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Discordant immunohistochemistry in an unusual MLH1 gene variant in a case of Lynch syndrome

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ABSTRACT

Immunohistochemistry for mismatch repair proteins and microsatellite instability testing are recommended screening methods for Lynch syndrome. They have a good sensitivity and specificity, allowing for directed genetic testing and diagnosis. We report a case of Lynch syndrome with retained MMR protein expression who later showed an *MLH1* gene variant on genetic testing (Next Generation Sequencing) requested because of the clinical presentation of metachronous colonic and endometrial carcinoma. This report makes the case for strong clinical suspicion and directed genetic testing despite initial screen negative results.

1. Background

Lynch syndrome accounts for 10% of endometrial cancers under the age of 50 years (Resnick et al., 2009). In 50%, the sentinel cancer is endometrial and, in the rest, colorectal (Lu et al., 2005). It has an autosomal dominant inheritance with incomplete penetrance. It is characterized by germline mutations in DNA mismatch repair genes (*MLH1, MSH2, MSH6* and *PSM2* or deletion of *EPCAM*). The incidence of these mutations in the general population is 1 in 279 (Resnick et al., 2009). Most international societies like National Comprehensive Cancer Network, Manchester International Consensus Group and National Institute for Health and Care Excellence now recommend universal screening of all endometrial and colorectal cancers for mismatch repair (MMR) status in resource-rich settings owing to the high frequency of these mutations (National Comprehensive Cancer Network; Crosbie et al., 2019; The National Institute for Health and Care Excellence, 2017). Immunohistochemistry (IHC) and microsatellite instability (MSI) testing are the most commonly used screening methods with good sensitivity and specificity (Shia, 2008). Screening in such patients has important diagnostic, therapeutic and prognostic implications on both patient as well as the family members. However, in rare instances, discordance between screening and genetic testing results have been described (Bartley et al., 2012). We report a case which was negative on screening by IHC, but underwent genetic testing based on clinical suspicion and diagnosed as Lynch syndrome due to MLH1 gene variant.

2. Case report

A 50-year-old patient, presented in July 2020 with postmenopausal bleeding for duration of two months. She had on and off spotting per vaginum without any history of vaginal discharge or contact bleeding. She had no history of irregular menstrual cycles and had attained menopause at 41 years. There was no history of infertility or polycystic ovarian syndrome and she had a 22-year-old daughter. In the family history, five members of her family including her maternal grandmother (colon cancer at 70 years), maternal aunt (endometrial cancer at 55 years), two first cousins on paternal side (both had endometrial cancer at 50 years) and paternal uncle (prostate cancer at 60 years) were affected with cancers involving colon, endometrium and prostate (Fig. 1). Her past history revealed that she had been diagnosed with well-differentiated adenocarcinoma of ascending colon in 2019 at 49 years of age, staged T2N1M0, for which right hemicolectomy and ileo-colic anastomosis was performed followed by 8 cycles of adjuvant chemotherapy consisting of oxaliplatin and capecitabine. MMR IHC (including MLH1, PMS2, MSH2 and MSH6) on the colorectal specimen showed retained expression at that time. The immunohistochemistry for MMR proteins was repeated on the hysteroscopic endometrial biopsy showing well-differentiated adenocarcinoma of endometrium, grade 2, with

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same results as before (Fig. 2 panel). Thereafter, she underwent staging laparotomy with total abdominal hysterectomy, bilateral salpingo-oophorectomy and lymph node sampling. The diagnosis was confirmed on the hysterectomy specimen. In view of the strong family history and also the association with previous right sided colon cancer at 49 years and present endometrial cancer at 50 years of age, the patient was tested for Lynch syndrome panel of genes by next-generation sequencing (NGS). She was found to have mutation in exon 3 of *MLH1* (ENST00000231790.2) gene, c.306G > T(p.Glu102Asp), a rare pathogenic variant associated with Lynch syndrome.

Currently patient is under regular follow-up for annual colonoscopy, which has, so far been normal. Her family members have been counseled for genetic testing and the need for regular follow-up with colonoscopy and endometrial evaluation annually if found positive.

3. Discussion

Screening for Lynch syndrome serves several purposes including establishing of a diagnosis, prognosticating the disease as well as preventing cancers among patients and family members by targeted surveillance. The traditionally used clinical criteria like Bethesda and Amsterdam, have their own limitations such as low sensitivity and reliance on family history (Syngal et al., 2000). Acceptable methods of screening today are tissue-based testing for mismatch repair status by Polymerase Chain Reaction-based MSI testing or by IHC staining for MMR proteins. Each method has its own strengths and limitations. MSI testing is highly reproducible, has a sensitivity of 93%, needs minimal amount of tissue and picks up cases of Lynch syndrome which are missed on IHC (Shia, 2008). On the other hand, MSI needs microdissection of both normal and abnormal tissue followed by molecular analysis and hence, is not readily available. MSI testing also has a lower sensitivity in detecting MSH6 mutations. Another major drawback is that, it does not pinpoint to the specific affected gene. In contrast MMR IHC is simple, inexpensive, easily available, with a sensitivity of 92% (Shia, 2008). It may reveal the exact gene affected and help in directed genetic testing. However, IHC results may be difficult to interpret due to variable tissue fixation, hence, needing an experienced pathologist. It may show lower sensitivity in detecting MLH1 mutations, especially when the antibody against PMS2 is not part of the panel (Shia, 2008). There may be a small proportion of cases with mis-sense mutations causing production of non-functional MMR proteins which may retain immunostaining. This may result in false negative screening results, as happened in the index case and is one of the drawbacks of IHC, albeit infrequent (Shia, 2008; Giardiello et al., 2014; McCracken and Neff, 2018).

In almost all cases, both methods of screening are highly concordant with each other and also with genetic testing results. However, there have been infrequent instances proving the contrary. A study by Bartley et al revealed that immunostaining may be retained despite MSI-high status in around 11.8% of cases and of these, 2.2% are attributed to be due to mis-sense mutations mentioned above. In this study of 646 cases, out of the 12 patients with discordant results, only one patient was detected to have pathologic MMR variant in *MLH1*gene i.e. 0.15% (Bartley et al., 2012). This would mean that identifiable MMR mutations are extremely rare in patients having retained immunostaining.

Our patient was detected to have *MLH1* gene variant which resulted in substitution of Aspartic acid for Glutamic acid at codon 102 (p. Glu102Asp; ENST00000231790.2) which causes abnormal splicing and thereby loss of function (National Center for Biotechnology Information). An in vitro study carried out on yeast cells by Takahashi et al. in 2007 for this particular variant revealed that MLH1 expression was over 75% but protein was functional in only 56.1% (Takahashi et al., 2007). This differential loss of function may also explain the incomplete penetrance of the disease. All previously reported cases with this particular pathologic variant had loss of MLH1 expression on IHC (National Center for Biotechnology Information; Whitworth et al., 2016). However, uniquely in our case there was retention of MLH1 protein expression on IHC specimen of colorectal and endometrial cancer specimens consistently. In such situations, with a strong family history and clinical suspicion, a negative screening might point towards a mis-sense mutation and it would be rational to proceed with NGS on germline DNA. Indeed, such patients may qualify directly for germline testing as per recent guidelines – which was delayed in the index case (SGO, 2014). In the absence of pathognomonic mutations of Lynch syndrome, other differentials including Lynch like syndrome, Familial Colorectal Cancer Type X syndrome and POLE/POLD1 mutations must also be kept in mind (Jm and Stoffel, 2015).

In the context of an established diagnosis of Lynch syndrome, the responsibility of the clinician would widen to include first degree relatives at risk of having similar variants, given this is an autosomal dominant condition. Germline testing for Lynch panel of genes is indicated in first degree relatives. Due to the increased risk of endometrial, ovarian and colorectal cancers in those who test positive, they must further be offered risk-reducing surgeries such as total abdominal hysterectomy, bilateral salpingo-oophorectomy and colectomy, deferring which surveillance by annual colonoscopy (starting at age 25) and annual endometrial evaluation (starting at age 30 or 5 years prior to the onset of earliest endometrial cancer in the family) would be advisable (Syngal et al., 2015).

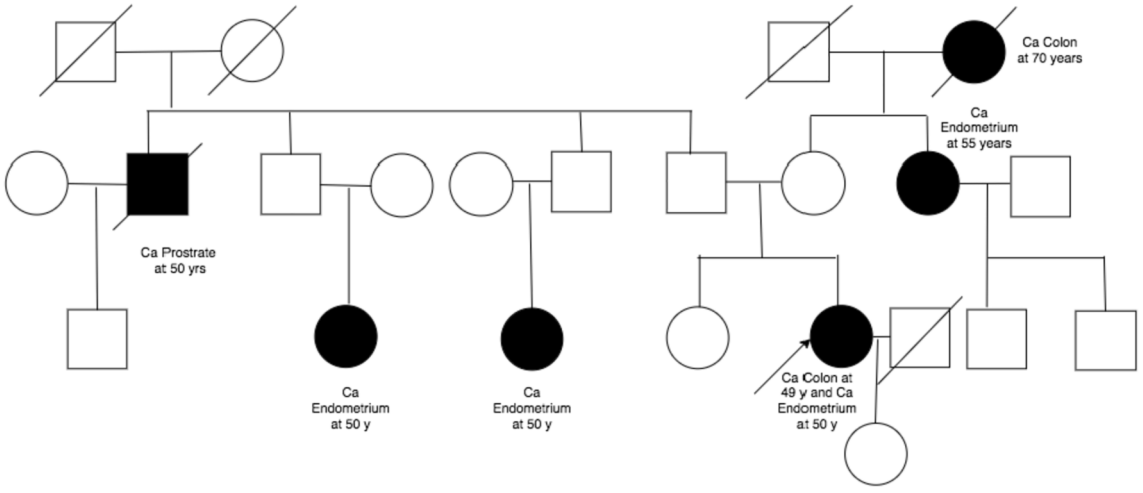

Fig. 1. Pedigree chart showing affected individuals in patient's family across three generations.

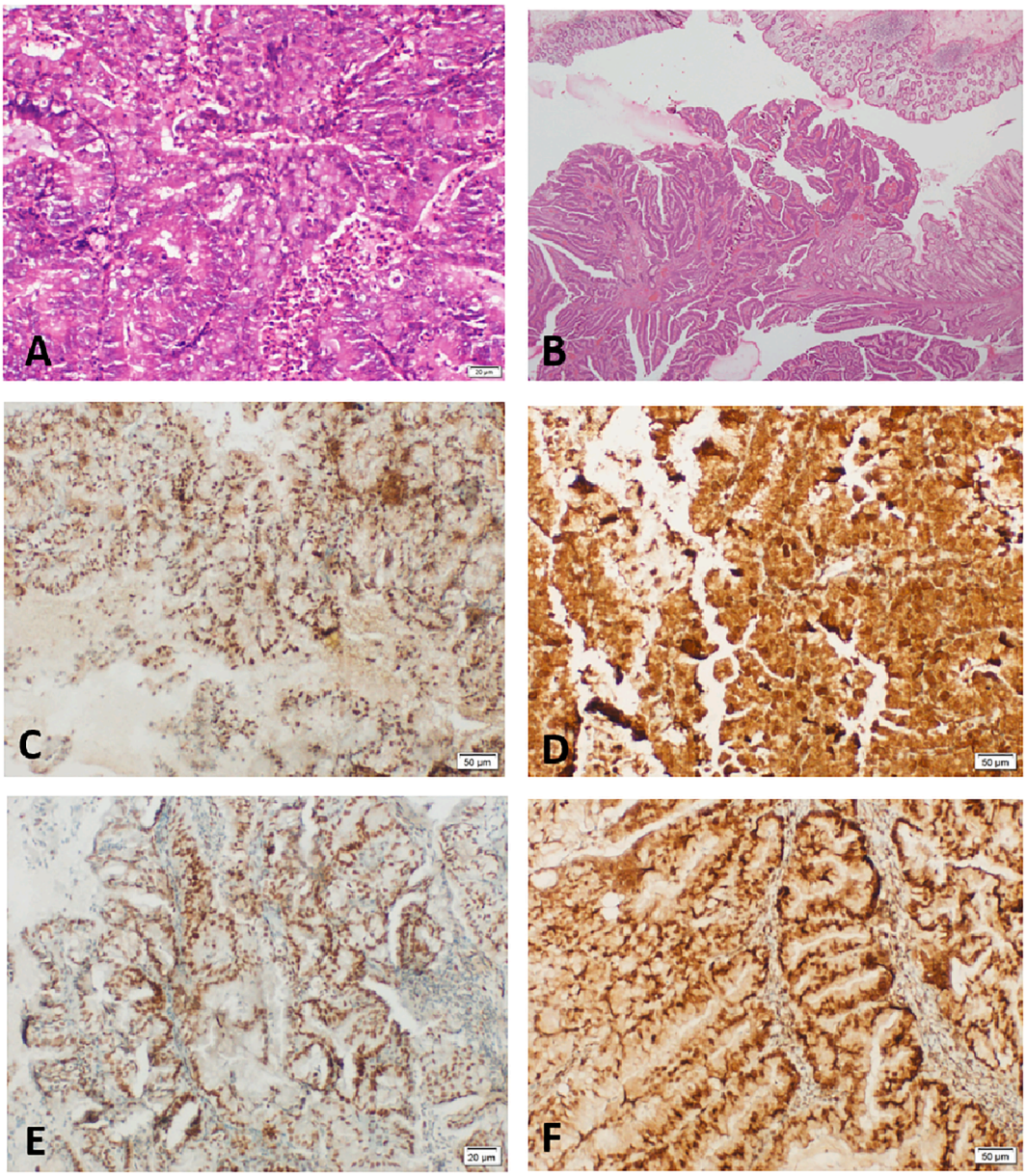

Fig. 2. Image panel with A) endometrial biopsy and B) colorectal histopathology specimen showing adenocarcinoma; C) MLH1, D) PMS2, E) MSH2 and F) MSH6 immunohistochemistry showing retained expression. (A, B Hematoxyline-eosin stain; CF, immunoperoxidase stain).

4. Conclusion

Preliminary negative screening for lynch syndrome in a patient with strong family history and clinical suspicion warrants further evaluation by NGS. Latest society recommendations even advise genetic testing forthwith. Conferring a definite diagnosis would help in clinical decision-making regarding surveillance protocols and risk-reducing surgery for the patient and family members. It would also add significantly in deciding post-operative chemotherapy regimens and counseling regarding prognosis of the disease. Of particular interest is the recent approval of Pembrolizumab (anti-programmed cell death-1 therapy) for MMR deficient metastatic colorectal cancer (Le et al., 2017). The drug has been shown to be efficacious in non-colorectal solid tumors as well, such as endometrial cancer and may dictate the choice of post-operative chemotherapy in the future (Rousset-Rouviere et al., 2021).

CRediT authorship contribution statement

Prerana Nagabhushana: Conceptualization, Writing – original draft. **Snigdha Kumari:** Conceptualization, Supervision, Writing – review & editing, Visualization. **Minakshi Rohilla:** Conceptualization, Supervision. **Radhika Srinivasan:** Investigation, Supervision, Writing – review & editing. **Aashima Arora:** Conceptualization. **Pulkit Rastogi:** Investigation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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