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Pre-analytical Associated Factors of Foreign DNA Detection Beneath Deceased's Fingernails

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Abstract:

Objective: To assess the pre-analytical factors including hand-side, representative collection technique and individual forensic physician for foreign deoxyribonucleic acid (DNA) detection in specimen collected from deceased's fingernails.

Material and Methods: This cross-sectional study, of an initial 164 samples from the fingernails of both hands, of 82 deceased caseworks; from 2010 to 2018, at the Forensic Unit, Faculty of Medicine, Prince of Songkla University. The autosomal short tandem repeats profile fingernail DNA results, pre-analytical factors, and the deceased's characteristics were obtained from the records. The fingernail DNA outcomes were evaluated and ranked into five groups, i.e., high-level profile, low-level profile, or residual profile; if foreign alleles of more than 11 alleles, 4 to 11 alleles, or less than 4 alleles were detected, respectively. The non-specific profile group consisted of foreign DNA being detected; however the peak signals were below the decisional threshold. The unidentified group consisted of no foreign DNA being detected. The full model underwent both directional stepwise model selection, and the resulting model with the lowest Akaike information criterion was selected as the final model. The final model was analyzed by ordinal logistic regression for significant associated factors: at a 95% confidence level.

Results: The representative collection technique is an associated factor, via the use of fingernail swabs (adjusted odds ratio (OR_a)=13.44, 95% confidence interval (CI)=2.89–62.45), and had a larger effect size than using fingernail cuttings (OR_a=6.84, 95% CI=1.47–31.86).

Conclusion: At post-mortem examination, for the collection of foreign DNA from fingernails, the use of fingernail swabs, as a collection technique, is of particular interest.

Keywords: associated factor, evidence, fingernails, foreign DNA, forensic DNA, specimen collection

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Introduction

The most crucial objective of a post-mortem examination after a homicide death is to identify the assailant(s). This may require evidence collected by a physician, which can support the judgement in the determination of sentencing[1].

By law, unnatural deaths and deaths that occur during imprisonment are required to undergo a post-mortem examination. The process of examination begins with external examination and specimen collection for laboratory investigation. In the specimen collection process, the physician will determine the method for deoxyribonucleic acid (DNA) collection from contact sites (e.g., fingernails, vagina, rectum, or other sites). The following techniques are considered for collecting specimens from fingernails: swabbing, clipping, or scraping. Even though the collection technique may influence the detection rate, the optimal sample collection method is unclear[2]. After specimen collection, the fingernail samples are sent to a laboratory for collection of exogenous cells via techniques, such as soaking, swabbing, or scraping, and the DNA is extracted by either silica-based extraction or organic extraction[3].

Previous studies have reported a 25% to 33% prevalence of foreign DNA; wherein, the DNA that may have been derived from an assailant(s), has been deposited beneath the victim's fingernails and casework[4,5]. Detection of foreign DNA could result in many possible alleles of autosomal short tandem repeats (STRs) profiles. Indeed, in the studies reported, foreign DNA has been detected among acquaintances living in the same household[6-8].

In a low prevalence of foreign DNA detection predicament, pre-analytical enhancement by collecting as much foreign DNA as possible beneath a victim's fingernails is invaluable; especially in an atmosphere of ambiguous methods of specimen collection. Therefore, this study aimed to assess the pre-analytical factors; including the hand-side, representative collection technique, and forensic physician, which were determined along with the deceased's characteristics.

Material and Methods

Study setting

A cross-sectional study was conducted in the Forensic Unit of the Faculty of Medicine, Prince of Songkla University, Thailand. The inclusion criterion was any deceased persons with a post-mortem examination by the institution's forensic physicians; from January 2010 to October 2018. The exclusion criterion was missing either the right-hand or left-hand fingernail DNA data. This study was approved by the Institute of Ethics Committee of the Faculty of Medicine, Prince of Songkla University (REC 60-028-05-1).

Specimen collection, and preservation

The decision on the collection of the fingernail specimens depended on the discretion of the forensic physician and/or inquiry official in general. Information regarding the post-mortem findings, digital photographs of the deceased, and a list of specimens collected including sites and collection technique were recorded. In this study, specimens for foreign DNA investigation from underneath the fingernails were grouped according to the representative collection technique: swabs from underneath the fingernails; cuttings from the fingernails; or both swabs and cuttings from the fingernails. The decision to use either swabs, cuttings, or both methods for fingernail collection depended on the discretion of the forensic physician; after having taken into account the condition of the deceased's fingernails. The collected specimens were consistently stored in separate containers, based on the hand side from which they were obtained. The specific techniques used for specimen collection were left to the discretion of the individual forensic physician. In terms of fingernail swab collection, these included decisions regarding which

finger(s) to sample, whether to use individual swabs for each finger and combine them in a single container, or a single swab for all fingers on a hand. For fingernail cuttings collection, the specific collection procedures also involved deciding whether to use the same or different nail clipper/scissors for both hands and all fingers, whether to clean or change the nail clipper/scissors between the collection of each fingernail, and whether to store the same hand fingernails in the same container or separate containers for each finger. Furthermore, for both swabs and fingernail collection, the decision to use both swabs and cuttings for fingernail collection, on a particular hand or both hands as well as the order in which these methods were employed, were also at the discretion of the forensic physician when collecting specimens. Additionally, separate specimens; such as blood, costal cartilage, or molar tooth (depending on the degree of decomposition) from the deceased, were collected for the purpose of obtaining their autosomal STRs profile. This profile can be used to compare the autosomal STRs profile results obtained from the fingernails, so as to determine the presence of foreign DNA. All specimens were sent separately to the laboratory and stored at a temperature of −20 °C until a request from the forensic physician was received.

DNA profile detection technique

Manual DNA extraction was conducted using 5% chelating resin (Chelex® 100 Resin, Bio-Rad Laboratories Inc.) and Proteinase K (Invitrogen™, Thermo Fisher Scientific Inc.) as an extracting solution. In the case of fingernail cuttings, the cuttings were submerged in deionized water and vortexed (Vortex-Genie® 2, Scientific Industries, Inc.), with the speed setting at position 7 for 1 minute; after which the supernatant and sediments were then collected. In the case of fingernail swabs, the whole swab was directly submerged into the extracting solution and then spun manually for 20 rounds. After extraction, the supernatant was measured for DNA template concentration via the UV-Vis Spectrophotometry method (NanoDrop™ One, Thermo Fisher Scientific Inc.) and adjusted to a concentration of 4 ng/µl. Polymerase chain reaction was then performed, using an AmpFlSTR® Identifier® Plus Kit (Applied Biosystems®, Thermo Fisher Scientific Inc.), analyzed using a 3130 Genetic Analyzer (Applied Biosystems®, Thermo Fisher Scientific Inc.), and reported in standard 16-loci autosomal STRs; based on the collection technique and the hand-side. The decision of whether to investigate Y-STRs in cases involving female victims was at the discretion of the forensic physician. There was no institution's policy prohibiting the investigation of Y-STRs in these cases.

Interpretation of the DNA profile outcomes

The fingernail DNA results were compared with the deceased's DNA profile. If foreign DNA was detected, the rank was determined as a high-level profile (HL) when >11 foreign autosomal STRs alleles were detected; as a low-level profile (LL) when 4–11 foreign autosomal STRs alleles were detected, or as a residual profile (RS) when <4 foreign autosomal STRs alleles were detected[4]. The rank was determined as a non-specific profile (NS) when a non-deceased person's DNA was detected, but the peak signals were below the laboratory technician's decisional threshold. If only the deceased's DNA profile was detected, the rank was determined as a single profile (SP). If no DNA was detected, the rank was determined as undetected (X).

Data collection and definition of the factors

Pre-analytical factors

Pre-analytical factors are factors that are related to the method of specimen collection including hand-side, representative collection technique, and forensic physician. The hand-side and the collection technique were taken from the DNA results, with specimens from the right hand and left hand being collected, processed, and reported separately.

The collection technique that yielded the maximum foreign alleles results was recorded as the representative collection technique that should be considered in analysis in this study; for example, if the DNA results from fingernail swabs yielded 12 foreign alleles, and those from fingernail cuttings yielded 10 foreign alleles, "swab" was recorded as the representative collection technique that yields HL. If the DNA results from a fingernail swabs yielded 10 foreign alleles and those from fingernail cuttings also yielded 10 foreign alleles; therefore, having an equal number of foreign alleles being counted: "both" was recorded as the representative collection technique that yields LL.

The forensic physician factor was the institution's physician with the Thai Board of Forensic Medicine accreditation from the Medical Council of Thailand who was responsible for post-mortem examination and specimen collection. With the data mostly taken from post-mortem examination reports, in cases where the post-mortem examination report was not available, the data was taken from the deceased's registration database. The individual forensic physicians were identified as "Physicians A–E".

The deceased's characteristics

The deceased's characteristics were factors that were not related to specimen collection and included: age, gender, fingerprints, hand-covering material, gunshot-related death, suspected physical assault, immersion-associated death and level of decomposition. The age and gender were mostly taken from the same source as the forensic physician. The fingerprints, hand-covering material, gunshot-related death, suspected physical assault and the level of decomposition were taken by reviewing the deceased's photographs, and were conducted by the principal investigator. If there was any case of doubt that particular factor was recorded as: "not available" (NA). A fingerprint was defined by ink-staining at any of the fingertips, and the hand-covering material was defined by the covering material on both hands. While a gunshot-related death was defined by the evidence of a gunshot wound at any body site, suspected physical assault was defined by any signs of physical assault; such as defense wounds, multiple blunt force traumas of the head, multiple stab wounds, strangulation, or fingernail marks at any body site. An immersion-associated death was determined by the inquiry officer's document; wherein, it described that the deceased was found in water. The level of decomposition was defined by any sign of decomposition at any stage: 1st day was defined by a greenish tinge of the abdominal wall, 2nd day was defined by marbling or skin coming off the body, 3rd day was defined by a full bloating stage, more than 3rd day was defined by beginning of the subsiding of a bloating stage or beyond, formalin-fixation was defined by formalin-fixed deceased persons.

Statistical analysis

The data were analyzed by R version 3.4.3 (The R Foundation for Statistical Computing). The prevalence was manually calculated by constructing point estimates and 95% confidence intervals (CIs)[9]. Ordinal logistic regression was used to control for confounding factors and to calculate the odds ratios (ORs), 95% CIs, and p-values for the association between foreign DNA detection and pre-analytical factors or the deceased's characteristics[10]. The outcomes on fingernail DNA data were managed by the SP and X profiles being merged into unidentified (U). The remaining profiles were ranked by the foreign DNA outcome, from the highest level to the lowest level of detection; i.e., HL, LL, RS, NS, and U. The univariate analysis results were then reported as crude OR (OR_c), 95% CI, and p-value. A set of models was constructed by starting with a full model, followed by both directional stepwise model selection, and the best-fit model was determined by using the lowest Akaike information criterion (AIC) for a final model[11]. The multivariate analysis results of

the final model were then reported as adjusted OR (OR_a), 95% CI, and p-value. The NA data were treated as they were during univariate analysis, and by being omitted during both directional stepwise model selection and multivariate analysis. The statistical significance of associated factors was determined by using a 95% confidence level.

Results

The fingernail DNA results of 164 samples, from 82 deceased persons, were obtained from both hands. As shown in (Table 1), the overall demographic data demonstrated that fingernail cuttings were the common representative collection technique that yielded the best possible foreign DNA detection on the right hand (52.4%) and on the left hand (58.5%). More than half of the data was contributed by one forensic physician (56.1%). The majority of the deceased had no hand-covering material (96.3%). The most common factor related to death was suspected physical assault (63.4%), and more than half of the deceased had no signs of decomposition (52.4%). Table 2 presents the HL profiles among 82 deceased persons, whether found on either their right or left hand. Out of the total deceased persons, the HL profiles were observed in 10 cases (12.2%) on either their right of left hand, and in 3 cases (3.7%) on both hands simultaneously. Of the ten deceased persons with HL profiles subgroup, the majority were detected on the right hand (80.0%), with half being detected on the left hand (50.0%). More than half of these cases were collected by one forensic physician (60.0%), and all of the deceased persons in this subgroup had no evidence of gunshot injury (100.0%). Additionally, the HL profiles were detectable on the second day of decomposition in 20.0% of these cases. The prevalence of the DNA results are also reported in (Table 3).

The univariate analysis results of 164 samples are shown in Table 4. The significant pre-analytical factors for foreign DNA detection were from the representative collection technique; as the fingernail swabs showed statistical significance (OR_c=3.10, 95% CI=1.13–8.53). Either the right-hand-side (OR_c=1.02, 95% CI=0.53–1.94) or the highest effect size for forensic physician E (OR_c=8.17, 95% CI=0.90–74.22) did not show statistical significance.

The multivariate analysis results of the final model from 148 samples, after NAs being omitted, are shown in Table 5. The significant pre-analytical factors for foreign DNA detection were from the representative collection technique and the forensic physician. The representative collection technique as an associated factor, by using fingernail swabs (OR_a=13.44, 95% CI=2.89–62.45), had a larger effect size than using fingernail cuttings (OR_a=6.84, 95% CI=1.47–31.86). The forensic physician was also an associated factor, by forensic physician E (OR_a=36.19, 95% CI=2.47–531.09). The significant deceased's characteristics factors for foreign DNA detection were also determined. The associated factors were male gender (OR_a=3.75, 95% CI=1.33–10.57), the use of paper bags as the hand-covering material (OR_a=63.18, 95% CI=3.31–1206.95) as well as suspected physical assault (OR_a=2.97, 95% CI=1.03–8.59).

Discussion

Foreign DNA beneath the deceased's fingernails is substantial evidence that can be used in the judicial process of a criminal case. However, it is frequently difficult to achieve a foreign DNA profile[4,6]. The objective of this study was to report the pre-analytical associated factors for detecting foreign DNA beneath the deceased's fingernails casework. In the univariate analysis, only one factor among the set of factors was considered at a time. The representative collection technique was identified as a significant factor, as fingernail swabs showed statistical significance. However, due to the potential presence of confounding factors, a multivariate analysis is necessary to accurately assess their impact. The multivariate analysis began by initially including all factors in the model, followed

Table 1 Demographic data of the total deceased

Demographic	Total
Deceased persons (n)	82
Representative collection technique (%)	
Both	Lt. 15 (18.3), Rt. 16 (19.5)
Cuttings	Lt. 48 (58.5), Rt. 43 (52.4)
Swabs	Lt. 19 (23.2), Rt. 23 (28.1)
Forensic physician (%)	
A	6 (7.3)
B	17 (20.7)
C	3 (3.7)
D	46 (56.1)
E	10 (12.2)
Age in years, median (IQR)	32.5 (24.8–47.0)
Gender (%)	
Female	35 (42.7)
Male	47 (57.3)
Fingerprints (%)	
No	20 (24.4)
Yes	61 (74.4)
Not available	1 (1.2)
Hand-covering material (%)	
None	79 (96.3)
Paper bag	1 (1.2)
Plastic bag	2 (2.4)
Gunshot-related death (%)	
No	70 (85.4)
Yes	12 (14.6)
Suspected physical assault (%)	
No	30 (36.6)
Yes	52 (63.4)
Immersion-associated death (%)	
No	67 (81.7)
Yes	15 (18.3)
Level of decomposition (%)	
No	43 (52.4)
1st day	11 (13.4)
2nd day	13 (15.9)
3rd day	10 (12.2)
>3rd day	3 (3.7)
Formalin-fixation	1 (1.2)
Not available	1 (1.2)

Lt.=left hand, Rt.=right hand

Table 2 Demographic data of the high-level profiles

Demographic	Total
Deceased persons (n)	10
Representative collection technique (%)	
Both	Lt. 0 (0.0), Rt. 1 (10.0)
Cuttings	Lt. 4 (40.0), Rt. 4 (40.0)
Swabs	Lt. 1 (10.0), Rt. 3 (30.0)
Forensic physician (%)	
A	0 (0.0)
B	2 (20.0)
C	0 (0.0)
D	6 (60.0)
E	2 (20.0)
Age in years, median (IQR)	33.0 (28.0–47.0)
Gender (%)	
Female	5 (50.0)
Male	5 (50.0)
Fingerprints (%)	
No	2 (20.0)
Yes	8 (80.0)
Not available	0 (0.0)
Hand-covering material (%)	
None	9 (90.0)
Paper bag	0 (0.0)
Plastic bag	1 (10.0)
Gunshot-related death (%)	
No	10 (100.0)
Yes	0 (0.0)
Suspected physical assault (%)	
No	3 (30.0)
Yes	7 (70.0)
Immersion-associated death (%)	
No	9 (90.0)
Yes	1 (10.0)
Level of decomposition (%)	
No	7 (70.0)
1st day	1 (10.0)
2nd day	2 (20.0)
3rd day	0 (0.0)
>3rd day	0 (0.0)
Formalin-fixation	0 (0.0)
Not available	0 (0.0)

Lt.=left hand, Rt.=right hand

Table 3 The prevalence of the DNA results from fingernails

Sample groups	HL % (95% CI)	LL % (95% CI)	RS % (95% CI)	NS % (95% CI)	SP % (95% CI)	X % (95% CI)
All samples (164 samples)	7.9 (3.8–12.1)	3.0 (0.4–5.7)	1.2 (0.0–2.9)	19.5 (13.4–25.6)	51.2 (43.6–58.9)	17.1 (11.3–22.8)
Left hand only (82 samples)	6.1 (0.9–11.3)	4.9 (0.2–9.5)	1.2 (0.0–3.6)	19.5 (10.9–28.1)	51.2 (40.4–62.0)	17.1 (8.9–25.2)
Right hand only (82 samples)	9.8 (3.3–16.2)	1.2 (0.0–3.6)	1.2 (0.0–3.6)	19.5 (10.9–28.1)	51.2 (40.4–62.0)	17.1 (8.9–25.2)
Both hands concordance (82 deceased persons)	3.7 (0.0–7.7)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	15.9 (7.9–23.8)	45.1 (34.4–55.9)	13.4 (6.0–20.8)
Gunshot-related death excluded (140 samples)	9.3 (4.5–14.1)	3.6 (0.5–6.6)	1.4 (0.0–3.4)	20.7 (14.0–27.4)	51.4 (43.1–59.7)	13.6 (7.9–19.2)
Suspected physical assault (104 samples)	8.7 (3.3–14.1)	3.8 (0.2–7.5)	1.9 (0.0–4.6)	15.4 (8.5–22.3)	51.0 (41.4–60.6)	19.2 (11.7–26.8)

HL=high level profile (>11 foreign autosomal short tandem repeats alleles detected), LL=low level profile (4–11 foreign autosomal short tandem repeats alleles detected), RS=residual profile (<4 foreign autosomal short tandem repeats alleles detected), NS=non-specific profile, SP=single profile, X=undetected, CI=confidence interval

Table 4 Univariate analysis results for the association between foreign DNA detection and pre-analytical factors or deceased's characteristics

Pre-analytical factors		
	OR_c (95% CI)	**p-value**
Hand-side		
Left hand	Ref	Ref
Right hand	1.02 (0.53–1.94)	0.957
Representative collection technique		
Both	Ref	Ref
Cuttings	1.42 (0.55–3.67)	0.470
Swabs	3.10 (1.13–8.53)	0.030
Forensic physician		
A	Ref	Ref
B	6.70 (0.78–57.53)	0.085
C	6.71 (0.48–94.16)	0.160
D	4.86 (0.60–39.32)	0.141
E	8.17 (0.90–74.22)	0.064

Table 4 (continued)

The deceased's characteristics		
	OR_c (95% CI)	**p-value**
Age		
Years	1.01 (0.99–1.03)	0.211
Gender		
Female	Ref	Ref
Male	1.28 (0.66–2.50)	0.464
Fingerprints		
No	Ref	Ref
Yes	1.74 (0.76–3.96)	0.191
Hand-covering material		
None	Ref	Ref
Paper bag	4.55 (0.56–37.01)	0.159
Plastic bag	9.89 (1.92–51.04)	0.007
Gunshot-related death		
No	Ref	Ref
Yes	0.25 (0.07–0.88)	0.032
Suspected physical assault		
No	Ref	Ref
Yes	0.88 (0.46–1.71)	0.709
Immersion-associated death		
No	Ref	Ref
Yes	0.61 (0.24–1.50)	0.282
Level of decomposition		
No	Ref	Ref
1^{st} day	0.54 (0.20–1.50)	0.238
2^{nd} day	0.27 (0.09–0.86)	0.028
3^{rd} day	0.33 (0.10–1.06)	0.064
$>3^{rd}$ day	0.27 (0.03–2.36)	0.238
Formalin-fixation	0.00 (0.00–0.00)	0.000

OR_c=crude odds ratio, CI=confidence interval, Ref=reference

Table 5 Multivariate analysis results of the final model for the association between foreign DNA detection and pre-analytical factors or deceased's characteristics

Pre-analytical factors		
	OR_a (95% CI)	**p-value**
Representative collection technique		
Both	Ref	Ref
Cuttings	6.84 (1.47–31.86)	0.016
Swabs	13.44 (2.89–62.45)	0.001
Forensic physician		
A	Ref	Ref
B	4.03 (0.38–42.75)	0.249
C	5.54 (0.34–90.20)	0.232
D	2.85 (0.29–28.38)	0.373
E	36.19 (2.47–531.09)	0.010

Table 5 (continued)

The deceased's characteristics		
	OR_a (95% CI)	**p-value**
Gender		
Female	Ref	Ref
Male	3.75 (1.33–10.57)	0.013
Hand-covering material		
None	Ref	Ref
Paper bag	63.18 (3.31–1206.95)	0.007
Plastic bag	2.53 (0.25–25.69)	0.434
Gunshot-related death		
No	Ref	Ref
Yes	0.05 (0.00–0.45)	0.009
Suspected physical assault		
No	Ref	Ref
Yes	2.97 (1.03–8.59)	0.046
Level of decomposition		
No	Ref	Ref
1st day	0.13 (0.02–0.74)	0.023
2nd day	0.36 (0.10–1.35)	0.133
3rd day	0.08 (0.01–0.44)	0.005
>3rd day	0.31 (0.03–3.35)	0.339
Formalin-fixation	0.00 (0.00–0.00)	0.000

OR_a=adjusted odds ratio, CI=confidence interval, Ref=reference

by both directional stepwise model selections. This involved systematically excluding one factor at a time and evaluating the AIC of the resulting model. The models were ranked based on their AIC values, with the model having the lowest AIC being selected for the next round. In each subsequent round, the process continued by excluding any remaining factors and including back previously excluded factors, until a final model with the lowest AIC was determined. As a result, the final model may not include all factors that existed in the initial model. The results of the final model revealed that the representative collection technique was a significant pre-analytical factor, with fingernail swabs having a much larger effect size compared to fingernail cuttings. The difference in effect size observed between fingernail swabs and fingernail cuttings may be attributed to differences in the extraction methods used; as various exogenous cell collection techniques have been shown to yield significantly different amounts of exogenous DNA[3]. Additionally, minimizing the presence of fingernail endogenous DNA is also important[3,12]. Therefore, it is worth noting that in Hebda et al.'s study, different collection techniques were compared, including fingernail soaking, fingernail swabbing, and fingernail scraping[3]. The fingernail soaking method yielding higher DNA quantities than the swab method, which seems to contrast with the results reported in this study[3]. However, when considering the detection of foreign DNA, the swab method resulted in a major profile of exogenous material, while the soak method resulted in a major profile of fingernail DNA[3]. Therefore, when comparing collection techniques, the swab method may be the best overall method, similar to the results reported in this study; eventually[3]. Further explanation by Hayden et al.'s experimental study, which analyzed 41 mocked fingernail samples after removal of consensual partner contamination for comparison of the effectiveness of different extraction methods, including swabbing from

fingernails, PBS-soaked fingernails and PrepFiler® lysis buffer-soaked fingernails, on the maximization of foreign male DNA recovery and minimization of female endogenous DNA in multiple aspect results, including the number of foreign male alleles detection[12]. The results of that study showed that the average number of foreign alleles was equal between swab samples and lysis buffer samples, and both were higher than PBS samples[12]. However, based on the log-likelihood ratio across all ethnic groups, the swabbing sampling method was found to be the best method; as it resulted in higher average relative fluorescence units (RFU) compared to lysis buffer samples, despite having an equal average number of foreign alleles[12]. This is because a lower RFU can increase the chance of allelic dropout[12]. Additionally, co-extraction and differential degradation of fingernail endogenous DNA were higher in the lysis buffer samples[12]. Therefore, similar to the result of this study, fingernail swabs showed the best result[12]. Confirming the results observed in the casework of this study, the reported prevalence of foreign DNA was significantly lower than that in the study by Nurit et al. on victim samples[4]. The reported prevalence of HL in all samples in this study was 7.9%, 95% CI=3.8%-12.1%. This means that if one made an inference to the population level of the casework, and fingernail DNA is collected from deceased individuals requiring a post-mortem examination using the same procedures and tools as in this study, it can be estimated, with 95% confidence, that the true population prevalence of HL; either from the right or left hand is likely to fall between 3.8% and 12.1%. Even in subgroup analysis, by excluding the gunshot-related death cases, the reported prevalence in this study was still significantly lower than that reported by Nurit et al.[4]. This was due to the majority of the representative collection technique, which yield the best possible result in this study, was fingernail cuttings compared with Nurit et al. study that used fingernail swabs as the primary collection technique[4]. This explains the lesser rate of foreign DNA detection in this study. The evidence suggests that in casework, fingernail swabs from the deceased's fingernails may play an important role in foreign DNA detection.

The forensic physician may also be a significant pre-analytical factor, possibly due to high variations in the specimen collection techniques among individual forensic physicians. For example, in the fingernail cuttings technique, some physicians use nail clippers, while others use scissors; some collect the cuttings on a clean cloth first, then place these cuttings into a plastic bag, while others place the cuttings directly into a plastic bag. In the fingernail swabs technique, some forensic physicians use a regular, clean cotton swab soaked with regular tap water in the fingernail swabs technique; whereas, some forensic physicians just use dry swabs when the deceased's fingernails are already moist. Furthermore, the decision on choosing either the fingernail swabs or fingernail cuttings method for collecting specimens depended on the forensic physician. These details contribute to a high variation in collection techniques among individual forensic physicians, and since there is no consistently recorded information it may play an important role in this study as a confounder. Therefore, the findings of this study are also related to the forensic physician factor, which may be caused by their different in-depth specimen collection techniques.

An association exists between foreign DNA detection and the deceased's characteristics. In this study, the rare use of paper bags to cover the deceased's hands, as recommended by the National Institute of Justice, was also discovered[13]. This also contradicts the recommendation of Nurit et al., who stated: "The majority of the bodies submitted to the mortuary had their hands protected by bags"[4]. This is an essential issue to consider in a practical situation since the hand-covering material is an associated factor for foreign DNA detection. Gender was also found to be an associated factor; confirming the findings of Dowlman et al., who suggested that there were higher odds of obtaining HL profiles from a male[7]. This resulted from the fact that generally, males are more powerful than females,

so leading to more vigorous force that increases the rate of the detection of foreign DNA[5]. However, the Dowlman et al. study found no statistical significance, which contrasted with this study[7]. Gunshot-related death was found to be a protective factor, confirming the assumption of Nurit et al., who stated that: "a physical struggle may not have taken place in gunshot cases"[4]. Additionally, suspected physical assault was found to be an associated factor consistent with the findings of Nurit et al. who suggested that the prevalence of DNA mixtures is much higher in murder victims[4]. The level of decomposition was significant, and this factor required inclusion in an ordinal logistic regression analysis model because standard STRs are prone to failure in degraded forensic samples[14].

It should be noted that this study was conducted as an observational study on casework, which is a much different context from the experimental study of Hayden et al.[12]. In Hayden et al.'s study, DNA profiles of volunteers and their consensual partners were available, while in this study unknown sources of foreign DNA were detected as there were no available DNA profiles of suspects or consensual partners systematically recorded for comparison. Therefore, the foreign DNA detected in this study may have been contaminated by their consensual partners as well. Additionally, DNA contamination from consensual partners can be deposited beneath fingernails even after showering[12]. Analyzing an electropherogram peak or elevated stutter can be helpful in addressing this issue[12]. This may be reflected in the NS profiles observed in this study. However, this study focused on pre-analytical factors that occurred outside the laboratory, so it did not examine this aspect.

Limitations

This study had four limitations. First, being a cross-sectional study, the in-depth technique for specimen collection of individual forensic physicians was not systematically recorded, which could introduce a confounding effect and therefore warrants further study. Second, the results on NS profiles were reported based on the decision of the laboratory technicians. Third, this study did not involve an analytical step, which also has an essential role in detecting foreign DNA. Finally, the hand-covering material factor may have conferred a type I error, due to the fact that the majority of the deceased had neither paper bag nor plastic bags coverings on their hands before being transferred to the hospital mortuary.

Conclusion

The detection of foreign DNA is crucial as evidence that can be used in a court of law; however, it can be challenging to detect. During a post-mortem examination, the use of fingernail swabs as the collection technique is of particular interest.

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Conflict of interest

The author declares that there are no conflicts of interest.

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