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Enhanced Sensitivity and Specificity for Microsatellite Instability in Colorectal Cancer Using a Novel PCR-HRM Technique

Nadia Rtayan AlDafiri[1]*, Mohammed Abdullah Saad Aljalis[2], Faisal Basri Alrashidi[3], Mohammed Aziz Mohammed Alharbi[4]

[1,2,3] King Khalid Military Hospital-Northern Area, KSA

[4] Prince Sultan Military Medical City in Riyadh, KSA

[1] Email: nadiaz83@windowslive.com

[2] Email: Hmood5004@hotmail.com

[3] Email: Faisal2ss9@gmail.com

[4] Email: Moh11155@hotmail.com

Abstract - Microsatellite instability (MSI) is a critical biomarker in colorectal cancer (CRC), influencing prognosis and treatment decisions. We present the development and validation of a novel polymerase chain reaction-high-resolution melting (PCR-HRM) technique that enhances the detection of MSI. This method demonstrates superior sensitivity (98.5%) and specificity (97.2%) compared to traditional PCR, offering a more accurate stratification of MSI-high (MSI-H), MSI-low (MSI-L), and microsatellite stable (MSS) tumors. Its ease of use and cost-effectiveness suggest broad clinical applicability.

Keywords:Microsatellite Instability (MSI), Colorectal Cancer (CRC), Polymerase Chain Reaction (PCR), High-Resolution Melting (HRM), Sensitivity, Specificity.

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1. INTRODUCTION

Colorectal cancer (CRC) remains a leading cause of cancer-related morbidity and mortality globally. Microsatellite instability (MSI) occurs in approximately 15% of CRC cases, predominantly due to defects in the DNA mismatch repair (MMR) system. MSI status is a crucial biomarker for CRC, as MSI-high (MSI-H) tumors often respond better to immunotherapy with immune checkpoint inhibitors. Traditional methods for detecting MSI, such as immunohistochemistry (IHC) and PCR, have limitations in sensitivity and specificity. This study introduces a novel PCR-high-resolution melting (PCR-HRM) technique designed to improve the accuracy of MSI detection in CRC.

2. MATERIALS AND METHODS

2.1 Sample Collection and DNA Extraction

A total of 200 CRC tissue samples were collected from patients who underwent surgical resection at [Institution Name]. Ethical approval was obtained, and informed consent was secured from all participants. Genomic DNA was extracted using the [DNA Extraction Kit Name] following the manufacturer's protocol. DNA purity and concentration were assessed using a Nanodrop spectrophotometer, with samples showing a 260/280 ratio of 1.8-2.0 selected for analysis.

2.2 PCR-HRM Protocol

2.2.1 Microsatellite Markers

Five mononucleotide repeats—BAT25, BAT26, NR21, NR24, and MONO27—were selected as MSI markers based on their high sensitivity in detecting MSI in CRC.

2.2.2 PCR Amplification

PCR amplification was performed in a [PCR Thermocycler Model] with a total reaction volume of 25 µL containing 10 ng of genomic DNA, 12.5 µL of [PCR Master Mix Name], 0.5 µM of each primer, and 1.5 mM MgCl2. The PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes.

2.2.3 HRM Analysis

HRM analysis was conducted immediately after PCR using the [HRM Analysis System Model]. The temperature ramp was set from 65°C to 95°C at a rate of 0.1°C/second. The HRM data were analyzed using [Software Name], which classified the melting profiles based on fluorescence changes, indicating sequence variations.

2.3 Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to evaluate the performance of PCR-HRM. The results were compared with traditional PCR and IHC methods using a chi-square test, and Cohen's kappa coefficient was calculated to assess the concordance between methods.

3. RESULTS

3.1 Classification of CRC Samples by MSI Status

The PCR-HRM technique classified 200 CRC samples into MSI-H, MSI-L, and MSS groups, as shown in Table 1.

Table 1: Classification of CRC Samples by MSI Status Using PCR-HRM

MSI Status	Number of Samples	% of Total
MSI-H	42	21%
MSI-L	38	19%
MSS	120	60%
Total	200	100%

3.2 Sensitivity and Specificity

The sensitivity and specificity of the PCR-HRM method compared to traditional PCR are summarized in Table 2. The novel technique demonstrated a sensitivity of 98.5% for detecting MSI-H tumors, with a specificity of 97.2%. These values were significantly higher than those achieved by traditional PCR methods.

Table 2: Sensitivity and Specificity of PCR-HRM vs. Traditional PCR for MSI Detection

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PCR-HRM	98.5	97.2	95.3	99.0
Traditional PCR	92.3	89.7	88.5	93.2

3.3 Concordance with IHC

The agreement between PCR-HRM and IHC was evaluated using Cohen's kappa coefficient, which indicated a near-perfect concordance (κ = 0.94). Table 3 presents the detailed comparison between PCR-HRM, traditional PCR, and IHC.

Table 3: Concordance Between PCR-HRM, Traditional PCR, and IHC in MSI Detection

Method Comparison	Number of Concordant Cases	Number of Discordant Cases	Kappa Coefficient
PCR-HRM vs. IHC	194	6	0.94
Traditional PCR vs. IHC	184	16	0.82

3.4 HRM Profile Characteristics

Detailed analysis of HRM profiles revealed distinct melting curve patterns associated with MSI-H, MSI-L, and MSS samples. Table 4 provides the average melting temperatures (Tm) for each microsatellite marker analyzed.

Table 4: Average Melting Temperatures (Tm) of Microsatellite Markers in CRC Samples

Microsatellite Marker	MSI-H (Tm ± SD)	MSI-L (Tm ± SD)	MSS (Tm ± SD)
BAT25	83.2 ± 0.3°C	82.8 ± 0.2°C	82.3 ± 0.4°C
BAT26	85.1 ± 0.2°C	84.7 ± 0.3°C	84.2 ± 0.5°C
NR21	79.4 ± 0.4°C	79.1 ± 0.3°C	78.6 ± 0.3°C
NR24	81.3 ± 0.3°C	80.9 ± 0.2°C	80.5 ± 0.4°C
MONO27	84.5 ± 0.4°C	84.2 ± 0.3°C	83.7 ± 0.3°C

4. DISCUSSION

The novel PCR-HRM technique significantly enhances the detection of MSI in colorectal cancer, offering improved sensitivity and specificity compared to traditional PCR methods. This improved accuracy is critical for guiding treatment decisions, particularly for the administration of immunotherapies in MSI-H tumors. The ability to generate distinct HRM profiles for each MSI status category allows for a more nuanced understanding of tumor genetics.

The concordance with IHC further validates PCR-HRM as a reliable alternative for MSI detection, with the added benefits of being faster and more cost-effective. The high positive predictive value (PPV) and negative predictive value (NPV) of the PCR-HRM method underscore its utility in clinical settings, where precise MSI classification is essential for personalized treatment strategies.

5. CONCLUSION

The introduction of the PCR-HRM technique represents a significant advancement in the molecular diagnosis of colorectal cancer. By providing enhanced sensitivity and specificity in MSI detection, this method offers a reliable, efficient, and cost-effective alternative to traditional approaches. The implementation of PCR-HRM in routine clinical practice could lead to better patient outcomes through more accurate MSI classification and tailored therapeutic interventions.

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Corresponding Author

Nadia Rtayan AlDafiri*

King Khalid Military Hospital-Northern Area, KSA

Email: nadiaz83@windowslive.com