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DNA typing of debris from fingernails

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Summary. In 3 series paired volunteers were asked to gently scratch each other with the fingernails to produce superficial abrasions only of the stratum corneum. In a 4th series scratch marks were produced in the skin of cadavers but additionally including the deeper epidermal layers. Debris was removed using a thorough technique in series 1 and 2 and a careful technique in series 3. After DNA extraction, the debris was typed using the STR systems HUMACTBP2 (SE33), HUMTH01 (TC11) and HUMVWFA31 (VWA). In the material obtained from series 1 (i.e. scratching with no prior cleaning of the nails) and series 2 (i.e. cleaning of the nails prior to the experiment) the debris was removed with a sharp instrument and only the DNA pattern of the person who carried out the scratching could be detected. In the 3rd series extraneous material was removed very carefully from under the fingernails to avoid contamination with DNA from the nails. In 71% of these cases DNA patterns of the person who had been scratched or mixed DNA patterns of both persons could be detected. In the experiments with postmortem skin the DNA pattern of the cadaver could be detected in all cases. These results show that in crime cases where the perpetrator has been scratched by the victim, sufficient material can be obtained from under the fingernails for DNA typing if removal of the particles is carried out with sufficient care.

Key words: Fingernails – Scratched epidermal particles – PCR-VNTRs – Short tandem repeats

Zusammenfassung. In 3 Versuchsreihen erzeugten freiwillige Probanden jeweils gegenseitig oberflächliche Kratzverletzungen, die ausschließlich auf das Stratum corneum der Epidermis beschränkt waren. In einer vierten Versuchsreihe wurden Kratzverletzungen auch der tieferen Epidermisschichten an Leichenhaut hervorgerufen. Die Epithelschüppchen unter den Fingernägeln wurden mit verschiedenen Techniken asserviert und nach DNA-Extraktion unter Anwendung der STR-Systeme HUMACTBP2 (SE33), HUMTH01 (TC11) und HUMVWFA31 (VWA) individualisiert. In den beiden ersten Versuchsreihen (1. Kratzen mit zuvor ungereinigten Fingernägeln, 2. Asservieren der Epithelschüppchen mit scharfem Instrument und intensiver Reinigung vor und nach dem Kratzen) konnten lediglich Merkmale des Kratzenden festgestellt werden. Da bei der dritten Versuchsreihe besonderer Wert auf eine vorsichtige Asservierung der Epithelpartikel gelegt wurde, ohne dabei gleichzeitig Schüppchen der Fingernägel abzukratzen, gelang es in 71% der Fälle, die DNA des Gekratzten oder ein Mischmuster des Kratzenden und Gekratzten darzustellen. Die Versuche an Leichenhaut ergaben in allen Fällen das Muster des Verstorbenen. Die Ergebnisse belegen, daß bei entsprechenden Spurenfällen die Untersuchung eines sorgfältig präparierten Fingernagelschmutzes in einem hohen Prozentsatz erfolgreich sein kann und somit zur Identifizierung eines Tatverdächtigen geeignet ist.

Schlüsselwörter: Fingernagelkratzspuren – Epidermispartikel – PCR-VNTRs – Short Tandem Repeats (STRs)

Introduction

Violent crimes are not infrequently associated with a superimposition of multiple actions of aggression and defense. During the defense actions, especially by the victim, the perpetrator often suffers from scratch injuries. This is mainly true for rape as well as for deaths associated with mechanisms of suffocation. Scratch injuries are preferentially located on the head, on the arms and sometimes on the trunk. Classically, a scratch injury is an excoriation, defined by loss of the Stratum corneum and the superficial epidermial layers. Usually, no bleeding occurs and the scratch is transformed postmortem to a dessication of the skin or in a living person to a scab which heals leaving no scars. Deeper scratch injuries associated with bleeding can also occur. In addition to these types very superficial lesions only of the Stratum corneum are more commonly found which are very difficult to recognize morphologically. – In relevant cases the medico-legal analysis is mainly directed towards the fingernails of the victim. Special care is taken if there are fresh fractures or cracks in the nails. In such cases microtechniques are applied to recover small fragments of epi-

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dermis from the other remnants. Despite a high expectation there is only a very low success rate in this field of investigation.

It was the aim of the present investigation to apply recent DNA technologies in order to check whether (epidermal) DNA of the scratched person can be detected under the fingernails of the scratcher independent of the problematical morphological proof of epidermis. Therefore, we decided to experimentally cause and check very superficial scratches which morphologically did not give rise to marks or only very superficial marks.

Materials and methods

Experimental design

The experimental design was subdivided into 4 series. In 3 series, volunteers were arranged pairwise and asked to superficially scratch the "partner" while in the 4th series scratch experiments were performed on the skin of male cadavers. In the following, the scratcher is synonymous with "victim" and the injured person with the "perpetrator". The persons were asked to cause only very superficial lesions resulting in a red coloration of the skin with no discharge from the injury. Only the middle and the ring fingers were used. In each series, two very similar experiments were performed with one difference: in the first experiment scratching was performed with uncleaned fingernails, in the second experiment with cleaned fingernails. The following regions were selected for scratching: extensor area of the forearm, extensor area of the upper arm, posterior neck, scalp (Table 1). The remnants under the fingernails were removed with a small pair of scissors. In series 1 and 2 the cleaning procedure applied was quite extensive leading to abrasion of the fingernails and the skin. In the third series the removal of particles was carried out very carefully to avoid abrasion of the surface of the fingernail.

In addition to the scratch experiment, the volunteers in the first two series were also asked to recover material by scratching themselves with the blade of a scalpel. These injuries were also very superficial causing only reddening of the skin.

Skin abrasions on cadavers were caused in the early postmortem period (under 24 h) leading to a classical excoriation (with subsequent dessication of the skin).

In all experiments the material recovered from under the fingernails of both fingers was pooled.

DNA extraction and PCR based typing

In series 1 and 4 DNA was extracted after proteinase K lysis by phenol chloroform and precipitated by ethanol (Brinkmann et al. 1991). The DNA was resuspended in 50 µl Aqua bidest. In the other two series, Chelex extraction was performed according to Walsh et al. (1991) with a minor modification: the extraction volume consisted of 150 µl of 5% Chelex solution and 50 µl of proteinase K (2mg/ml).

The PCR assay contained 5 µl of the phenol chloroform extraction and 10 µl of the Chelex extraction (further conditions for HUMTH01, Edwards et al. 1992 and HUMACTBP2 – Polymeropoulos et al. 1992; Wiegand et al. 1993). The PCR conditions for HUMVWFA31 (Kimpton et al. 1992) were as follows:

PCR protocol

1 U Taq-Polymerase (Promega, USA), 0.3 µ*M*/primer 100 µ*M* dNTPs, 2 µl 10 × buffer (Promega, USA), distilled water added to a final volume of 25 µl, oil overlay.

Primer 1: 5′-CCC TAG TGG ATG ATA AGA ATA ATC-3′
Primer 2: 5′-GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG-3′

Temperatures (30 cycles):
94°C – 1 min
50°C – 1 min
72°C – 1.5 min

Thermocycler: Triothermoblock, Biometra/Germany

Gels: Polyacrylamide urea gels (6%, 10 *M* urea), piperazin diacrylamide as cross-linker, formate 80 m*M*; 20 cm separation distance, 2% agarose plugs, 29 m*M* CHES (discontinuous PAGE according to Allen et al. 1989).

Electrophoresis. 1000 V, 40 mA, initially with 5 W and ramping at 5 W per hour up to 15 W; silver staining (Budowle et al. 1991).

Results

In the 4 series a total of 121 samples were investigated. The scratch injuries were always as originally planned with two exceptions where epidermal abrasions were caused although only very superficial ones had been

Table 1. Results of the scratch series using the STR systems HUMTHO1, HUMACTBP2 and HUMVWFA31. (Results are to read as follows: e.g. 2nd column: 1a) 10 out of 11 amplifications led to interpretable results in HUMTH01. The numbers in parentheses show the success rate of PCR typing)

System		Series						Sum
		1a) Sc Phe-Chl pos./sum	1b) F Phe-Chl pos./sum	2a) Sc Chelex pos./sum	2b) F Chelex pos./sum	3) F Chelex pos./sum	4) F Phe-Chl pos./sum	
HUMTH01	(*n* =)	10/11	7/15 V	12/12	8/12 V 1/12 M	6/ 8 S		44/ 58 (76%)
HUMACTBP2	(*n* =)	5/11	4/ 9 V		8/12 V	3/ 8 S 2/ 8 M	2/3 S 1/3 M	25/ 43 (58%)
HUMVWFA31	(*n* =)				8/12 V	5/ 8 S 1/ 8 M		14/ 20 (70%)
Sum	(*n* =)	15/22 (68%)	11/24 (46%)	12/12 (100%)	25/36 (69%)	17/24 (71%)	3/3 (100)	83/121 (69%)

Sc = recovery of epidermal material using a scalpel
F = scratching with fingernails
Phe-Chl = phenol chloroform extraction
Chelex = Chelex 100 extraction
pos. = successful typing
V = band pattern of victim detected (scratching person)
S = band pattern of suspect detected (scratched person)
M = mixed stain containing the pattern of both victim and suspect

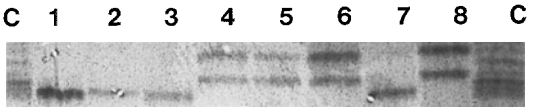

Fig. 1. HUMTH01 amplification, patterns of material collected from fingernails of the "victim" compared to blood from the "suspect" (series *3*, scratch area: head and neck). *(C)* = allelic ladder. *(1–3)* = fingernail debris of "victim 1" with uncleaned (*1*; neck) and cleaned fingernails (*2, 3*; neck and head). *(4–6)* = fingernail debris of "victim 2" with uncleaned (*4*; neck) and cleaned fingernails (*5, 6*; neck and head). *(7–8)* = blood DNA of the two volunteers

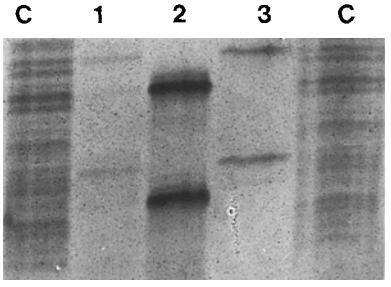

Fig. 2. HUMACTPB2 amplification patterns: scratch experiment on the skin of male cadaver (series *4*, Table 1; scratch area: right arm). *(C)* = allelic ladder. *(1–3)* = mixed pattern of fingernail debris *(1)* with alleles from the "victim" *(2)* and the "suspect" *(3)*

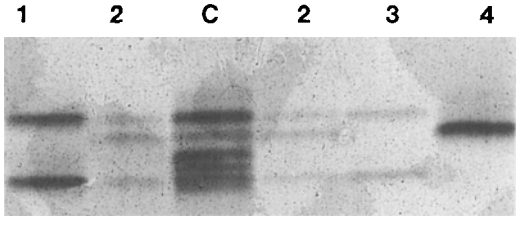

Fig. 3. HUMTH01 typing: murder case with a micro-bloodstain under the fingernails of the victim. The stain was 2 years old. Chelex extraction. *(C)* = allelic ladder. *(1)* = victim. *(2)* = mixed stain on the fingernails of the victim containing blood from the suspect and epidermal cells from the victim. *(3)* = bloodstain from the victim on the clothes of the victim. *(4)* = suspect

planned. On average, the PCR based analysis was successful in 69% (Table 1). In the first two series we regularly detected the pattern of the scratcher (with one exception). In the third series either the DNA pattern of the scratched person (Fig. 1) or a mixed DNA pattern from both persons was recovered in the positive cases. – The scratch experiments performed on postmortem skin had a 100% success rate and always included the DNA information derived from the scratched person (Fig. 2).

From the systems applied HUMTH01 and HUMVWFA31 appeared to be slightly more successful than HUMACTBP2 (Table 1).

Discussion

The applicability of STR systems has considerably enhanced the possibilities of DNA analysis in biological stains (Brinkmann 1992). Based on the exceptional sensitivity of these systems, even minute stains such as scratched remnants under fingernails offer a realistic chance of successfully highlighting the pattern of the scratched person. This was applied in a rape case and the DNA pattern of the suspect could be demonstrated under the fingernails of the victim (Fig. 3). Especially important is the method of preparation. A careful but not extensive cleaning procedure is the most successful because more extensive procedures lead to an excess of DNA from the fingernails of the scratcher. This can give rise to allelic drop-out or to a quantitative suppression of the other contributor. Chelex extraction was at least as efficient as phenol chloroform extraction but is much less time-consuming and is therefore to be recommended.

The aforementioned observations could have an impact on the practice of investigation of relevant crime cases where either scratch contacts have probably occurred due to the pattern of findings or due to the circumstances of the case. In such cases we recommend the following procedure:

- the remnants under fingernails should be carefully but not extensively removed with an appropriate tool (e.g. wood or plastic toothpicks);
- the remnants recovered should be investigated even if there is no morphological evidence of skin fragments;
- if the material recovered from different fingers has to be pooled, both hands should be treated separately. Pooling can enhance the chance to recover sufficient template DNA.

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