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Pitfalls in interpreting autosomal InDel markers profiling: a study on mutations in tumoural specimens

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Highlights

- In tumoural tissues genetic alterations could be also at the InDels markers.
- This is the first work investigating genomic instability in tumour tissue samples when using InDel markers.
- In 170 samples analysed we found incorrect genotyping in 27% of them.
- It is necessary to exercise great caution in InDel genotyping of neoplastic tissues in forensics.

Keywords: InDel markers; Microsatellite instability; Loss of heterozygosity; Tumoural specimens

Dear Editor,

In the last decade, human diallelic InDel polymorphisms have become promising genetic markers in forensic identification cases and biogeographic inference of biological samples [1,2]. InDels can be used successfully in highly degraded DNA samples and may play an important role as an effective supplement for existing genetic markers in personal identification. InDels can also be a good choice for population studies, parentage testing, missing person identification and ancient DNA analysis [3-5].

In special cases, the biological samples that are used for identification purposes can come from tumour biopsies stored in biobanks when they turn out to be the last source of biological material available [6]. Nevertheless, it is known that carcinogenesis is a multistep process in which cells accumulate genetic alterations as they progress to a more malignant phenotype [7, 8]. Genomic instability within the tumour tissue can be expressed with different modalities: chromosomal instability (CIN), microsatellite instability (MSI) and instability associated with Nucleotide-Excision Repair (NIN) [9, 10].

The study was conducted by comparing genetic profiles obtained from healthy tissue, tumour tissues (colorectal, gastric, breast and hepatic cancer) and, in some cases, (clorectal) metastatic hepatic tissue belonging to the same individuals. . The aim of the study was to determine, in particular, whether there are neoplasms, among those analysed, which are more subject to variation in 38 InDel markers and if there are InDel polymorphisms, among those analysed, which are more subject to alterations attributable to the genomic instability phenomena which characterize many neoplasms.

The study was performed on 170 tissue samples obtained from 71 individuals. The samples were taken at the time of surgery and stored – in anonymized form - at -80 ° C in two different biobanks. Both healthy and pathological frozen samples were stored at -80°C. Genomic DNA was obtained from samples using the QIAamp DNA Micro Kit (Qiagen, GmbH, Hilden, Germany). All samples were genotyped for 38 InDels using the InDelPlexkit (GENOMICA, S.A.U., Grupo Zeltia, Madrid, Spain) according to the manufacturer's recommendations. PCR reaction was conducted using the GeneAmp® PCR System 9700. Capillary electrophoresis was performed on ABI 3130 Genetic Analyzers (Applied Biosystems) and allele designation was made using GeneMapper ID-X software (Applied Biosystems).

The association between tumour and mutation type was tested with chi-square test. The level of significance was set at the 5% level. Genomic instability was identified by comparing the genotype at each InDel locus in the frozen tumour sample with the genotype obtained from the respective healthy frozen tissue. The main InDel alteration in the analysed frozen tumour samples was LOH and MSI.

We determined 38 InDel markers for each of the 170 samples available, for a total of 6460 loci analysed. Amongst the 66 samples that presented altered genotypes in tumour specimens, 16 hepatic, 11 gastric, 12 breast and 27 colorectal samples showed MSI or complete LOH events and these specimens (27%) can therefore be incorrectly genotyped, thus complicating the forensic evaluation.

Amongst the 3762 markers genotyped in the 99 tumour tissues samples analysed, 231 (6.1%) showed genetic alterations in terms of LOH and among these 43 were in hepatic tumour tissues, 31 in gastric tumour tissues, 38 in breast cancer tumours and 119 in colorectal tumours. The percentage of samples with loss of heterozygosity is substantially similar for different types of cancer and the comparison is not statistically significant ($p = 0.3975$). MSI was observed 58 times (1.5%). All breast cancer tissues showed genetic alterations in at least one marker. Only five samples did not show any alterations.

Furthermore, a comparison between DNA profiles of colorectal tumour tissue samples and their respective liver metastasis was performed and, among genetic alterations, 41 LOH and 6 MSI were observed in metastatic samples showing new alterations not exhibited by the primary tumour.
The samples showing the highest number of alterations were a breast cancer one (10 LOH and 1 MSI) and a colorectal one (4 LOH and 8 MSI).
Based on our results we could infer that InDels seem to be most affected by mutational events with respect to STR mutations in tumour specimens, it would be optimal to choose only the most stable markers for forensic typing and population studies. In order to obtain more reliable results and to be able to interpret them, it would therefore be appropriate to perform histological analysis before forensic genotyping and set up PCR multiplexes that investigate different InDel loci in order to strengthen the robustness of genetic information.

AUTHOR STATEMENT

Pamela Tozzo: Investigation, Data Curation, Methodology, Writing - Original Draft,

Anna Chiara Frigo: Validation, Formal analysis

Luciana Caenazzo: Conceptualization, Resources, Writing - Review & Editing, Supervision

Declarations of interest: none

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Table 1. Comprehensive number of genetic alterations found in the tumour tissue samples analysed.

TISSUE	TYPE OF GENETIC VARIABILITY		TOTAL
	LOSS OF HETEROZIGOSITY	MICROSATELLITE INSTABILITY	
HEPATIC	43	16	59
GATRIC	31	9	40
BREAST	38	9	47
COLORECTAL	119	24	143
TOTAL	231	58	289