A preliminary study targeting exon 12 *MSH2* mutations in FFPE rectal tumors: feasibility and challenges

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Rectal cancer constitutes a major burden of colorectal malignancies in Sri Lanka. Despite growing disease burden, Sri Lanka lacks molecular data and genomic profiles, particularly on Mismatch repair (MMR) gene mutations. *MSH2* is a frequently mutated MMR gene, associated with microsatellite instability-high (MSI-H) tumors. This pilot study explores the feasibility and limitations of detecting *MSH2* exon 12 mutations using formalin-fixed paraffin-embedded (FFPE) tissue the most accessible archival resource in local clinical settings. Twelve FFPE tumor samples and four control blocks were sectioned and processed using the QIAamp DNA FFPE Tissue Kit. DNA was quantified via NanoDrop spectrophotometry and agarose gel electrophoresis. Primers targeting exon 12 (471 bp) were designed using Primer-BLAST, BioEdit, and IDT OligoAnalyzer. PCR optimization was initially conducted with high-quality blood-derived DNA, then adapted for FFPE DNA using incremental changes in annealing temperature, $MgCl_2$ concentration (2.0-3.5 mM), and template pre-treatment (dilution, 95 °C incubation). Amplified products were visualized, gel-purified using low-melting agarose, and sequenced via Sanger method. Blood DNA consistently yielded specific 471 bp products. In contrast, FFPE DNA yielded smeared products or weak bands, often accompanied by primer-dimers. Gel-purified products from FFPE samples were successfully extracted but sequencing chromatograms showed poor peak resolution, baseline noise, and unreadable sequences, confirming insufficient template quality for Sanger sequencing. Amplification and sequencing of *MSH2* exon 12 from FFPE rectal tumor DNA proved technically unviable due to extensive fragmentation and chemical modifications inherent to formalin fixation. Large-amplicon PCR from FFPE was not consistently achievable despite multi-parameter optimization, indicating that prolonged/formalin fixation and block age are dominant constraints on amplifiability in this setting. Future efforts should employ standardizing fixation, enforcing block-age thresholds shorter amplicon designs, FFPE-specific polymerases, and enzymatic repair steps to enable reliable mutation detection from archival tissue. By systematically confronting the technical barriers of FFPE tissue genomics in a Sri Lankan context, this pioneering study not only highlights the urgent need for protocol innovation but also lays the foundation for accessible, population-scale molecular diagnostics in underrepresented cancer cohorts.

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