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ANALYTICAL ASPECTS OF HEREDITARY NONPOLYPOSIS COLORECTAL CANCER (HNPCC) OR LYNCH SYNDROME: AN OBSERVATIONAL SURVEY ON INDIAN POPULATION

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ABSTRACT

Background: Subjects having Lynch syndrome do not show any distinct features apart from increased evidence of adenocarcinomas. As Lynch syndrome lacks any distinguished phenotypic feature, its diagnosis is challenging.

Aims: The present study aimed to reveal the clinical presentation of Lynch syndrome and to assess if there is a successive decrease in the age of presentation with the generations. The study also aimed to assess if MLH1 mutation acts as a founder mutation for Lynch syndrome and if genetic alteration constitutes the clinical presentation of the disease.

Materials and Methods: The Study included 12 families (40 subjects) having colorectal cancer under the age of 50 years or at least two relatives (first/second degree) who had a history of colorectal cancer at any age The TaqMan SNP genotyping assay was done in all the subjects and controls to assess the missense mutation in gees (MSH1, MLH2, MSH6, and PSM 2). The collected data were statistically analyzed.

Results: Only 1 individual among the controls was carrying MLH1 mutation. Concerning the haplotype analysis, in the selected 12 families a region of genes in a range of 1-3 Mb was characterized with crossovers present. A shared haplotype was observed between upstream as well as downstream haplotypes in the assessed families. This sharing of haplotype implied that in different families compared, there are mutation events different from the recombination, occurring in the markers of these families. The NREM model was applied along with the COX-R model and these models showed that there was a constant decrease in the consecutive generation regarding the mean age of first-time cancer diagnosis. This decrease in the present study was shown to be by 2.2 years in consecutive generations in the whole study cohort.

Conclusion: The results of the present study conclude that the clinical presentation of Lynch syndrome depends on the gene mutated, environmental factors, and personal factors (age and

gender). Although the Lynch syndrome incidence is lower globally, with the recent advances in diagnostic criteria and surveillance, more cases with different clinical pictures are identified recently. There was also seen a decrease in the age of detection of the first cancer in Lynch syndrome in successive generations.

Keywords: CRC, Founder mutation, genetics, Haplotype, Lynch syndrome, mismatch repair genes, Missense mutation, MLH 1. MSH2.

Keynote: Although genetic heterogeneity is associated with Lynch syndrome, a great variation is seen in the phenotypic presentation of the disease. This heterogeneity can be attributed to environmental alterations and genetic variations. This heterogeneity makes the diagnosis as well as treatment for Lynch syndrome difficult. In clinical representation, Lynch syndrome shows a very high risk for colorectal cancer in addition to ovarian cancer, cancer of the biliary tract, pancreas, brain, urinary tract, and stomach. Rarely, associated lesions of the skin can be encountered.

INTRODUCTION

Lynch Syndrome, which was earlier termed Hereditary Non-Polyposis Colorectal Cancer (HNPCC), is a hereditary carcinoma travelling in autosomal dominant form from generation to generation.[1] Lynch syndrome accounts for approximately 3-5% of the colorectal cancers that exist. The main characteristics of Lynch syndrome are associated with high tumour incidence in the ascending colon, early onset of colorectal cancer, a high number of extracolonic manifestations (small bowel, endometrium, renal pelvis/ureter cancers), and excessive metachronous and synchronous tumours.[2] Colorectal cancers associated with Lynch syndrome, are characterized by adenocarcinoma in the ratio of 1:1.[3]

Henry Lynch in 1966 was the first to describe Lynch syndrome as the syndrome following the autosomal dominant fashion with an average onset age of less than 45 years, having approx. equal prevalence in males and females, and associated strongly with the adenocarcinomas of the endometrium, colon, and stomach.[4]

Based on the criteria for its identification (Amsterdam criteria I and II), the association of small bowel/uterine pelvis or endometrial cancer, and association with the families and relatives, Lynch syndrome was originally explained as a familial clustering entity.[5] However, later the familial adenomatous polyposis was excluded and Bethesda Guidelines were proposed in 1997 and were later revised in 2004.[6] These guidelines were described based on tumour phenotype to identify patients at high risk of developing Lynch syndrome. Bethesda guidelines also recommended the MSI (microsatellites) testing criteria.[7] MSI are the repeated sequences found in tumour s with instability in their genome. The general guidelines of Amsterdam criteria and Bethesda guidelines do not fit all the subjects with Lynch syndrome. Also, the families which meet these guidelines and criteria may not show the genetic alterations in the mismatch repair genes (MMR).[8]

Although genetic heterogeneity is associated with Lynch syndrome, a great variation is seen in the phenotypic presentation of the disease. This heterogeneity can be attributed to environmental alterations and genetic variations.[9] This heterogeneity makes the diagnosis as well as treatment for Lynch syndrome difficult. In clinical representation, Lynch syndrome

shows a very high risk for colorectal cancer in addition to ovarian cancer, cancer of the biliary tract, pancreas, brain, urinary tract, and stomach. Rarely, associated lesions of the skin can be encountered. These include keratoacanthomas and sebaceous lesions, which might be indicative of Muir Torres (a rare variant associated with Lynch syndrome).[10,11]

Subjects having Lynch syndrome do not show any distinct features apart from increased evidence of adenocarcinomas. As Lynch syndrome lacks any distinguished phenotypic feature, its diagnosis is challenging. 12 Hence, a detailed history of the family and relatives concerning cancer, in a suspected subject is an important factor to reach a definitive diagnosis of Lynch syndrome. This makes the diagnosis and decoding of the pattern of disease easy for the clinician and the patient. To identify the inheritance pattern, the family pedigree for 2-3 generations along with age at the time of death and ethnicity should be judged.[13]

According to recent diagnostic criteria, Lynch syndrome is confirmed in patients with genetic rearrangements, point mutations, and germline mutations in five genes which are considered Mismatch repair genes (MMR). These genes are MutL homolog 1 (*MLH1*), MutS homolog 2 (*MSH2*), MutS homolog 6 (*MSH6*), post-meiotic segregation increased 1 (*PMS1*), and post-meiotic segregation increased 2 (*PMS2*).[14] In the recent past, another germline (EPCAM) is linked with Lynch syndrome, as a chromosome for EPCAM is located adjacent to that for MSH2. However, genetic testing is available widely for Lynch syndrome, it is limited to MLH 1 and MSH2 gene, as these two are the most frequently mutated. Early diagnosis is warranted despite low incidence globally, to reduce associated mortality and tumour s in affected individuals as well as family members.[15,16]

The present study was conducted to reveal the clinical presentation of Lynch syndrome and to assess if there is a successive decrease in the age of presentation with the generations. The study also aimed to assess if MLH1 mutation acts as a founder mutation for Lynch syndrome and if genetic alteration constitutes the clinical presentation of the disease.

MATERIALS AND METHODS

The present study was carried out at and utilizing the data obtained from different cancer institute of India. All the study subjects were asked for verbal as well as written informed consent. The mean age group of the study subjects was 43.6 years with the age range of 38 years to 54 years. A total of 40 subjects were included for the trial from 12 families. All the study subjects had MLH1 mutation with associated Lynch syndrome. To assess the prevalence a cohort of 250 individuals from previous records was collected, and individuals visiting the OPD (n=200) for routine check-up purposes were randomly included to serve as the controls for the study. Taqman SNP genotyping assay[17] was done in all the subjects and controls to assess the MLH1 mutation. Microsatellites (19) which were polymorphic and were present around the MLH1 gene were subjected to PCR for amplification using electrophoresis. The patients were regularly assessed from the time they first presented with cancer.

The subjects were enrolled in the study based on the following inclusion criteria where the tumour tissue from a colorectal cancer patient was available to be analyzed for MSI mutation, colorectal cancer was diagnosed in one of the members from included 12 families before the age of 50 years or if at least two relatives (first/second degree) had a history of colorectal cancer at any age. In subjects where tissue was not available then for the screening of the

mutation, at least one member should be present. The included 12 families with 20 subjects met these inclusion criteria and hence, were included in the study. Among the study subjects, there were individuals with no positive family history or relatives with colorectal cancer. These subjects were detected with colorectal cancer before the age of 50 years.

A complete medical and family history was recorded for all the subjects, after which they were divided, based on the Amsterdam criteria either I or Amsterdam criteria II or as a non-Amsterdam family. Those included in Amsterdam criteria were the families which had 3 individuals diagnosed with colorectal cancer including one relative and others younger than 50 years of age or had other associated cancer as per the Amsterdam criteria II. Among included 40 subjects 28 fulfilled the Amsterdam criteria and the remaining 12 were considered non-Amsterdam families. Ethical Clearance for the study was obtained from the institutional Ethical Review Board.

The MSI testing was done following the Bethesda guidelines from the dissected tumour tissues. At least one tissue sample from each family was taken. Because of suspicion about sporadic cancer in older individuals, the youngest member was chosen for tumour analysis from the family. MSI testing was done based on conventional MSI testing. MLH1, MSH2, MSH6, and PMS2 assessment was done using immune-histochemistry. The patient's records were thoroughly analyzed to detect the history of any surgery for cancer (hysterectomy/ polypectomy). For mutations, the described testing was done. The data stratification was done for age, gender, gene mutation, and primary cancer. The collected data were statistically analyzed.

RESULTS

Concerning the prevalence study included 250 cases from the previous medical records and 200 controls who visited the hospital OPD for routine check-ups were considered. Only 1 individual among the controls was carrying MLH1 mutation. Concerning the haplotype analysis, in the selected 12 families a region of genes in a range of 1-3 Mb was characterized with crossovers present. A shared haplotype was observed between upstream as well as downstream haplotypes in the assessed families. This sharing of haplotype implied that in different families compared, there are mutation events different from the recombination, occurring in the markers of these families. The mutation in the MLH1 gene was found in all the samples evaluated. The MLH1 mutation is found universally around the globe even though it shows a very low allele frequency. The isolated population showed a more marked effect of these mutations than the heterogeneous individuals.

The mean age of the study subjects when cancer associated with Lynch syndrome or colorectal-associated cancer was 50 years and this reference age of the first diagnosis was considered in this assessment. When this age was assorted based on the mutated genes i.e., MLH1, MSH2, MSH6, and PMS2 genes, the mean age was different for all the mutated genes. For these genes, the mean age was 49 years, 51 years, 56 years, and 62 years respectively for MLH1, MSH2, MSH6, and PMS2 genes. The NREM model was applied along with the COX-R model and these models showed that there was a constant decrease in the consecutive generation regarding the mean age of first cancer diagnosis. This decrease in the present study was shown to be by 2.2 years in consecutive generations in the whole study cohort. The stratification of the study subjects when done based on the genes which were

mutated, then for the effects of anticipation, the assessed confidence interval for PMS2, and MSH2 was shown to be away from null values, whereas, the anticipation effect was less obvious for MSH6 and MLH1 gene. The anticipatory effects of genes in the consecutive generation are summarized in Table 1 and Table 2 based on NREM and COX-R criteria respectively.

As in various previous studies in the literature, the tumour spectrum in subjects with lynch syndrome was analyzed in the present study along with the tumour frequencies of various tumour s associated with Lynch syndrome. The relative frequencies of various tumours were analyzed except for endometrial cancer and colorectal cancer, with these being the most common cancers. In the study cohort, the most common cancer among males was the cancers of the urinary tract, small bowel cancer, and gastric cancer. While in females, skin cancer, and ovarian cancers were found commonly.

Concerning the genes where a mutation in Lynch syndrome is found, the number of study subjects with PMS2 Had few mutations and hence this gene was not studied/investigated further in the study. Patients having mutated gene MSH6 showed a 9% higher risk of developing cancer of the upper GI tract. The subjects with MLH1 mutation had a higher number of small bowel, pancreas, and gastric cancers. Study subjects with MSH 2 gene mutation had a high frequency of ovarian (females), skin (females), urinary tract, small bowel, and urinary tract cancer.

DISCUSSION

The present study aimed to reveal the clinical presentation of Lynch syndrome and to assess if there is a successive decrease in the age of presentation with the generations. The study also aimed to assess if MLH1 mutation acts as a founder mutation for Lynch syndrome and if genetic alteration constitutes the clinical presentation of the disease... Concerning the genes where a mutation in Lynch syndrome is found, the number of study subjects Same as mentioned above. Patients having mutated gene MSH6 showed a 9% higher risk of developing cancer of the upper GI tract. The subjects with MLH1 mutation had a higher incidence of small bowel, pancreas, and gastric cancer. Study subjects with MSH 2 gene mutation had a high frequency of ovarian (females), skin (females), urinary tract, small bowel, and urinary tract cancer.

MSH 2 mutation was seen in the study subjects, the similar results were shown by the other studies conducted in the population of Portugal in the study by Pinheiro M et al in 2013.[18]This study showed recurrent mutations. In the present study as well as the study by Pinheiro M et al[18] the MSH2 mutation acted as the founder mutation in compliance with the MLH1 mutation. These findings were also consistent with the findings by Tomsic J et al in 2012.[19]The evaluation of the risk for developing cancer in Lynch syndrome can be easily assessed by considering these recurrent and founder mutations as a tool for risk assessment, in addition to Single nucleotide polymorphism seen in the shared haplotypes as found in the present study. These shared haplotypes with a missense mutation of the MLH1 gene are found to be linked to extracolonic cancers, and this finding was shared between the present study and the study by Minde DP et al in 2011.[20]

The present study also showed MSH2 mutation to be highly linked with colorectal cancer in males and ovarian cancer in females. In the study cohort, it was also seen that a higher risk for ovarian cancer was associated with a missense mutation in genes MSH2 and MSH 6, whereas, was not found associated with MLH1 mutation. A similar rise in ovarian cancer was

seen associated with MSH2 mutation in the study by Stuckless S in 2007.[21] Although ovarian cancer is less common than cervical cancer, its prevalence is higher in Lynch syndrome as confirmed by Coburn SB in 2017.[22]

Apart from the genetic implications, despite various advances in the diagnostic criteria, the mechanism behind these anticipations in genetics remains largely unknown. A molecular mechanism is required to break the lock of the mechanism behind the hereditary transmission of cancers in Lynch syndrome. Nowadays, surveillance for Lynch syndrome is started at an earlier age before the cancer diagnosis, and this might be the reason for the decrease in the age of cancer diagnosis in successive generations with Lynch syndrome.

The present study also indicated a relative increase in the cancers of the small bowel concerning Lynch syndrome. Also, an increase in the cancers of the bile duct, stomach, gall bladder, duodenum, and pancreas was seen. These findings were correlated with the findings of the study by Ramsoekh D et al[23] 2009 and Engel C et al[24] 2012 where an increased risk of bowel cancer was associated with Lynch syndrome. Recently, another prospective study by Dominguez-Valentin M et al[25] in 2020 was found consistent with the present study where upper GI tract cancer risk was associated with Lynch syndrome, and this increased risk was also linked to the genetic implications. The missense mutation in genes MHS2 and MLH1 was linked with the increased risk of upper GI tract cancers by 7% in Lynch syndrome.

Although ovarian cancer is less prevalent and more lethal in females when compared to uterine cancer, breast cancer, and cervical cancer, this was contradicted in the present study. However, the results of the present study depicted these findings, where a higher prevalence of ovarian cancer was found in females with Lynch syndrome and mutation in genes MSH2 and MSH6. Similar findings were reported by the study ofMøller P et al[26] in 2018where subjects with MSH2 and MSH6 mutations had a higher prevalence of ovarian cancer in premenopausal age by 17% compared to controls, and MSH2 mutation, besides, was found to be associated with increased uterine cancer.

Concerning the association of skin cancers with Lynch syndrome, non-melanoma cancer was found higher in females with Lynch syndrome. Muir-Torre is well linked to Lynch syndrome which presents as a malignancy of the GI tract and sebaceous gland. An increase in pancreatic cancer was seen in the study subjects. These findings were consistent with the findings of Bansidhar BJ et al[27] in 2012 and Kastrinos F et al[28] in 2009. Similar mutations were seen in the study of Møller P et al[26] in 2018. Whereas, contradicted results were reported by Barrow E et al in 2009 where authors reported the mutation in different germline mutations.

CONCLUSION

The results of the present study conclude that the clinical presentation of Lynch syndrome depends on gene mutation, environmental factors, and personal factors (age and gender). Although the Lynch syndrome incidence is lower globally, with the recent advances in diagnostic criteria and surveillance, more cases with different clinical factors are recently described in the literature. There was also seen a decrease in the age of detection of the first cancer in Lynch syndrome in successive generations. These findings are of particular importance for genetic counseling and better public health screening programs.

The missense mutation in genes such as MSH1, MLH2, MLH6, and PMS2 is associated with the different colorectal cancers based on the mutated gene. Also, associated cancers of the upper GI tract and female reproductive organs were highly associated with Lynch syndrome. Despite advances in diagnostic tools for Lynch syndrome, a definitive diagnosis of the syndrome is still lacking. It can be attributed to heterogeneity in the phenotype of study subjects and environmental factors. The role of genetics is also debated largely in literature, with few studies reporting no correlation of genetics with the clinical picture.

The decrease in the age of diagnosis in successive generations can be attributed to the improvement in diagnostic tools. The study has a few shortcomings including the smaller sample size, uniform sample with similar geographical and environmental factors of the study subject, similar study design for all the subjects, and similar uniform criteria for inclusion.

Future studies should include wider genetic involvement with larger study samples from different geographical areas and different environments. The subjects should be monitored for a longer duration to reach definitive conclusions.

REFERENCES

1. Peltomaki P, Olkinuara A, Nieminen TT. Updates in the field of hereditary nonpolyposis colorectal cancer. J Gastent Hepat. 2020;14:702-20.
2. Weinberg BA, Marshall JL, Salem ME. The growing challenge of young adults with colorectal cancer. Oncology (Williston Park) 2017;31:381–9.
3. Bhattacharya P, McHugh TW. Lynch syndrome Last updated October 6, 2017, StatPearls [Internet]. Treasure Island FL: StatPearls Publishing; 2018.
4. Cohen SA, Leininger A. The genetic basis of Lynch syndrome and its implications for clinical practice and risk management. Appl Clin Genet. 2014;7:147–58.
5. H.T. Lynch, C.L. Snyder, T.G. Shaw, C.D. Heinen, M.P. Hitchins. Milestones of Lynch syndrome: 1895–2015. Nat. Rev. Cancer. 2015;15:181-94.
6. Lynch PM. History Of Hereditary Nonpolyposis Colorectal Cancer Or "Lynch Syndrome. Revista Médica Clínica Las Condes. 2017;28:500-11.
7. Kawakami H, Zaanan A, Sinicrope FA: Microsatellite instability testing and its role in the management of colorectal cancer. Curr Treat Options Oncol 2015;16:30.
8. Giardiello FM, Allen JI, Axilbund JE, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-society Task Force on colorectal cancer. Am J Gastroenterol. 2014;109:1159–79.
9. Hahn MM, de Voer RM, Hoogerbrugge N, Ligtenberg MJ, Kuiper RP and van Kessel AG: The genetic heterogeneity of colorectal cancer predisposition - guidelines for gene discovery. Cell Oncol (Dordr). 2016;39:491–510.
10. 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.
11. Cox VL, Saeed Bamashmos AA, Foo WC, Gupta S, Yedururi S, Garg N, Kang HC. Lynch Syndrome: Genomics Update and Imaging Review. Radiographics. 2018; 38:483–99.
12. You YN, Lee LD, Deschner BW, et al: Colorectal cancer in the adolescent and young adult population. JCO Oncol Pract. 2020;16:19-27.

13. Kalady MF, Heald B. Diagnostic Approach to Hereditary Colorectal Cancer Syndromes. Clin Colon Rectal Surg 2015;28:205-14.
14. Liccardo R, De Rosa M, Izzo P, Duraturo F. Novel MSH2 splice-site mutation in a young patient with Lynch syndrome. Molecular medicine reports. 2018;17:6942–6.
15. Tutlewska K, Lubinski J, Kurzawski G. Germline deletions in the EPCAM gene as a cause of Lynch syndrome. Hered Cancer Clin Pract. 2013;11:9.
16. Mehta A. Gupta G. Lynch syndrome—It's time we start detecting it. J Curr Oncol. 2018;1:55-60.
17. Ding, R. Chen, G. Chen, M. Li, J. Wang, J. Zou, F. Du, J. Dong, X. Cui, X. Huang, Y. Deng, Z. Tang, 135 One-step colorimetric genotyping of single nucleotide polymorphism using probe-enhanced loop136 mediated isothermal amplification (PE-LAMP), Theranostics. 2019;9:3723-31.
18. Pinheiro M, Pinto C, Peixoto A, Veiga I, Mesquita B, Henrique R, et al. The MSH2 c.388_389del mutation shows a founder effect in Portuguese Lynch syndrome families. Clin Genet. 2013;84:244-50.
19. Tomsic J, Liyanarachchi S, Hampel H, Morak M, Thomas BC, Raymond VM, et al. An American founder mutation in MLH1. Int J Cancer. 2012;130:2088-95.
20. Minde DP, Anvarian Z, Rudiger SG, Maurice MM. Messing up disorder: how do missense mutations in the tumour suppressor protein APC lead to cancer? Mol Cancer. 2011;10:101.
21. Stuckless S, Parfrey PS, Woods MO, Cox J, Fitzgerald GW, Green JS, et al. The phenotypic expression of three MSH2 mutations in large Newfoundland families with Lynch syndrome. Familial cancer. 2007;6:1-12.
22. Coburn SB, Bray F, Sherman ME, Trabert B. International patterns and trends in ovarian cancer incidence, overall and by histologic subtype. Int J Cancer. 2017;140:2451-60.
23. Ramsoekh D, Wagner A, van Leerdam ME, Dooijes D, Tops CM, Steyerberg EW, et al. Cancer risk in MLH1, MSH2, and MSH6 mutation carriers; different risk profiles may influence clinical management. Hereditary cancer in clinical practice. 2009;7:17.
24. Engel C, Loeffler M, Steinke V, Rahner N, Holinski-Feder E, Dietmaier W, et al. Risks of Less Common Cancers in Proven Mutation Carriers With Lynch Syndrome. Journal of Clinical Oncology. 2012;30:4409-15.
25. Dominguez-Valentin M, Sampson JR, Seppälä TT, ten Broeke SW, Plazzer J-P, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. Genetics in Medicine. 2020;22:15-25.
26. Møller P, Seppälä TT, Bernstein I, Holinski-Feder E, Sala P, Gareth Evans D, 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.
27. Bansidhar BJ. Extracolonic manifestations of lynch syndrome. Clin Colon Rectal Surg. 2012;25:103-10.
28. Kastrinos F, Mukherjee B, Tayob N, Wang F, Sparr J, Raymond VM, et al. Risk of pancreatic cancer in families with Lynch syndrome. JAMA. 2009;302:1790-5.

TABLES

Mutated Gene	NREM (anticipation effects in consecutive generation)	p-value
MLH1	-1.84	0.042
MSH2	-2.68	0.006
MSH6	-1.67	0.384
PMS2	-7.21	0.018

Table 1: Anticipatory effects of genes in the consecutive generation (based on NREM)

. Mutated Gene	COX-R (anticipation effects in consecutive generation)	p-value
MLH1	0.125	0.142
MSH2	0.268	0.003
MSH6	-0.007	0.985
PMS2	-0.592	0.064

Table 2: Anticipatory effects of genes in the consecutive generation (based on COX-R)