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TECHNICAL NOTE

Changes in colour of different human tissues as a marker of age

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Abstract This study deals with age estimation based on colour changes of human tissue from the intervertebral discs, Achilles tendon and rib cartilage. The investigated colour changes are the result of the accumulation of non-enzymatic browning products in the tissue. Samples of excised tissues were photographed with a digital camera and the pictures were evaluated using the image analysis processor Lucia G 4.11 processor. The values of the intensities of the RGB channels (MeanRed, Mean Green, MeanBlue) and parameters from the IHS system (Mean-Saturation, HueTypical, HueVariation, BrightVariation and MeanBrightness) were evaluated. The results confirm that colour changes of some tissues depend on ageing and are a good tool for age estimation.

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Introduction

Age estimation in forensic medicine is an important procedure in the identification of an unknown dead body or of a part of the human body. There are many methods of age estimation according to the teeth [1–7] or skeleton [8–13] where the evaluation of morphological parameters or chemical changes of proteins bring quite reliable results in adults and children. However, little attention was paid to the soft tissues, most probably because there are few valuable age-related changes that can be seen. One of such changes is the colour. In forensic medicine, colour changes were studied for different purposes [14–18] and for age estimation [19–23]. One of the causes of colour changes is the glycation of proteins, which gives advanced glycation end products (AGEs). Their accumulation causes a yellow-to-brown tint of the tissue [24–26]. This can be registered because if any colour intensity is reduced, e.g. by material absorption, a supplementary colour starts to prevail, e.g. should a substance absorb a blue portion from the colour spectrum the object seems to be yellow. Colour changes caused by ageing and the utilisation of this effect for age estimation were studied only on hard dental tissues. The change of dentin colour [19] and tooth enamel was used as one of the morphological parameters for age estimation [2, 20]. The colour changes of dental root surface were investigated by means of image analysis [22, 23]. It was found that the colour of dental root changes from chalky white in the young to yellow in middle age to yellow–brown in old people depending on the individual age.

Two different objective approaches can be employed in the colour changes estimation: the use of a colorimeter or spectrophotometer [14–18] or an image processor, which evaluates the object's image from a digital camera. The image analysis of the object's picture obtained by a digital camera was used for the purposes of this study.

Materials and methods

The excisions of the intervertebral discs, Achilles tendon and rib cartilages obtained from dead bodies autopsied in the Institute of Forensic Medicine and Toxicology, First Faculty of Medicine, Charles University and General Teaching Hospital in Prague were used in this study. The post-mortem interval did not exceeded 3 days. The excised tissues were rinsed in water to remove residues of blood, then shortly dried in air, put into plastic bags and stored at −80°C. The total amount of samples consisted of 151 intervertebral discs excisions (106 men and 45 women), 163 Achilles tendons (119 men and 54 women) and 52 samples of rib cartilage (35 men and 17 women). All procedures were carried out according to current acts of law and ethical standards.

The colour of the samples was evaluated using an image processor Lucia G 4.11 (Lucia Imaging, CZ) [27]. Kaiser equipment (Germany) was used for the lighting and photography. Objects to be measured were placed on the contrast homogenous pad made up from grey plasticine. The evaluated objects were illuminated by a strictly defined source of light—two light fluorescent lamps "Osram Dulux L" with a colour temperature of 5,000 K. The sources of light were 40 cm above the objects and the camera was placed in the middle as high as the photographed objects so they were visible in the field of sight with sufficiently distinguishable details. The system was calibrated on the white standard—a plate made up from barium sulphate (used for the calibration of spectrophotometers). Photographs were made with a digital camera "Canon EOS 300D" and transformed to an image processor where the measurement was carried out. A macro program that enabled the automatic processing of the whole sequence of pictures was created. Mean values of the intensities of the RGB channels (image processor parameters: MeanRed, Mean Green, MeanBlue) ranging from 0 to 255 were evaluated for each object. The following image processor parameters from the IHS system were evaluated: MeanSaturation (expresses the purity of light), HueVariation (expresses the homogeneity of coloration), HueTypical (meaning the characteristic tint), BrightVariation (expresses the homogeneity of coloration from the point of brightness) and MeanBrightness (the parameter that expresses the average value of brightness). Statistical evaluation was performed using the software MS Excel and Statistica 6.1 CZ (StatSoft, USA). Mean values with 95% confidence interval and correlation coefficients were calculated for the values of the intensities for all three RGB channels and IHS parameters. Evaluation of the relationship of the measured values on age was performed on exponential transformation.

Results and discussion

The results proved age-related colour changes in examined tissues. The colour changes are most probably the result of the accumulation of AGEs during life like N^{ϵ}-(carboxymethyl) lysine, N^{ϵ}-(carboxyethyl)lysine or pentosidin and chemically unidentified compounds, which result in protein-bound browning or fluorescence and cross-linking [28]. The colour changes and correlation coefficient are different in different tissues (Table 1). They are most conspicuous in the rib cartilage then in the intervertebral discs, while in the Achilles tendon nearly no relation with age was registered. The accumulation of products of non-enzymic browning absorbs mainly the blue portion from white light. That is the reason why the mean blue parameter evaluated by image analysis has good correlation with age. The hue modifies with age (hue typical parameter) from white in young persons—15–20 years of age, to yellow in middle age persons and then a yellow–brown hue starts to prevail that can be seen by the naked eye mainly on the rib cartilages. The distribution of accumulated substances in the tissue is not uniform, which is documented by the mean saturation parameter. It reflects the saturation of the tissue with these substances and is concurrently accompanied with darkening of the tissue as shown by the mean brightness parameter. Other studied parameters like hue variation, bright variation give information about the distribution of substances in the tissue or other physicochemical properties but in relation with age, they have little importance.

The graphs of the average values of the monitored parameters in particular age decades illustrate the proper usefulness of this method for age estimation. This is evident from Figs. 1, 2, 3 and 4 that show the dependence of colour

Table 1 Correlation coefficients of different parameters with age and in different tissues

Tissue	Correlation coefficient of parameters with age			
	Mean blue	Hue typical	Mean saturation	Mean brightness
Rib cartilage	−0.87	−0.69	0.90	−0.87
Intervertebral disc	−0.82	−0.69	0.80	−0.78
Achilles tendon	−0.47	0.03	0.54	−0.22

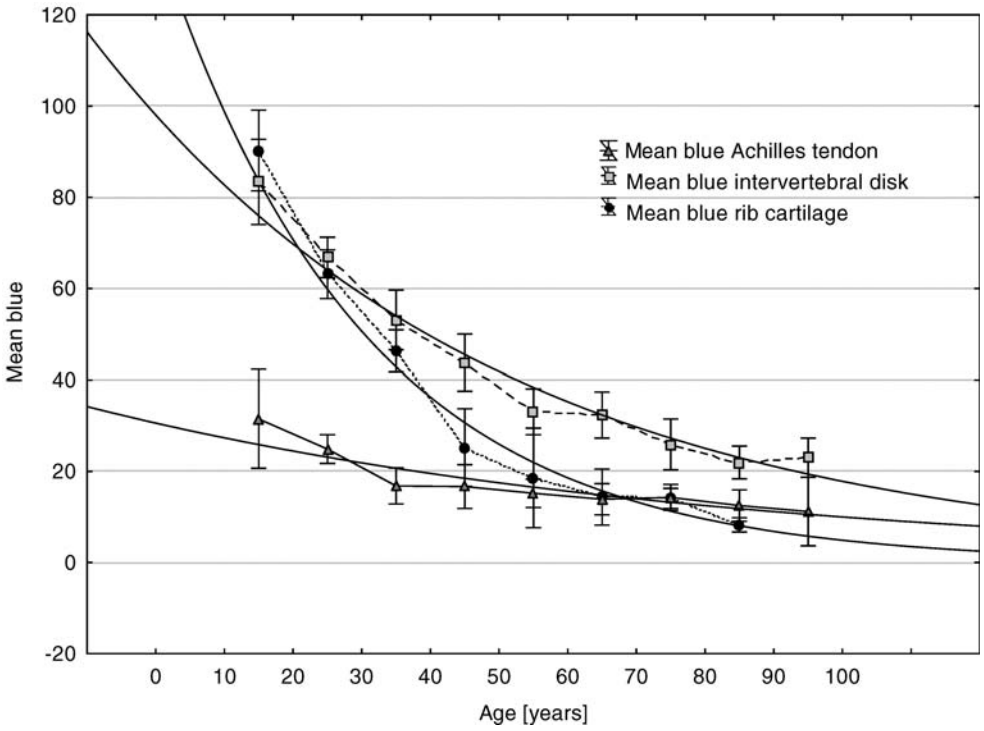

Fig. 1 Average values of the mean blue parameter (average± 0.95 interval of confidence). Mean blue Achilles tendon= $30.6037\exp^{-0.0112x}$. Mean blue intervertebral disk= $98.0548\exp^{-0.017x}$. Mean blue rib cartilage= $137.88\exp^{-0.0334x}$

and changes of other parameters on age in the analysed tissues: the best correlation of colour changes to age was found in the rib cartilages followed by the intervertebral discs and the lowest relationship was found in the Achilles tendon. This depends most probably on the amount of proteoglycans in the tissue that is highest in the rib cartilage. This opinion is supported by the observation of tissues formed almost exclusively by collagen (e.g. dura matter, pericardium) where no visible colour changes can be found.

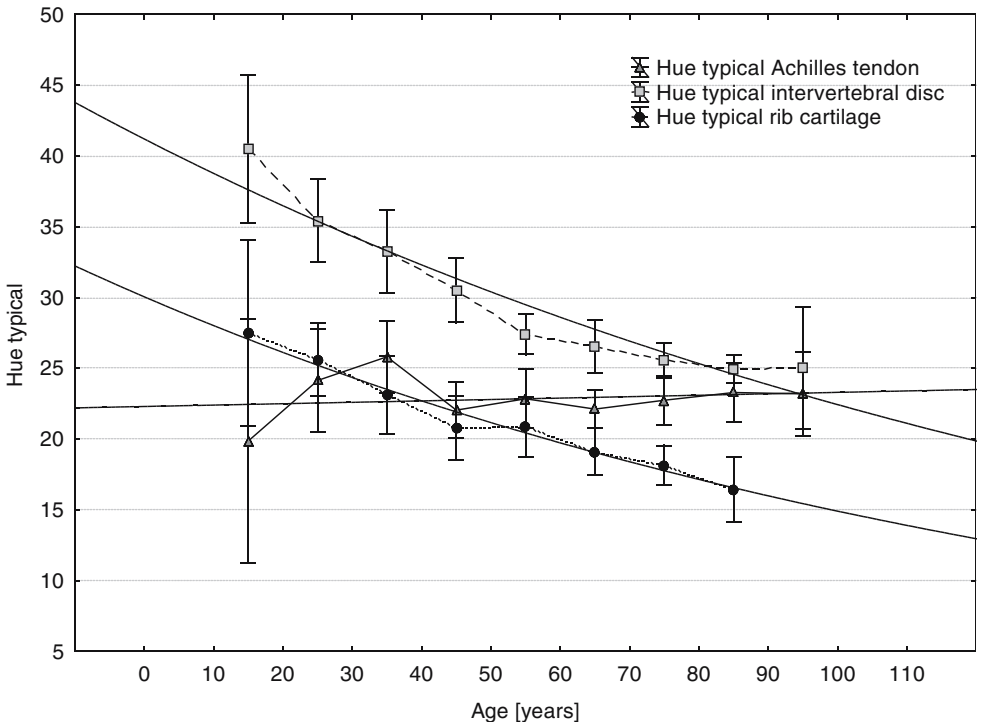

Fig. 2 Average values of the hue typical parameter (average± 0.95 interval of confidence). Hue typical Achilles tendon= $22.3084\exp^{0.0004x}$. Hue typical intervertebral disc= $41.2034\exp^{-0.0061x}$. Hue typical rib cartilage= $30.0534\exp^{-0.007x}$

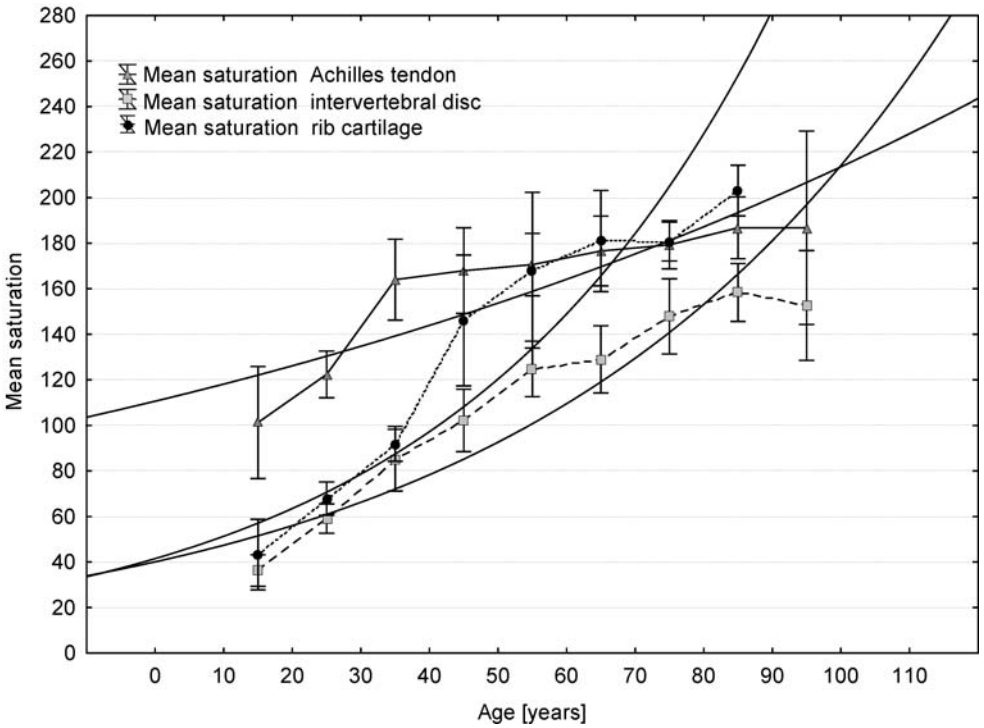

Fig. 3 Average values for the mean saturation parameter (average±0.95 interval of confidence). Mean saturation Achilles tendon= $110.6192\exp^{0.0066x}$. Mean saturation intervertebral disc= $40.0674\exp^{0.0168x}$. Mean saturation rib cartilage= $41.4763\exp^{0.0213x}$

Conclusions

This method was worked out for the purposes of age estimation in unidentified dead adult persons where other methods cannot be applied or only a part of the body was found. The importance of the method lies in rapid age estimation and in sorting out the persons according to age groups. The results of this study lead to a conclusion that reliable age estimation based on colour changes can be performed up to the age of 45. After 45 years of age, the spread of values increases, which makes the usability of this method low in this time period. Age estimation can be performed especially from the rib cartilage where colour changes are most evident. However, this conclusion still

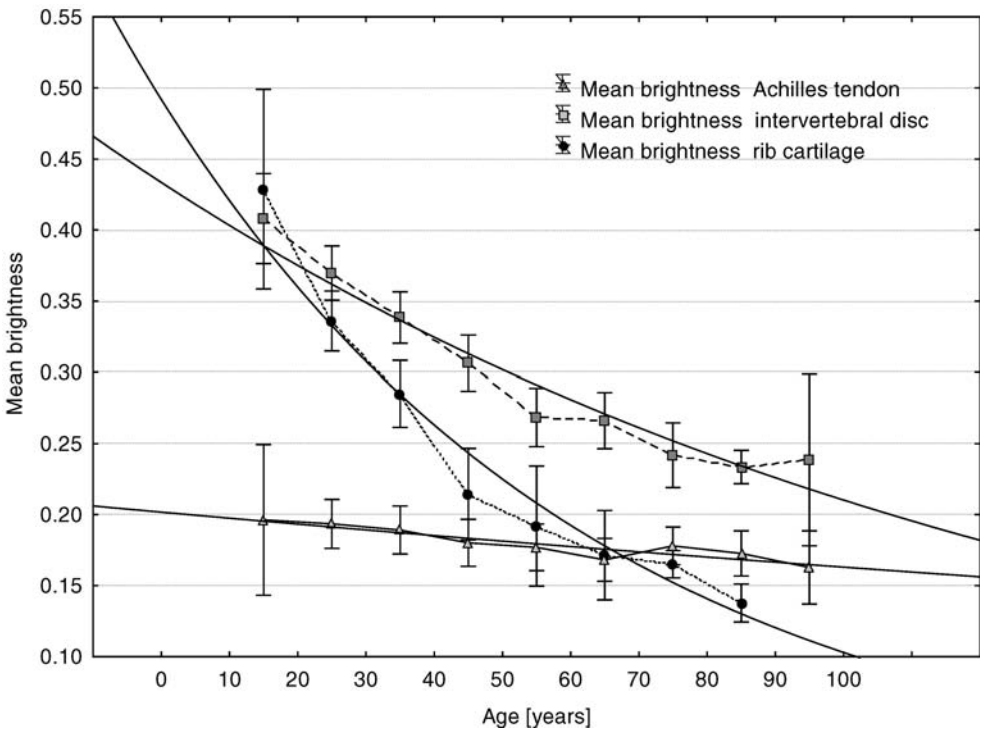

Fig. 4 Average values of the mean brightness parameter (average±0.95 interval of confidence). Mean brightness Achilles tendon= $0.2017\exp^{-0.0021x}$. Mean brightness intervertebral disc= $0.4335\exp^{-0.0072x}$. Mean brightness rib cartilage= $0.4919\exp^{-0.0156x}$

needs confirmation based on a larger group. The best colour analysis is feasible by the mean blue, mean saturation and mean brightness parameters.

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