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Estimation of Intra and Inter-Individual Variability of Post Mortem Skin Hydration by Corneometry تقديـر تبايـن رطوبـة الجلـد بعـد الوفـاة يف الفـرد الواحـد و بني األفـراد عـن طريـق قيـاس الطبقـة

القرنيـة

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Abstract

Kinetics of post mortem skin desiccation, potentially useful for post mortem interval (PMI) determination, is poorly understood. We therefore propose a preliminary study to assess intra- and inter-individual variability of post mortem skin hydration using a valid method, such as corneometry. Corneometer CM-825® (Courage & Khazaka electronic GmbH, Cologne, Germany) measures the stratum corneum hydration (SCH) by measuring its electrical capacitance, given in arbitrary units (A.U.). To assess intra- and interindividual variability of SCH, we evaluated SCH at 19 body sites for 30 subjects with known cause of death at 6 ± 2 hours post mortem (hpm). Thirty subjects (20 males and 10 females) were selected. Mean age was 73.6 ± 9.1 years, mean BMI was 23.9 ± 5.2 kg/m². There is a significant effect of body site on SCH values. Inter-individual variability appears to be significant, depending on the considered body site. Biological sex does not influence studied parameters, but age, BMI and cause of death influence SCH values, at various degrees depending on the considered body site. This study highlights the significant intra- and interindividual post mortem variability in skin hydration levels. The intra-individual variability of this parameter means that the skin should not be considered as a homogeneous surface in future studies of post mortem skin desiccation.

 Keywords: Forensic sciences, thanatology, anthropology, post mortem interval, skin, skin hydration, stratum corneum, skin capacitance, corneometer CM-825®.

الستخلص

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لا يزال فهم حركية جفاف الجلد بعد الوفاة، والتي قد تكون مفيدة في تحديد فترة ما بعد الوفاة، محدودًا. لذلك، نقترح دراسة أولية لتقييم التباين داخل الفردية وخارجها لرطوبة الجلد بعد الوفاة باستخدام طريقة صالحة، مثل القياس القرين. يقيس جهاز القياس القرين & Courage® (-825CM electronicGmbH Khazaka، كولونيا، أملانيا(رطوبة الطبقة القرنية عن طريق قياس السعة الكهربائية لها، معطاة بوحدات اعتباطية (.A.U). لتقييم التباين داخل الفردية وخارجها يف رطوبة الطبقة القرنية، قمنا بتقييم رطوبة الطبقة القرنية يف 19 موقًًعا بالجسم لـ 30 موضو ًًعا معروف سبب الوفاة لديهم بعد 6 ± 2 ساعة من الوفاة. تم اختيار ثالثني موضو ًًعا)20 ذكًًرا و 10 إناث(. كان متوسط العمر 73.6 ± 9.1 سنة، ومتوسط مؤشر كتلة الجسم 23.9 ± 5.2 كجم / م ؒ. هناك تأثير كبير لموقع الجسم على قيم رطوبة الطبقة القرنية. يبدو أن التباين بين الأفراد كبير، اعتمادًا على موقع الجسم المعتبر. لا يؤثر الجنس البيولوجي على المعلمات المدروسة، ولكن العمر ومؤشر كتلة الجسم وسبب الوفاة يؤثرون على قيم رطوبة الطبقة القرنية، بدرجات متفاوتة اعتمادًا على موقع الجسم المعتبر. تسلط هذه الدراسة الضوء على التباين الكبير داخل الفردية وخارجها يف مستويات رطوبة الجلد بعد الوفاة. يعني التباين داخل الفردية لهذا المعامل أنه لا ينبغي اعتبار الجلد سطحًا متجانسًا في الدراسات

الكلمات املفتاحية: علوم األدلة الجنائية، علم التثنية، الأنثروبولوجيا، فترة ما بعد الوفاة، الجلد، رطوبة الجلد، الطبقة القرنية، سعة الجلد، مقيسا القرنية -825CM®.

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The significant inter-individual variability of this parameter probably partly explains the variations in observed PMI for this phenomenon and seems to be a major challenge in using this method to determine PMI.

1. Introduction

The skin is an extremely interesting organ to study in forensic medicine. It is a large organ easily accessible for non-invasive or minimally invasive measurements and samples, compatible with the respect of human dignity. Paradoxically, however, it has been relatively little studied, even though it is certainly the organ most observed by forensic pathologists [1, 2].

In living individuals, skin hydration is maintained relatively constant by transudation and sweat production. After death, these processes are interrupted and post mortem skin drying process (desiccation) can occur, notably influenced by air movements, ambient temperature, ambient relative humidity, body site, age of the subject, clothing and the presence of a skin abrasion and/or epidermic loss [3]. Historically, the link between skin desiccation and post mortem interval (PMI) has been first described by Galloway [4]. But it is only recently that authors have begun to systematically study the link between PMI and the extent of skin desiccation [5, 6].

It has been pointed out that the lack of knowledge about the post mortem desiccation of the human body may lead to an underestimation of the PMI in forensic practice as it interrupts putrefaction [5–8]. Moreover, it was also noted that the conditions under which skin desiccation occurred remained poorly understood ; and that the terms "mummification" and "desiccation", as well as the differences between indoor and outdoor skin drying processes, suffered from a lack of clear characterisation and definition in the forensic context [7, 8]. For example, Ceciliason et al, in contrast to the Galloway's historical description, describes not one but two distinct possible patterns المستقبلية لجفاف الجلد بعد الوفاة. يبدو أن التباين الكبير بين الأفراد لهذا المعامل يفسر جزئيًا الاختلافات في فترة ما بعد الوفاة الملاحظة لهذه الظاهرة، ويبدو أنه تحدٍ رئيسي في استخدام هذه الطريقة لتحديد فترة ما بعد الوفاة.

for indoor desiccation of human bodies: i) a "leatherlike" pattern, beginning at the fingers and toes pads, with brownish/blackish skin discolouration and an opaque and thickened appearance of skin ; ii) a "parchment-like" pattern, characterised by the appearance of yellowish/orange skin discolouration "patches" on the trunk, arms and thighs, with a translucent and refined appearance of skin [8].

However, it should be noted that the possible influence of the post mortem desiccation phenomenon has not been considered in recent studies on the determination of the postmortem interval by analysing the expression level of various protein or RNA markers of skin cells, namely immunohistochemical detection of HMGB1 and related proteins (Beclin1 and RAGE) [9], protein and mRNA levels of the apoptosis-related proteins Bax and Bcl-2 [10], the expression of skin-specific mRNA marker late cornified envelope 1C (LCE1C)[11], the expression of matrix metalloproteinase-9 (MMP-9) and long non-coding fatty acid oxidation (lncFAO) RNA [12], or the description of skin histological changes [13–15].

To better understand the different processes involved in post mortem skin desiccation, we believe that it could be beneficial to opt for an objective and quantitative approach through measurement of skin's bioelectrical parameters, rather than a clinical assessment which is subjective and qualitative in nature, as it has been suggested in literature [16, 17]. Especially as the literature suggests a link between PMI and the electrical properties of skin, both in non-human proxies [18] and, more recently, in human donor bodies placed in outdoor conditions [19, 20], in the form of a progressive decrease in electrical impedance. The choice of a paraclinical method, rather than a laboratory method, also seems appropriate to us, as such a method has the advantage of being directly usable in the field in a forensic context.

In living subjects, skin hydration can be assessed objectively and quantitatively in a validated way through its electrical capacitance, specifically with the Corneometer CM-825® (Courage & Khazaka electronic GmbH, Cologne, Germany). This device **of this parameter. Indeed, inter-indivio** measures the electrical capacitance of the skin at a depth of 30 to 45 μ m, which is deemed to correspond **buse of PMI estimation, as intrinsic biological di** to the average thickness of the stratum corneum [21]. This method, usually referred to as corneom- position ra etry, would have several advantages for the study of skin desiccation process as it is non-invasive, validated in vitro and in vivo in multicentre studies

[21–23] and potentially used wirelessly with a laptop at the bedside of the deceased by any examiner in forensic context, Figure 1 [24]. However, this device has not yet been used on deceased subjects.

Before considering the link between PMI and skin hydration level, however, we feel it is important to assess the inter- and intra-individual variability of this parameter. Indeed, inter-individual variability of parameters significantly impacts the accuracy of PMI estimation, as intrinsic biological differences among individuals can lead to inconsistent decomposition rates [16, 17, 25]. The influence of the subject's characteristics is also highlighted in the future research prospects for several promising skin techniques mentioned above [11,13,26]. Consequently,

Figure 1- Photograph of the wireless version of the Corneometer CM-825®, accompanied by the device that must
be connected *be connected*

the choice of a parameter that is too variable would not be relevant in forensic practice, even if it were shown to vary with the PMI. All the more so as it is generally not possible, in a deceased subject encountered in forensic practice, to know a posteriori the basal antemortem value of a parameter that is not a constant between individuals.

The question of intra-individual variability is also critical insofar as dermatological medical literature teaches us that human skin cannot be considered as a homogeneous whole. The skin has both morphological and functional specificities from one body site to another [27–30]. As well as choosing a skin parameter to study for PMI determination, we also need to determine the body site where we want to study the skin.

Therefore, in the larger context of understanding the post mortem skin desiccation process and before going any further in the study of skin electrical properties for PMI determination, we feel it is necessary to determine basal post mortem stratum corneum hydration (SCH) values at multiple anatomical sites all over the body, within a population with known parameters to quantify the post mortem intra-individual (influence of body site) and inter-individual (influence of subject characteristics) variability of this parameter.

2. Material & Method

2.1. Corneometer CM-825

Corneometer CM-825® (Courage & Khazaka electronic GmbH, Cologne, Germany) is a 22 cm surface probe with a flat surface of 49 mm² inside which is a capacitor subjected to an alternating current of 0.9 to 1.2 Mhz. When the skin is attached to this capacitor as a dielectric, it changes its capacitive reactance depending on its dielectric constant (K), which in turn is a function of the skin's moisture content [31]. This instrument does not generate galvanic current, performs a measurement in 1 second and has an inaccuracy rate of ±3% following manufacturer [24]. The maximal measurement depth over 30 μ m according to the producer [24] and up to 45 μ m according to in vitro measurements by independent source [12]. The level of hydration is not given in absolute electrical capacitance units (Farads (F)), but indirectly, in arbitrary units (a.u.) on a scale from 0 to 120, from the driest to the most hydrated [21]. A skin is considered as normally hydrated >40 a.u., as dry between 30 and 40 a.u. and as "very" dry <30 a.u. [21]. The probe is very sensitive for dry and very dry skin conditions, but less sensitive for high levels of skin hydration [21].

The device is factory calibrated by an in vitro method with a reference pad impregnated with a calibration solution (saturated aqueous NaCl solution). The maximum reference hydration value of 120 is obtained by measuring the hydration level of this impregnated filter pad. The minimum hydration value is determined by covering the surface of the impregnated pad with a layer of 15 μ m thick polyurethane foil [31].

As a result of its operating principle, there is an influence of probe application pressure and any interposed substance (including hairs) between the studied skin and the probe. The influence of probe application pressure is partially cancelled by means of a spring system theoretically enabled with a force between 1.1 and 1.8 Newton, with measurement realization indicated by an acoustic signal [24]. To avoid influence of any interposed substance, skin at measurement site must be cleaned and dried before and between measurement(s) by dabbing it with a soft towel [32]. Influence of excessive hairiness can be cancelled out by gentle shaving with a non-electric razor [33].

2.2. Measurements

A panel of 19 different body sites were chosen for SCH measurements in order to compare body segments to each other, central areas to extremities, fat to nearby lean areas and finally dependent and non-dependent areas: forehead, nose, cheekbone, cheek, sternum, pectoral region, umbilicus, latus, dorsum, scapula, lumbus, paralumbar region, arm (anterior and posterior), index fingertip, thigh (anterior and posterior) and leg (anterior and posterior), Figure 2.

To study the effect of location using a linear mixed model (GLMM), the nineteen sites studied were grouped according to the body segment to which they belonged: the head (forehead, nose, cheekbone, cheek), anterior thorax (pectoral region and sternum), anterior abdomen (umbilicus and latus), posterior thorax (dorsum and scapula), posterior abdomen (lumbus and paralumbar region), the upper limb (arm and index) and the lower limb (thigh and leg).

We also chose to examine the possible correlation between three different arbitrary anatomical ratios of SCH and subject characteristics : one "hypostasis" ratio independent of prone position's pressure sites (umbilicus/lumbus ratio), one "fat-lean" ratio comparing an area with a thick subcutaneous fat with an area with a thin or non-

paralumbar region (12), arm (anterior (13) and posterior (14)), index fingertip (15), thigh (anterior (16) and posterior (17)) and leg (anterior (18) and posterior (19)). **Figure 2 -** Diagram showing the selected body sites: forehead (1), nose (2), cheekbone (3), cheek (4), sternum (5), pectoral region (6), umbilicus (7), latus (8), dorsum (9), scapula (10), lumbus (11),

existent subcutaneous fat (umbilicus/forehead ratio) and one "center-to-ends" ratio comparing a central area covered by clothing with an uncovered extremity (umbilicus/index ratio).

Measurements were performed outside of the mortuary fridge in a room with ambient conditions of 20-21°C and 45-55% relative humidity (RH) in less than ten minutes to prevent too much variation in skin temperature. No measurement was made under direct sunlight or any heating device. To avoid occlusion phenomenon, the repeated measurements were made using adjacent skin areas. To avoid influence of any interposed substance, particularly condensation, skin at measurement site was gently cleaned and dried before measurement(s) by dabbing it with a soft cotton towel. To obtain the most repeatable values possible while eliminating the influence of the operator hand pressure, the measurements were carried out using a low and increasing pressure until the minimum threshold for obtaining the acoustic signal from the device was reached. The SCH value retained for each body site is the average of three consecutive measurements made on one site at one second interval. The calibration of the device was checked before and after the study was carried out, in accordance with the manufacturer's instructions.

2.3. Subjects and environment characteristics

All subjects were selected from the mortuary of the University Hospital and studied in the controlled environment of the mortuary's fridges, at 6 ± 2 hours post mortem (hpm). According to the hospital's protocol, deceased patients remain in their room for an average duration of 2 hours after their death, to allow the family to pay their respects, before entering the mortuary. Selection criteria were the following: age >60 years, skin phototype II, no previous autopsy, natural death, no dermatological pathology at measurement site.

The age criterion was chosen to homogenise the population insofar as the comorbidities and cause of death of the young deceased differed significantly from the usual population of subjects dying in hospital from a natural cause of death.

Given the exploratory nature of the study, as the post-mortem variability of the parameters was not yet known, the sample size of 30 subjects was chosen on the basis of 2 factors: firstly, by comparison with the sample sizes used in studies of this parameter in living subjects in literature [17- 19]; secondly, based on a realistic estimate of the number of subjects meeting the inclusion criteria that could be recruited in one season, as some studies mention seasonal variations in SCH [20-22]. In this case, our subjects were recruited during one autumn in Belgium.

Age, biological sex (male or female), body mass index, time of death and cause of death were registered. The body mass index (BMI), expressed in units of kg/m^2 , is defined as the body weight divided by the square of the body height. All subjects selected wore the same standardized outfit, made of an hospital gown and an adult diaper. Following usual morgue's protocol, all subjects received the same post mortem grooming and were stored in the same manner: in an opened plastic bag with a covering of one thin white cotton sheet.

Six categories of cause of death were arbitrarily established for statistical analysis: heart failure, cancer, infection, stroke, internal bleeding and other.

In accordance with the hospital's internal procedure, the deceased is placed in a refrigerated mortuary cell within 3 hours of death. The refrigerated mortuary system measures 240 cm x 210 cm x 204 cm and involves 4 individual mortuary cells, where each deceased person is placed in an open plastic body bag on a stainless steel tray measuring 210 cm x 60 cm, then covered with a white cotton sheet.

Cooling is provided by means of a conventional condensation/expansion/evaporation refrigeration cycle, with an evaporator located in the cell providing cooling by capturing heat and transferring it to a refrigerant, while the heat is extracted via a ventilated air-cooled condenser located above the cell. The stability of the environment is controlled on the basis of temperature only, not humidity, using an electronic temperature controller which is given minimal and maximal reference values of 1 and 6 °C respectively. In the event of an anomaly, an audible alarm alerts the staff of the mortuary. No incident was reported during the study period and no alarm was triggered for the subjects recruited.

To ensure that the system is working properly and that the values supplied are credible, two independent continuous 72 hours-long registrations of temperature (T°) and relative humidity (RH) of fridges were made to obtain local reliable mean T° and RH and gave following mean values: 3.95°C and 3.79°C of T° with 83.63% and 86.94% RH. To find out more about the body's "microenvironment", under the sheet and inside the open body bag, one 2 hours-long registration of T° and RH was also made under cotton sheet, next to a corpse with PMI < 6 hpm and gave 4.43° C of T° and 69.21% RH. These values appear to be consistent with the alleged controls.

2.4. Statistics

The investigated parameters were summarized using the mean, standard deviation (SD), median, interquartile range (Q1-Q3), and range (Min-Max). The normality of the parameters was checked using descriptive (mean-median comparison) and graphical techniques (histogram, Quantile-Quantile plot) and the Shapiro-Wilk test. The qualitative parameters have been summarized by means of numbers and percentages.

Associations between SCH values and qualitative sociodemographic variables (biological sex and cause of death) were tested by means of a one-way analysis of variance (ANOVA-1) (if normally distributed) or the nonparametric Kruskal-Wallis (KW) (if the normality of the distribution is not met). Associations between SCH values and quantitative sociodemographic variables (age and BMI) were tested by means of a Pearson (R) (if normally distributed) or nonparametric Spearman correlation (RS) (if the normality of the distribution is not met). The 95% reference intervals were sought for the measured parameters and were plotted.

Ratios of interest were compared to value 1 using the Student univariate test or the Wilcoxon univariate test in the case of skewed distribution.

The effect of location on the studied parameters was assessed using a linear mixed model (GLMM) to allow both fixed and random effects, with the assumption that there is non-independence in the concerned parameters. Pairwise comparisons were performed, and the results were represented using Least Squares Means (LSM) and standard error (SE).

The uncertainty level is set at $\alpha = 5\%$ (p < 0.05). The software used were SAS version 9.4 and RStudio.

2.5. Ethical considerations

The study has been performed with agreement of the local Ethics Committee. The data collection was strictly non-invasive and did not interfere with the normal funeral process or with usual morgue's protocols. Measurements were strictly made outside of visiting hours. Measurements outside the refrigerated compartment took an average of ten minutes, a time that does not result in any alteration to the body. The absence of ante-mortem opposition to the experiment was verified in the national register before recruitment into the study. Informed consent was not sought from the family of the deceased. Each patient has been anonymized. All data have been numerically stored and managed in an GDPR (General Data Protection Regulation of the European Union) compliant manner.

Table 1- Subjects characteristics (n=30).

3. Results

3.1. Influence of subject's characteristics

Thirty subjects (20 males and 10 females) were selected. Mean age was 73.6±9.1 years, mean BMI was 23.9±5.2 kg/m². The characteristics of the subjects are shown in Table 1.

Age is significantly and positively correlated with SCH values on the anterior arm (RS=0.38, p=0.040) (nonparametric correlation of Spearman) and anterior thigh (R=0.36, p=0.049) (nonparametric correlation of Spearman) (Appendix I), but do not influence other studied parameters.

BMI is positively correlated with SCH value in the forehead (R=0.37, p=0.041) (parametric correlation of Pearson) and with the umbilicus/index ratio (RS=0.40, p=0.027) (nonparametric correlation of Spearman), and negatively correlated with SCH values in pectoral region (R=-0.38, p=0.041) (parametric correlation of Pearson), anterior arm (RS=-0.37, p=0.045), index (RS=-0.47, p=0.0088) and anterior thigh (RS=-0.46, p=0.011) (nonparametric correlation of Spearman) (Appendix I).

Biological sex (Appendix II) does not influence studied parameters.

Cause of death influences the umbilicus/ forehead ratio ($p = 0.014$, $n=30$) (ANOVA-1), with lower values in cardiac deaths (0.83±0.27, n=12) compared with cancer $(1.31±0.34, n=4)$, infection $(1.45\pm0.40, n=4)$ and stroke deaths $(1.14\pm0.51, n=4)$ n=4), but no other studied parameter (Appendix III).

Table 2 - Reference ranges and coefficient of variation for studied parameters (n = 30).

Variable	Mean $(\pm SD)$ (a.u.)	CV (%)	95% reference interval (a.u.)
Forehead	$33.52 \ (\pm 13.41)$	40.02	$7.24 - 59.80$
Nose	$31.59 \ (\pm 16.44)$	52.04	$-0.63 - 63.81$
Cheekbone	55.61 (± 14.02)	25.21	$28.13 - 83.09$
Cheek	$35.23 (\pm 14.00)$	39.73	$7.79 - 62.67$
Sternum	$47.55 \ (\pm 10.53)$	22.15	$26.91 - 68.19$
Pectoral region	40.93 (± 10.78)	26.34	$19.80 - 62.06$
Umbilicus	$30.96 (\pm 7.06)$	22.80	$17.12 - 44.80$
Latus	$31.58 \ (\pm 8.13)$	25.74	$15.65 - 47.51$
Arm (anterior)	$37.66 \ (\pm 10.24)$	27.18	$17.59 - 57.73$
Index	$37.50 \ (\pm 14.65)$	39.06	$8.79 - 66.21$
Thigh (anterior)	$32.01 (\pm 8.48)$	26.49	$15.39 - 48.63$
Leg (anterior)	$30.88 \ (\pm 10.61)$	34.35	$10.08 - 51.68$
Dorsum	49.29 (\pm 16.88)	34.24	$16.21 - 82.37$
Scapula	$42.06 (\pm 13.17)$	31.31	$16.25 - 67.87$
Lumbus	$39.38 \ (\pm 16.05)$	40.75	$7.92 - 70.84$
Paralumbar region	$36.53 (\pm 12.82)$	35.09	$11.40 - 61.66$
Arm (posterior)	$34.16 (\pm 10.83)$	31.71	$12.93 - 55.39$
Thigh (posterior)	$31.76 \ (\pm 7.88)$	24.82	$16.32 - 47.20$
Leg (posterior)	$32.84 \ (\pm 9.33)$	28.40	$14.55 - 51.13$

The comparison between the different ratios and the unit reveals that umbilicus/lumbus hypostasis ratios is significantly less than 1 (p $= 0.0012$) at 6 hpm while the other ratios are not statistically significantly different from 1 ($p > 0.05$) (Appendix IV).

3.2. Inter- and intra-individual variability

With regard to the inter- and intra-individual variability of SCH values, given in arbitrary units (a.u.), Table 2 shows the values of the coefficients of variation (CV) as well as the 95% reference intervals for the studied parameters.

Comparing the mean results with the values defined as normal or pathological in adult skin (>40 a.u. = "normally hydrated"; $30-40$ a.u. = "dry"; < 30 $a.u. = "very dry")$, it appears that fourteen of the nineteen sites studied show pathological values, with skin that can be locally considered "dry" on the forehead, nose, cheek, umbilicus, latus, arm, index, thigh, leg, lumbus and the paralumbar region. The remaining five sites (cheekbone, sternum, pectoral region, dorsum and scapula) can be considered as normally hydrated.

Inter-individual variability appears to be ranging from 22.15 to 52.04% depending on the considered body site. A representation of these intervals and dispersion of the data is shown in Figure 3.

There is a significant effect of body site on SCH values ($p < 0.0001$). The differences between the two-by-two locations (grouped by body segments) are included in Table 3. The segments that do not differ from each other are: i) the anterior abdomen from the lower limb; ii) the posterior abdomen from the head and upper limb.

4. Discussion

These last twenty years, slowly growing literature highlighted the potential of skin as a reliable tissue for PMI estimation, utilizing methods such as histology [13–15], immunohistochemistry [9], electrical [18–20] or mechanical property assessment [26] and molecular analysis [10–12]. But most of these studies use animal models [9,10,12,18,26] or very reduced sample sizes of three [14], four [19], five [20], six [11] and eight [13] subjects, which respectively does not take into account the specific characteristics of human

Figure 3 - *Graphical representation of 95% reference intervals.*

Table 3- Intra-individual variability: body site influence on SCH (n = 30).

Upper left corner of the diagonal: multiple comparisons p-value

Lower right corner of the diagonal: mean difference (LSMEANS ± SE) between two locations.

skin and the significant inter-individual variability in skin properties. And yet, a recent study carried out in the forensic field and on a larger population of 102 subjects reveals significant heterogeneity in the post mortem evolution of the skin, for reasons that are still poorly understood [7]. Our study provides some interesting insights to answer the question of heterogeneity in the chronology and nature of the appearance of the post-mortem phenomenon of skin desiccation, by revealing significant inter- and intra-individual variation in the hydration of the most superficial layer of the skin.

It appears that age does not affect all parts of the body in the same way, with a positive correlation with SCH values on the anterior arm and the anterior thigh. This correlation diverges from various studies, which report a tendency for SCH values to decrease with age, in areas with or without photoexposure, from the age of 18 to 70 years [34,35]. Age does not significantly influence the SCH at the other seventeen body sites studied. These results are consistent with the findings of a recent metaanalysis, which found no significant age-related differences in SCH in forehead, cheeks, neck, forearm and the back of the hand [27]. However, the absence of age influence can be explained by our selection criteria, which homogenises our population in terms of age.

We do not observe significant influence of biological sex on SCH values, providing there is an asymmetrical distribution of the sexes within our population. In literature, the influence of biological sex on SCH values is open to debate. In their recent meta-analysis, Samadi et al [27] also conclude that there is no influence of biological sex on SCH values of multiple body sites, contrary to several other studies [36,37].

An effect of BMI on the post mortem desiccation process has been suggested in the literature, either directly or indirectly : i) desiccation would occur more frequently on smaller bodies, which have a higher ratio of skin surface area to body weight (and therefore water content) [1] ; ii) the tendency of extremities (such as fingers, ears and nose) to dry out could be linked to the smaller amount of underlying soft tissue, including subcutaneous fat [38]. However, the results of recent studies do not support these hypotheses: it seems that obesity is associated with lower SCH values and higher rate of transdermal water loss at various body site [39–42]. Henceforth, their lower basal level of skin hydration and their impaired skin barrier function suggest a more rapid post mortem skin desiccation process in obese individuals. Our study also provides an argument in favour of a correlation between BMI and lower SCH values at various body sites, as it is negatively correlated with SCH values in pectoral region, anterior arm, index and anterior thigh. But above all it emphasises that this relationship cannot be generalised to the skin as a whole. For example, our study shows a positive correlation between BMI and forehead's SCH value. This observation could be explained by the increased sebum production in obese individuals [42]. Indeed, sebum production is one of the main determinants of SCH [28] and is reputed to be high on the forehead [43].

About the influence of cause of death, our study shows that cardiac deaths are negatively associated with the umbilicus/forehead SCH ratio ($p = 0.014$), compared with cancer, infection, and stroke deaths. The occurrence of ante-mortem veinous congestion of the upper body segment due to congestive heart failure could explain this observation.

Our results highlight a significant intra-individual variability according to body site in deceased subjects. As suggested in the literature for living individuals, hydration varies according to body site, depending on stratum corneum lipids (including ceramides, cholesterol, and free fatty acids) [44,45], natural moisturizing factors (NMFs) [34], endogenous humectants like glycerol and aquaporin-3 [46], the degree of occlusion [28,47], sebaceous gland density [28,47], degree of corneocyte maturation [47], stratum corneum thickness [30,48], vascular supply and microcirculation [27,49], exposure to environmental factors (like UV radiation and pollution) [34] and microenvironmental conditions, such as relative humidity and temperature [50]. For instance, the cheek exhibits a strong correlation between ceramide profiles and skin electrical capacitance [45] and facial skin in general, particularly around the eyes, is thinner and has a higher hydration state but a poorer barrier function compared to other body parts like the trunk and limb [47]. Facial skin also generally maintains higher NMF levels compared to other body parts to assure constant hydration despite a strong exposure to environmental factors [34].

The stratum corneum and the epidermis thicknesses seem particularly crucial, especially as it introduces a possible bias in what the probe is measuring. The Corneometer CM-825® measures the electrical capacitance of the epidermis from 30 to 45 μ m [21,24]. However, the stratum corneum thickness is sometimes less than 45 μ m. For example, it is about 17 μm for the cheek, 23 μm for the palmar forearm, 29 μm for the back of the hand, 173 μm at the thenar eminence [17] and 12 μ m at abdominal level [51]. That could influence the result of the measurements insofar as the proportion of water (as a percentage of mass in relation to 100 grams of wet tissue) increases as one goes from the surface to the depth of the epidermis, with a variable rate of 20 to 40% on the surface (stratum corneum), and a stable rate of 70% for the basal stratum granulosum (independently of the body site) [52]. As a result, the values measured by corneometry actually reflect – depending on the chosen body site – sometimes the most superficial portion of the stratum corneum, sometimes the stratum corneum as a whole, and sometimes a portion of the epidermis, whose thickness is estimated between 61 and 84 μ m at the umbilic [51], between 101 and 157 μ m at forehead and between 59 and 95 μ m at cheek [53].

It is interesting to note that very close and clinically similar body sites show very different mean SCH values. For example, the skin of the cheek (35.23±14.00 a.u.) was indeed significantly different from that in the skin over the cheekbone (55.61±14.02 a.u.). This demonstrates the importance of precisely defining the body site studied for future studies using this method.

Hypostasis could influence the post mortem evolution of skin hydration, as all body fluids move according to gravity [54]. It has also been suggested that firm contact between the skin and a garment or surface prevents water evaporation and therefore delays post mortem desiccation [55]. The methodology of our study does not allow us to distinguish the respective influence of these two phenomena. Nevertheless, the hypostasis influence must be put into perspective: at 6 ± 2 hpm, when hypostasis is already clinically evident, the cheekbones remain the most hydrated site in our study, despite being a high and protruding body site in supine position.

Despite a relatively homogeneous group due to our selection criteria, and the lack of influence of age and biological sex, our study suggests a significant inter-individual variability, with coefficients of variation (CV) ranging from 22.15% (sternum) to 52.04% (nose). This variability could be explained by genetic factors [56–58]; ante mortem drug treatment [34]; comorbidities [27], and especially chronic renal failure [59] and diabetes mellitus [60]; chronic exposure to environmental factors like UV radiation and pollution [35]; nutritional status [34]; the vitamin C, collagen and probiotic content of the diet [61]; abnormalities in aquaporin-3 expression [61]; circadian rhythm [61] and chronic stress [62]. We can also mention the influence seasonality [45] and clothing [63,64], but the influence of these factors must have been reduced in our study in consequence of our methodology.

The influence of local skin temperature seems critical, as studies have shown a link between the temperature of a biological tissue and its electrical properties [18,65]. However, the influence of this factor was possibly limited by the choice of a short and fixed PMI and by the standardisation of clothing, ambient temperature, and relative humidity (RH). However, we cannot exclude the influence of this parameter and it would be interesting to quantify the exact relationship between skin temperature and SCH measurement. It will be therefore the subject of a further specific study.

5. Conclusion

The use of the Corneometer CM-825® (Courage & Khazaka electronic GmbH, Cologne, Germany), as an inexpensive, non-invasive, objective and quantitatively validated instrument, certainly offers several advantages for purely academic studies of the post mortem kinetics of skin hydration values. In this case, despite our relatively small sample size,

the choice of short PMIs and the lack of investigation into several subjects characteristics, we were able to easily identify and quantify post mortem intra- and inter-individual variability in skin hydration levels, which could partially explain the PMI heterogeneity of the onset of skin desiccation, as well as the morphological variations of this phenomenon.

The choice of a parameter that is relatively independent of the subject's intrinsic characteristics is essential in PMI research. In fact, in practice, the body of the deceased is subject to the influence of a large number of extrinsic factors, the most important of which are certainly environmental conditions, clothing and the cause of death. All of these factors, whether intrinsic or extrinsic, will compromise the accuracy of any potential PMI determination technique, which is critical in a forensic context. For that reason, the choice of stratum corneum hydration, measured by corneometry, seems questionable.

However, it seems to us that highlighting the significant inter- and intra-individual heterogeneity of skin provides a crucial element in research into the determination of PMI by skin analysis. Indeed, it is very important to stress, for future academic studies on this subject, that human skin should not be considered as a homogeneous surface, and that particular care must be taken in choosing the body site, whatever the parameter being studied.

With regard more specifically to research into the post mortem drying of skin, the evidence of the heterogeneity of skin hydration provided by our study suggest that there are probably several distinct desiccation patterns to identify, depending on considered body site and subject's characteristics.

For future research into understanding the post mortem skin desiccation, we feel it is important to determine the exact relationship between skin temperature and the indirect measurement of its hydration via its electrical properties; and to determine whether or not there is a link between SCH and short PMIs, prior to the clinically evident phenomenon of skin desiccation or mummification.

Conflict of interest

The authors declare no conflicts of interest.

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130 Estimation of Intra and Inter-Individual Variability of Post Mortem Skin Hydration by Corneometry

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1. Appendices

Appendix I. Correlation between subject traits & parameters (n = 30).

SCH site	Age	BMI
Forehead*	$R = -0.12974 (p = 0.4944)$	$R = 0.37493 (p = 0.0412)$
Nose**	$R_s = 0.27470$ (p = 0.1418)	$Rs = -0.27751 (p = 0.1376)$
Cheekbone*	$R = -0.21573$ ($p = 0.2522$)	$R = 0.06570 (p = 0.7301)$
Cheek*	$R = -0.22328 (p = 0.2356)$	$R = 0.14712 (p = 0.4379)$
Sternum*	$R = -0.33230 (p = 0.0728)$	$R = 0.13387$ ($p = 0.4806$)
Pectoral r.*	$R = 0.00223$ ($p = 0.9907$)	$R = -0.37622$ ($p = 0.0405$)
Umbilicus*	$R = 0.14420 (p = 0.4471)$	$R = 0.11877$ ($p = 0.5319$)
Latus*	$R = -0.35584 (p = 0.0536)$	$R = 0.20515 (p = 0.2768)$
Arm (anterior)**	$R_s = 0.37763$ (p = 0.0397)	$Rs = -0.36831 (p = 0.0452)$
Index**	$R_s = 0.06883$ (p = 0.7178)	$R_s = -0.46974$ (p = 0.0088)
Thigh (anterior)**	$R_s = 0.36244$ (p = 0.0490)	R_s = -0.46039 (p = 0.0105)
Leg (anterior)**	$R_s = 0.10559$ (p = 0.5787)	R_s = -0.03694 (p = 0.8463)
Dorsum**	$R_s = 0.04700$ (p = 0.8052)	$R_s = 0.01157$ (p = 0.9516)
Scapula**	$R_s = 0.00200$ (p = 0.9916)	$Rs = -0.11437 (p = 0.5473)$
Lumbus**	R_s = -0.03520 (p = 0.8535)	$R_s = 0.29862$ (p = 0.1090)
Paralumbar r.**	$R_s = 0.09022$ (p = 0.6354)	$R_s = -0.04918$ (p = 0.7964)
Arm (posterior)**	R_s = -0.03988 (p = 0.8342)	R_s = 0.01313 (p = 0.9451)
Thigh (posterior)**	$R_s = 0.07909$ (p = 0.6778)	R_s = -0.01135 (p = 0.9525)
Leg (posterior)**	$R_s = 0.23524$ (p = 0.2108)	R_s = -0.11838 (p = 0.5333)
Umbilicus/Lumbus*	$R = 0.09802$ ($p = 0.6063$)	$R = 0.07505 (p = 0.6935)$
Umbilicus/Forehead*	$R = 0.23202 (p = 0.2173)$	$R = -0.26193 (p = 0.1620)$
Umbilicus/Index**	$R_s = -0.05970$ (p = 0.7540)	$R_s = 0.40454$ (p = 0.0266)

* Parametric correlation of Pearson

** Nonparametric correlation of Spearman

Abbreviations : BMI = Body Mass Index ; Pectoral r. = Pectoral region ; Paralumbar r. = Paralumbar region.

Appendix II. Association between biological sex and studied parameters.

Appendix II Continued.

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136 Estimation of Intra and Inter-Individual Variability of Post Mortem Skin Hydration by Corneometry

Appendix III - Association between studied parameters and cause of death

Appendix III Continued.

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138 Estimation of Intra and Inter-Individual Variability of Post Mortem Skin Hydration by Corneometry

Appendix III Continued.

Appendix III Continued.

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140 Estimation of Intra and Inter-Individual Variability of Post Mortem Skin Hydration by Corneometry

Appendix III Continued.

Appendix IV. Ratio analysis (n = 30).

