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Loss of heterozygosity (LOH) – implications for human genetic identification

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Abstract: The aim of this study was assessment of possible effects of loss of heterozygosity on human genetic identification of histolopathogical tissue sections. DNA templates were extracted from tumour tissue specimens excised from oncological patients and from reference blood samples. AmpFlSTR Identifiler PCR Amplification Kit and ABI 310 Genetic Analyzer (Applera) were used to obtain genetic profiles. Frequency of LOH was calculated for respective samples. Fisher's exact test was performed for statistical analysis. Forty-two percent of the 101 cancer cases analysed were found to possess alterations of the microsatellites manifesting with allelic loss. The most frequently altered loci were D3S1358 and D18S51. The alteration was detected in 47% of cases with larynx carcinoma, 44% of cases with uveal melanoma, 60% of cases with cervical cancers, one case of liposarcoma G3 and one case od neurofibrosarcoma. No LOH was found in liposarcoma G1, dermatofibrosarcoma and cystosarcoma protuberans in either primary or recurrent tumours. In benign tumours (lipoma and fibroma) LOH was also absent. During genotyping of DNA extracted from histopathological tissue sections caution should be taken when non-match or exclusion based on few discrepancies is concluded.

Key words: histolopathogical tissue sections, loss of heterozygosity, forensic genotyping

Introduction

Short Tandem Repeats (STRs) represent highly polymorphic microsatellite markers in human genome that have tandemly repetitive sequence elements of 2-7 bps length, located approximately every 10-15 kbs. Genetic instability is reflected in alterations in the patterns of these polymorphic repeat segments and is thought to occur when there has been a damage to the cell mismatch repair (MMR). Examination of loss of heterozygosity (allelic loss, LOH) using the specific microsatellite markers is a method of choice in assessing tumour suppressor genes (TSGs) localisation in human genome. This loss of normal allele in heterozygotic locus, may result from different mechanisms, including chromosomal deletion, mitotic recombination (MR), gene conversion, point mutation, or intragenic allele inactivation [1]. Consequently, the locus may become homozygous due to mitotic recombination, gene conversion or chromosome loss with reduplication; hemizygous due to deletion or chromosome loss, complex heterozygote due to introduction of another point mutation at the locus, or may remain heterozygous (with relation to nucleotide sequence), if one allele is inactivated intragenically [2]. Typically, LOH analysis involves comparison of allelic profiles of microsatellite markers generated by amplification of DNA from matching normal and tumor samples. An implementation of commercially available multiplex PCR kits and automated fluorescence based DNA detection technology allow increased sensitivity, unmatched accuracy and high throughput of samples for forensic casework analysis and kinship tests [3,4].

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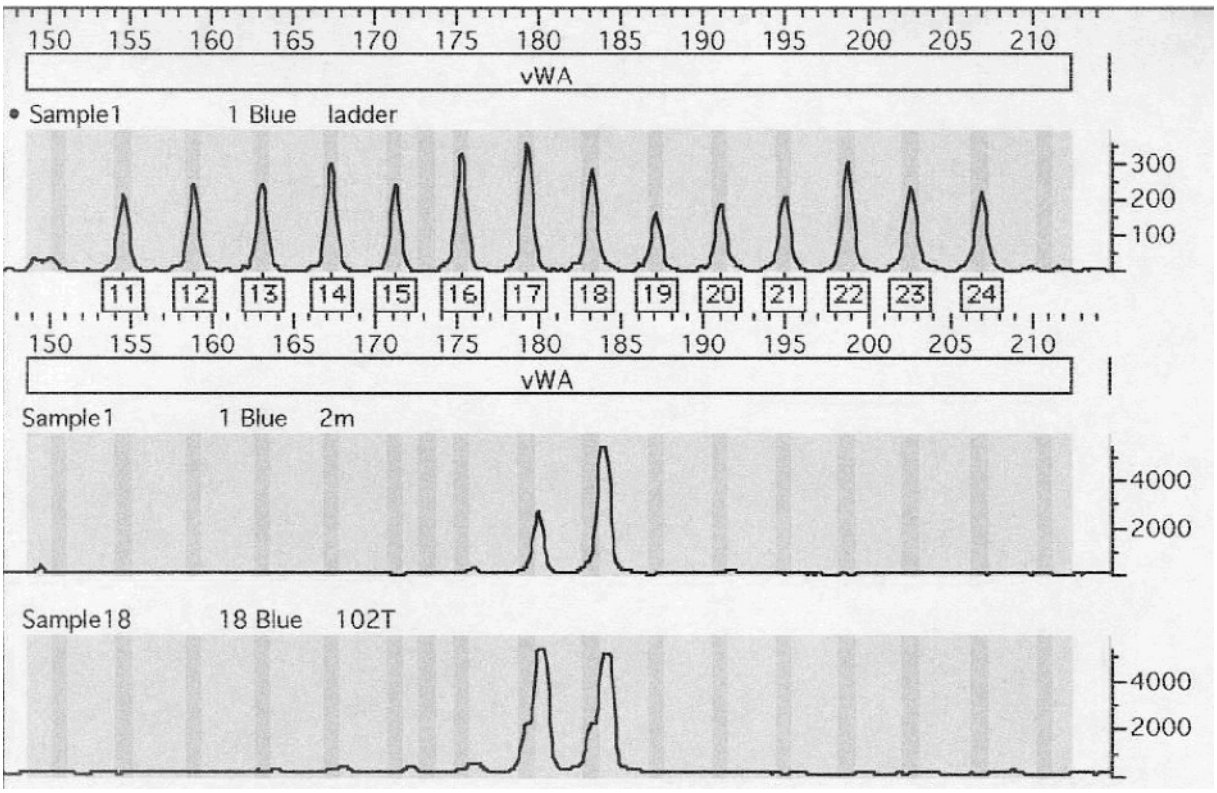

Fig. 1. Electrophoregram of pLOH at locus vWA. Upper panel – allelic ladder (Sample 1); lower panel – reference blood DNA showing a profile of alleles 17 and 18 (Sample 18); middle panel – larynx carcinoma DNA showing partial loss of allele 17 (Sample 1).

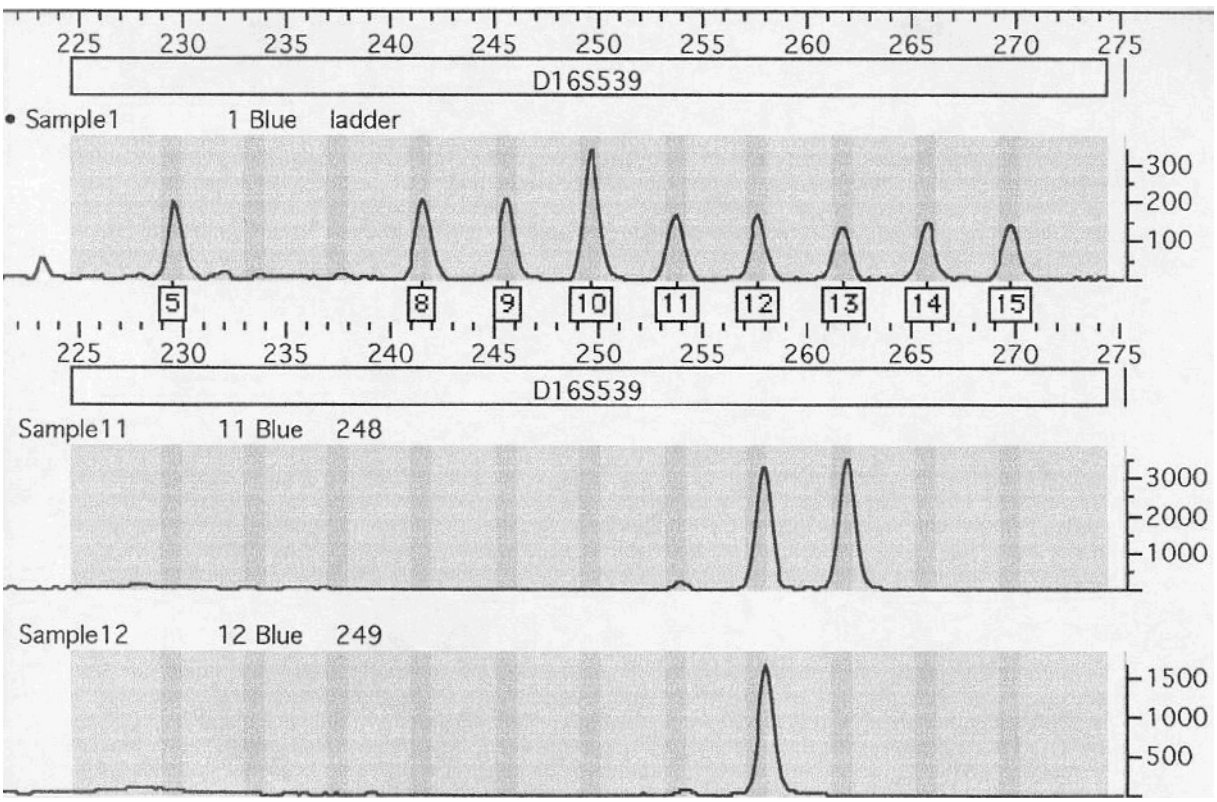

Fig. 2. Electrophoregram of LOH at locus D16S539. The upper panel – allelic ladder (Sample 1); middle panel – reference blood DNA showing a profile of alleles 12 and 13 (Sample 11). The lower panel – liposarcoma DNA showing complete loss of allele 13 (Sample 12).

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Table 1. Marker characteristics

Marker	Chromosomal location	
D2S1338	2q35-37.1	
TPOX	2p23-2pter	intron 1 of human tyrosine hydroxylase gene
D3S1358	3p21.31	
FGA	4q28	intron 30 of human alpha fibrinogen gene
CSF1PO	5q33.3-34	intron 6 of human c-fms proto-oncogene for CSF-1 receptor gene
D5S818	5q21-q31	
D7S820	7q21.11	
D8S1179	8q24.13	
TH01	11p15-15.5	intron 1 of human tyrosine hydroxylase gene
VWA	12p12pter	intron 40 of von Willebrand factor
D13S317	13q22-q31	
D16S539	16q24-ter	
D18S51	18q21.3	
D19S433	19q12-13.1	
D21S11	21q11.2-q21	

Multiplex AmpFlSTR Identifiler PCR Amplification Kit (Applied Biosystems) has been validated to produce rapid and robust amplification of multiple DNA loci from biological samples [5]. Amplification of microsatellite markers will yield one or two major allele peaks, depending upon whether the individual is homozygous or heterozygous for that marker. Loss of an allele in the tumor sample that is found in the corresponding normal sample indicates LOH. High incidence of LOH is regarded as a unfavourable prognostic factor, accompanying aggressive nature of the tumour and indicates involvement of certain genome regions in cancerogenesis. Genetic establishment of paternity in deficient cases, the identity of unknown human corpses or remains is usually based on comparisons with DNA profiles obtained from reference samples collected from female or male relatives. When no such samples are available a direct comparison with genetic data of DNA derived from personal items can be efficient, assuming sufficient quantities of DNA can be recovered from a personal effect and its sole use by the victim can be assured. Use of histolopathogical tissue sections as a source for DNA may be necessary in such cases as well as if mix-up of tissue specimens or medical malpractice is suspected. Due to increasing incidence of tumours, according to the Polish National Cancer Registry, availability of histolopathogical tissue is consequently higher. A significant part of this archived material includes solid tumors known to retain genetic alterations in defined genes but also in repetitive sequences of non-coding DNA regions. Therefore, alterations of STRs used in forensic casework are also possible. In this view, loss of heterozygosity (LOH) and microsatellite instability (MSI) are the most important characteristics of many tumors [6]. The aim of the study was assessment of possible effects of LOH at tetranucleotide STR loci in four different tumor types on human genetic identification.

Material and methods

Sample material. The study material comprised the following tumour specimens: larynx carcinoma from 45 patients treated at the Otolaryngological Department, Medical University of Bialystok, uveal melanoma from 16 patients treated at the Ophthalmological Department, Medical University of Bialystok, cervical carcinoma from 20 patients and sarcoma from 20 patients treated at the Regional Center of Oncology in Bialystok. The specimens were collected during surgical procedures and verified histopathologically. Reference blood samples were collected from all the patients. The material was stored at -20°C until examination.

Genetic analysis. Standard organic procedure was used for DNA extraction with additional microcolumn purification (QIAquick, Qiagen) when necessary. Fluorescent multiplex PCR was used according to the manufacturer's recommendations to amplify 15 tetranucleotide-repeat microsatellite loci contained in the commercially available AmpFlSTR Identifiler PCR Amplification Kit (Applied Biosystems) (Table 1). The research panel includes 13 STRs that constitute the STR standardization project – CODIS announced in 1997 by the FBI and adopted afterward by forensic DNA analysts worldwide [7]. Genotyping was performed in a 310 ABI Prism Genetic Analyzer (Applied Biosystems) using GeneScan v3.7 and Genotyper v3.7 software. The analyses were performed twice for reproducibility. LOH was defined as a decrease (at least 50%) in peak height of an allele compared with that of the other determined after comparison of normal and pathologic DNA.

Statistical analysis. Frequency of LOH was calculated for respective samples. Statistical comparisons were carried out using Fisher's exact test with SPSS software package v.11.0 (SPSS Inc., Chicago, USA). *P*-values of 0.05 or less were considered as statistically significant.

Results

Forty-two percent of the 101 cancer cases analysed were found to possess alterations of the microsatellites manifesting with allelic loss. Partial or complete loss of one allele (pLOH or LOH) could be observed in our tumour samples (Fig. 1 and Fig. 2). The most frequently altered loci were D3S1358 and D18S51. Distribution of LOH incidence over the fifteen STR loci in all investigated cancer types is displayed in Table 2. The alteration was detected in 47% of cases with larynx carcinoma: 1 patient (2%) displayed LOH at 5 loci, 4 patients (9%) at 4 loci, 7 patients (16%) at

Table 2. Distribution of LOH over the fifteen STR loci in all investigated cancer types.

Type of cancer	D2S1338	TPOX	D3S1358	FGA	CSF1PO	D5S818	D7S820	D8S1179	TH01	VWA	D13S317	D16S539	D18S51	D19S433	D21S11
larynx carcinoma	-	-	6 13%	2 4%	8 18%	5 11%	-	1 2%	-	3 7%	4 9%	-	7 16%	-	-
uveal melanoma	3 19%	1 6%	1 6%	-	-	-	-	1 6%	-	-	4 24%	1 6%	-	-	-
cervical cancer	-	-	8 40%	-	-	-	-	1 5%	4 20%	1 5%	-	1 5%	6 30%	2 13%	1 5%
sarcoma	-	-	-	-	-	-	-	1 5%	-	-	-	1 5%	1 5%	-	-

3 loci, 4 patients (9%) at 2 loci, 5 patients (11%) at 1 locus. Allelic loss frequency was statistically significant in high G grade cancers (P=0.0032). The highest LOH frequency was found in the tumor cases where the neighbouring cervical lymph nodes were affected (P=0.0041). LOH was found in 44% of cases with uveal melanoma: 1 patient (6%) displayed LOH at 3 loci, 2 patients (13%) at 2 loci, 4 patients (25%) at 1 locus. LOH was detected in 60% of cases with cervical cancers: 3 patients (15%) displayed LOH at 3 loci, 4 patients (20%) at 2 loci, 5 patients (25%) at 1 locus. In all cases with poor prognostic histopathologic factor (G3) the alteration was present in all three loci (P=0.0084). Allelic loss was detected at D8S1179 and D16S539 in one case of liposarcoma G3 and at D18S51 in one case od neurofibrosarcoma. No LOH was found in liposarcoma G1, dermatofibrosarcoma and cystosarcoma protuberans in either primary or recurrent tumours. In benign tumours (lipoma and fibroma) LOH was also absent.

Discussion

Application of microsatellite loci as forensic genetic markers is common due to their abundance in the genome, extreme polymorphism and amplification by the PCR reaction [8,9]. STR markers can be also used to detect a change in length of a microsatellite allele resulting from either insertion or deletion of repeat units during DNA replication and failure of the DNA mismatch repair system to correct these errors referred to as microsatellite instability (MSI) [10,11]. Involvement of certain genome regions in cancerogenesis may result in LOH/MSI genotype at a locus of interest displaying allelic drop-out and/or multiple allele peaks [12,13]. Contrary to quasimonomorphic mononucleotide repeat markers, polymorphic tetranucleotide repeat markers used in our study are reported to be less sensitive and specific to MSI alterations in tumor samples with mismatch repair defects [14,15]. Nonetheless, the use of tumor tissue for forensic applications is questionable. In the present study LOH was found in 21 out of 45 cases (47%) of larynx carcinoma. Our findings are in contrast with those of El-Naggar *et al.* who reported LOH in 87% of patients suffering from head and neck squamous cell carcinoma [16]. According to these authors, in non-invasive cancers LOH was observed on chromosomes 9p, 9q, 11q, 17p, 18p, while in invasive cancers on chromosomes 3p, 8p, 9p, 9q, 11q. Obviously, our goal involved the system of loci accepted commonly for human forensic identification [17] that did not match exactly the research panel used by the latter authors. It should be pointed however, that in the both studies comparable results were obtained for LOH incidence on chromosomes 3p and 18q. Also Veltman *et al.* reported LOH at 18q21 in pre-malignant laryngeal lesions, which suggest involvement of this chromosomal region in the laryngeal carcinogenesis [18]. Malignant uveal melanoma was documented by Metzelaar-Blok *et al.* to display recurring abnormalities particularly on chromosome 6q [19]. In contrast to the frequency of 6q loss, other authors observed LOH at loci on four other chromosomes (1, 11, 16, 17) in only 5% of cases [20]. Scholes at al. reported LOH in 63% of tumors, the majority involving chromosome 3 (52%) [21]. According to these authors, the second most common alteration (21%) was LOH at 13q14 and at 13q12.3-q13 – an RB1 intragenic marker, with retention of a more centromeric 13q marker (near BRCA2). In our material LOH was found in 7 out of 16 cases (44%), most frequently at 13q22-q31 (24%), which corresponds with the latter findings. Chromosome 6p is reported the most frequently to display LOH in patients with cervical carcinoma [22-24]. Other most common sites of LOH are 3p,

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11q, 17p, 18q. LOH was frequently found at 3p21.1 (41%) [24]. About 40% of squamous cell carcinoma of uterine cervix displayed LOH at 11q23.3 and 11q22 [25,26]. On chromosome 17p13.3 LOH was found in about 35% of specimens [24,25]. Kozlowski *et al.* demonstrated LOH in 75% cases of squamous cell carcinoma G2 and G3 [27]. According to these authors, eight of nine cervical cancers (83.3%) in clinical stage III showed LOH. In these samples LOH was also present on two or three other chromosomes (3p, 11p, 12p, 18q and 21q). In our material LOH was detected in 60% of cases with cervical cancers. Few studies have investigated the loss of heterozygosity and microsatellite instability in soft tissue sarcomas. Taubert *et al.* analyzed samples of human soft tissue sarcomas to determine the status of the chromosomal region 12q14-15, which contains the MDM2 gene encoding the well-known counterpart of the tumor suppressor p53 [28]. Of the 88 soft tissue sarcoma samples, 24 (27%) showed evidence of LOH at markers representing 12q14-15, and 12 (14%) showed evidence of MSI. According to these authors, the alterations occur early in soft tissue sarcoma progression and possibly define a subgroup of soft tissue sarcoma. In the material analyzed here, we found no evidence of LOH at the chromosomal region 12p12pter which contains intron 40 of the human von Willebrand factor gene, associated with the endothelial structure [29]. In the present study the changes were distributed over fourteen out of fifteen STRs analysed, but the most affected were D3S1358 and D18S51. This prevalence is in line with the comprehensive results from 118 solid tumors, 46 lymph node metastases, and 16 distant metastases analysed with the AmpFlSTR Profiler by Poetsch *et al.* [30]. The presented data as well as reports of other authors suggest that genetic instability resulting from defective human DNA mismatch repair mechanism in human carcinomas may cause interpretation difficulty during forensic genotyping and DNA profile matching [31-33]. We suggest that during genotyping human DNA extracted from histopathological tissue sections caution should be taken when non-match or exclusion based on few discrepancies is concluded.

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