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Lynch-like Syndrome and its Molecular Approaches: A Brief Report and Literature Review

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Abstract

Lynch syndrome (LS) predisposes individuals to early-onset colorectal and other Lynch-associated cancer. This disorder is an autosomal dominant genetic disturbance caused by germline mutations in one of the mismatch repair genes. Different clinical and molecular criteria are used to diagnose LS. Microsatellite instability testing and immunohistochemistry are two widely used methods for the molecular screening of LS-associated cancers. According to the immunohistochemistry and Microsatellite instability testing, we introduce three Persian families with Lynch-like syndrome (LLS) who met clinical Amsterdam-II criteria and their probands were mismatch repair deficient. In the case of immunohistochemistry-MLH1 absent, *BRAF*-V600E mutation was evaluated to rule out the sporadic colorectal cancer cases. No pathogenic germline variants were found by next generation sequencing method. Multiplex ligation-dependant probe amplification technique was done to find large in/dels within *MLH1/MSH2* genes of the probands. A two-exon deletion within *MLH1* gene was eventually identified in one of the patients. Finally, we have represented a molecular pipeline to diagnose LLS based on literature review and the introduced cases.

Keywords: Lynch syndrome, Colorectal cancer, Neoplastic syndromes, Hereditary, Mismatch repair gene

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Introduction

Lynch syndrome (LS) is the most frequent hereditary cancer condition, predisposing people to colorectal and endometrial cancers at an early age, as well as malignancies of the stomach, ovary, urinary system, hepatobiliary tract, pancreas, small intestine, brain, skin, and breast.[1] This disorder which was previously

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known as Hereditary non-Polyposis Colorectal Cancer (HNPCC), is an autosomal dominant genetic disturbance caused by germline mutations in one of the mismatch repair (MMR) genes, usually *MLH1, MSH2, MSH6*, and *PMS2*.[2]

LS is diagnosed using a variety of clinical and molecular criteria, including the Amsterdam I/II criteria, which were developed based on a strong family history of early-onset colorectal and extra colonic malignancies. Revised Bethesda is the most recent diagnostic proposal, and it is more sensitive for clinical selection of LS patients.[3] Patients who meet the criteria are nominated for molecular evaluations. Microsatellite instability testing (MSI) and immunohistochemistry (IHC) of MMR proteins are two widely used methods for the molecular evaluations of MMR deficiency in LS-associated cancers.[4] The primary molecular findings are confirmed by the detection of germline pathogenic variants in MMR genes using next generation sequencing (NGS).[5]

The patients who meet clinical criteria with no DNA-MMR-deficiency, are classified as familial colorectal cancer (CRC) type X.[6] Currently, there is no specific guideline for the patients who meet the clinical criteria and positive molecular testing (MSI & IHC) for LS, but no germline mutations are found in the MMR genes by NGS.[4]

The presence of a causative mutation in a family has a key role to determine the risk of LS-associated cancer in those blood relatives who are identified carriers. For these at-risk family members, a comprehensive screening and preventive approach is being explored. Individuals that are negative are at danger for the whole population.[4] Intensive management might help these people live longer.[7]

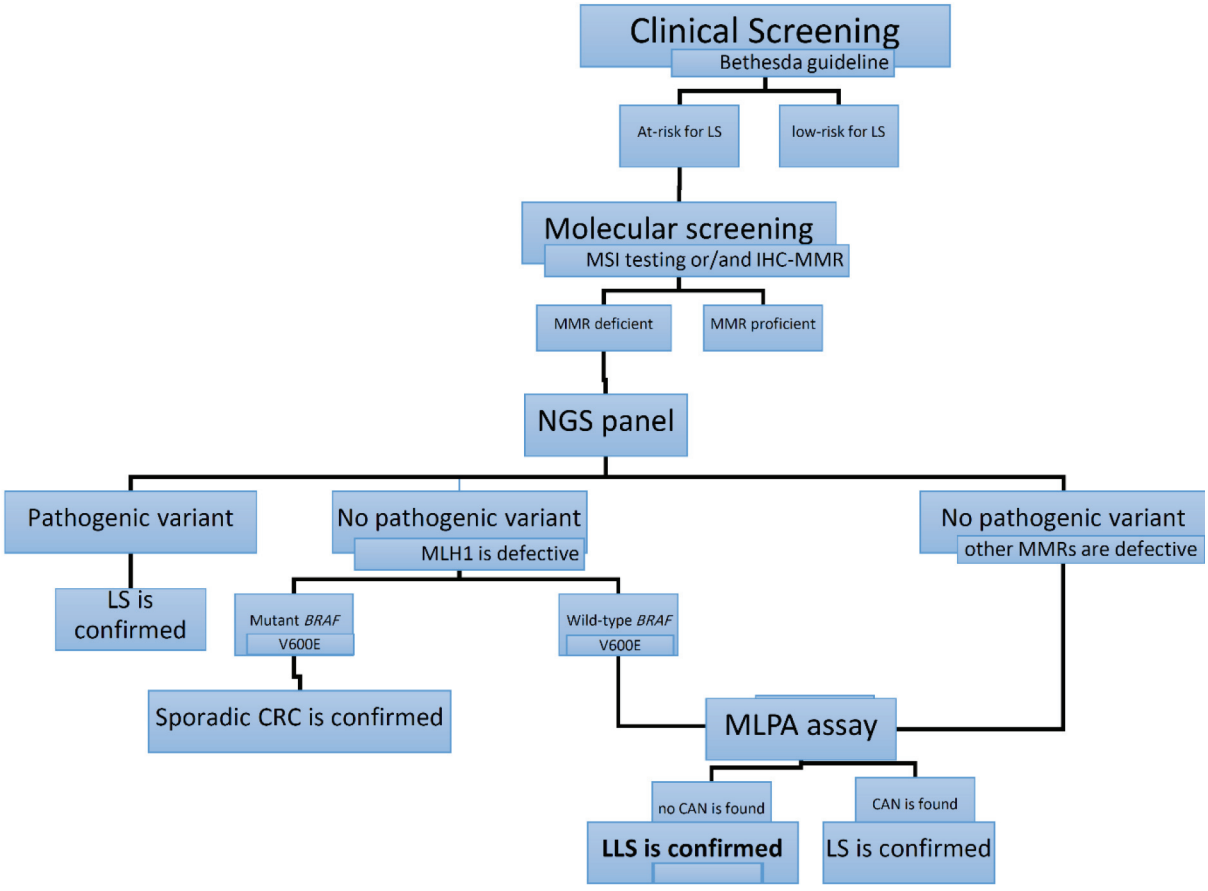

Chart 1. This chart depicts the recommended pipeline for the evaluation of LLS.
LLS: Lynch-like syndrome; LS: Lynch syndrome; MSI: Microsatellite instability testing; MMR: Mismatch repair: IHC: Immunohistochemistry; NGS: Next generation sequencing; CRC: Colorectal cancer

Despite intact MMR genes -according to NGS result, positive clinical criteria and MMR deficiency in molecular testing is suggestive for Lynch-like syndrome (LLS) in terms of unknown non-spot variants and pathogenic genetic rearrangements. Three affected probands with LLS were molecularly presented with highlighting the fact that there is much more to be learned about this genetic condition.

Materials and Methods

This is a brief report study and a literature review in which the molecular approaches of LLS were explained. Inform consent was obtained from all patients and ethical approval was provided by the research ethical committee of Isfahan University of Medical Sciences (ethics code: IR.MUI.REC.1396.1.054). For clinical screening of at-risk cases, the Amsterdam II criteria (Figures 1-3) were utilized, which included at least three relatives with an LS-associated cancer, of which one is a first-degree relative of the other two, the existence of LS-associated cancer in at least two consecutive generations, the diagnosis of at least one patient with an LS-associated cancer before the age of 50, and the exclusion of familial adenomatous polyposis (FAP) in CRC (CRIHC assessment of MSH2, MLH1, MSH6 and PMS2 proteins was accomplished by Novocastra kit (Leica Biosystems, Leica Microsystems Inc, Buffalo Grove, US) on the surgical normal and tumor tissues of the at-risk patients. MSI testing was performed by Promega Kit (MSI Analysis System, Version 1.2). The peaks were assessed using GeneMarker software version HID V3.00.

In the next step, mutation analysis was carried out on the genomic DNA of MMR-deficient patients by NGS Illumina HiSeq 2500 platform (Otogenetics CRC NGS panel, Atlanta, Georgia, USA).

After NGS DATA analysis, Multiplex Ligation-dependent Probe Amplification (MLPA) assay was then performed on uncertainty genomic DNA. SALSA MLPA kit P003 B1 (MRC Holland, Amsterdam, Netherlands), including *MLH1* and *MSH2* probes, was used to survey these genes. Coffalyser.Net Software (MRC Holland, Amsterdam, Netherlands) was applied to perform the data analysis via comparing the peak height patterns among samples.

Results

An affected woman with 33 years old (patient 1) and two affected men with 31 and 45 years old (patients 2 and 3) are presented. The Amsterdam II criteria were satisfied by all three cases. IHC-MMRs for MLH1 and PMS2 proteins were absent in two instances (patients 1 and 2),

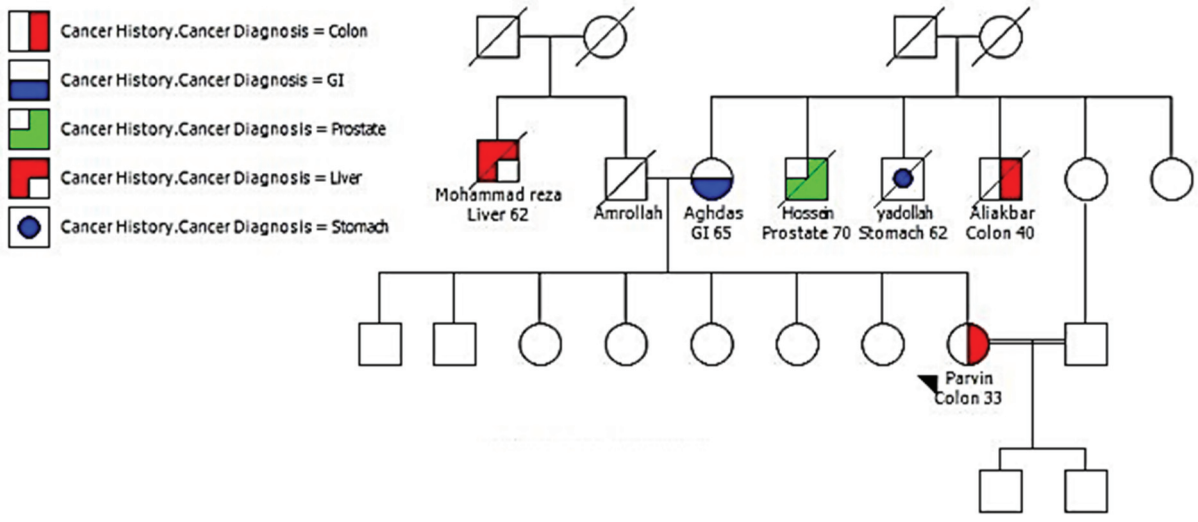

Figure 1. This figure shows the pedigree of an at-risk family for the Lynch syndrome with a 33-year-old female proband.
GI: Gastrointestinal

whereas MSH2 and MSH6 proteins were absent in one case (patient 3). Using MSI testing, all three patients showed instability in more than two markers and they were identified as MSI-H based on manufacturer's Promega kit protocol.

After the mutation analysis using NGS panel, two likely pathogenic variants were identified in patient 2, as 'chr1:45797505 C>G' in *MUTYH* and 'chr2:48010488 G>A' in *MLH1*. In patient 1, one likely pathogenic variant was found as 'chr1:45797505 C>G' in *MUTYH*. After analyzing all obtained variants, no pathogenic variants were detected.

Moreover, the tumor DNA extracted from three patients was evaluated regarding *BRAF* hot-spot mutations, particularly *BRAF* V600E, using sanger sequencing and no mutation was detected.

Regarding strong suspicion for the presence of chromosomal rearrangements and large indels within MMR genes, the deletion of 7th and 8th exons of *MLH1* gene was identified in one of the patients (Figure 4). No Gene Copy Number Alterations (CNA) were seen in *MLH1* and *MSH2* genes in either of the other two patients.

Discussion

CRC is the most frequent kind of gastrointestinal cancer and one of the main causes of cancer mortality throughout the globe. Both sporadic and inherited forms of this illness have been seen. The most frequent hereditary CRC disease is known as 5 LS. The prevalence of numerous genes implicated in the DNA MMR pathway, as well as a broad spectrum of mutations, has made diagnosing HNPCC more difficult.[4]

The contribution of MMR genes in LS has a frequency distribution pattern as 42% for *MLH1*, 33% for *MSH2*, 18% for *MSH6* and 7.5% for *PMS2*.[8] In Peltomäki et al. study, a missense mutation was introduced as the most common

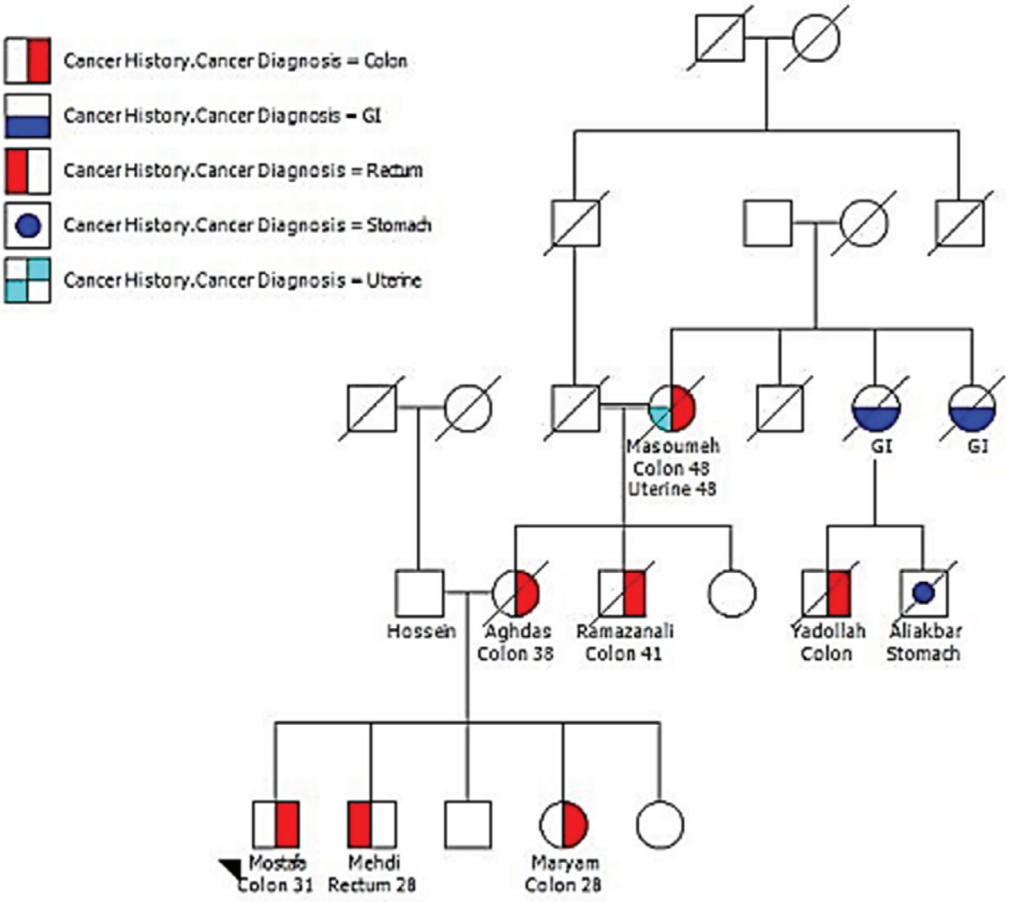

Figure 2. This figure shows the pedigree of an at-risk family for the Lynch syndrome with a 31-year-old male proband.
GI: Gastrointestinal

mutation of *MLH1* in LS, while *MSH2* germline mutations led to complete loss of gene expression.[9] The important point to remember about MMR genes is that the expression of sub-genes like *MSH6* and *PMS2* is dependent on the expression of main genes like *MLH1* and *MSH2*, thus if MLH1 or MSH2 expression is lost in a tumor, PMS2 or *MSH6* expression will be lost as well. Meanwhile, the primary germline mutations of *MSH6* and *PMS2* genes are only associated with the lack of their proteins without affecting the expression of other MMR proteins.[10] This could explain PMS2 and MSH6 IHC defect in these three cases.

The sensitivity of IHC in the diagnosis of MMR defects is about 95%. False intact protein staining may be seen in IHC in terms of the presence of dysfunctional proteins (protein-truncating or large in-frame deletion gene-mutations) which have still immunogenic activity.[11] In a study was done by Lindor et al., out of 818 MSI-H tumor specimen with *MLH1* mutation, 27 cases showed intact IHC.[12] In the introduced cases other evidences, such as MSI testing demonstrated that IHC-MMRs findings would not be false.

MSI phenotype is caused by an MMR-deficient system. Previous research has shown that cancer cells in LS are unable to repair single-based mispaired/unpaired and insertions-deletions of tandem repeat sequences, resulting in MMR deficiency. The phenotype of microsatellite instability is caused by an MMR-deficient system.[13] Recent guidelines provided a recommendation for the molecular evaluation of LS and proposed a consensus MSI testing panel.[14] Although MSI testing is a highly sensitive test to detect LS, the sensitivity in cases caused by *MSH6* mutation is just 86%.[4]

Since defective expression of MLH1 and MSH2 proteins had been already confirmed by IHC and MSI testing, it was reasonable to observe

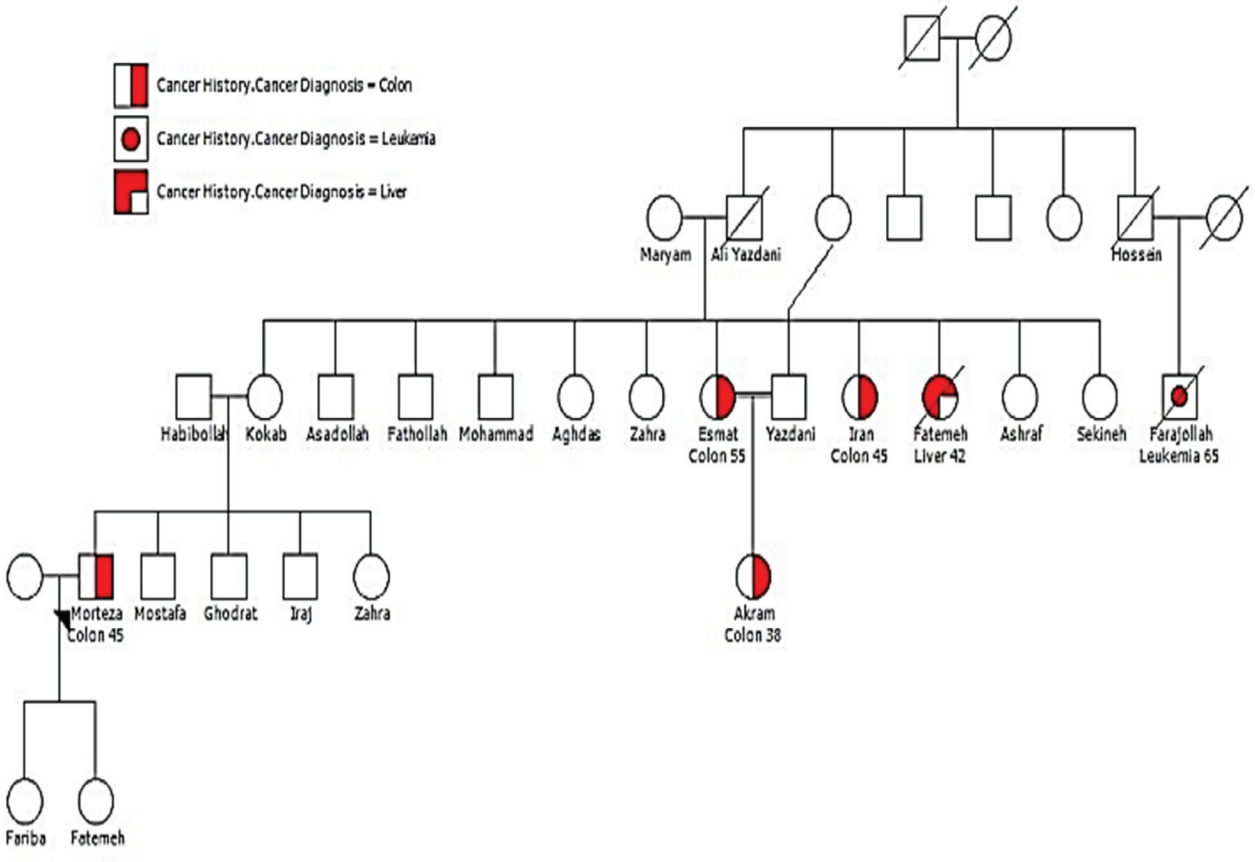

Figure 3. This figure shows the pedigree of an at-risk family for the Lynch syndrome with a 45-year-old male proband.

germline mutations in these two genes. Meanwhile, no pathologic variants were found in MMR genes of these cases which could explain why they were classified as LLS patients. A small number (1.85%) of total mutations of *MLH1* gene is due to the variants of non-coding regions or complicated intronic rearrangements. As a result, traditional NGS systems will be unable to detect these changes.[15] Morak et al. looked at 37 CRC patients whose IHC findings revealed a deficiency in at least one MMR protein, but NGS analysis revealed no known mutations.[16] In NGS investigations, around one-third of suspected LS patients had no pathogenic mutation.[17] The variants of uncertain significance (VUS) are the major challenges against the molecular diagnosis of LS.[18] According to International Society for Gastrointestinal Hereditary Tumors (InSiGHT),

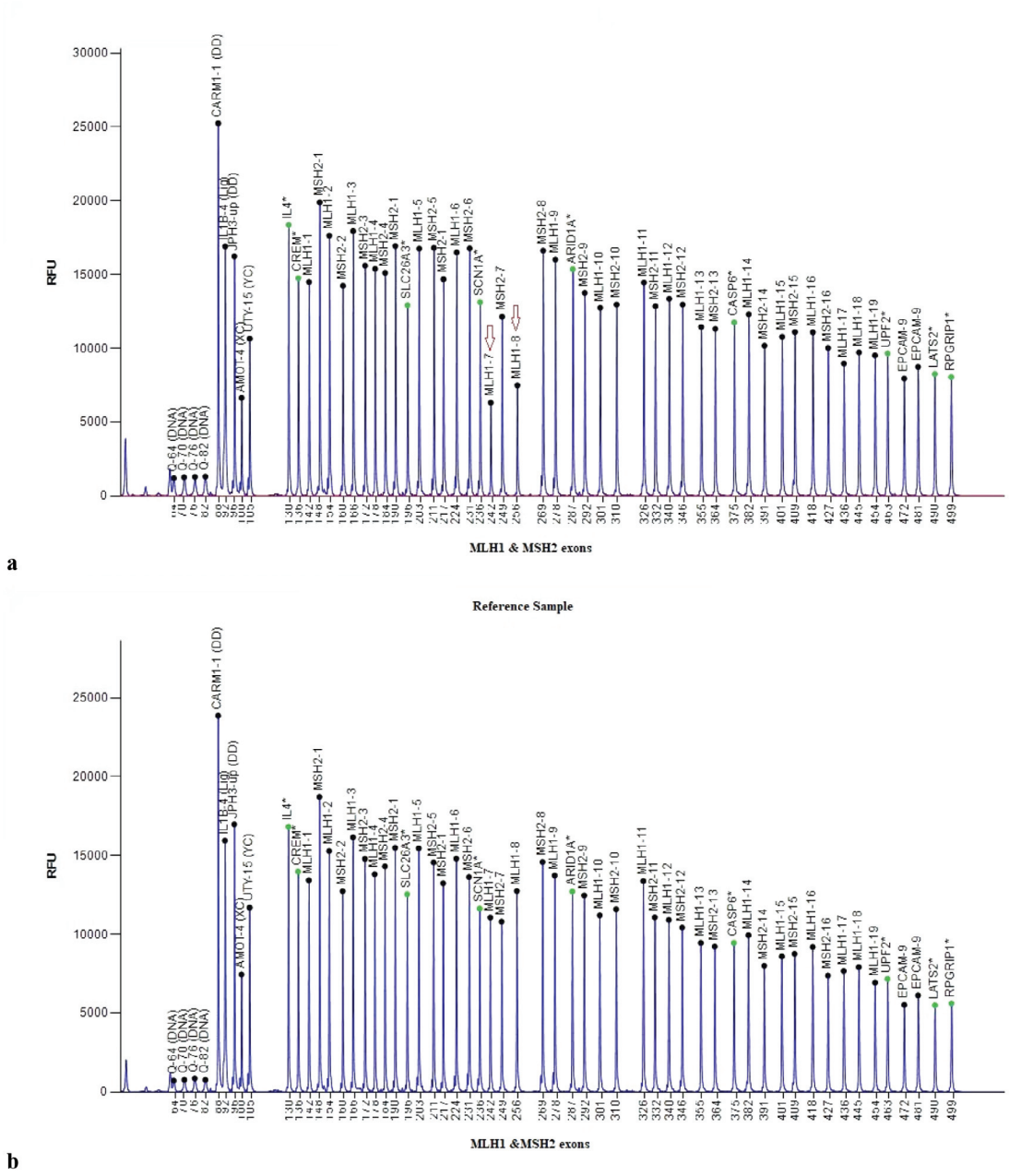

Figure 4. This figure shows the MLPA result of 31-year-old man. A deletion was observed in 7th and 8th exons of MLH1 gene (a) compared with reference sample (b).
MLPA: Multiplex legation-dependent probe amplification, RFU: Relative fluorescent units

family history should be considered to determine the pathogenicity VUS.[19] The whole genetic variation in the MMR genes remain unrealized and further work is still required to determine the pathogenicity of VUS.

Because IHC revealed a faulty MLH1 protein in two of the three cases described, the possibility of sporadic CRC in these individuals was raised. The MSI-H phenotype in CRC sporadic type is caused by hyper-methylation of the *MLH1* gene promoter, which may be detected using the BRAFV600E mutation as a surrogate marker.[20] The familial history and genetic pedigrees are strongly in favor of LS in the reported patients which the wild type *BRAF* confirms it. Bessa et al., investigated 1222 CRC patient of which 119 cases presented MSI and MMR deficiency. Although *BRAF* mutation were detected in 22 patients of 119 MMR deficient cases, *BRAF* ^{V600E} was not found in none of the patients with specified germline mutation.[21]

There is a possibility of genomic rearrangements, such as gene-flanking deletions, inversions, duplications, or translocations within MMR genes which are difficult to detect. In a study which was performed by MLPA and oligo-array on the patients similar to the introduced cases, a duplication of whole promoter region and exon 1-19 of *MLH1* gene was identified.[16]

MLPA of the *MLH1* and *MSH2* genes, whose absence of expression had previously been established, was used to investigate probable CNA alterations. This is a quantitative multiplex PCR method for determining relative CNA changes that standard diagnostic approaches may overlook.[22] After data analyses using bioinformatics tools, a large deletion within exon 7-8 of *MLH1* gene was detected in one patient. Meanwhile, no CNA changes were found in the other two cases.

The presence of MSI status without MMR defect has not been explained so far.[23] Unknown effective genes in MMR pathway or presence of the mutations in regulatory areas of MMR genes are one of likely reasons to explain this condition. Further studies with new technologies are needed to demonstrate other mechanisms in the genome which are involved in DNA repair.[4]

Recent study was conducted by Aruma et al. highlighted the importance of the diffusion of MMR proteins and their different electrostatic affinity for DNA.[24] This hypothesis is proposed that post transcriptional altering can change MMR proteins function and cause MSI status.[23]

In the reported cases, MMR gene epimutation was likely occurred and transmit to the next generation, not recognizable by conventional approaches. Hitchins et al. indicated that hyper-methylation of one allele of *MLH1* in somatic cells (a germ-line epimutation) predisposes individuals for developing cancer in HNPCC pattern.[25] A large duplication in the vicinity of *MLH1* gene was previously reported leads to the autosomal dominant inheritance of *MLH1* epimutation.[26] In up to 10% of LS patients with no identified MMR gene mutation, *MLH1* epimutation was detected.[17]

In certain LS patients, germline deletions in the MSH2 neighboring gene epithelial cell adhesion molecule (EPCAM) might result in MSH2 epimutation.[27] MLPA, which is related with MSH2 epimutation, is used in standard molecular testing for LS to screen for EPCAM.[4]

Conclusion

No disease-causing genetic change can be defined in 10%-20% of LS suspected cases. Based on the evidence, LS cannot be ruled out for the presented cases, but there is no molecular evidence to confirm it. Based on obtained data, the following pipeline is recommended for the molecular approach of LLS cases:

1. Clinical screening of patients based on the standard criteria (Bethesda guideline)
2. Molecular screening (MSI testing or/and IHC-MMR) for clinically at-risk patients
3. The evaluation of NGS panel for MSI-H or *MMR* deficient cases
4. The evaluation of *BRAF* V600E mutation in *MLH1* defective patients with no pathogenic variant in NGS (to rule out the sporadic CRC cases)
5. Using MLPA assay on the patients with wild type *BRAF*

LLS is diagnosed for MMR deficient patients with no pathogenic variant, wild type *BRAF*, and no CAN in MLPA (Chart1).

Conflict of Interest

None declared.

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