as being positive screen for LS (Figure 1).

Of the 322 CRCs, 189 cases had sufficient information to allow for evaluation of the Amsterdam II criteria. Nine cases met the Amsterdam II criteria (4.76%), 4 of whom were FCCTX (Table 1). Additionally, 211 cases had enough information for the Bethesda guidelines, and 104 cases of these were positive (49.3%) (Table 2). The predictivity of the Amsterdam II criteria for LS and each dMMR complex (MLH1 vs. MSH2) is outlined in Table 1. The sensitivity of the Amsterdam II criteria for LS was 21.74%, which was increased to 33.33% for MSH2 complex. The sensitivity of the revised Bethesda guidelines for LS was better than the sensitivity of the Amsterdam II criteria. It was 69.56%, which increased to 83.33% for MSH2 complex (Table 2). But its positive predictive value (PPV) was only 15.39%, which decreased to 4.80% for MSH2 complex. Overall, the study revealed a poor sensitivity for the Amsterdam II criteria and a poor PPV for the revised Bethesda guidelines.

The mean age of patients with dMMR was 56.06 ± 14.622 years.

Table 1. Predictivity of Amsterdam II Criteria for LS in the study considering 4-panel\2-panel MMR and BRAF mutation testing as the gold standard in 189 CRCs.

Gold Standard	Amsterdam II Criteria	Positive	Negative	Sensitivity Specificity PPV
4-panel MMR and BRAF mutation testing	Positive	5	18	21.74% 97.60% 55.55%
	Negative	4	162	
MLH1 Complex and BRAF mutation testing	Positive	3	12	25% 96.55% 33.33%
	Negative	6	168	
MSH2 Complex and BRAF mutation testing	Positive	2	4	33.33% 96.20% 22.22%
	Negative	7	176	

Table 2. Predictivity of Bethesda guidelines for LS in the study considering 4-panel\2-panel MMR and BRAF mutation testing as the gold standard in 211 CRCs.

Gold Standard	Bethesda Criteria	Positive	Negative	Sensitivity Specificity PPV
4-panel MMR and BRAF mutation testing	Positive	16	7	69.56% 53.20% 15.39%
	Negative	88	100	
MLH1 complex and BRAF mutation testing	Positive	10	5	66.66% 52.04% 9.61%
	Negative	94	102	
MSH2 complex and BRAF mutation testing	Positive	5	1	83.33% 51.70% 4.80%
	Negative	99	106	

Table 3. Association of LS status in cases screened negative for LS vs. those positive for LS with age, gender and location of CRC, Amsterdam II, revised Bethesda, history of CRC in FDR, history of CRC in SDR, and FHC (*n* = 322).

	Negative LS (*n* = 293) No. of cases (%)	Positive LS (*n* = 29) No. of cases (%)	P-value
Age (*n* = 320)			
< 50 years (*n* = 118)	107 (36.8)	11 (37.9)	0.902
≥ 50 years (*n* = 202)	180 (63.2)	18 (66.1)	
Gender (n = 322)			
Female (*n* = 159)	150 (51.2)	9 (31)	0.038
Male (*n* = 163)	143 (48.8)	20 (69)	
Location of CRC (*n* = 238)			
Proximal (*n* = 29)	25 (11.4)	4 (21.1)	0.263
Distal (*n* = 209)	194 (88.6)	15 (78.9)	
Amsterdam II (*n* = 189)			
Absent (*n* = 180)	162 (97.6)	18 (78.3)	0.002
Present (*n* = 9)	4 (2.4)	5 (21.7)	
Revised Bethesda (*n* = 211)			
Absent (*n* = 107)	100 (53.2)	7 (30.4)	0.039
Present (*n* = 104)	88 (46.8)	16 (69.6)	
History of CRC in FDR (n = 195)			
NO (*n* = 179)	163 (93.7)	16 (76.2)	0.018
Yes (*n* = 16)	11 (6.3)	5 (23.8)	
History of CRC in SDR (n = 199)			
No (*n* = 186)	168 (95.5)	18 (78.3)	0.009
Yes (*n* = 13)	8 (4.5)	5 (21.7)	
FHC (*n* = 189)			
Absent (*n* = 138)	125 (75.3)	13 (56.5)	0.057
Present (*n* = 51)	41 (24.7)	10 (43.5)	

LS = Lynch Syndrome, CRC = Colorectal Cancer, FDR = First Degree Relatives, SDR = Second Degree Relatives, FHC = Family History of Cancer

Mean age of 9 dMMR cases with only loss of MSH2 or MSH6 was lower than those 24 cases with loss of MLH1 or PMS2 (49.33 ± 9.165 vs. 58.58 ± 15.617 years; *P* = .047).

Table 3 compares demographic and clinicopathological variables between cases screened negative LS vs. those who were screened positive for LS.

Most CRCs with positive LS were older than 50 years (62.07%), and among 29 CRCs who screened positive for LS, 19 had information about the location of the CRCs, most of which were distal (78.95%; Table 3).

Discussion

To the best of our knowledge, this is the first study to investigate the results of IHC screening for LS using a universal strategy in Iran. The results of the study revealed dMMRs in 10.24% of CRCs in northeastern Iran. Positive IHC screening for LS reached 9% after excluding BRAF/epigenetic dMMRs. Previous studies performed in the capital of Iran, Tehran, and in Malaysia have reported dMMRs of 13.99% and 9.9% for CRCs, respectively,[20,24] which demonstrate that our study is in line with previous findings.

An interesting finding of the study was that 2-antibody panels were as effective as 4-antibody panels. A 4-antibody panel, which includes the 4 most commonly affected proteins (MLH1, MSH2, MSH6 and PMS2), is generally used to detect dMMR, but Shia *et al.* showed that a 2-antibody panel composed of PMS2 and MSH6 is as effective as the current 4-antibody panel for detecting dMMR.[25] In our study, all MLH1- and MSH2-abnormal cases were also abnormal for PMS2 and MSH6, respectively. Of 33 subjects with dMMR, 24 had loss of PMS2, and 9 had loss of MSH6, revealing that a 2-antibody panel (PMS2 and MSH6) can be a sufficient and cost-effective approach. A previous study performed in Iran also described that among 19 tumors with loss of MLH1, 15 had a simultaneous loss of PMS2, and 12 tumors of 24 tumors with loss of MSH2 had simultaneous loss of MSH6 expression (20).

Two previous studies performed on LS prevalence in Iran addressed 2,580 and 449 CRCs that respectively fulfilled 2% and 4.7% of the Amsterdam II criteria.[19,21] These rates are consistent with our estimated prevalence of 4.76% from the current study and are in contrast to a study from north of Iran where LS was reported to be as prevalent as 10.9%.[26] Small sample size and potential selection bias towards familial cases may explain the unusually high prevalence of LS in the latter study.

Overall, the sensitivity and specificity of Amsterdam II criteria for a diagnosis of LS were 21.74% and 97.6%, respectively, in our study, consistent with other reports.[27-30] If we had used the Amsterdam II criteria as a pre-selection of IHC screening similar to the studies performed in Iran,[21,26,31] we would have missed 78.26% of the cases who were screened positive for LSs.

Most studies have suggested high sensitivity and low specificity for revised Bethesda guidelines to detect LS.[13,15,30] Bethesda guidelines reached a relatively good sensitivity (69.56%) and a fairly poor specificity (53.20%) in our series to detect LS. Its PPV was poor (only 15.39%); in other words, if we chose this model as pre-selection for IHC screening, we should have tested 104 CRCs to detect only 16 positive LS.

Overall, these studies indicate that the Amsterdam II criteria and revised Bethesda guidelines are not sufficiently reliable to detect dMMR. A universal strategy for IHC screening or MSI testing alone performs better but may be too expensive.[15] A localized screening model has been proposed to detect dMMR cases to screen for LS based on the clinical and demographic characteristics of each population, and available resources.[30] Based on our results, we propose that a 2-antibody panel testing may perform as well as a 4-antibody panel.

In this study, 2.11% of patients were estimated to belong to the FCCTX category. The cause for increased risk of CRC in FCCTX families remains unknown.[23] Clinical management of these families should be similar to that for established LS patients.[1,22,23,27]

Several studies have challenged universal screening for LS, proposing that the selective strategy of only screening high-risk individuals is similar to a universal screening strategy to identify LS.[14,21,26,31] Our data strongly suggest that if we limited our screening to early–onset or positive FHC, we would have missed 18 cases of LS (62.07%) and 13 cases of LS (56.52%), respectively. Indeed, patients in our dMMR group were older than the pMMR group (mean age of 56.06 ± 14.622 versus 55.47 ± 14.618 years), although it was reverse between positive and negative LS groups (53.72 ± 13.812 versus 55.71 ± 14.683). Additionally, it is interesting that in our study, only 11 of 33 patients with dMMR were aged under 50 years. If we had limited the study to early-onset cases, similar to the studies performed in Iran.[21,31] we would not have detected 66.66% of the dMMR cases. A recent study from China also found MSI CRCs to be older than the MSS group.[8]

We also observed a significant correlation between the history of CRC in FDR\SDR and LS status (Table 3). Based on these findings, we suggest that in areas where IHC staining is not available, patients with a history of CRC in FDR\SDR should be referred to tertiary centers for IHC of their MMR.

Our study had some limitations. First, it was performed using a relatively small sample size from one province in northeastern Iran, and the results need to be confirmed in larger studies. We were not able to contact and therefore include all consecutive CRCs. In the future, a comprehensive registry of all CRCs will be necessary for larger multicenter studies to investigate the prevalence of LS and optimal screening strategies in Iran.

Methodologically, we are currently limited in performing germline mutation analysis in our suspected LS patients and as such, we could not differentiate between true Lynch and Lynch-like patients.

In conclusion, we estimated the prevalence of patients at risk of LS to be 9% in northeast Iran. We recommend not to rely on screening only high-risk patients based on clinical criteria to find LS families because clinical criteria such as Amsterdam II were shown to be too poor in sensitivity in the study area. The study revealed that IHC screening for MMR with at least a 2-antibody panel (PMS2, MSH6) should be conducted for newly diagnosed CRCs. Larger multicenter studies are needed to design a localized prediction model to screen for LS in Iran. Germline-mutation assessment of suspected LS patients need to be incorporated in our clinical practices in Iran.

Competing Interests

The author(s) declare that they have no competing interests.

Authors' Contributions

Designed the experiments: LG AE. Performed the experiments: KGK AKH. Conducted the sample collection: MRKH AE. Analyzed the data: LG BH FB. Wrote the paper: LG BH FB. All authors read and approved the final manuscript.

Acknowledgments

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