Forensic Science International
89 (1997) 15–20

Forensic Science International

The quantitative alteration of the DNA content in strangulation marks is an artefact

Wolfgang Grellner*, Mark Benecke

Institute of Forensic Medicine, University of Cologne, Melatenguertel 60-62, D-50823 Koeln, Germany

Received 22 January 1997; accepted 14 April 1997

Abstract

It is frequently difficult to prove the vitality in cases of fatal hanging. Using impulse cytophotometry, Müller et al. (Krim. Forens. Wiss. 44, 1981) reported on possibly vital alterations of the DNA content in five strangulation marks (hypodiploidy). The objective of the present study was to perform a re-evaluation by means of current methods of quantitative DNA analysis. The investigation included 24 cases of hanging (typical, $n=11$; atypical, $n=13$). DNA was extracted by use of phenol/chloroform and quantitated photometrically in relation to the tissue weight. In addition, skin specimens were examined histologically and their water content was determined. The mean DNA content of strangulation marks was 3.2 μg DNA/mg tissue, that one of uninjured skin (control) 1.4 μg DNA/mg tissue. The ratio of DNA content between strangulation grooves and control skin varied from 1.2 to 5.2 (average: 2.4) implicating apparently elevated DNA levels in strangulation marks. Histology revealed typical changes with absence of leukocyte infiltration suggesting mechanical compression of DNA containing layers. The additional determination of the water content emerged - in dependence on the macroscopic appearance, dried or non-dried - reduced values for strangulation marks (average: 42% vs. 66%). Statistical analysis demonstrated a significant correlation between elevated DNA content and reduced water content of strangulation marks. Conclusion: the markedly elevated DNA content of strangulation marks is interpreted as an artefact due to drying of the skin with a reduction of the water content (and weight) in the course of mechanical cutaneous alterations (compression). Therefore, quantitative changes in the local DNA content of the groove have no importance as a sign of vitality in fatal strangulations.
© 1997 Elsevier Science Ireland Ltd.

Keywords: Strangulation marks; Hanging; Vitality; DNA content; Water content; Histology

*Corresponding author

0379-0738/97/$17.00 © 1997 Elsevier Science Ireland Ltd. All rights reserved.
PII S0379-0738(97)00069-8

1. Introduction

In many cases the course of agony in fatal strangulations is comparatively short. Particularly in typical hanging, death occurs within minutes. So-called "classical" signs of asphyxia or suffocation such as congestion and petechiae may be absent. Thus, it is frequently difficult to prove the vitality of this event. For decades forensic scientists have searched for adequate criteria to differentiate vital from postmortem hanging using various starting points such as local macroscopical, histological and chemical changes, indirect lesions and systemic (shock) reactions.

In 1981 the German forensic scientists Müller et al. [1] reported on a possibly vital alteration of the DNA content in five strangulation marks (significant amount of hypodiploid cells compared with control skin) detected by use of impulse cytophotometry. Objective of the present study was to perform a re-evaluation by means of current methods of quantitative DNA analysis in a greater group of fatal strangulations.

2. Material and methods

The investigation included 24 cases of fatal hanging (20 males, 4 females; mean age: 47.8 years) consisting of typical (n=11) and atypical (n=13) forms of hanging. The macroscopic appearance of the strangulation mark in particular, the state of drying was registered. In each corpse two samples from the strangulation mark measuring approximately 1.0×0.5 cm were collected. They were divided up into native specimens for DNA quantitation and determination of the water content and specimens for fixation in 4% buffered formalin with subsequent embedding in paraffin and H&E histology. Uninjured skin of the neck served as a control.

2.1. DNA extraction and quantitation

Skin samples were frozen at −20°C for at least 1 h. Aliquots of approximately 40 mg were sliced into 10–20 pieces by use of a sterile scalpel and incubated over night at 37°C (water bath) with 510 μl lysis buffer (0.01 M Tris–HCl, pH 8.0; 0.01 M EDTA; 0.1 M NaCl; 2% SDS) containing 10 μl proteinase K solution (Boehringer, c=20 mg/ml) [2]. On the next day, another 10–20 μl proteinase K solution were added; the incubation continued at 54°C until the tissue was clearly disrupted. Extraction of DNA was performed by use of the phenol/chloroform method [3]. After vigorous mixing of the lysate with 500 μl phenol saturated with 1 M TE (10 mM Tris–HCl, pH 8.0; 1 mM EDTA), the samples were centrifuged at 8,000 r.p.m. for 10 min (Eppendorf centrifuge). The supernatant was separated. The lower phase (debris and phenol) was mixed with 100 μl TE and centrifuged as previously (re-extraction). This supernatant was added to the supernatant of the first centrifugation step, mixed with 1 ml chloroform (Merck) and centrifuged as above. The resulting supernatant was again transferred and 1 ml 100% ethanol was added. After gentle mixing, tubes were centrifuged at 14 000 r.p.m. for 30 min. Ethanol was removed and the DNA pellet was washed once with 1 ml 70% ethanol.

Then samples were dried over night under sterile conditions (LaminAir HB 2448, Heraeus) and dissolved by addition of 0.5 ml 1 M TE. DNA solution was stored at 4°C.

For DNA quantitation 10 aliquots (1–3 μl) of each sample were measured at 260 nm and 280 nm in a 2-beam UV photometer (Shimazdu UV-160) against 1 M TE (correct ratios 260/280 nm of 1.75–2.0). The average values were calculated according to the formula given by Sambrook et al. [3] and set in relation to the tissue weight.

2.2. Determination of the water content

In the further course of the study the water content of skin samples was determined by drying them at 130°C to constant weight. The weight loss was defined as the water content.

Statistical analysis was performed by paired *t*-test.

3. Results and discussion

Most of the strangulation marks revealed the typical macroscopic appearance (very firm and yellow–brown dried skin tissue). In several cases the strangulation mark showed only paling and slight cutaneous drying (when broad and smooth strangulating tools were used).

Histologically, the more or less typical changes of strangulation marks were present as described in the literature: extreme compression or even condensation of the epidermal layers with elongated cells and partial loss or abrasion of the stratum corneum (Fig. 1).

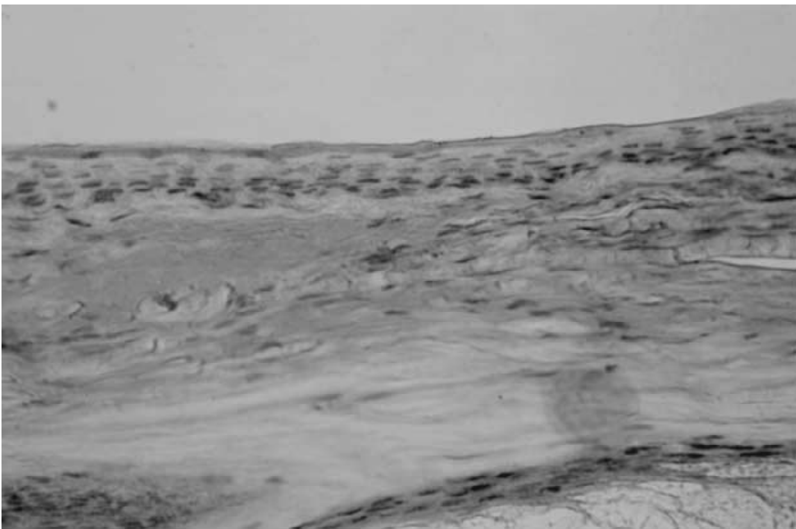

Fig. 1. Strangulation mark, histologically: loss of stratum corneum, marked epidermal condensation with elongated keratinocytes. H & E, original magnification ×500.

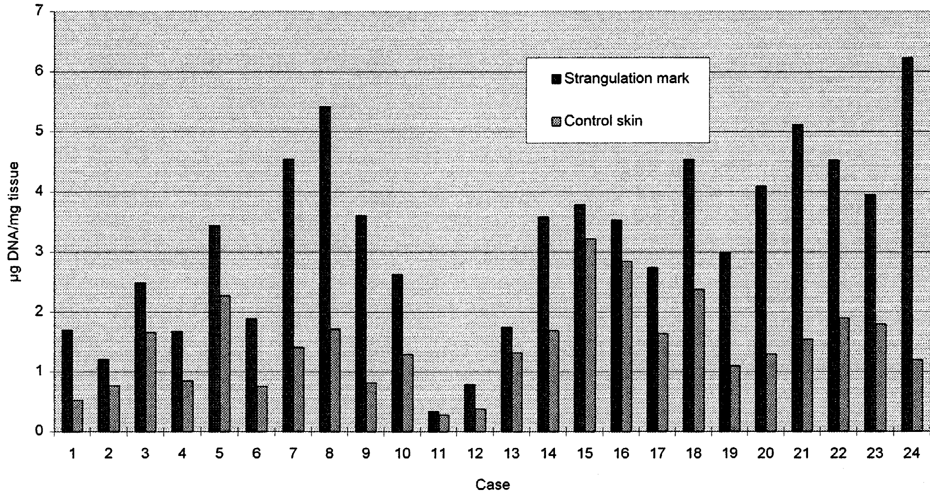

Fig. 2. DNA content of strangulation marks (I).

The degree of histological alterations was dependent on the macroscopic appearance: normal skin anatomy was most severely disturbed in solid and dried strangulation marks.

Leukocyte infiltration as a possible source of DNA was not present in any case. This observation was not unexpected, as the duration of agony in fatal hanging is too short for inflammatory reactions on the cellular level.

The results of quantitative DNA analyses are summarized in Fig. 2 and Table 1. The absolute values exhibited great interindividual variation, in particular in strangulation marks, with a range from 0.3 to approximately 6 μg DNA/mg tissue. However, in all 24 cases the DNA content of strangulation marks was higher than in control skin which is also reflected by the average values of 3.2 $\mu g\ mg^{-1}$ for strangulation grooves and 1.4 $\mu g\ mg^{-1}$ for uninjured neck skin. The ratio of DNA content between strangulation marks and control skin varied from 1.2 to 5.2 averaging at 2.4. Thus, the DNA content of strangulation marks was doubled compared with control skin. This result is in contrast to the observations of Müller et al. [1] who registered hypodiploidy of cutaneous cells in strangulation marks. Thus, reduced DNA levels could rather be expected than increased values. However, we did not establish karyograms and therefore cannot judge the parameter of diploidy in detail.

For a further clarification, we additionally started to determine the water content of

Table 1
DNA content of strangulation marks (II)

DNA content ($\mu g\ mg^{-1}$)	Strangulation marks	Control skin
Average	3.2	1.4
Range	0.34–6.23	0.28–3.22

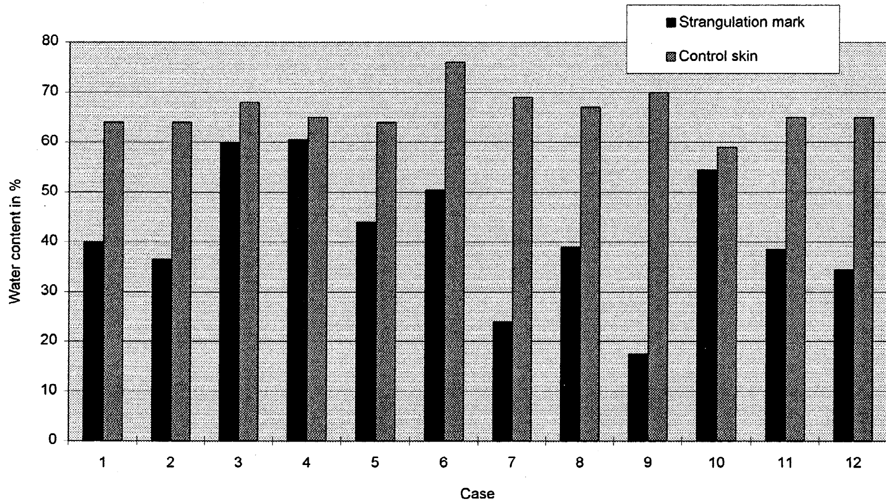

Fig. 3. Water content of strangulation marks (I).

tissues (Fig. 3 and Table 2). Interindividual variation was again present, but not as marked as in DNA content. In all cases the water content of strangulation marks was lower than in control skin which is reflected by average values of 42% for strangulation marks and 66% for control skin with ranges from 17–61% and 59–76%, respectively. The ratio of water content between control skin and strangulation marks varied from 1.1 to 4.0 with an average of 1.8. Thus, the water content of control skin was double as high compared with strangulation marks.

There was an obvious relation between the water content of the strangulation marks and their macroscopic appearance - dried or non-dried. We did not observe significant dependencies on the duration of postmortem interval: all samples were collected within several days on fresh corpses. Possible effects, however, would influence both control skin and strangulation grooves. The ratios would not be changed.

In Fig. 4 both the ratios of DNA content (strangulation mark by control skin) and water content (control skin by strangulation mark) are summarized. The closer the respective values (symbols) lie together the higher is the proportionality between both parameters. Only in three cases there are greater differences, but the tendency - as mentioned above - is identical: high DNA content is associated with low water content. Statistical analysis revealed a significant correlation.

We draw the conclusion that the DNA content of strangulation marks in fact is

Table 2
Water content of strangulation marks (II)

Water content (in %)	Strangulation marks	Control skin
Average	42%	66%
Range	17–61%	59–76%

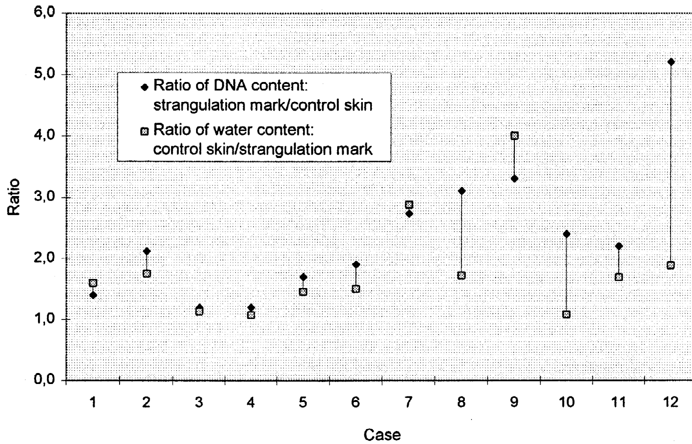

Fig. 4. Summary of DNA and water content (ratios).

markedly elevated when compared with control skin. However, this result must be interpreted as an artefact due to drying of the skin being accompanied by a reduction of water content and weight in combination with mechanical cutaneous alterations (compression). Therefore, quantitative changes in the local DNA content of the groove have no importance as a sign of vitality in fatal strangulations. Possible qualitative alterations in the sense of a DNA shifting on the single cell level (e.g. hypodiploidy of certain cells according to [1]) are not excluded by the present investigation and need further clarification.

Acknowledgments

We would like to thank Mrs. Heike Schilbach for excellent technical assistance in determination of the water content.

References

[1] E. Müller, A. Simon, R. Weidhase, Impulscytophotometrische Messungen der DNS dermaler Zellen in vitalen und postmortalen Strangmarken, Krim. Forens. Wiss. 44 (1981) 53–56.

[2] P. Gill, A.J. Jeffreys, D.J. Werrett, Forensic application of DNA ''fingerprints'', Nature 318 (1985) 577–579.

[3] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning, A Laboratory Manual, Vol. 3, 2nd ed., Laboratory Press, Cold Spring Harbour, 1989, pp. E.3–E.5.