CASE REPORT

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Dandruff as a Potential Source of DNA in Forensic Casework*

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ABSTRACT: Dandruff is a clinical alteration of the skin that consists histologically of orthokeratotic clumps with minute parakeratotic foci found in inflammatory pathologies such as seborrheic dermatitis and psoriasis. Therefore, some nucleated cells should be found in dandruff and hence there is a possibility that forensically typeable DNA could be extracted from dandruff.

Because of a particular case in which we were involved, a study was carried out to determine whether or not DNA could be extracted from dandruff, and if the two most widely used extraction techniques (Chelex and organic) would be applicable.

Results show that sufficient quantities of DNA (more than 30 to 40 ng) can be obtained from as little as 1.0 to 1.5 mg of dandruff. Both methods yield DNA, although the organic procedure seems to yield more (72.5 ng Chelex vs. 183.3 ng organic). All the DNA samples extracted were typed correctly for the loci HUMTH01 and HUMvWA.

Therefore, dandruff can be considered a potential source of DNA for forensic identification.

KEYWORDS: forensic science, dandruff, DNA typing, polymerase chain reaction, HUMTH01, HUMvWA

Dandruff is produced as a pathological desquamation of the skin, and histologically consists of orthokeratotic clumps with minute parakeratotic foci. Dandruff is often found in inflammatory pathologies such as seborrheic dermatitis and psoriasis (1).

Based on the etiology, where inflammatory pathologies are involved, it is presumed that some nucleated cells could be found in dandruff; therefore, in some cases, where sufficient quantities of dandruff could be obtained, DNA for the polymerase chain reaction (PCR) might be obtained for DNA analysis.

Because of a missing body case in Granada, Spain, a preliminary set of studies was performed to determine: (i) the potential to obtain typeable DNA from dandruff; and (ii) whether the Chelex-100 and/or organic phenol:chloroform extraction method is useful to recover forensically typeable genetic material from dandruff.

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Materials and Methods

Dandruff sampling—Dandruff samples, ranging between 1.0 and 2.3 mg of total weight, were obtained from 14 different donors. All the samples were directly recovered from the head of the donor by scratching with the hand protected with sterile gloves. Dandruff flakes were collected on sterile plain white paper and then transferred inside sterilized paper envelopes, where they were stored in a refrigerator (+4°C) until used.

DNA extraction—Since there were no known references for extracting DNA from dandruff, the two most widely used extraction procedures for blood were employed. These are the Chelex-100 and the organic phenol:chloroform methods.

Chelex extraction—The classical procedure (2) with slight modifications was used. Basically, 5% Chelex-100® was directly added to 1.0 to 1.5 mg of dandruff without previous washing/incubations steps. The protocol is as follows: using a sterile brush, 0.5 to 1 mg of dandruff was placed in to a 1.5 mL microcentrifuge tube. To the tube, 50 to 100 μL of 5% Chelex-100® were added directly. The tube was incubated at 56°C for 30 min. The tube was vigorously vortexed for 10 s, then incubated in a boiling water bath for 8 min. The tube was then vortexed vigorously for 10 s and placed in a microcentrifuge and subjected to maximum speed centrifugation (13,000 *g*) for 3 min. The quantity of extracted DNA was estimated using the slot-blot hybridization assay (3).

Phenol-chloroform extraction—The organic extraction protocol previously described (4) was used. Minor variations from this protocol are the following. Using a sterile brush, 1.0 to 1.5 mg of dandruff was added to a 1.5 mL microcentrifuge tube. To the sample were added 200 μL of stain-extraction buffer (10 mM Tris-Hcl, pH 8.9; 10 mM EDTA; 0.1 M Na Cl; 2% SDS), 8 μL 1 M DTT, and 5 μL of 10 mg/mL Proteinase K. The tube was incubated at 56°C overnight (12 to 14 h). Then, an equal volume (215 μL) of phenol/chloroform/isoamyl-alcohol 25:24:1) was added to the extract. The tube was vortexed vigorously for 10 s and then subjected to centrifugation (2500 *g*) for 10 min. The aqueous phase was transferred to a Microcon®-100 concentrator. The DNA was recovered in 100 μL of TE^{-4} (10 mM Tris•Cl, pH 8.0, 0.1 mM EDTA). The quantity of extracted DNA was estimated using the slot-blot hybridization assay (3).

DNA typing—Approximately 3 ng of DNA were successfully

amplified for the loci HUMTH01 and HUMvWA according to previously reported methods (5,6).

Results and Discussion

Apparently DNA extraction methods designed for blood or epithelial cells also are applicable to extracting DNA from dandruff. Both the Chelex-100 and organic methods proved to be useful for extracting a sufficient quality and quantity of DNA. Amounts of dandruff ranging between 1.0 to 1.5 mg of dandruff yielded 183.3 $\pm$28.9 ng ($n = 7$) with the organic protocol, and 72.5 $\pm$37.1 ng with Chelex ($n = 6$). All genotypes from dandruff perfectly matched the known controls obtained from the saliva of donors (data not shown).

It is known that the frequency of seborrheic dermatitis ranges between 3% and 5% (7). The adult form is more common in men than in women, and there does not appear to be any racial predilection (7).

In a particular case, it would be very difficult to estimate accurately the amount of DNA (ng) that could be extracted from a given amount of dandruff (mg) because of: (*a*) the limited amount of this biological material that might be found at a crime scene or serve as an exemplar. We found that the amount of dandruff that could be obtained from our sample of 14 individuals ranged between 0.8 and 6.2 mg, and these amounts are difficult to collect and handle. (*b*) The drastic influence of inflammatory processes (psoriasis, spongiotic psoriasiform dermatitis, etc.), which could facilitate the release of nucleated cells (basically neutrophils and/or lymphocytes) that adhered to the non-nucleated desquamation tissues. Thus, it has been reported that a large number of parakeratotic cells associated with some infiltrated polymorphonuclear leukocytes (PMNLs) were found in the scales obtained from 11 individuals complaining of dandruff (8), making it possible for these authors to demonstrate the presence of neutrophil chemotactic anaphylotoxins in human dandruff.

Regardless, at least 30 to 40 ng of DNA were recovered from dandruff with either extraction procedure. The data support that dandruff can be a reliable source of biological material in some casework scenarios, and this material could be collected and stored along with hairs, nails, and other biological specimens.

It has been suggested that accidental contamination from foreign sources could be problematic in forensic science (9), and dandruff could be a potential contaminant in some cases. Therefore, depending on the case situation, we would recommend wearing haircaps.

In our particular case, DNA typing from dandruff obtained from clothes in the house of a missing person was compared with the DNA obtained from a set of bones. The results were consistent with the presumed identity. Further, mock case analyses performed in our laboratory gave always positive and conclusive results using dandruff as the only source of DNA.

Further studies are underway to determine the influence of medication and cosmetic products on the recovery of a sufficient quality and quantity of DNA from dandruff and to determine if the relationship between the amount of dandruff recovered and the amount of DNA expected can be roughly predicted.

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