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Biography: Mutated hMLH1 Gene in Bangladeshi Gastric Cancer Patients: Its Relevance to Clinicopathological Factors

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Abstract: Like all cancers, Gastric cancer (GC) is also known to be of genetic origin. Genes are mutated in every steps of cell division, among them hMLH1 is a DNA mismatch gene which plays important role during carcinogenesis. This study was chalked out to find out the status of mutated hMLH1 Gene and its clinicopathological relevance among Bangladeshi gastric cancer patients. It was a cross sectional study conducted during January 2015 to April 2016. Tissue extracted from gastrectomy specimen of carcinoma stomach patients sent to specified laboratory. In the laboratory after DNA extraction, PCR, sequencing and analysis was done. In this study out of 19, exons 7 and 8 and introns 8 and 9 were studied after primer designing for hMLH1 gene mutation. After analysis, mutated gene were matched with clinicopathological parameters like age, sex, location of tumour, types and grade of tumour to see their relevance. Out of 45 patients, mutation was found in 15 patients (33.3%). Most gene alteration was found in elderly patients, more in male. Antral growth had more (80.0%) mutation and in ulceroproliferative type (66.7%) ($p < .005$). Mutation was common in intestinal type (73.3%) of GC. hMLH1 gene mutation found more in the moderately differentiated carcinoma patients. Factors like age, sex, morphology, Lauren's type, grading, personal habits etc. might play pivotal role in the development of gene mutation. Multi-centre large study are required to extract more relevant information in this regard.

Keywords: Hmlh1 Gene Mutation, Gastric Cancer, Clinicopathological Relevance

1. Introduction

Gastric cancer (GC) is the third leading cause of cancer related death and fifth most common malignant neoplasm across the world [1]. It is well established fact that the prognosis of stomach cancer is generally poor, because it affects the much elderly group of people and a good number of the patients get metastasis when they present [2]. The average five-year survival rate for stomach cancer is reported to be less than 10 percent [3]. Almost 300 genes are linked to outcomes in stomach cancer with both favourable and unfavourable genes. Unfavourable genes are high expression

genes- related to poor survival and favourable genes are are responsible for longer survival when they are associated with the disease process [4]. It is now widely accepted that gastric cancer develops through accumulation of different genetic and epigenetic alteration involving oncogene activation, tumour suppressor genes mutations, DNA mismatch repair genes mutation and genomic instability [5-8].

Cells contain many normal genes that are involved in regulating cell proliferation. Some of these genes can be mutated who promote uncontrolled cell proliferation. The normal forms of these genes are called proto-oncogenes, while the mutated, cancer-causing forms are called oncogenes. Mutations that converts normal genes to proto-oncogenes typically increase the activity of the encoded protein or increase the expression of the normal gene. Only one copy of the gene needs to be mutated in order to promote cancer [9, 10].

Tumour suppressor genes can be defined as genes which encode proteins that normally inhibit the formation of tumours. Their normal function is to inhibit cell proliferation, or act as the "brakes" for the cell cycle. Mutations in tumour suppressor genes contribute to the development of cancer by inactivating that inhibitory function [11].

DNA mismatch repair is a system for recognizing and repairing erroneous insertion, deletion, and misincorporation of bases that can arise during DNA replication and recombination, as well as repairing some forms of DNA damage.

The genomic instability pathways mentioned in the literature till date are of two types: chromosomal instability (suppressor pathway) and microsatellite instability (mutator pathway)[11, 12]. In gastric cancer, Microsatellite instability (MSI) is caused by mutations in the main DNA mismatch repair (MMR) genes hMLH1 and hMSH2, and less frequently in hMSH6, hPMS1 and hPMS2, MED1, RAD50, BLM, ATR, MRE11 genes.

hMLH1 gene:

Official name of hMLH1 gene is mutL homolog 1 gene. It is composed of 19 exons spanning in a region of 57360bp. MLH1 is a protein involved in the mismatch repair process after DNA replication.

The MLH1 gene is a member of a set of genes known as the mismatch repair (MMR) genes. It provides instructions for making a protein that plays an essential role in DNA repair [13]. This protein helps to fix mistakes that are made when DNA is copied (DNA replication) in preparation for cell division. The hMLH1 protein joins with another protein called PMS2 (produced from the PMS2 gene), to form a protein complex. This complex coordinates the activities of other proteins that repair mistakes made during DNA replication. The repairs are made by removing a section of DNA that contains mistakes and replacing the section with a corrected DNA sequence. The inactivation of these genes result in increased genetic instability, and in turn leads to an increased rate of mutation in the process of 'gatekeeper' genes that regulate cell proliferation and death. Impairment of MMR can occur [1] by mutational inactivation of one or two MMR genes or [2] by epigenetic inactivation of MMR genes. In gastric cancer, functional inactivation of MMR is mainly caused by latter [14]. In Bangladesh we don't have any study of any of the genes linked to gastric cancer. The aim of this study was to see and document the status of alteration of hMLH1 gene in gastric adenocarcinoma and to find its relationship with different clinical and pathological factors of the affected patients.

2. Materials and Methods

This was a cross sectional study conducted at Department of Surgical Oncology of National Institute of Cancer Research & Hospital (NICRH), Dhaka from January 2015 to April 2016. Laboratory facilities were taken from department of Genetic Engineering and Biotechnology, Dhaka University under a Memorandum of understanding (MOU) with the said department. Study population were the Patients of adenocarcinoma stomach who were admitted in for surgical intervention. Every alternate patients were enrolled for this study. Out of 92 admitted patients for surgery 45 patients were finally selected for the study. Ethical approval was taken from the institutions ethical approval committee.

2.1. Procedure

Normal and tumor tissues were extracted from the specimen immediately after resection. The specimen surface was washed with saline fluid prior to fragment extraction to avoid DNA contamination. Areas of tissue extraction from the specimen were demarcated for routine pathological examination. Only tissue fragments containing suspected tumor tissue were included for hMLH1 analysis. Normal areas were used as controls. Tissues were stored at -80°C before DNA purification and extraction. After that tissue was sent to Department of Genetic Engineering and Biotechnology, Dhaka University for PCR, sequencing and analysis.

2.2. PCR Amplification

Oligonucleotide primers for hMLH1 from the long arm of chromosome 18 was designed on the basis of published sequences (D18S55, D18S58, D18S61, D18S64, and D18S69). PCR-based dinucleotide repeat assays was carried out in 96-well plates for 30 cycles; each cycle was carried out at 95°C for 30 seconds, 50°C for 1 minute, and 70°C for 1 minute. Two volumes of stop buffer (95% of formamide, 20 uM sodium hydroxide, and 0.05% bromophenol blue and xylene cyanate) was added at the end of the amplification, plates were boiled in a water bath for 10 minutes at 100°C, and the samples was loaded onto 7% polyacrylamide gels containing 32% formaldehyde and 5.6 M urea.

2.3. Sequencing

Primers for MLH1 exon 7 and 8 was used after adjusting the proper primer designing. After DNA sequencing data analysis were done. After finding the genetic changes it was

matched with clinicopathological profile like age and sex, blood group, tumor characters, types, morphology and location of the tumor. Environmental factors like smoking and nutritional factors like extra salt intake was taken into consideration to find association with the gene changes. Data was compiled in written in structured data sheet and later analysed by using Standard software.

2.4. Primer Sequences of Hmlh1 Gene

MLH1_5 Forward	5 ′-GCTCTGACATCTAGTGTGTG-3 ′
MLH1_5 Reverse	5 ′-TGAAGACTTAGCAACACGA-3 ′

2.5. Statistical Analysis

Result of the study was calculated and analyzed by standard statistical method and was presented in forms of tables. For analysis of data, SPSS for Windows (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY:IBM Corp.) software was used. To see the association between categorical variables Chi squared test (or Fisher's Exact test when applicable) was performed. A value of $P < 0.05$ was considered statistically significant in all analyses.

Logistic regression analyses were performed to ascertain the effects of patients' age, gender, location of the tumour, morphology, Lauren's type, grading and smoking habit on the likelihood that patients have hMLH1 mutation. The logistic regression model was statistically significant ($\chi^2_{(df=6)} = 2.890$, $p < .05$).

3. Result

Mean age of the participating 45 patients was 53.91 years (Fig 1) Leading representation was from elderly patients (aged >60 years) followed by 41-50 years age group (p=.05). But no significant difference was observed in other age groups when the patients were stratified on the basis of hMLH1 mutation status. Gender distribution showed frequencies of mutations were male 11/34 vs 4/11 in number. (Table 1). Among the smokers (60%), 53.3% of the mutation of hMLH1 was found (p=0.05). It was evident from the above table that more patients developed cancer in antrum of the stomach (88.9%) followed by cardia (6.67%). However, no significant difference (p=.023) was noted between location of tumor and hMLH1 mutation status. Regarding morphology (48.9%) of the tumor, significant co relation was found in ulcero proliferative variety with gene mutation (p=.036). Most of the mutation found in intestinal variety (73.3%), but no significant association was noted between tumor type and hMLH1 mutation status (p=.454). Tumor grading had no significant association with the mutation.

Table 1. *Correlation between hMLH1 mutation and clinicopathological parameters of ca stomach patients.*

	Presentn (%)	**Absentn (%)**	**Total**	**p-value**
Age group				
≤30	0 (0.0)	3 (10.0)	3 (6.7)	-
31-40	2 (13.3)	5 (16.7)	7 (15.5)	-
41-50	3 (20.0)	8 (26.7)	11 (24.5)	0.05
51-60	4 (26.7)	4 (13.3)	8 (17.8)	0.705
>60	6 (40.0)	10 (33.3)	16 (35.5)	0.405
Total	15 (100.0)	30 (100.0)	45 (100.0)	0.420
Sex				
Male (n,%)	11 (73.3)	23 (76.7)	34 (75.6)	
Female (n,%)	4 (26.7)	7 (23.3)	11 (24.4)	
Total (n,%)	15 (100.0)	30 (100.0)	45 (100.0)	
Smoking				
Yes (n,%)	8 (53.3)	19 (63.3)	27 (60.0)	
No (n,%)	7 (46.7)	11 (36.7)	18 (40.0)	0.05
Total (n,%)	15 (100.0)	30 (100.0)	45 (100.0)	
Location				
Cardia	2 (13.3)	1 (3.3)	3 (6.7)	
Body	1 (6.7)	1 (3.3)	2 (4.4)	0.233* (NS)
Antrum	12 (80.0)	28 (93.4)	40 (88.9)	
Total	15 (100.0)	30 (100.0)	45 (100.0)	
Morphology				
Ulcerative (n,%)	1 (6.7)	4 (13.3)	5 (11.1)	
Proliferative (n,%)	10 (66.7)	8 (26.7)	18 (40.0)	
Ulcero-proliferative (n,%)	4 (26.7)	18 (60.0)	22 (48.9)	0.036*(S)
Total (n,%)	15 (100.0)	30 (100.0)	45 (100.0)	
Lauren's type				
Intestinal (n,%)	11 (73.3)	25 (83.3)	36 (80.0)	
Diffuse (n,%)	4 (26.6)	5 (16.7)	9 (20.0)	0.454*(NS)
Total (n,%)	15 (100.0)	30 (100.0)	45 (100.0)	
Grading	1 (6.7)	1 (3.3)	2 (4.4)	0.576*(NS)
	6 (40)	16 (53.3)	22 (48.9)	
	8 (43.3)	13 (53.3)	21 (46.7)	
	15 (100.0)	30 (100.0)	45 (100.0)	

* Fisher's Exact Test; NS= Not significant

Table 2. *Logistic regression estimates of the effects of different background characteristics on hMLH1 mutation.*

Variables in the Equation	Coefficient (β)	OR [(Exp (β)]	p-value
Age			
<50 years (r)	-	1.00	
≥50 years	-1.280	0.94	0.064
Gender			
Female (r)	-	1.00	
Male	1.102	3.01	0.372
Location			
Cardia (r)	-	1.00	
Body	2.355	10.53	0.258
Antrum	1.469	4.35	0.489
Morphology			
Ulcerative	-	1.00	
Proliferative	0.385	1.47	0.771
Ulceroproliferative	1.812	6.12	0.045*
Lauren's type			
Intestinal	-	1.00	
Diffuse	-0.287	0.75	0.807
Grading			
Well differentiated (r)	-	1.00	
Moderately well differentiated	-0.722	0.49	0.745
Poorly differentiated	-0.628	0.53	0.503
Smoking habit			
No (r)	-	1.00	
Yes	-0.333	0.72	0.765

r = Reference category; OR= Odds Ratio; *significant at the p level of 0.05

Logistic regression analysis showed that in model explained 37.5% (Nagelkerke R^2) of the variances in hMLH1 mutation and correctly classified 66.7% of cases. Male patients were three times more likely to develop hMLH1 mutation than female patients. Lesions located in the body and antrum of the stomach have 10.5 & 4.3 times more chances to exhibit hMLH1 mutation respectively. Patients with ulceroproliferative morphology have 6 times more chance to express hMLH1 mutation than other morphology.

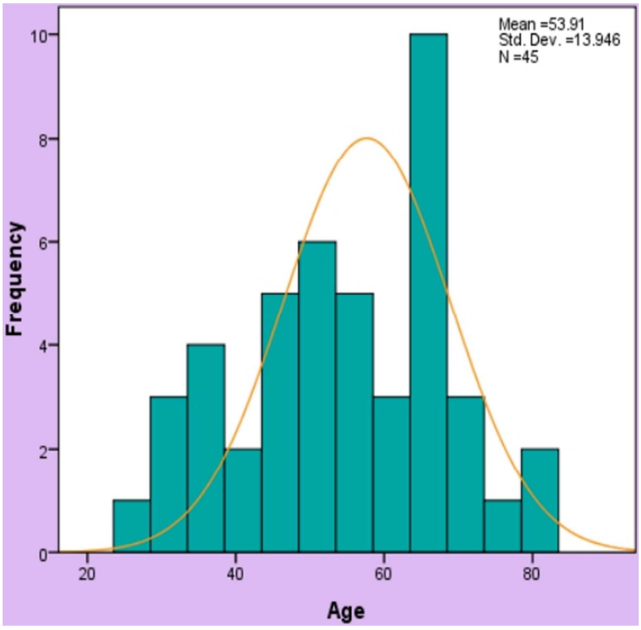

Figure 1. *Shows the age distribution of the patents. The mean age of the patients was 53.9 (±13.95) years.*

4. Discussion

To know the clinico-pathological relevance of altered hMLH1 gene in gastric cancer patients this study was conducted. In this series, 45 patients diagnosed as adenocacinoma of stomach were included. Mutation of hMLH1 gene was detected by PCR. Out of 19, exons 7 and 8 and introns 8 and 9 were studied. Mutation was found in 15 patients.

The current study shows that majority were >60 years of age and 6 mutations occurred in that age group followed by 51-60 years age group with 4 mutation. However, no significant difference was observed when the patients were stratified on the basis of hMLH1 mutation status (p=0.405). Though little series was found, in a study it was reported higher frequency of hMLH1 in gastric cancer patients aged between 50 and 59 years (five out of 65; 7.7%) than in controls (0.0%). However, it was not significantly associated with the risk of gastric cancer (P = 0.069) [15].

It has been also seen that out of 34 male patients 11 (73.3%) had features of hMLH1 mutation while among 11 female patients that number was 4 (26.7%). No significant difference was observed in this regard (p>0.5). Wenxian Zhi et al. in their study documented in their findings that an association between the MLH1 variation 2101 C>A and gastric cancer risk in males, and this may be of biological significance. It could be tentatively suggested that the MLH1 2101 C>A variation might increase the risk of gastric cancer in males [15].

Our study finding revealed that about two-third of the patients (60%) were smokers which had significant association with mutation. Xian-Qiu et al reported that there

were more regular smokers and drinkers in the gastric cancer patients (45.2 and 33.4%, respectively) than in the controls (29.1 and 25.0%, respectively), and the differences were significant (P<0.001 and P=0.002, respectively)[14].

It is evident that more patients developed cancer in antrum of the stomach (88.9%) followed by cardia (6.67%). Mutation found mainly in antral lesions 12 (80%). However, no significant difference was noted between location of tumour and hMLH1 mutation status. (p=0.233). Ping Liu and his group in a study showed that gastric cancer with MSI had a tendency to be located in the distal stomach compared to gastric cancer with MSS [16)].

About morphology, most of the tumours were ulcero-proliferative type (48.9%). The next common type was proliferative (40%) but mutation found mainly in proliferative type 10 (66.7%). However, no significant association was noted between tumour type and hMLH1 mutation status (p=0.036).

In the present study, intestinal type was common (36.80%) and mutation was also common in these group (11, 73.3%). In diffuse type group this mutation was only 26.7% but statistically these difference was not significant (p=0.454). There was no association between MSI and Lauren's classification in a study by Wirtz [17].

In our study majority of patients had moderately differentiated carcinoma (48.9%) then poorly differentiated carcinoma (46.7%) but MLH1 gene mutation was found mainly (53.3%) in poorly differentiated carcinoma patients. Study found no association was observed between MSI status and tumour grade [16, 17].

Limitation of the study:

There are very limited study found in the literature especially linked to clinical factors. Some studies are available on MSI. One study expressed that no association was observed between MSI status and age, gender, tumor grade, tumor location or lymph node spread. Gastric cancer with MSI had a tendency to be located in the distal stomach compared to gastric cancer with MSS. (Ping Liu,) In a study done in Slovenia regarding tumourogenesis that nucleotide changes, pathogenic mutations, and polymorphisms of the gene hMLH1 may have an important role in the development of gastric cancer with the MSI phenotype [18, 19].

5. Conclusion

This study showed that the occurrence of hMLH1 gene mutation in GC patients is prevalent in more than on third of the gastric cancer of Bangladeshi patients. The mutated gene doesn't have much corelation with clinicopathogical factors like tumour size, location, tumor grading but has relationship with morphology of the tumors. It has association with smoking. Future studies with larger sample sizes are needed to determine whether MMR-related gene like hMLH1 expression can be considered a useful marker in the early prediction of gastric cancers. In addition, future research will find out the relevance of other risk factors with the gene mutation.

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Conflict of Interest

All authors went through and approved the manuscript. This manuscript is not under consideration elsewhere. On behalf of all the authors, I confirm that there are no associated conflicts of interest.

References

[1] Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. Cancer incidence and mortality worldwide: Sources, methods and major Patterns in GLOBOCAN 2012. Int. J. Cancer.136:359-386.

[2] Orditura M, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM, et al. Treatment of gastric cancer . World Journal of Gastroenterology. 2014 20 (7): 1635–49.

[3] Cabebe EC, Mehta VK, Fisher G, Talavera F, Movsas M, McKenna R, et al. Gastric Cancer. Medscape Reference. WebMD. Archived from the original on 7 April 2014. Retrieved 4 April 2014.

[4] Mathias U, Cheng Z, Sunjae L, Evelina S, Linn F, Gholamreza B, Rui B, Muhammad A, Zhengtao L (2017-08-18). A pathology atlas of the human cancer transcriptome. Science. 357 (6352):

[5] Tahara E. Genetic pathways of two types of gastric cancer. IARC Scientific Publications 2004. 157: 327–349.

[6] Bandla S, Pennathur, Luketich AJD. Comparative genomics of esophageal adenocarcinoma and squamous cell carcinoma. Annals of Thoracic Surgery 2012. 93:1101–1106

[7] Smith MG, Hold GL, Tahara E & El-Omar EM. Cellular and molecular aspect of gastric cancer. World Journal of Gastroenterology 2006. 12: 2979–299.

[8] Ong CAJ, Lao-Sirieix P & Fitzgerald. Biomarkers in Barrett's esophagus and esophageal adenocarcinoma: predictors of progression and prognosis. World Journal of Gastroenterology 2010. 16:5669–5681.

[9] Hanahan D, Weinberg R. The Hallmarks of Cancer. Cell 2000. 100: 57-70.

[10] Nagini S. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. World J Gastrointestinal Oncology 2012. 4:156-169.

[11] Lauren P. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma, An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965. 64:31–49.

[12] Tamura G. Alterations of tumour suppressor and tumour-related genes in the development and progression of gastric cancer. World J Gastroenterology 2006. 12: 192–8.

[13] Wenxian Z, Binshuang X, Lifeng W, Nong X, Qiong H, Yaping W, et. al. The MLH1 2101C>A (Q701K) variant increases the risk of gastric cancer in Chinese males. BMC Gastroenterology 2011. 11:133.

[14] Available at https://ghr.nlm.nih.gov/gene/MLH1 accessed on 22.02.2018

[15] Xian-Qiu X, Wei-Da G, Shi-Zhi W, Zheng-Dong Z, Xiao-Ping R, Feng R et.al. Polymorphisms of mismatch repair gene hMLH1 and hMSH2 and risk of gastric cancer in a Chinese population. Oncology Letters 2012. 3: 591-598.

[16] Ping L, Xiao-Yong Z, Yun S, Dao-Fu Z. Microsatellite instability in gastric cancer and pre-cancerous Lesions. World Journal of Gastroenterology 2005; 11: 4904-4907.

[17] Wirtz HC, Muller W, Noguchi T, Scheven M, Ruschoff J & Gabbert HE, et al. Prognostic value and clinicopathological profile of microsatellite instability in gastric cancer. Clin Cancer Res 1998. 4:1749-1754.

[18] Hudler P, Voulk K, Liovic M, Repse S, Juvan R, Komel R. Mutations in the hMLH1 gene in Slovenian patients with gastric carcinoma. Clin Genet 2004: 65: 405–411.

[19] Peltomaki P. DNA mismatch repair and cancer. Mutat Res 2001: 488: 77–85.