ELEVATED MICROSATELLITE ALTERATIONS AT SELECTED TETRA-NUCLEOTIDE REPEATS (EMAST) TESTING IN COLORECTAL CANCER USING THE COST-EFFECTIVE QIAXCEL ADVANCED PLATFORM

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Abstract – Objective: Elevated Microsatellite Alterations at Selected Tetranucleotide repeats (EMAST) is a type of microsatellite instability occurring in about 60% of colorectal cancers (CRCs) and is associated with metastases and decreased patient survival. Fluorescent capillary sequencers, as most common EMAST detection technique, are relatively time-consuming and expensive. This study was conducted to examine the prevalence of EMAST in Iranian CRC patients using QiaXcel Advanced platform without the need to fluorescent primers, a technique that overcomes the main shortcomings of fluorescent capillary sequencers.

Patients and Methods: EMAST status was analyzed in 183 Iranian FFPE (Formalin Fixed Paraffin Embedded) CRC samples using QiaXcel Advanced system (Qiagen, Hilden, Germany) based on capillary electrophoresis. D20S82, D20S85, D9S242, D8S321, and MYCL1 were included as common tetranucleotide markers.

Results: A total of 75 patients (41.0%) had EMAST+ CRC. D20S85 was the most frequent marker (29.5%). Coincidence of instability at D8S321 and D20S85 markers were the most frequent type of instability (5.5%) and the distal colon had the highest number of EMAST+ cases (34.3%).

Conclusions: Using QiaXcel Advanced system, EMAST were observed in 41.0% of Iranian CRCs. Due to the importance of EMAST in cancer progression, this fast and cost-effective PCR-based method can improve the clinical management of CRC that may further modify the patient outcome.

KEYWORDS: Prevalence, Colorectal neoplasms, EMAST, QiaXcel Advanced system.

INTRODUCTION

Microsatellite instability (MSI) refers to the hypermutability of simple tandem repeats in the human genome caused by mutations in somatic mismatch repair gene (MMR) that finally leads to DNA slippage. MSI is detected by profiling the Bethesda markers, which often include two mononucleotides (BAT25 and BAT26) and three dinucleotides (D5S346, D2S123 and, D17S250) microsatellites loci. This panel classified MSI status as MSI-H (High-frequency MSI), MSI-L (low-frequency MSI) and MSS (Microsatellite stable). Recently, a different form of MSI at specific tetranucleotide repeats has also been documented in a plethora reports termed elevated microsatellite alterations at selected tetranucleotide repeats (EMAST), occurring at loci containing (AAAG), (ATAG), or (CTTT) repeats. EMAST has been reported in several solid tumors but because of
great impact of microsatellite instability on CRC biology, the vast majority of EMAST-related reports has been focused on CRC. In CRC, EMAST is found most frequently in up to 60% sporadic cases and associated with poor tumour differentiation. The diversity of reported frequencies could be due to the type and number of markers and also the thresholds used in EMAST evaluation. Compared to the fact that 15% to 20% of sporadic tumors are MSI-H, EMAST is more frequent than MSI-H in CRC. Unlike MSI-H, EMAST is associated with poor prognosis and defines a unique molecular subtype of CRC. The common EMAST testing technique is fluorescent PCR-based assays followed by capillary electrophoresis sequencing of resulting fragments to distinguish between fluorescent dyes. Capillary sequencing device is not available universally in laboratories. QiaXcel Advanced system (Qiagen, Hilden, Germany) can detect and measure PCR-amplified DNA and RNA fragments. To explore the landscape of EMAST, the prevalence of tetranucleotide markers and the distribution of EMAST across the different sections of the large intestine in Iranian CRC patients with different genetic background from other populations, we developed a rapid and effective screening method with QiaXcel Advanced system without need to fluorescent primers. It was previously shown that this system can be used for MSI testing in CRC and endometric cancers but was not yet used for the analysis of EMAST in colorectal tumors.

**EMAST analysis**

Briefly, DNA was extracted from microdissected tumour and (normal adjacent tissue) NAT using the QIAamp Tissue Kit (Qiagen GmbH, Hilden, Germany). EMAST analysis was performed using a panel of 5 tetranucleotide markers: D20S82, D20S85, D9S242, D8S321 and MYCL1. PCR performed with previously described primers without fluorescent labels. Amplification reactions (25 μl) were prepared subjected to PCR amplification: initial incubation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 40 s, 62°C for 40 s, and 72°C for 40 s, and a final incubation at 72°C for 5 min. PCR products were analyzed on the QiaXcel system (Qiagen, Hilden, Germany) using the QiaXcel DNA High-Resolution Kit and QX Alignment Marker 15 bp/600 bp. Separation was performed with OM500 method, customized protocol at 5 KV voltage using 10 seconds (samples) and 500 seconds separation time. Samples were classified as “EMAST+” when two or more than two of the analyzed markers were unstable and “EMAST-” when only one or none of microsatellite locus was unstable comparing with NAT. EMAST status data were analyzed with two investigators blinded to patient outcomes.

**Statistical analysis**

All data were statistically analyzed using the Statistical Package for the Social Sciences, version 21.0 (SPSS 21.0, IBM, Armonk, NY, USA).

**Results**

**EMAST evaluating and prevalence**

A typical gel image for evaluating instability at selected tetranucleotide is presented in Figure 1. EMAST+ tumors were identified in 75 cases (41.0%) and EMAST- tumors in 108 (59.0%) cases. Various patterns of distribution of unstable markers were observed in the diagnosis of 75 EMAST+ CRCs. No case had instability at 5 markers (1). Concurrent instability at D8S321 and D20S85 markers were the most frequent type of instability (n=10; 5.5%). Of 183 FFPE CRC samples, 134 (73.2%) cases were located at proximal and 49 (26.8%) cases were located at distal colon. The distribution of EMAST+ tumors across the different sections of the large intestine are presented in Figure 3. EMAST+ were predominantly located in the colon (n=60; 80%) compared to rectum (n=15; 20%) and the tumours located at distal colon had the highest number of EMAST+ cases (n=63; 34.3%).

**Patients and methods**

**Patients selection**

This study evaluated 183 CRC patients who underwent R0 resection (defined as no microscopic residual tumour cells at the longitudinal and circumferential resection margins) for colorectal cancer at Taleghani Hospital and Shohada Hospital, Shahid Beheshti University of Medical Sciences (Tehran, Iran) between September 2010 and March 2017. Formalin-fixed, paraffin-embedded (FFPE) tissues of the CRCs (tumour tissues and normal adjacent tissue) were retrieved from the pathology archives of the Taleghani and Shohada Hospital and were reviewed to be CRC tumour by our pathology laboratory. The tumour location was obtained from Clinical data of the patients. Ethical approval for this study was obtained from the Medical Ethical Committee of Department of Cancer prevention of Gastroenterology and Liver Disease Research Institute (RCGLD).
DISCUSSION

The prevalence of EMAST phenotype in Iranian CRCs using QiaXcel Advanced system was 41.0%. Previously the QiaXcel Advanced system was used for MSI testing in CRC\(^2\) and endometric cancers\(^13\), but to our knowledge so far it has not been used for EMAST analysis in colorectal cancer. Förster et al\(^13\) compared QiaXcel advanced system and IHC results for MSI testing in colorectal cancer and stated this rapid and economic typing can be used in the pathology laboratories without capillary sequencing device\(^14\). MSI testing for human colorectal cancers using the QiaXcel Advanced system could serve as an economic and acceptable tool for rapid diagnostics in laboratories that do not have access to a high throughput unit\(^13\). Few researchers have studied the prevalence of EMAST in CRC. However, the prevalence of EMAST, there was disputed due to the limited number of studies and heterogeneity in...
the studied characteristics. Approximately 10.60-64.8% of CRCs\(^6,7,9,11,15-26\) are diagnosed as EMAST\(^\). However, the reported prevalence varies according to ethnicity, instability at the number of markers for defining EMAST and the EMAST markers that were used for the detection\(^6,7,9,15-26\). EMAST CRCs were not accounted for Iranian patients so far. In the present study, which was conducted in Iran, EMAST phenotype was identified in 41.0% of cases. This percentage is higher than the reported prevalence by Lee et al\(^{25}\) in Korean population (22.0%)\(^{25}\) and slightly equals another study from Korea (44.44%)\(^7\). In western countries the prevalence of EMAST is between 11%-60%\(^\ 6, 20, 22, 23\). Lee et al\(^{25}\) identified D9S242 as the most frequent locus (72.7%) and in the other studies D20S82 had a higher frequency (30%)\(^{20}\) and (91.7%)\(^{23}\), but in our result D20S85 was the most frequent unstable marker (29.5%). Unlike former studies\(^7, 24, 26\) there was not much difference between the frequency of 5 tetranucleotide markers used in our study (20-29%) (Figure 2). Indeed, some studies have

**Fig. 2.** Patterns of distribution of number of unstable tetranucleotide markers and EMAST markers frequency in 183 CRC patients.

**Fig. 3.** Distribution of EMAST\(^*\) tumors across the different sections of the large intestine in 183 CRC patients.
suggested that EMAST+ tumors characteristically occur in proximal or right-angle colon lesions\textsuperscript{15-18} and others revealed EMAST+ tumors occur in distal or left colon\textsuperscript{6, 10, 22} that is similar to our result with more frequency of EMAST+ tumors in sigmoid. Due to the association of EMAST phenotype with metastases\textsuperscript{27} and reduced survival in CRC patients\textsuperscript{28}, EMAST could be a biomarker for CRC with more frequency of EMAST+ tumors in sigmoid progression\textsuperscript{29}. Furthermore, patients with EMAST CRCs respond well to 5-FU based chemotherapy\textsuperscript{22} and further evaluation may provide the foundation for EMAST as a predictive biomarker in CRC.

**CONCLUSIONS**

Using QiaXcel Advanced system EMAST phenotype accounts for 41.0% of Iranian CRCs. QiaXcel system as an easy and effective technique may be used in pathology institutes for routine diagnostics of EMAST testing in colorectal cancers and can improve personalized therapeutic strategies with more effectiveness and lower toxicity in the clinical management of CRC that may further modify the patient outcome.

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**CONFLICT OF INTEREST**

The Authors declare that they have no conflict of interests.

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