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High Frequency of Microsatellite Instability among Non-Metastatic Gastric Cancer

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ABSTRACT

Background: Microsatellite instability (MSI) is considered a key factor in carcinogenesis and a genetic alteration pattern in many types of cancers such as gastric cancer (GC). Although the role of MSI in colorectal cancer (CRC) is well known, its prognostic impact on GC has not been clearly defined. The assessment of MSI in GC has not been documented in the Iranian population yet. Therefore, this study analyzed the association of MSI status with GC in Iranian patients.

Materials and Methods: We compared the frequency of MSI at 5 loci from formalin-fixed paraffin-embedded (FFPE) gastrectomy specimens, between metastatic and non-metastatic cases of GC (N = 60). A panel of five quasi-monomorphic markers and a single dinucleotide marker with linker-based fluorescent primers was used.

Results: MSI was observed in 46.6% of cases, including MSI-high (H) (33.3%) and MSI-Low (L) (13.3%). Moreover, the most unstable and stable markers in our study were NR-21 and BAT-26 accordingly. MSI-H and MSI were seen more frequently in non-metastatic tumors (p= 0.028 and p= 0.019, respectively).

Conclusion: The current study showed MSI status more frequently in non-metastatic GC which may reflect a good prognostic factor in GC like CRC. Although, larger and more comprehensive studies are needed to confirm this statement. A panel consisting of NR-21, BAT-25, and NR-27 mononucleotide markers appears to be reliable and useful markers for detecting MSI in GC in Iranian patients.

Keywords: Gastric cancer; Microsatellite instability; Metastatic; Fragment analysis

INTRODUCTION

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer death in the world with a 5-year survival rate of 29.6% [1,2]. Based on the findings of genetic and gene expression studies by the Cancer Genome Atlas (TCGA), GC is divided into four genomic subtypes, including tumors positive for Epstein–Barr Virus (EBV) infection, MSI tumors, genomically stable tumors (GS) and tumors with chromosomal instability (CIN)[3]. MSI has been a major issue in cancer research [4]. MSI is defined as the presence of alternate-sized repetitive DNA sequences that are not seen in the corresponding germline DNA [5]. Deletions are the most allelic shifts. Majority of these allelic shifts occur in 3´ UTR compared to coding regions[6]. The diagnosis of MSI-positivity often has important clinical implications in a variety of cancers, including

determination of prognosis, familial cancer risk assessment, and identifying susceptible individuals to developing cancer[7-9]. These tumors may share similar cancer genetic pathways and may lead to similar clinical outcomes[10]. Moreover, these tumors are reluctant to respond to common cancer treatments[11].

Historically, two distinct strategies have been used to determine MSI status and mismatch repair (MMR) role: 1) MSI analysis to determine instability in microsatellite markers and 2) immunohistochemistry (IHC) to identify loss of MMR protein expression[12]. Combination of MSI and IHC assessment can be best interpreted compared to the assessment of each factor alone. Separate evaluation of MSI and IHC is also performed to adapt to clinical situations. IHC cannot detect somatic and germline mutations[13]. PCR-based MSI analysis is a functional test with approximately 100% reproducibility. It can detect dMMR (deficiency mismatch repair) tumors with genetic defects beyond four MMR genes [12].

In contrast to CRC, few MSI studies have been performed on GC and clinical significance of MSI status in GC remains controversial. Some reports have shown associations between MSI-H in GCs and intestinal type histology, antral tumor location, prominent lymphoid infiltration, lower prevalence of lymph node metastasis, older age, and better prognosis [14, 15]. But, in other studies, the clinico-pathological characteristics in GCs were not significantly different between MSI-H and MSI-L/S tumors, and the findings were even the opposite[5]. Hence, the current study was designed to investigate the MSI and its relationship with clinico-pathological features of patients with GC.

MATERIALS AND METHODS

Subjects

This retrospective cohort study was approved by the Medical Ethics Committee of Tehran Azad University of Science and Research (IR.IAU.SRB.REC.1397.109) and conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

Examination of specimens from GC patients was performed based on the protocol by the College of American Pathologists [16]. All patients were treated with FLOT (Docetaxel, oxaliplatin, leucovorin, and 5-fluorouracil) protocol. Biological materials were provided by the Iran National Tumor Bank, which is funded by Cancer Institute of Tehran University of Medical sciences for Cancer Research.

All histologically confirmed Gastric Adenocarcinoma (GAC) patients that underwent curative total or partial gastrectomy in the Cancer Institute of the Imam Khomeini Hospital, Tehran, Iran from 2008 to 2018 were selected. Patients with insufficient data in their medical records were excluded from the study. Type of surgery, age at diagnosis, sex, size and tumor location, uni or multifocal Histological grade, stage, Lauren's classification, Lymphovascular or Perineural invasion, as well as number of involved Lymph nodes, and Metastasis status of the samples were reviewed. Sixty FFPE blocks, including 30 metastatic and 30 non-metastatic cases were recruited. Overall survival (OS) was measured from the date of primary diagnosis to death, or the last follow-up date. Disease-free survival (DFS) time was calculated from the date of primary diagnosis to disease recurrence, death, or the last follow-up date. All samples were sectioned for making slides. Samples were evaluated using the hematoxylin and eosin (H&E)-staining. Slides were reviewed by a pathologist and tumor and matched adjacent normal tissues (representing either cancer or margin cells) were punched for DNA extraction.

MSI status assessment using PCR-based methods

Genomic DNA was extracted using QIAamp DNA FFPE Tissue Kit (Qiagen, Germany). Both quality and quantity of extracted genomic DNA samples were evaluated using Nanodrop and subsequent gel electrophoresis. The primers that were used to amplify were described previously[17]. To reduce the cost for adding a fluorescent label at the end of each forward primers, a M13 linker was added to the 5´ end of forward primers (Table 1). Hence, in the second run of PCR, all PCR products were labeled using two universal fluorescently labeled M13 primers. PCR reaction was designed to perform in two separate steps. In the first run, the linker was added to all PCR products, and the second run was performed using 5´ fluorescently labeled M13 primer. The following two M13 linkers with different

dyes were used; 6-FAM for BAT-26, NR-22, NR-27, D3S1260 and HEX for BAT-25, NR-21.

The first PCR step was performed in a total volume of 15 µl, including 20-26 ng of DNA, 7.5 µl Master Mix Hot Start and 0.5 µmol/L of each primer. The PCR program initiated with denaturation in 15 minutes at 95°C followed by 25 cycles of denaturation at 95°C for 30 seconds, annealing at 62°C in 60 s, extension at 72°C for 60 second, and a final extension at 72°C for 20 minutes. The second PCR step was carried out as follows: 2 µl PCR product was mixed with 2 and 1 µmol/L of Forward Primer FAM and HEX, respectively. Then the mixture was added to 0.5 µmol/L of each Reverse primer, and 10 µl Master Mix Hot Start to reach the total volume of 20 µL for PCR reaction. The second PCR program included denaturing at 95°C for 15 minutes followed by 25 cycles of denaturing at 95°C for 30 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 10 minutes. Fragment analysis was done using ABI genetic analyzer 3500. Data were analyzed by Gene Marker V1.85 program. According to the Bethesda guidelines, the instability of two or more microsatellite markers were considered MSI-H.

Instability of one or no marker was considered MSI-L and MS-stable (MSS), respectively[18].

Statistical analysis

Clinical and pathological data were analyzed with IBM SPSS software for Windows®, version 26.0 (SPSS, Chicago, IL, USA). The association between MSI and categorical clinic-pathological variables was assessed using the Chi-squared, Fisher's exact or Monte Carlo tests. The student's *t*-test was used to compare continuous variables. The patient OS and DFS were calculated by Kaplan-Meier method, and a comparison between different subgroups was analyzed using Log-rank test. Cox regression model was used for assessment the hazard ratio (HR) from Univeriate and multivariate analysis. A P-value less than or equal to .05 was considered significant.

RESULTS

MSI analysis using quasimonomorphic markers

A pentaplex panel consisting of BAT-25, BAT-26, NR-21, NR-22, and NR-27 mononucleotide markers was used to determine MSI status via multiplex PCR. The D3S1260 dinucleotide marker was recruited to ensure that the tumor and normal samples matched with each other. Fragment analysis results are demonstrated in Figure 1. We established a quasi-monomorphic variation range (QMVR) for each marker based on matched normal DNA, as previously described [19], the mean size of each marker was ± 3 bp (Table 2). Accordingly, a microsatellite marker was considered unstable when its size did not fall within the QMVR. The mean size observed for each marker in the tumor sample is as follows: *NR27* (99-100bp), *NR21* (121-122bp), *BAT26* (132-133bp), *BAT25* (139-140bp), *NR22* (159-160bp).

In this study, NR-21 marker had the highest instability (21/60, 35%), while BAT-26 marker showed no instability. The instability in other markers were 18/60 (30%) for BAT-25, 15/60 (25%) for NR-27 and 15/60 (25%) for NR-22 (Table 1). MSI-H, MSI-L and MSS were detected in 20 (33.3%), 8 (13.3%), and 32 (53.3%) of the examined patients, respectively. Thus, instability was seen in 46.6% (28/60) of the tumors.

Clinico-pathological features

The medical records of all 60 patients were carefully examined. Majority of the patients (73.3%, 44/60) were male and 26.7% (16/60) were female. The mean age of the patients was 60.72 ± 13.14 years old (24 to 87 years old). The mean tumor size was 4.45 ± 2.92 cm (1 to 19 cm). The most common sites of metastasis were liver, peritoneum, lung, and bone. Clinico-pathological data and MSI status of the patients are shown in Table 3. No significant association was found between clinico-pathological parameters and MSI-H. MSI-H was more frequently observed in lower third gastric tumors (60 %, 36/60) and among non-metastatic tumors compared to metastatic patients with GC (70 %, 42/60 vs. 40%, 18/60, p= 0.028).

Clinico-pathological features were also compared pairwise between MSI-H and MSI-L, MSI-L and MSS, and MSI and MSS. Only a significant relationship was found between MSI and MSS, indicating that non-metastatic tumors were more unstable (p= 0.019).

Survival analysis

The survival analyses are presented in Figure 2. During the study period (36 months), 38 patients (63.3%) either were relapsed or died. The mean OS of the subjects was 17.58 (95% CI: 13. 62 -21.53) months. The mean DFS was 17.19 (95% CI: 13.16-21.21) months. In the patients, OS and DFS were correlated with a tumor size ≤ 6 cm (p=0.001), the location of the tumor in the middle or lower parts (*p*=0.03), tumor stage I or II (p=0.001), intestinal tissue type (p=0.01 and p=0.008, respectively), tumors without metastasis (p=0.001,) and without lymphovascular invasion (p=0.006). As shown in Table 4 and 5, these associations were also confirmed by univariate mode in the Cox regression model. Moreover, OS was associated with tumor stage (P=0.004) and metastasis tumors (*P*=0.04), and DFS was correlated with tumor stage (*P*=0.005) in the multivariate mode. Furthermore, the OS and DFS were more in patients with MSI-H compared to MSI-L/MSS, but these differences were no significant (mean: 22.55, 95% CI: 15.53- 29.57, P = 0.12 and mean 22: 95% CI: 14.76-29-24, P = 0.12, respectively). No correlation was seen between OS and DFS with MSI-H in the Cox regression model by univariate and multivariate analyzes (P>0.05).

Table 1: Sequence of oligonucleotide primers used

Primer	*Sequence*	*Product size*
BAT25	F: 5′-CAGGAAACAGCTATGACCTCGCCTCCAAGAATGTAAGT-3′ R: 5′-TCTGCATTTTAACTATGGC*TC-3′*	*142-144 bp*
BAT26	*F: 5′-TGTAAAACGACGGCCAGTTGACTACTTTTGACTTCAGCC-3′* *R: 5′-AACCATTCAACATTTTTAACCC-3′*	*136-137 bp*
NR21	*F: 5′-CAGGAAACAGCTATGACCTAAATGTATGTCTCCCCTGG-3′* *R: 5′-ATTCCTACTCCGCATTCACA-3′*	*118-119 bp*
NR22	*F: 5′-TGTAAAACGACGGCCAGTGAGGCTTGTCAAGGACATAA-3′* *R: 5′-AATTCGGATGCCATCCAGTT-3′*	*158-160 bp*
NR27	*F: 5′-TGTAAAACGACGGCCAGTAACCATGCTTGCAAACCACT-3′* *R: 5′-CGATAATACTAGCAATGACC-3′*	*104-105 bp*
D3S1260	*F: 5′-TGTAAAACGACGGCCAGTCTACCAGGGAAGCACTGTAG-3′* *R: 5′-CATGTACCTGAGCACCTACTG-3′*	*191-209 bp*
M13-HEX	*5′-CAGGAAACAGCTATGACC*	
M13-FAM	*5′-TGTAAAACGACGGCCAGT*	

Table 2: Sizes of the alleles for each marker

NR27			*NR21*			*BAT26*			*BAT25*			*NR22*		
Size(bp)	No.	%	Size(bp	No.	%	Size(bp)	No.	%	Size(bp)	No.	%	Size(bp)	No.	%
92	-	-	106	-	-	129	-	-	129	-	-	144	-	-
93	-	-	107	-	-	130	-	-	130	-	-	145	-	-
94	2	3.3	108	2	3.3	131	2	3.3	131	3	5	146	4	6.6
95	0	0.0	109	0	0	132	14	23.3	132	8	13.3	147	0	0.0
96	0	0.0	110	4	6.6	133	30	50	133	4	6.6	148	1	1.6
97	0	0.0	111	13	21.6	134	12	20	134	0	0.0	149	1	1.6
98	4	6.6	112	2	3.3	135	2	3.3	135	0	0.0	150	1	1.6
99	7	11.6	113	0	0.0	136	-	-	136	0	0.0	151	1	1.6
100	26	43.3	114	0	0.0	137	-	-	137	0	0.0	152	0	0.0
101	6	10	115	0	0.0				138	5	8.3	153	7	11.6
102	2	3.3	116	0	0.0				139	9	15	154	0	0.0
103	0	0.0	117	0	0.0				140	23	38.3	155	0	0.0
104	0	0.0	118	0	0.0				141	5	8.3	156	0	0.0
105	0	0.0	119	1	1.6				142	0	0.0	157	0	0.0
106	0	0.0	120	6	10				143	0	0.0	158	0	0.0
107	2	3.3	121	18	30				144	0	0.0	159	11	18.3
108	1	1.6	122	14	23.3				145	2	3.3	160	23	38.3
109	0	0.0	123	-	-				146	0	0.0	161	6	10
110	5	8.3	124	-	-				147	1	1.6	162	5	8.3
111	5	8.3							148	-	-	163	-	-
112	-	-							149	-	-	164	-	-
113	-	-												
unstable	15	25%		21	35%		0	0%		18	30%		15	25%
stable	45	75%		39	65%		60	100%		42	60%		45	75%

Table 3: Correlation between MSI status and clinic-pathological background in patients with GC

Variable		Total N=60	MSI-L/S n=40	MSI-H (n=20)	P
Age	≥60	24 (40.0%)	16 (40.0%)	8 (40.0%)	0.999‡
	>60	36 (60.0%)	24 (60.0%)	12 (60.0%)	
Gender	Male	44 (73.3%)	30 (75.0%)	14 (70.0%)	0.680
	Female	16 (26.7%)	10 (25.0%)	6 (30.0%)	
Tumor size	6cm≤	50 (83.3%)	33 (82.5%)	17 (85.0%)	0.999‡
	>6 cm	10 (16.7%)	7 (17.5%)	3 (15.0%)	
Tumor location	Upper	14 (23.3%)	11 (27.5%)	3 (15.0%)	0.683
	Middle	14 (23.3%)	9 (22.5%)	5 (25.0%)	
	Lower	32 (53.3%)	20 (50.0%)	12 (60.0%)	
Focal	Unifocal	57 (95.0%)	38 (95.0%)	19 (95.0%)	0.999‡
	Mutifocal	3 (5.0%)	2 (5.0%)	1 (5.0%)	
Histological grade	Well to moderately	36 (60.0%)	25 (62.5%)	11 (55.0%)	0.576
	Poor	24 (40.0%)	15 (37.5%)	9 (45.0%)	
Stage	I/II	24 (40.0%)	15 (37.5%)	9 (45.0%)	0.576
	III/IV	36 (60.0%)	25 (62.5%)	11 (55.0%)	
Lauren's classification	Intestinal	34 (56.7%)	22 (55.0%)	12 (60.0%)	0.700†
	Diffuse	23 (38.3%)	15 (37.5%)	8 (40.0%)	
	Mixed	3 (5.0%)	3 (7.5%)	0 (0.0%)	
Lymphovascular invasion	Absent	17 (28.3%)	11 (27.5%)	6 (30.0%)	0.839
	Present	43 (71.7%)	29 (72.5%)	14 (70.0%)	
Perineural invasion	Absent	21 (35.0%)	14 (35.0%)	7 (35.0%)	0.999
	Present	39 (65.0%)	26 (65.0%)	13 (65.0%)	
Lymph node involved	Absent	20 (33.3%)	14 (35.0%)	6 (30.0%)	0.699
	Present	40 (66.7%)	26 (65.0%)	14 (70.0%)	
Metastasis status	Non-Metastatic	30 (50%)	16 (40%)	14 (70.0%)	0.028*
	metastatic	30 (50%)	24 (60%)	6 (30.0%)	

*Significant differences ‡ Fisher test was used for comparison †The Monte Carlo test was used for comparison

Table 4: Evaluation of OS using Cox regression model in univariate and multivariate mode

Prognostic factor	Univariate HR (95% CI)	P	Multivariate aHR (95% CI)	P
Age 60≤ y vs >60 y	1.34 (0.7-2.58)	0.38		
Male vs Female	1.48 (0.74-2.96)	0.27		
Tumor size 6cm≤ vs >6 cm	3.3 (1.58-6.89)	0.001*	1.75 (0.76-4.01)	0.19
Tumor location, upper vs middle or lower	2.07 (1.04-4.14)	0.04*	1.3 (0.56-3.02)	0.55
Focal unifocal vs mulltifocal	2.1 (0.64-6.92)	0.22		
Grade, well to moderately vs poor	1.2 (0.63-2.28)	0.59		
Stage, I/II vs III/IV	8.4 (2.93-24.08)	0.001*	6.45 (1.81-23.27)	0.004*
Lauren's classification, intestinal vs diffuse or mixed	2.23 (1.16-4.29)	0.02*	1.13 (0.53-2.43)	0.76
Lymphovascular invasion, Yes vs No	3.24 (1.29-8.59)	0.01*	1.15 (0.37-3.57)	0.82
Perineural invasion, Yes vs No	1.61 (0.79-3.25)	0.19		
Lymph node involved, Yes vs NO	1.63 (0.77-3.46)	0.21		
Metastasis, Yes vs No	3.56 (1.73-7.31)	0.001*	2.45 (1.1-5.95)	0.04*
MSI, MSI-H vs MSI-L/MSS	0.58 (0.28-1.21)	0.15	0.81 (0.37-1.83)	0.62

* Significant difference

Table 5: Evaluation of DFS using Cox regression model in univariate and multivariate mode

Prognostic factor	Univariate HR (95% CI)	P	Multivariate aHR (95% CI)	P
Age 60≤ y vs >60 y	1.32 (0.69-2.53)	0.41		
Male vs Female	1.48 (0.74-2.96)	0.26		
Tumor size 6cm≤ vs >6 cm	3.17 (1.59-6.6)	0.002*	1.69 (0.74-3.89)	0.21
Tumor location, upper vs middle or lower	2.08 (1.04-4.16)	0.04*	1.15 (0.5-2.64)	0.74
Focal unifocal vs multifocal	2.08 (0.63-6.9)	0.23		
Grade, well to moderately vs poor	1.19 (0.63-2.28)	0.59		
Stage, I/II vs III/IV	8.27 (2.88-23.74)	0.001*	6.6 (1.72-21.44)	0.005*
Lauren's classification, intestinal vs diffuse or mixed	2.26 (1.17-4.37)	0.02*	1.12 (0.52-2.41)	0.77
Lymphovascular invasion, Yes vs No	3.33 (1.8-29.6)	0.01*	1.18 (0.3-39.62)	0.77
Perineural invasion, Yes vs No	1.61 (0.79-3.27)	0.19		
Lymph node involved, Yes vs NO	1.64 (0.77-3.49)	0.2		
Metastasis, Yes vs No	3.29 (1.62-6.84)	0.001*	2.08 (0.86-5.04)	0.1
MSI, MSI-H vs MSI-L/SS	0.59 (0.28-1.21)	0.15	0.8 (0.35-1.79)	0.58

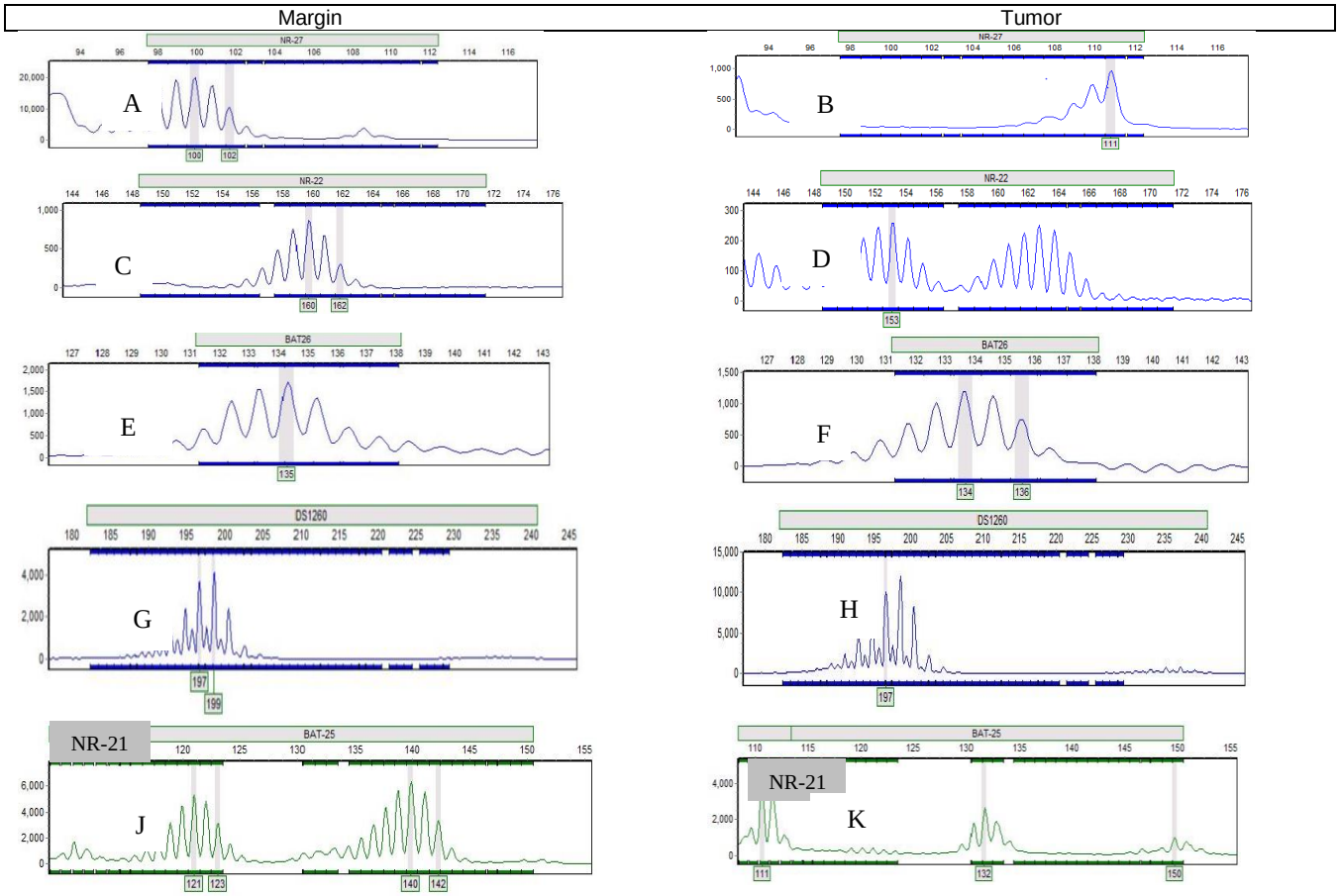

Figure 1. Fragment analysis results using Gene Marker software. A panel consisting of 5 mononucleotide markers was used to determine MSI by multiplex PCR. The X-axis shows the size of the part and the Y-axis shows the fluorescence intensity. The D3S1260 dinucleotide marker was used to match tumor sample H with margin G. Note the displacement in the size of the amplified products in the tumor specimens compared to its normal size. An example of a displaced locus in tumor samples B, D, K is compared with a normal sample in A, C, J respectively. The BAT-26 marker was not variation (E, F).

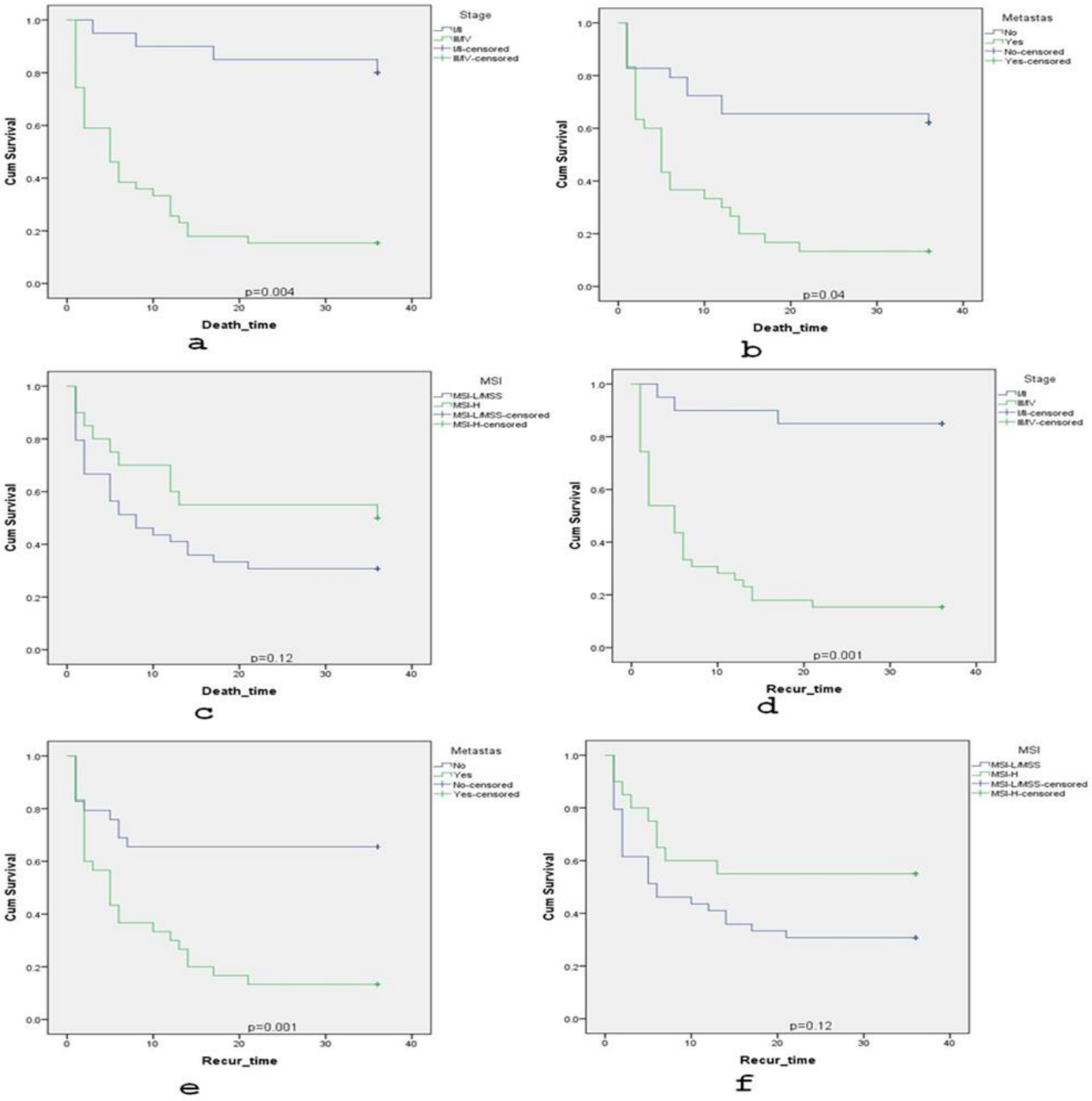

Figure 2. Kaplan-Meier curves for overall-survival (OS) and disease-free survival (DFS). (a) Kaplan-Meier curves for OS according to ypTNM stage. (b) KaplanMeier curves for OS according to metastatic status. (c) Kaplan-Meier curves for OS according to MSI. (d) Kaplan-Meier curves for DFS according to ypTNM stage. (e) KaplanMeier curves for DFS according to metastatic status. (f) Kaplan-Meier curves for DFS according to MSI

DISCUSSION

GC is one of the most prevalence malignancy in Iran and both genetic and environmental risk factors play a role in the etiology of the diseases [20]. Hence, study of the genetic risk factors to find favorable biomarker could be of interest. MSI as one of the most interesting DNA biomarkers has been well studied in CRC, but there is a paucity of data regarding GC, specifically among Iranian patients with GC. The pentaplex system is widely used in various demographic analyzes around the world and has a confirmed suitability [21]. Several studies have shown that mononucleotide markers have higher or equivalent specificity and higher sensitivity for detecting MSI-H compared to dinucleotides markers, and they are more informative and easier for interpretation [22]. In addition, mutations in the MSH6 gene often do not result in changes in dinucleotide markers [19]. This study was conducted to study MSI among the Iranian GC patients in both metastatic and non-metastatic samples.

We found that the NR-21 and BAT-26 were the most unstable and stable markers to determine the MSI phenotypes, respectively. While some of these markers (e.g., BAT-25 and BAT-26) have shown to be polymorphic in certain ethnic groups, this polymorphism could result in false data [22, 19]. Hence, studying these markers in each ethnic group might help to reduce the false positive or negative data[19,23]. To eliminate these errors, we evaluated the marginal sample along with tumor samples to check markers instability.

Based on previous data from a wide range of global populations, BAT-25, BAT-26, NR-21, NR-22, and NR-27 markers in germline DNA are highly monomorphic. The NR-21 in Oceania, NR-21, BAT-25, NR-27 and BAT-26 in the Middle East, NR-21, NR-27 and BAT-25 in South and Central Asia, BAT-26 and NR-21 in USA, NR-21 and NR-27 in East Asia have been reported polymorphic markers [24, 25]. Thus, the result of our study was consistent with that from Middle East populations. These findings were also very close to the results of the studies conducted in East, West and Central Asia and Oceania. BAT-26, the most frequent monomorphic marker in our study. In Asian populations, BAT-26 is also reported to be monomorphic, except in the north India where it shows some genetic variation[24, 25]. To our best of knowledge, there is no published data regarding the MSI in GC among Iranian patients. On the contrary to the findings of the current study, several studies on Iranian patients with CRC have reported NR-21 as the most unstable marker[26,27.] But Farahani have identified BAT-26 as the most unstable marker[28]. Although laboratory methodology is effective in determining the instability of markers, there is no consensus on the use of these panels as the criteria for classifying MSI tumors. In concordance with the current study, many authors consider the minimum of 2 to 3 out of 5 unstable markers in determining the MSI-H phenotype [29].

The incidence of MSI varies greatly in different types of tumors[3]. The highest incidence rate of MSI is reported in the endometrium[30]. The MSI-H phenotype accounts for 5 to 50% of all GCs with significant differences within ethnic groups. The incidence of MSI-H in GC differs between Asian and European populations[31,32]. In our study, the frequency of MSI and MSI-H was 46.6% and 33.3%, respectively. Higher rates of MSI (58.3%) have been reported in patients with gastric cancer in China. Several factors, including the use of different MSI panels or differences in the clinical features of the patient population could be attributed to the reported rate [33].

Due to heterogeneity, the prognosis of MSI varies in different cancers. The prognosis of MSI-H is poor in breast and endometrial cancers, while it is good in most cases of GCs 34. The current research showed that lower gastric tumors were more associated with MSI-H-GC patients compared to MSI-L/S patients (60% vs 50%, P= 0.683). This feature is clinically important. According to TCGA group calculations, 85% of instabilities in the stomach occur in antrum and body regions[35]. In addition, MSI-H was significantly detected in non-metastatic tumors (p= 0.028). But no correlation was found between MSI and other clinico-pathological features. Similarly, Huang et al., [10] reported that MSI-H only tended to be located in distal parts of the stomach and less lymph node involvement. Other studies in GC have shown that MSI-H tumors are usually associated with

female sex, larger tumor size, well or moderately differentiate among all these features, the association between the onset of cancer at an older age and the MSI-H phenotype can be seen in most studies. Presence and increased methylation of the hMLH1 gene is associated with aging. Methylation of hMLH1 reduces the expression of hMLH1 and is the main cause of microsatellite instability in sporadic GC cases[36]. According to the study by Polom et al., [37] the prognosis of GC is more affected by the patient's age compared to MSI situations. Although 60% (36/60) of the patients in our study were over 60 years old, there was no significant difference between MSH and MSI-L/S. It has been argued that MSI-H GC has a better prognosis due to its correlation with earlier TNM stage at diagnosis (stages I–II) [37, 38]. In this study, MSI-H patients had lower stages compared to MSI-L/S patients, but this difference was not statistically significant. Although MSI-H was prevalent among stage 1/2 cancers, it seems that the reason for this finding in GC is not clear. Also, we examined the most important modes of change in microsatellites such as MSI-L. No significant relationship was found between clinical features and MSI, except in the case of MSI to MSS, where non-metastatic tumors were significantly more unstable (p= 0.019).

Overall, it is believed that determining the prognosis of GC patients is not possible based on a sole marker or absolutely based on MSI situation. Prediction of GC prognosis is influenced by factors including age, tumor grade and stage, MSI patterns, and chemotherapy treatment. Therefore, the correlation between MSI and GC prognosis is not clear. Hence, MSI- H cannot be considered as an independent factor in determining the prognosis of GC patients[39]. In this study, variables OS and DFS did not show a significant relationship with MSI. In concordance with our study findings, in the study Meiying [40] et al., MSI-positive patients have no trend to have a longer OS than MSI-negative patients in these tumors. But Polom et al.,[37] found an association of MSI with good overall survival.

Limitations

One of the limitations of our study was small sample size. We did not have access to blood and fresh samples. One of the strong points of this research was the preparation of samples using punch method and as a result very high confidence of the purity of the samples.

CONCLUSION

The current study could be the documented report between MSI and GC among Iranian patients. Detecting MSI-high in non-metastatic tumors may reflect a good prognosis as reported in CRC. However, a comprehensive study and more samples might be needed to clarify this statement. In addition, it seems that a panel consisting of NR-21, BAT-25, and NR-27 mononucleotide markers could be the most reliable and useful markers for detecting MSI in the population of Iran.

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CONFLICT OF INTEREST

All authors have no conflict of interest to declare.

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