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Xanthochromia of the skull bone associated with HbA1c

T. Schäfer [a,*], M. Klintschar [a], R. Lichtinghagen [b], I. Plagemann [c], A. Smith [d], E. Budde [d], L. Hagemeier [a]

[a] Institute of Forensic Medicine, Hannover Medical School (MHH), Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[b] Institute for Clinical Chemistry, Hannover Medical School (MHH), Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[c] Institute of Food Chemistry, Leibniz Universität Hannover, Callinstraße 5, 30167 Hannover, Germany
[d] Institute of Biometry, Hannover Medical School (MHH), Carl-Neuberg-Str. 1, 30625 Hannover, Germany

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ABSTRACT

The color of the surface of 105 skull bones (part of the parietal bone) was determined using a portable spectral colorimeter (spectro color®). By this means it was possible to characterize the color objectively according to the $L^*a^*b^*$ color system defined by the "International Commission de l'Eclairage" (CIE). Biochemical markers of carbohydrate metabolism, HbA1c from venous blood, and glucose/lactate concentrations from vitreous humor, were also determined, for assessment of the ante-mortem plasma glucose concentration using Traub's sum formula. As biochemical markers for lipid metabolism disorder, cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were all determined from venous blood. There is a significant correlation of bone yellowing with HbA1c ($p < 0.001$) and age ($p < 0.001$). The literature asserts a significant correlation between diabetic condition and yellowing of the skull bone. Despite efforts to find the substance responsible for the yellowing of the bone in chronic metabolism disorder, no significant correlation was found between bone color and lipoproteins/bone extracted lipid acids.

1. Introduction

Exploration of the cause of death of a human by post-mortem examination is the second cornerstone of forensic medicine, following reliable confirmation of death. Certain life-threatening and fatal diseases cannot be detected by autopsy and subsequent investigation, however, although in some cases a few indications can be collected. This is true in particular for metabolic diseases that are possible causes of death, but which may remain unrecognized during life and cannot be accurately inferred post-mortem because of the onset of putrefaction. It is particularly important to find tests for the indicators of such diseases. In addition to various metabolic disorders that can lead to life-threatening diseases or complications, diabetes mellitus (DM) and its complications play a key role in forensic practice as a result of their high prevalence [1,2]. Type 2 DM is the most common type (accounting for more than 90% of cases), and involves impairment of insulin secretion and insulin resistance, resulting in increased levels of blood glucose [3]. In addition, between 0.7% and 2.1% of all adults suffer from undiagnosed DM. Cohort studies in the age range from 55 to 74 years show that for almost every person known to have diabetes there is another with unrecognized diabetes [4,5]. According to a meta-analysis, in 2008 approximately 347 million people worldwide suffered from diabetes [2], or about 5% of the world's population [6]. In 2015 published research reveals a prevalence of diabetes of even 12–14% among US adults [7]. These figures show the importance of diagnosis of diabetes mellitus – in particular diagnosis of unrecognized DM – as part of post-mortem examinations.

Post-mortem diagnosis of diabetes mellitus presents some difficulties, even among known diabetics pathognomonic histological changes are seen in only 30% of cases [8]. In the medico-legal routine at least, the Traub formula [9] is applicable by summing the glucose and lactate concentrations, combined to a sum value redefined by Sippel and Möttönen [10], but is somewhat controversial [11–16]. Another problem is that glucose and lactate concentration is difficult to determine in biochemistral routine because of analytical problems due to the viscosity of vitreous humor [17,18]. Probably the most reliable parameter which is applicable post-mortem is HbA1c, which represents non-enzymatically glycated proteins. Elevated blood glucose level sustained for at least 6–8 h leads to a significant increase in HbA1c, which

* Corresponding author. Tel.: +49 5115368891.
E-mail address: derschaefer@gmx.net (T. Schäfer).

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considering the half-life of erythrocytes enables detection of hyperglycemia over the previous 3–4 months [19]. The reliability of HbA1c as a post-mortem marker for diabetes has been demonstrated in various studies [20,21], only recently Keltanen et al. [22] confirmed that HbA1c is a valuable tool in medico-legal routine.

A HbA1c $\geq$ 6.5% is evidence for a DM diagnosis and a HbA1c $<$5.7% is evidence against it [23].

In view of the close relationship between metabolic syndrome and diabetes mellitus, the detection of lipid metabolism parameters appears to be a good way to proceed in identifying possible dyslipidaemia, but interpretation of post-mortem lipids in the blood is, however, difficult [24–27], and the results have to be considered in conjunction with other findings and external circumstances.

Legal practitioners and pathologists have long discussed whether there is a relation between discoloration of the skull and diabetes mellitus. Schmorl [28] was the first who asserted this relation in 1928, that led Bornstein [29] in 1929 to evaluate subjectively the color of the human cranial bone of 266 deceased persons. Like Schmorl, he came to the conclusion that those who suffered from DM during their lifetime showed a particular yellowing of the skull. The discoloration in diabetics was described as "pronounced yellow brown". Krug and Zschoch [30] were the first to examine whether the difference in color could be measured objectively with a reflectance photometer, in 1964. They examined parts of the frontal bone of the cranium of 100 deceased persons, including 34 diagnosed diabetics. They found that in cases of DM only a "reduced brightness" could be measured, which caused the subjective impression of a stronger yellow tone but did not find a difference in the actual color tone. They supposed that the substance responsible for the difference in diabetic patients was concentrated in the bone following dietary differences, but did not produce evidence. Recently, in 2001, Schäfer [31] considered this issue. He was the first to use a colorimeter that could reproduce the color of the surface of the bone based on objective values of the "International Commission de l'Eclairage" (CIE) $L^*a^*b^*$ developed by the CIE in 1976, which standardized human observation of color for the first time [32,33]. Schäfer [31] noted in his study that, in contrast to the results of Krug and Zschoch [30], there was no difference in brightness or coloration between the cranial bones of diabetics and non-diabetics. However, the number of diabetic patients was relatively low: 8 cases in 124 total individuals. Nevertheless he found a correlation between an increased b-value (and thus yellowing) and the age of the deceased, but failed to explain this in his study.

2. Materials and methods

2.1. Bone samples and eligibility criteria

A total of 105 bone samples of horizontal slices of the parietal bone were collected during autopsy in due consideration of inclusion and exclusion criteria. Adults of all ages with or without secondary diseases (including diabetes mellitus) were included. Exclusion criteria were head injury or intracerebral hemorrhage, malnutrition, jaundice, hematopoietic disease, treatment of tetracycline, putrefaction and a post-mortem interval of more than 10 days (240 h).

The samples were taken using an oscillating saw (Aesculap®, type G-6100-05) performing a parallel saw cut 1 cm above the typical circumferential cut applied to open the neurocranium. The parietal region has been chosen for different reasons. On the one hand the sampling was easy to integrate into the autopsy procedure, on the other hand the skull in this region shows an even thickness and contains very little trabecular bone. The bone samples, which measured at least 5 cm in length and 1 cm in width were manually stripped of any adhesive tissue and cleaned by use of a humid cloth. To avoid light reflection from the bone surface the samples were then air-dried.

2.2. Bone surface color analysis

The samples were examined by means of a portable spectral reflex photometer (Hach-Lange GmbH, type: spectro color®; see Fig. 1). By this means the color of the bone surface was measured objectively.

The spectro color® measures according to the standard DIN 5033, 5036 and 6174 and can resolve the color into the $L^*a^*b^*$ color space [34]. The L^* value represents the brightness and is represented exclusively by positive values (0 = black to 100 = white). Positive values of a^* represent red hues, and negative values of a^* represent green hues. Negative values of b^* represent blue hues, and positive values represent yellow hues. The range of values of a^* and b^* values is in principle infinite [35].

The light source for the "spectro color®" is a tungsten filament lamp, which produces a standard light (D65) and covers the spectral range from 400 nm to 700 nm at a resolution of 10 nm. To prevent distortion of the color value by gloss, an integrated sphere (Ulbricht sphere) is used for diffuse illumination of the sample. The reflected light is measured at a viewing angle of 8° relative to the sample [35]. To avoid distortion due to local stains or dents (of vasculature on the inner surface) ten individual measurements of the outer surface were made on each sample (see Fig. 1, small picture).

2.3. Biochemical investigation

Concentrations in the vitreous humor of glucose (Gluco-quant Glucose/HK, Roche Diagnostics) and lactate (Lactate Gen.2, Roche Diagnostics) were determined, and the sum formula of Traub was applied. The HbA1c concentration was determined in venous blood (Tina-quant Hemoglobin A1c III, Roche Diagnostics), as well as blood lipids (CHOD-PAP, Roche Diagnostics, SAS-3 cholesterol profile Kit, Helena Biosciences Europe).

2.4. Statistical analysis

The primary aim of this study was to assess the yellow color of the skull as measured by the value of b^* (as dependent variable)

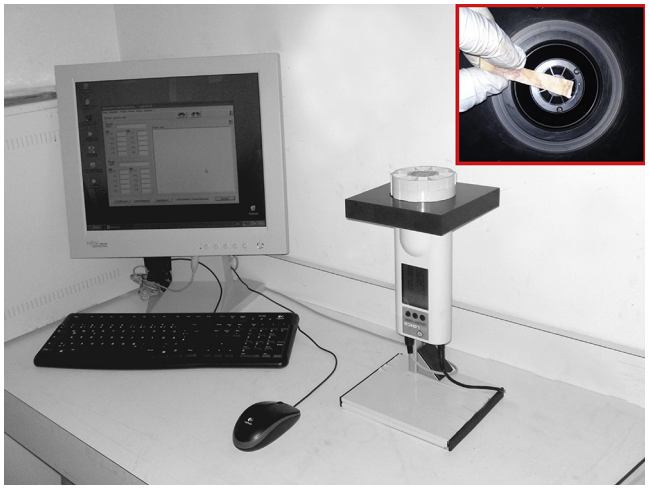

Fig. 1. Experimental set-up with portable reflex photometer and personal computer. Small picture: measuring field of the photometer with applied bone sample.