Forensic Science International 228 (2013) 176.e1–176.e6

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Forensic Anthropology Population Data

Reconsideration of bone postmortem interval estimation by UV-induced autofluorescence

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ARTICLE INFO

Article history:
Received 8 December 2011
Received in revised form 11 July 2012
Accepted 10 March 2013
Available online 3 April 2013

Keywords:
Forensic osteology
Skeletal remains
Bone
Postmortem interval
UV-fluorescence
Fluorescence spectrometry

ABSTRACT

Assessing the UV-fluorescence of a freshly cut cross section of the compact parts of a bone is often recommended as a first step to estimate the postmortem interval (PMI) of skeletal remains. Opinions differ concerning the cause of fluorescence and on how to categorize fluorescent properties as well as the significance of fluorescent characteristics in correlation with the PMI. In this study we evaluated the UV-fluorescence of over 200 bones with known PMI to reassess the diagnostic value of this method for differentiating between historical and recent skeletal remains. It could be shown that there is a correlation between the PMI and fluorescence colour, but not with fluorescence intensity. Furthermore, the quality of two UV-fluorescence test possibilities based on fluorescence colour was assessed by calculating the individual test efficiency, sensitivity and specificity. The results showed that blue bone fluorescence, as well as blue fluorescence combined with other colours (mainly yellow) does not allow the observer to draw any conclusions about sample age. Only overall yellow fluorescence may indicate a historical specimen. But still, 2% of all forensically relevant samples were falsely excluded, making bone fluorescent properties inappropriate as the sole criterion for deciding whether a specimen is included or excluded for further forensic investigation.

1. Introduction

Although substantial research has been conducted in this field [1–10], reliable dating of skeletal remains continues to pose one of the key questions in forensic science today. Accidental discovery of human bones is a frequent occurrence, e.g. in the course of earth removal during construction work, archaeological excavations or building developments. The initial concern that arises with respect to such a find is whether it is historical or recent (PMI shorter than about 50–60 years) and thus falls into the field of forensics.

Recently Ramsthaler et al. [11] tested several practically applicable methods for estimating the PMI of skeletal remains. Among other things they confirmed that the long established method of evaluating the UV-fluorescence of a freshly sawn bone cross section is suitable for excluding a forensically relevant PMI. Overall, they found a good correlation between the extent of reflection on the cross section and the PMI. Like others before [1,4,12], they recommend this method to discriminate between modern and ancient samples.

However, the actual cause of autofluorescence of bone cross sections under UV-light and the described change in colour or reduction of fluorescence intensity is still widely unknown. Fresh bone exposed to long-wave UV-light fluoresces as a light blue colour. Older material is thought to exhibit additionally or exclusively only brown, yellow and grey fluorescence [13,14]. Recent studies showed that the visually assessed colour impressions of "blue" and "yellow" created by ultraviolet light, in fact, represent two distinct shades of fluorescent colour emitting measurably different wavelengths [15,16].

While the protein component of bone tissue, due to chemical specifications of the collagen molecule, plays a major role in triggering the blue fluorescence observed in fresh bone [17–21], the modification of the mineral phase together with the loss of a major proportion of collagen could be an explanation for the yellow or brown fluorescence in older bone material [15,22].

Independent of the observed colouring, there are also differences in fluorescence intensities leading to visually darker colour impressions while wavelength maxima stay the same. Blue fluorescing bone thus appears dark blue, yellow fluorescence turns into brown. Most of the older studies [1,4] usually describe the differences observed in fluorescence colour (blue or yellow), correlating their finds with the PMI. They rarely consider intensity changes (dark or light blue, brown or yellow) as an additional factor for absolute colour appearance. Ramsthaler et al. [11] see a correlation between the PMI and "complete reflection", "reduced reflection" and "no reflection" of fluorescence, supposedly referring

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http://dx.doi.org/10.1016/j.forsciint.2013.03.013

to the occurrence of blue fluorescence rather than intensity differences. As those categories are not further explained in detail, the actual definition of fluorescence reflection remains unclear.

Furthermore, there are different conclusions regarding the significance of fluorescent characteristics in correlation with the PMI in literature: while Berg and Specht [1] as well as Knight and Lauder [4] postulate that blue fluorescence in whole bone cross sections and the absence of differing colours can only be observed up to a PMI of 100–150 years, Facchini and Pettener [12] suggest increasing the limit up to 200–350 years. According to Yoshino et al. [23] fluorescent colouration is not as reliable for dating as the successive reduction in fluorescence intensity.

The practical application of evaluating bone fluorescence in forensics seems disputable concerning the inconsistencies becoming apparent when comparing the correlation depicted between fluorescence and the PMI among different studies. The sometimes unclear descriptions of the concept used for judging the fluorescence appearance and the classification of fluorescence that does not take the difference between colour and fluorescence intensity into account make it difficult to find clear guidelines for forensic problems.

For this reason, we investigated the fluorescence of a large sample set comprising 213 bones with known PMI in order to re-evaluate the potential of this method for forensic practice. The particular aim of this study was to reinvestigate to what extent the method can reliably differentiate between forensically relevant versus historical bone finds using a clear description of bone fluorescence, focusing on the observed colour and intensity separately.

2. Materials and methods

In order to cover a large inhumation time span ranging from ancient to modern remains, two major sample sets with a total of 213 bones were investigated. For the more recent and forensically relevant time period, 58 long bone samples (38 femora, 15 tibiae and 5 humeri) were collected from abandoned graves at a modern cemetery, with a postmortem interval ranging from 8 to 60 years. Detailed documentation of individual data for each grave was available through cemetery records, so the PMI could be traced back to the day. The burial environment ranged from open grassy fields to woodland areas with different soil consistencies and textures, corresponding to typical conditions found in temperate regions. Samples were subsequently stored at −18 °C immediately after removal from the grave until further processing.

The second sample set comprised 155 archaeological specimens composed of 79 human bones (all femora except for one skull) found at different sites throughout Germany and 76 horse bones (mainly from the extremities; exceptions: 4 scapulae and 1 pelvis) excavated in Central and Eastern Europe. Time elapsed since death was 90–4500 years (human remains) and 2000–15,000 years (horses). Individual dating was done by carbon 14, the assignment of grave goods or historical documentation. For details about the investigated specimens refer to the data provided as supplementary material.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.forsciint.2013.03.013.

Cross sections or pieces of 0.5 cm × 1 cm were prepared from each bone using a band saw (Metabo) or a multi tool equipped with a common corundum cutting wheel (Proxxon). One side of the cross section was then ground and polished with fine sandpaper (1200 grit) on a circular grinder (Struers) to even the surface in order to avoid reflection artefacts. Furthermore, degreasing of the modern samples proved to be a necessary step, since remnants of bone fat and adipocere partly produced large, bright orange or blurry areas covering parts of the cross section during the initial UV-examination. Defatting was carried out in a Soxhlet apparatus for 48–72 h using diethyl ether (Roth) as a solvent.

After drying was completed, samples were then viewed under a common UV-light source (Kisker UVL-4L) emitting long-wave (365 nm) UV-light in a darkened room.

A three-stage index system was established [15] to classify the observed fluorescence colouring (proportion of blue versus yellow/brown). Since fresh bone fluoresces as a blue colour, an index value of 3 was attributed to specimens with a predominantly blue fluorescing cross section (approx. 85% or more blue). No distinction was made between light blue and dark blue. Samples exhibiting a mixed colouration between blue and yellow/brown were classified as 2 (15–84% blue), while bones with no or very little blue, but predominantly brown and/or yellow colour were given an index-value of 1 (less than 15% blue).

For estimating the correlation between fluorescence index and PMI we categorized the samples in PMI classes as displayed in Table 1, following the model of Ramsthaler et al. [11]. The gap between PMI classes 1 (8–60 years) and 2 (90–150 years) is due to a discontinuity in the sample set which is, however, not relevant for the investigation of the problem, because forensic research terminates approx. 50 years postmortem.

To determine whether the colour of UV-fluorescence can help distinguish between remains that are recent (PMI $<$ 60 years, PMI class 1) or historical (PMI $>$ 90 years, PMI classes 2–4) we have to assess the power of the UV test. To do so, we classified the 3-stage UV-index into two groups. This can be done in two ways, resulting in two different test-sets (A and B):

1. We assume that index value 3 (predominantly blue fluorescence) is an indicator for a forensically relevant PMI shorter than 60 years, and mixed and yellow fluorescence (index values 1 and 2), in contrast, suggest a forensically irrelevant PMI of more than 90 years (Test A).
2. We assume that blue and mixed fluorescence are indicators for a forensically relevant PMI shorter than 60 years, and only yellow fluorescence is indicative for a forensically irrelevant PMI of more than 90 years (Test B).

To assess and compare the performance of these tests we calculated measures (quality indicators) analogous to those defined for medical testing. Table 2 shows a crosstab matrix displaying the potential outcome of these two tests. The measures were calculated as follows:

- Sensitivity (true positive rate, in %):

$$\frac{a}{(a+c)} \times 100$$

- Specificity (true negative rate, in %):

$$\frac{d}{d+b} \times 100$$

- Efficiency (percentage of the actual test result that was estimated correctly):

$$\frac{(d+a)}{\sum \text{samples}} \times 100$$

- False positive rate:

$$100\% - \text{specificity}$$

- False negative rate:

$$100\% - \text{sensitivity}$$

In order to determine fluorescence intensities, fluorescence spectrometrical measurements were carried out on a smaller sample set of 71 of the archaeological bones. Samples were mounted in a Spex Fluorolog 1680 0.22 m Double Spectrometer and measured punctually in front face geometry. The excitation wavelength was 366 nm, chosen similar to the one emitted by the UV-lamp used for visual fluorescence determination. Integration time was 0.5 s at steps of 1 nm. Wavelength detection started at 380 nm, which is the threshold for light visible to the human eye, and terminated at 702 nm. Results were compared to the spectrum of a fresh, bright blue fluorescing bone, which was also assessed and served as a positive control. Measurement artefacts, such as intensity differences of the excitation light source or background noise were eliminated mathematically using the software OriginPro 8.0.

3. Results

In forensically relevant samples, meaning samples younger than 60 years, one single case with an index value of 1 (yellow fluorescence) was observed, having a PMI of 25 years. About 48% showed mixed fluorescence, containing blue and other colours

Table 1
Classification of samples according to their PMI.

PMI [a]	*n*
8–60	58
90–150	37
151–1000	14
Over 1000	104