Colorectal Cancers: Recent Advancements in Diagnostic and Preventive Strategies of Hereditary Tumours (Lynch Syndrome)

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Abstract

Lynch syndrome, is one of the most common hereditary colorectal cancer predisposition conditions, is characterised by an increased risk to a spectrum of cancers, primarily colorectal and endometrial cancers. We highlight the urgent need to diagnose, explain diagnostic methodologies and several cancer risks reducing interventions in these patients and their relatives. We highlight the role of aspirin as a potent chemo preventive agent in Lynch syndrome patients and discuss patient groups where the dose and duration of the intervention may require personalisation. With new genetic technologies on the horizon, early detection of these patients coupled with targeted chemo preventive intervention could potentially lead to the reduction of the cancer burden in India.

Keywords: Lynch syndrome, colorectal cancer, aspirin, chemoprevention

Conflict of Interest: The authors declare no conflict of interest.

Source of Support: HS is funded by the Gujarat State Biotechnology Mission grant (667020).

Introduction

"He is a better physician that keeps diseases off us, than he that cures them being on us; prevention is so much better than healing because it saves the labour of being sick" – Thomas Adams, 1618

Cancer arising within the colon and rectum is called colorectal cancer (CRC). It is one of the most frequent cancers in the developed world, with a global incidence of about 1.9 million people every year.[1] In India, the incidence rate is estimated to be approximately 1 per 25,000 people in the population, which makes it one of the top 5 common cancers.[2]

A small proportion of monogenic syndromes account for ~3-5% of CRCs observed in the clinics.[3] Traditionally, the remainder is divided into "familial" and "sporadic". This is a false dichotomy as some of the familial cases are simply coincidental occurrence, while many of the isolated cases have a genetic predisposition, usually triggered by a combination of environmental and stochastic/ chance factors.[3] Those with an affected first-degree relative will be more likely on average to have a bigger genetic contribution, reflected in an even greater chance of a further relative being affected.

The identified genetic risk factors lie between the two extremes- rare mutations that substantially increase the risk of cancer and common mutations that confer individually small effects.[3] To date, 14 genes implicated in hereditary CRC syndromes have been identified, of which, 5 genes- *MLH1, MSH2, MSH6, PMS2* and *EPCAM* have been implicated in Lynch syn-

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drome (LS), formerly known as hereditary non-polyposis colorectal cancer.[4] Here, we give a brief overview of screening, diagnostics and cancer prevention strategies for LS. We also emphasize the importance of diagnosing LS patients in order to reduce the cancer burden.

What is Lynch Syndrome?

LS is characterised by predisposition to a wide variety of gastrointestinal and genitourinary tumours- colorectum, endometrium, stomach, small bowel, ovary, gallbladder, hepatobiliary, pancreas, urinary tract, kidney, brain, prostate and sebaceous skin lesions.[4] LS tumours often arise in young people (<50 years of age) and several factors affect an individual cancer risk- sex, age, the affected gene, family history of cancer and BMI.

The risk of cancer is highest with mutations in *MLH1* and *MSH2* genes and relatively lower (and with later age of onset) in patients with mutations in *MSH6* and *PMS2* genes (Table 1) [5]. Systematic testing of CRCs suggests that LS is a cause of CRC in ~1 in 30 patients. The World Health Organisation estimates the prevalence of LS in the general population to be ~1 in 125, thereby making it one of the most common genetic diseases in the world. [5]

With such high prevalence and availability of risk reducing chemo preventive interventions, detecting LS patients and their family members is of utmost priority.

How to Diagnose a Patient with Lynch Syndrome?

Several international guidelines recommend a 3-step molecular diagnostic pathology pathway towards LS diagnosis.[4,5,6,7] They are primarily centred around CRC patients, as CRC is the most common tumour detected in LS patients (Figure 1). These 3 steps are as follows:

1. **Microsatellite instability testing in tumour biopsy:** LS results due to a defect DNA mismatch repair (MMR) mechanism.[8,9] Defective MMR in a tumour prevents recognition and repair of addition and deletion of bases within repetitive regions in the DNA that naturally occur when DNA is making a copy of itself (i.e. replication). Repetitive regions of the DNA are known as microsatellite and come in different varieties based on their composition i.e. mononucleotide repeats (eg. AAAAAAA….) and dinucleotide repeats (eg. CACACACA….). The addition or deletion of the base within these repeats is known as microsatellite instability (MSI).[8,9] MSI can either be detected by a technique called polymerase chain reaction (PCR) followed by fragment length analysis (FLA) that detects addition/ deletion of bases in microsatellite regions or by immunohistochemistry (IHC) of MMR proteins.[5] PCR-FLA is observed to have superior sensitivity and specificity compared to IHC, hence is preferably used in CRC patients.[7] MSI is observed in ~1 in 6 CRC patients; these patients are likely to be LS as their tumour suggests a defect in MMR pathway. [8]
2. ***BRAF* V600E / *MLH1* hypermethylation testing in tumour biopsy:** Somatic p.V600E mutation in the *BRAF* gene occur in at least 85% of sporadic CRCs with MSI, but not in those with LS; thus presence of such mutations are highly predictive of tumours being of sporadic origin and not due to LS.[5,7] Alternatively, detection of *MLH1* gene promoter hypermethylation in tumour also provides good evidence that the tumour is sporadic in origin. However, due to the higher sensitivity and specificity of *BRAF* V600E testing, its use is preferred.[5,7]
3. **Germline gene panel testing in DNA from whole blood:** In patients who have MSI and *BRAF* V600E/ *MLH1* hypermethylation negative tumour, LS can be definitively diagnosed by constitutional MMR gene testing by next-generation sequencing (NGS) technology to identify the causative constitutional mutation. In cases with very young-onset cancers (eg. <35 years of age), LS is likely even in the absence of family history, there-

Table 1: Relative cumulative incidence of cancer by 75 years of age in Lynch syndrome carriers stratified by mutations in mismatch repair genes. Data adapted from [5]

Anatomical region	Population incidence	Relative cumulative incidence, by affected gene (95% CI)			
		MLH1	*MSH2*	*MSH6*	*PMS2*
Gynaecological	2.6%	19.1 (15.6-22.7)	25.3 (20.1-30.4)	20.8 3.3-28.2)	10.1 (0.3-20.0)
Colorectal	3.8%	12.1 (10.0-14.2)	11.3 (8.7-13.9)	3.9 0.9-7.0)	0
Upper gastrointestinal	1.9%	11.2 (8.2-14.3)	5.4 (2.1-8.6)	3.5 0.0-7.8)	0
Urinary tract	2.3%	3.5 (1.9-5.1)	10.8 (7.2-14.4)	4.8 0.7-8.8)	0

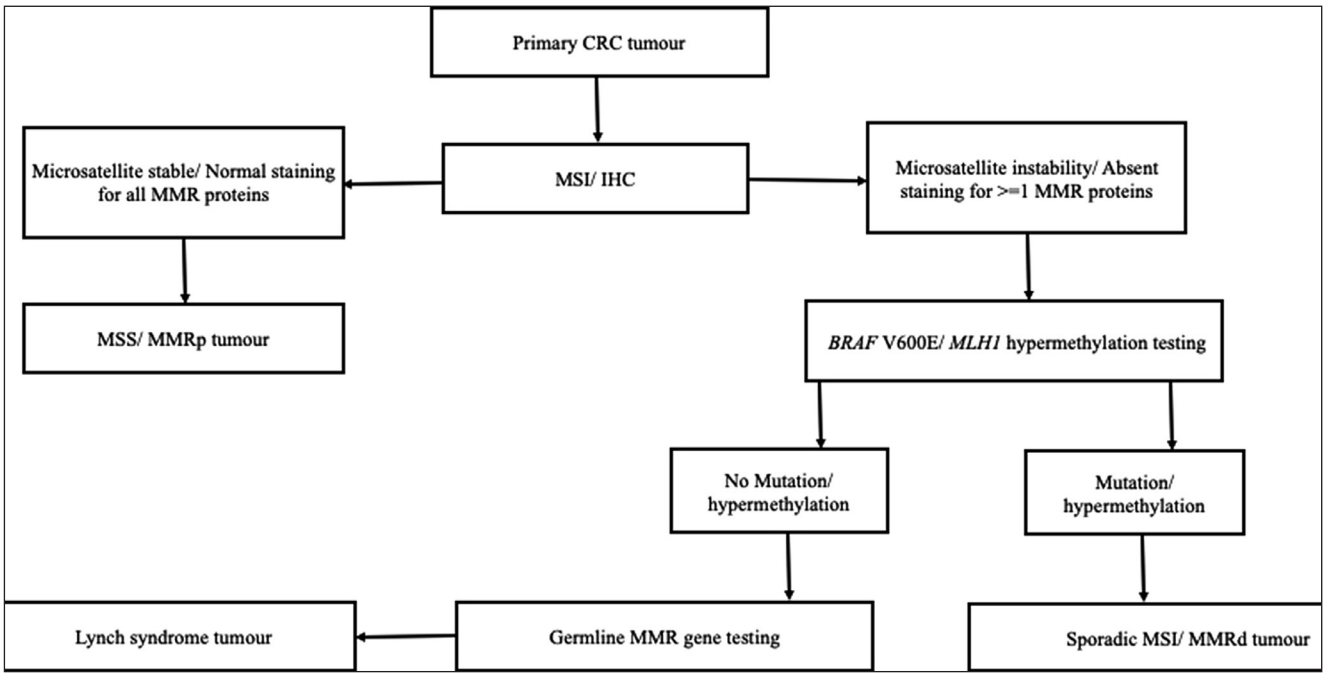

Figure 1: Molecular diagnostic pathway for Lynch syndrome detection in colorectal cancer patients

fore, over-reliance on family history could lead to poor diagnostic uptake.[5] It is often useful to have samples from more than 1 family member because a segregation study may be required to determine the pathogenicity of the detected mutation.

A recent analysis by the UK's National Institute of Health Research suggests universal deployment of this pathway across all CRC patients, regardless of age, gender and family history, is cost-effective in order to diagnose LS patients and subsequently reduce cancer burden.[10] This pathway though primarily designed for CRC patients is currently being optimised to be used in other cancers, for example, endometrial cancer (EC). Like CRC, ~3% of EC tumours arise due to LS although the diagnostic pathway only involves 2 steps- IHC of MMR proteins followed by germline gene panel testing in patients with one or more absence of MMR proteins in the tumour. [11] Furthermore, new NGS based MSI and *BRAF* V600E detection technologies are being introduced in the clinical setting to improve throughput and reduce per-sample costs, thereby improving detection of LS. [12]

Can Cancer Risk be Reduced in Lynch Syndrome Patients?

Currently, three main prevention strategies are being advised worldwide: lifestyle modifications, early detection of pre-neoplastic and neoplastic lesions by surveillance and regular use of aspirin.[3] Cigarette smoking increased body mass index, consumption of alcohol and red meat is associated with an increased risk of cancer in LS patients. In contrast, colonoscopic surveillance and regular aspirin use are associated with reduced cancer risk.

The latest guidelines by the European Tumour Hereditary Group and the European Society of Coloproctology suggest the utilisation of regular colonoscopic surveillance in order to reduce cancer incidence and mortality.[13] Particularly, they recommend 2-3 yearly colonoscopic interval in patients with mutations in *MLH1, MSH2* and *MSH6* genes; and 5 yearly colonoscopic interval in patients with mutations in the *PMS2* gene. Even for patients with prior CRC history and subtotal colectomy, biennial colonoscopies could be performed. Ideally, it is recommended that surveillance colonoscopies should be initiated at the age of 25 years for *MLH1* and *MSH2* carriers and 35 years for *MSH6* and *PMS2* carriers.

UK's National Institute of Health and Care Excellence and USA's the United States Preventative Services Task Force now recommends a daily intake of 75-100mg of aspirin in LS patients to reduce cancer risk by upto 50%.[14,15] These recommendations are based on the outcome of a recent randomised controlled trial (called CAPP2) that showed 600mg aspirin daily for 2-4 years was well tolerated and reduced CRC incidence by 50%, a benefit that began at 5 years following treatment initiation and persisted into the second decade.[16] For an average bodyweight above 70kg, a higher dose than currently in clinical use for other in-

dications may be required. Another trial called CAPP3 is currently being carried out in LS patients in order to determine the optimal dose and duration of aspirin treatment (www.capp3.org), results of which are anticipated to arrive in 2024.

What is the Current Status of Lynch Syndrome Detection in India?

Universal MSI and LS testing in all CRC patients across all cancer care facilities in India is not being carried out currently. However, some centres have introduced reflex testing in order to improve LS detection and cancer prevention. Indeed, the authors are currently running a government-funded prospective study to systematically assess the prevalence of MSI, *BRAF* V600E and LS in CRC patients in India. The results of the study are anticipated to be published by 2022 following which the authors would be conducting a cost-effectiveness analysis to assess the economic viability and utility of these molecular genetic technologies in a routine clinical oncology setting in India.

Conclusion

LS is one of the most common hereditary cancer predisposition syndromes and its diagnostic modalities involve 3-step serial genetic tests. Universal deployment of genetic testing for MSI and LS status in all CRC patients, regardless of age, sex or family history, is recommended in order to improve the diagnosis of LS. With low-cost cancer prevention interventions now available, detection of LS patients and subsequent cancer prevention is the need of the hour to reduce the cancer burden in India.

Acknowledgement: We are grateful to our collaborators Dr. Pankaj Shah, Dr. Mithun Shah, Prof. Suresh Advani, Dr. Liyana Thomas and Dr. Vipul Yagnik, and generously thank the patients for participating in our ongoing study on Lynch syndrome prevalence in colorectal cancer patients in India.

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