The image analysis of colour changes of different human tissues in the relation to the age. Part 1. Methodological approach

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Summary

The human age for medico-legal purposes is usually estimated from hard tissues like bones and teeth. Only little attention was paid to soft tissues most probably due to the lack of detectable age changes. This study deals with colour changes of human tissue from intervertebral discs, Achilles tendon and rib cartilage in the relation to the age. The image analysis of colour of investigated tissue samples was performed. The values of intensities of channels RGB (MeanRed, MeanGreen, and MeanBlue) and parameters from the IHS system (MeanSaturation, HueTypical, HueVariation, BrightVariation and MeanBrightness) were evaluated. The results confirm that colour changes of some tissues can be used for age estimation.

Key words: age estimation – colour changes of tissues – non-enzymatic browning – image analysis – AGE@s, Lucia G

Souhrn

Obrazová analýza změn barevnosti různých lidských tkání v závislosti na věku. Část 1. Metodický přístup

Pro účely soudního lékařství se určení věku provádí obvykle z tvrdých tkání jako jsou kosti nebo zuby. Jen malá pozornost byla věnována měkkým tkání, nejspíše pro nedostatek zjistitelných změn. Tato studie se zabývá změnou barevnosti lidských tkání z intervertebrálního disku, Achillovy šlachy a žeberní chrupavky v závislosti na stárnutí. Pro sledování změny byla použita metoda obrazové analýzy. Byly vyhodnoceny hodnoty intenzit kanálů RGB (MeanRed, MeanGreen, and MeanBlue) a parametry ze systému HIS MeanSaturation, HueTypical, HueVariation, BrightVariation and MeanBrightness). Výsledky potvrzují, že změna berevnosti některých tkání může být použita pro určení věku.

Klíčová slova: Určení věku – změny barevnosti tkání – neenzymatické hnědnutí – obrazová analýza – AGE's – Lucia G

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Introduction

Although the DNA profiling is the leading method in identification, the first question given to the forensic pathologist is "what is the age of the body?" mainly in cases of burning or fragmentation of the body. Different methods can be applied to solve this problem. There is extensive menu of methods for age estimation for medico-legal purposes, that can be divided into morphological and biochemical. The offer of morphological methods [1-19] prevails over biochemical ones considerably. [20-32]. Majority of the methods is based on assessment of hard tissues like bones or teeth but 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 a colour.

In forensic medicine, changes of colour were studied with the development of post-mortal lividities [33, 35], changes of skin colour with timing of injury [34], contusion [36], or colour of human skull [37], but in the relation to the age they were

not studied sufficiently. Tissue colour changes originate from an accumulation of pigments. This effect occurs both in certain diseases —e.g. jaundice, ochronosis, argyrosis— and normally (e.g. skin browning caused by melanin increase during sunbathing), but also as an aging phenomenon. For example, it is known that lipofuscine accumulation in tissues of the elderly persons causes state called brown tissue atrophy. The glycation of proteins is one of the biochemical reactions which causes colour products. These reactions result in advanced glycation end products (AGE@s) and their accumulation causes a yellow-to-brown tint of the tissue [38-40]. Investigations dealing with age colour changes and the utilisation of this effect for age estimation are scarce and related only to the hard dental tissues. The change of dentin colour [41] and tooth enamel were used as one of the morphological parameters for age estimation [42, 43]. The colour changes of dental root surface were investigated by means of image analysis [44, 45, 46]. It was found that colour of roots in young persons is chalky white; yellowish in persons of middle age while in elderly people is dark yellow until brownish.

The colour changes can be registered visually-subjectively or objectively by an instrument, i.e. a colorimeter or spectrophotometer [33, 34], or by using an image processor which evaluates the object's image from a digital camera. The colour of an object is a valuable feature that reflects the physicochemical properties of an object. Nevertheless, the colour is not a fixed characteristic of an object. Under constant conditions of colour assessment (i.e. the geometry of the lighting, temperature and setting of instruments), the final colour value depends on object's characteristics (e.g. chemical composition, transparency, reflexivity). The human eye perceives three basic colours (red, green, blue), and combines them to get other colours depending on a) dominant wavelength (i.e. "hue"), b) colour purity (i.e. "saturation") and c) intensity ("brightness"). Therefore, three variables are necessary to determine the colour of object under given conditions. Different systems (colour spaces) have been implemented in order to enable an objective characterisation of colour perception, e.g. RGB, IHS, CIE XYZ, CIE $L^* a^* b^*$ (L equals the luminance ranging from 0 for black to 100 for white, a* equals values ranging from -100 for green to +100 for red and b* means values ranging from -100 for blue to +100 for yellow) [33]. The CIE L* a* b* colour space is used in spectrophotometers while other apparatuses like digital cameras exploit colour space RGB, unambiguously transformable into an IHS system (I= intensity, H=hue, S=saturation). Digitalised image (raster) consists of picture points – pixels that contain the information on RGB channels intensity. Most frequently, it is expressed as intensities in the range between 0 and 255, i.e. 8 bites per channel. Therefore, a so-called 24-bit colour system is theoretically able to distinguish more than 16 million hues (256^3). White colour can be characterised by values R=255, G=255 and B=255 in this system. 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 it seems to be yellow.

The progress in the processing of digital images makes their evaluation more objective. The image analysis of the object's picture obtained by a digital camera was used for purposes of this study.

Material and methods

Materials

The excisions of intervertebral discs, Achilles tendons and rib cartilages obtained from dead bodies autopsied in the Institute of Forensic Medicine and Toxicology, 1st 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 immediately after excision 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). While preparing tissue excisions (especially intervertebral discs - IVD), it is necessary to keep all instruments and tissue free of blood because tissues are easily stainable even by trace amounts of blood and the investigated colour could be overlapped.

All procedures have been carried out according to current acts of law and ethical standards.

Methods

The image analysis of the object's picture was used as described previously [47]. The colour of samples was evaluated using an image processor Lucia G 4.11 (Laboratory Imaging, s.r.o., CZ) [48]. Kaiser equipment (Germany) was used for the lighting and photography. Objects (usually 12-15 excisions) to be measured were placed on the contrast homogenous pad made up from grey plasticine. The illumination of objects was accomplished by a strictly defined source of light – two light fluorescent lamps "Osram Dulux L" with a colour temperature of 5000° 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 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 intensities of RGB channels (image processor parameters MeanRed, MeanGreen and MeanBlue) ranging from 0 to 255 were evaluated for each object. The image processor parameters from the IHS system were also 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), MeanBrightness (the parameter that expresses the average value of brightness).

Numerical data from the image processor have been implemented into MS Excel table. Statistical evaluation has been performed using the software Statistica 6.1 CZ (StatSoft, USA). Mean values with confidentiality interval 95% have been calculated for values of intensities for all three RGB channels. Evaluation of relationship of measured values on age was performed on exponential transformation.

Discussion

The sets of samples show some non-homogeneity from the point of view of a number of persons and male/female ratio. However, according to our opinion it has only little importance because the sets are not compared each to the other. It is only stated that different types of tissues come in useful more or less for the age estimation according to age changes. In spite of low amount of rib cartilages it is worth to deal with these samples because the colour changes with the age on rib cartilages are meretricious and very good visible.

The evaluation of colour can be made by a spectrophotometer or by an image processor. The spectrophotometer CM-2600d (Minolta, Japan) was used in preliminary study (results not shown). The values obtained in that preliminary study showed greater variation than those obtained by image analysis. It was caused due to that measuring area of spectrophotometer overlapped the measured area on the tissue sample so that the errors from the background were involved in the measured values. It happened also that different values had been obtained in the repeated measuring of the same object and no reproducible procedure could be abided. That is the reason why the image analysis was used for the purposes of this study. Another disadvantage of the spectrophotometer is that it does not evaluate the colour non-homogeneity of tissue, variation in brightness etc. On the other hand, the evaluation of colour characteristics by image analysis is not so precise because it uses RGB (or the IHS system) which is the subset of L*a*b* colour space used by a spectrophotometer. Measuring with a colorimeter or spectrophotometer brings only average value from only one point while the evaluation by the image processor enables the measurement of a colour profile and so it provides much more information (the changes of intensities of values of RGB channels, changes of parameters in the IHS system) according to a chosen trajectory. These data are intercepted in the variation parameters like e.g. HueVariation, BrightVariation. Another advantage of image analysis is its simplicity and reproducibility. The reproducibility is assured by the standardized procedures: for all measurements, the same camera, objective, set up of camera, geometry of lighting, calibration of white. The fact that the same conditions of analysis were abided can be easily verified in the image processor immediately after measuring and a correction on calibrated colour can be made, if necessary.

Conclusion

The image processor for the analysis of digital pictures of age colour changes of human tissues was applied. This method is simple and does not require expensive instrumental equipment.

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References

1. **Gustafson G.:** Age determination on teeth. J Amer Dent Ass 1950; 41: 45–54.
2. **Johanson G.:** Age determination from teeth. Odontol Revy; 1973, 22 (Suppl. 21).
3. **Willems G.J.:** A review of the most commonly used dental age estimation techniques. J Forensic Odontostomatol 2001; 19(1): 9–17.
4. **Paewinsky E., Pfeiffer H., Brinkman B.:** Quantification of secondary dentin formation from ortopantomograms – a contribution to forensic age estimation methods in adults. Int J Legal Med 2005; 119: 27–30.
5. **Todd W.T.:** Age changes in the pubic bone. Am J of Physical Anthropology 1920; 3: 285–334.

6. **Brooks T.S.:** Skeletal age at death: The reliability of cranial and pubic age indicators. Am J Phys Anthropology 1957; 13: 567–597.
7. **Hanihara K., Suzuki T.:** Estimation of age from the pubic symphysis by means of multiple regression analysis. Am J Phys Anthropol 1978;48:233-240.
8. **Pasquier E., Luc de Saint Martin Pernot, Burduj V., Mounayer, Le Resr C., Colin D. Mottier D., Roux C., Baccino E.:** Determination of age at the death: assessment of an algorithm of age prediction using numerical three dimensional CT data from pubic bones. Am J Phys Anthropol 1999; 108: 261–268.
9. **Sinha A., Gupta V.:** A study on estimation of age from pubic symphysis. For Sci Int 1995; 75: 73–78.
10. **Hajniš K., Novák J.T.:** Srůst švů lebeční klenby. [Obliteration of cranial sutures] – in czech. Avicenum, Praha 1984.
11. **Iscan M.Y., Loth S.R., Wright R.K.:** Age estimation from the rib by phase analysis: white females. J For Sci 1984; 29: 1094–1104.
12. **Iscan M.Y., Loth S.R., Wright R.K.:** Metamorphosis at the sternal rib end: a new Method to estimate age at death in white males. Am J Phys Anthropol, 1984; 65: 147–56.
13. **Iscan M.Y., Loth S.R., Wright R.K.:** Age estimation from the rib by phase analysis: white males. J For Sci 1985; 30: 853–863.
14. **Iscan M.Y., Loth S.R.:** Determination of age from the sternal rib in white males: a test of the phase method. J For Sci 1986; 31: 122–132.
15. **Iscan M.Y., Loth S.R., Wright R.K.:** Racial variation in the sternal extremity of the rib and its effect on age determination. J For Sci 1987; 32: 452–466.
16. **Schmeling A., Schulz R., Reisinger W., Mühler M., Wenecke K-D., Geserick G.:** Studies on the time frame for ossification of the medial clavicular epiphyseal cartilage in conventional radiography. Int J Legal Med 2004; 118: 5–8.
17. **Schmeling A., Mühler M., Schmidt S., Reisinger W., Schmeling A.:** Studies on the time of frame for ossification of the medial epiphysis of the clavicle as revealed by CT scans. Int J Legal Med 2005; 119: 142–145.
18. **Camariere R., Ferrante L., Mirtella D., Cingolani M.:** Carpals and epiphyses of radius and ulna as age indicators. Int J Legal Med 2006; 120: 143–146.
19. **Camariere R., Ferrante L., Cingolani M.:** Age estimation in children by measurement of open apices in teeth. Int J Leg Med 2006; 120: 49–52.
20. **Helfman P.M., Bada J.L.:** Aspartic racemization in dentin as a measure of ageing. Nature 1976; 262: 279–281.
21. **Ritz S., Schutz H.-W., Schwarzer B.:** The extent of aspartic acid racemization in dentin: a possible method for a more accurate determination of age at death? Z Rechtzmedizin 1990; 103: 457–462.
22. **Ohtani S., Yamamoto K.:** Age estimation using the racemization of amino acid in human dentin. J For Sci 1991; 36: 792–800.
23. **Ritz S., Schutz H.-W., Pepper C.:** Postmortem estimation of age at death based on aspartic acid racemization in dentin; its applicability for root dentin. Int J Leg Med 1993; 105: 289–293.
24. **Ritz S., Schütz H.-W.:** Aspartic acid racemization in intervertebral discs as an aid to postmortem estimation in of age at death. J For Sci 1993; 38: 633–640.
25. **Ritz S., Turzynski A., Schütz H.W.:** Estimation of age at death based on aspartic acid racemization in noncollagenous bone proteins. For Sci Int. 1994; 69: 149–159.
26. **Pilin A., Čabala R., Herrmannová M., Pudil F., Gross R.:** Evaluation of age – possibilities in estimating the relation of D, L- forms of aspartic acid. II. chiral separation of amino acids from human dentin Soud Lék 1997; 42: 12–16.
27. **Pilin A., Čabala R., Pudil F., Bencko V.:** The use of the D-, L- aspartic ratio in decalcified collagen from human dentin as an estimator of human age J For Sci 2001; 46:1228–1231.
28. **Pilin A., Čabala R., Pudil F., Bencko V.:** Assessment of the ratio of the D- and L- form of aspartic acid in intervertebral disc as an indicator of age [in Czech] Soud. Lék. 2001; 46: 24–26.
29. **Pilin A., Čabala R., Pudil F., Bencko V.:** Assessment of the ratio of the D- and L- form of aspartic acid in tissue of the Achilles tendon as an indicator of age. [in Czech] Soud. Lék. 2001; 46: 27–29.
30. **Martin-de las Heras S., Valenzuela A., Vilaneuva E.:** Deoxypyridinoline crosslinks in human dentin and age estimation of age. Int J Legal Med 1999; 112: 222–226.
31. **Martin-de las Heras S., Valenzuela A., Overall C.M.:** Gelatinase A in human dentin as a new biochemical marker for age estimation J Forensic Sci 2000; 45(4): 807–811.
32. **Cloos P.A.C., Jensen A.L.:** Age-related de-phsophorylation of proteins in dentin: a biological tool for assessment of protein age Biogerontology 2000; 1: 341–356.
33. **Vanezis P., Trujillo O.:** Evaluation of hypostasis using a colorimeter measuring systém and its application to assessment of the post-mortem interval (time of death). For Sci Int 1996; 78: 19–28.
34. **Trujillo O., Vanezis P., Cermignani M.:** Photometric assessment of skin colour and lightness using a tristimulus colorimeter: reliability of inter and intra-investigato observations in healthy adult volunteers For Sci Int 1996; 81: 11.
35. **Bohnert M., Weinman W., Pollak S.:** Spectrophotometric evaluation of postmortem lividity. For Sci Int 1999; 99: 149–1586
36. **Bohnert M., Baumgartner R., Pollak S.:** Spectrophotometric evaluation of the colour of intra- and subcutaneous bruises. Int J Leg Med 2000; 113: 343–348.
37. **Schafer Th. A.:** The colour of the human skull. For Sci Int 2001; 117: 53–56.
38. **Hormel S.E., Eyre D.R.:** Collagen in the ageing human intervertebral disc: an increase in covalently bound fluorophores and chromophores. Biochim Biophys Acta 1991; 1078: 243–250.
39. **Brinkman Fraye E., Degenhardt T.P., Thorpe S.R., Baynes J.W.:** Role of the Maillard reaction in aging of tissue proteins. Advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins. J Biol Ch 1998.
40. **Bank R.A., Bayliss M.T., Lafeber F.P.J.G., Maroudas A., Tekoppele J.M.:** Ageing and zonal variation in post-translational modification of collagen in normal human articular cartillage. The age-related increase in non-enzymatic glycation affects Biochem J 1998, 300: 345–351.
41. **Ten Cate A.R., Thompson G.W., Dickinson J.B., Hunter H.A.:** The estimation of age of skeletal remains from the colour of roots of teeth. J Canad Dent Assn 1977; 43:83-86
42. **Solheim T.:** A new method for dental age estimation in adults. Forensic Sci Int 1993; 59: 137–147.
43. **Solheim T.:** Dental color as an indicator of age. Gerodontics 1988; 4: 114–118.
44. **Pudil F., Pilin A.:** The evaluation of morphological parameters on teeth for age estimation using computer image analysis. Proceedings of "European IOFOS Millennium Meeting", Leuven, Belgium; August 23.–26. 8. 2000.
45. **Lackovic K.P., Wood R.E.:** Tooth root colour as a measure of chronological age. J.Forensic Odontostomatol 2000; 18: 37–45.
46. **Valenzuela A.; Martin-de las, Heras S., Mandojana J.M., De Dios Luna J., Valenzuela M., Villanueva E.:** Multiple regression models for age estimation by assess-

ment of morphologic dental changes according to teeth source Am J Forensic Med Pathol 2002; 23(4)3.
47. **Pilin A., Pudil F., Bencko F.:** Changes in colour of different human tissues as a marker of age. Int J Legal Med 2007; 121: 158–162.
48. http://www.lim.cz/

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ZPRÁVY

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