European Geriatric Medicine 2 (2011) 140–144

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Research paper

Skin ageing and oxidative stress in a narrow-age cohort of older adults

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ARTICLE INFO

Article history:
Received 10 December 2010
Accepted 26 April 2011
Available online 21 June 2011

Keywords:
Skin ageing
Oxidative stress
Factor analysis

ABSTRACT

Purpose: Skin ageing is not a monolithic entity, but comprises several related sets of features. We sought to determine the number of related sets of skin ageing features and test whether these were associated with environmental exposures and measures of oxidative stress.
Subjects and methods: Facial skin ageing features were scored by three independent raters from photographs on a narrow-age, community-resident cohort at age 83 years. Smoking, sun exposure (indoor or outdoor occupation), social class, body mass index, 8-hydroxy-2'-deoxyguanosine (8-OHdG, a measure of oxidative DNA damage) and Trolox Equivalent Antioxidant Capacity (TEAC, a measure of antioxidant capacity) were measured as independent predictors of skin ageing. Skin ageing feature items with adequate inter-rater reliability were entered into an ordinal factor analysis to determine factor structure. Extracted factors were correlated with independent predictors of skin ageing.
Results: Two hundred and fourteen (102 male, 112 female) photographs were considered to be of adequate quality for rating by all three raters. Inter-rater reliability was acceptable (Kendall's $w > 0.6$) for ten of the 16 scale items. Three factors were extracted, relating to pigmented spots, wrinkles and facial sagging, respectively. Multivariate analysis showed sex ($P < 0.001$, partial $\eta^2 = 0.605$), BMI ($P = 0.004$, partial $\eta^2 = 0.063$) and social class ($P = 0.005$, partial $\eta^2 = 0.061$) to have significant effects on skin ageing. For participants with oxidative stress measures, in addition to sex and social class, 8-OHdG was positively associated with skin ageing ($P < 0.001$, partial $\eta^2 = 0.152$), but not TEAC ($P = 0.13$).
Conclusion: In this elderly cohort, skin ageing was associated with increased levels of oxidative stress.

1. Introduction

Skin ageing results from intrinsic biological ageing together with extrinsic exposures such as ultraviolet light and smoking. Evidence is increasing that biological ageing and photoageing of the skin share fundamental aetiological mechanisms [1]. Experimental studies indicate that oxidative stress is one likely candidate mechanism [2]. In vitro studies demonstrate that increased oxidative stress causes metalloproteinase-1-induced collagen fragmentation [3,4]. Antioxidant enzyme gene expression is reduced with concomitant elevation of protein oxidation on acute ultraviolet light exposure [5] and topical ascorbic acid reduces ultraweak photon emission, a marker of the antioxidant effect of the treatment, and increased anti-wrinkle effects [6]. Other putative anti-ageing treatments, including coenzyme Q10 and retinoids, have also been tried to ameliorate skin ageing. One study found that measures of oxidative stress and large-scale mitochondrial DNA deletions increased with chronological age, especially after 60 years [7]. However, to date there is a lack of epidemiological data linking in vivo oxidative stress measures to skin ageing.

There are several methodological obstacles to testing the relationship between in vivo oxidative stress measures and skin ageing. First, skin ageing is not a singular phenomenon, but comprises several factors [8]. Secondly, there is a need to address the contribution of ultraviolet light exposure in analyses. It is dubious whether older people can provide a reliable estimate of lifetime sun exposure and samples usually contain participants over a wide age range, which means they had different ultraviolet light exposures at different ages; randomised controlled trials of sun exposure can only cover shorter periods [9]. Using proxy measures for sun exposure, such as having an outdoor occupation [10], cannot adjust for this birth cohort effect. Thirdly, there is a question of rater reliability; some skin ageing scale items have greater inter-rater reliability than others [11]. Using unreliable items introduces considerable error variance thus, reducing power to detect true effects.

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doi:10.1016/j.eurger.2011.04.009

We were fortunate in having high quality facial photographs available from a cohort all born in the same year, resident in Scotland at age 11 and 83 years. This, together with occupational history, allowed us to control for photoageing cohort effects (i.e. in general participants experienced the same weather at the same age from childhood). Although narrow-age cohorts are suited to controlling for the effects of sun exposure, the lack of variance in age did not allow correlation of putative skin ageing features, such as wrinkles, with age within the cohort itself. Hence, first it was necessary to ascertain that skin ageing at 83 years can be explained in terms of those skin ageing factors identified at other ages. We then tested whether these skin-ageing factors were associated with measures of oxidative stress.

2. Methods

2.1. Sample

The sample comprised Lothian 1921 Birth Cohort (LBC1921) [12,13]. Potential participants were largely identified from the local Community Health Index. Consent was obtained in accordance with permission from the local Research Ethics Committee. Five hundred and sixty-nine participants (238 men, 331 women) were seen at age 79, 321 (145 male, 176 female) at age 83 and 191 (87 male, 104 female) at 87 years.

2.2. Measures

2.2.1. Facial photographs

The use of digital photographs allowed us to standardize lighting conditions, a potential confounder not often controlled for [8,9]. Photos of 314 participants were taken at mean age 83.24 (range 82.04–84.57) years with a Nikon E5700 Digital Camera [14]. Photographs were taken spread evenly across all seasons. Participants were instructed to maintain a neutral expression. Four to six images were taken of each participant under standardized lighting conditions, distance to the camera, and camera zoom. Images were rotated in Adobe Photoshop 10.0 so that both pupils and the line where the lips met were horizontally aligned.

2.2.2. Skin ageing variables

Variables available from facial photographs were taken from a validated skin ageing scale [8]: milia (cheek), milia (forehead), pigmented spots (cheek), pigmented spots (forehead), fine lines (cheek), fine lines (forehead), lines on lips, wrinkles (cheek), wrinkles (under eyes), wrinkles (upper lip), furrows between eyebrows, nasolabial folds, crows feet, facial tissue slackening, bags under eyes and drooping eyelids. Apart from milia, all variables were scored between one and three according to severity. We used photographs so that we could measure the reliability of each skin ageing scale item: this was done so that unreliable items would not introduce error into the factor analysis. Each variable was scored by three raters independently with standard instructions on zoom (200% for lines, else 100%) and cut-offs between few and many where appropriate. All raters used the same size computer monitor screen, model and settings in the same room. Raters were asked to rate whether each photograph was of adequate quality to measure all variables.

2.2.3. Correlate measures

Since many potential variables were available from the LBC1921 data set, with the likelihood of Type 1 statistical error if these were all included indiscriminately, independent correlate variables were chosen, a priori, as those designated as ''determinants of facial ageing'' found to be significant in the large Danish twin study of facial ageing [10]: sex, smoking, sun exposure (indoor or outdoor occupation), social class and body mass index (BMI, measured at age 79; for those attending at age 87, mean BMI of 25.94 kg/m^2 was unchanged from the mean of 25.93 kg/m^2 measured at age 79). Social class was derived from the participant's main occupation. Women were asked for their husband's occupation as well as their own, and assigned a social class based on the highest occupation of the household.

2.2.4. Measures of oxidative stress

For those participants seen at age 87, we had two measures of oxidative stress, serum 8-hydroxy-2'-deoxyguanosine (8-OHdG, a measure of oxidative DNA damage) [15] and Trolox Equivalent Antioxidant Capacity (TEAC, a measure of antioxidant capacity). The plasma 8-OHdG levels were determined by a competitive immunoassay (Assay Designs Stressgen, Ann Arbor, Michigan, USA). In brief, 8-OHdG monoclonal antibody and the sample or standard are added to 8-OHdG-precoated microtiter plate wells. The 8-OHdG in the sample or standard competes with immobilised 8-OHdG on the plate for 8-OHdG monoclonal antibody binding sites. Immobilised 8-OHdG is detected by a horseradish peroxidase-conjugated secondary antibody and developed with tetramethylbenzidine substrate and the absorbance read at 450 nm. The intensity of the absorbance is inversely proportional to the concentration of 8-OHdG (ng/mL). Plasma antioxidant capacity was measured by the method of Rice-Evans and Miller [16] and used in our laboratory for many years [17]. The vitamin E analogue, Trolox (Sigma Aldrich, Poole, UK) was used to produce a standard curve for quantification of the TEAC activity of plasma. The TEAC was calculated by defining the concentration (mM) of Trolox that had the equivalent antioxidant capacity to 1.0 mM of the plasma sample under investigation.

2.3. Statistical analyses

Inter-rater reliability (Kendall's w) was calculated for each skin-ageing item. A threshold of Kendall's $w > 0.6$ was taken as the cut-off to determine the subset of skin ageing items with adequate inter-rater reliability. Exploratory factor analysis using MPlus 5.21 was carried for each rater on the subset of skin ageing variables with acceptable inter-rater reliability to determine a consistent factorial structure across raters. This was followed by confirmatory factor analysis of the variable scores summed across all three raters to derive factor scores for subsequent modelling. For the items measured, confirmatory factor analysis had previously been performed for the validated skin scale, identifying four factors from 22 skin ageing features in 361 white women aged 18 to 80 years, labelled as milia, pigmented spots, wrinkles and sagging [8]. Factor scores were then entered into a multivariate general linear model and predictor variables entered as: those identified in the Danish twin study [10]; then oxidative stress variables. Predictor variables were only retained at $P < 0.1$ and an optimal model was defined as that where all predictor variables reached $P < 0.05$ and explained the greatest proportion of variance as measured by the sum of partial η^2. All models investigated main effects only to limit the number of hypotheses being tested.

3. Results

3.1. Sample description and inter-rater reliability

Three hundred and six photographs were available for rating, 214 (102 male, 112 female) were considered to be of adequate quality for rating by all three raters. Sixteen (8%) participants were current smokers, 98 (46%) ex-smokers and 99 (46%) non-smokers; 22 participants (10%) had had an outdoor occupation. There was no significant association between smoking status and indoor/

Table 1
Means and standard deviations for skin ageing predictor variables in the LBC1921 cohort.

	Men		Women	
	Mean	Standard deviation	Mean	Standard deviation
Body mass index (kg/m^2)	26.2	3.2	25.9	4.2
8-OHdG	25.8	5.7	21.5	5.9
TEAC	1.1	0.2	1.1	0.2

8-OHdG: 8-hydroxy-2'-deoxyguanosine; TEAC: Trolox Equivalent Antioxidant Capacity.

outdoor occupation ($\chi^2 = 0.28$, $P = 0.87$). Other sample characteristics are shown in Table 1. There was a good spread of scores across all severity levels for most variables and inter-rater reliability was acceptable for ten of the 16 scale items (Table 2).

3.2. Ordinal factor analysis of skin ageing variables with acceptable inter-rater reliability

Proceeding to exploratory factor analysis an acceptable solution in terms of goodness-of-fit (Root Mean Square Error of Approximation (RMSEA) < .06) was obtained with three factors for all three raters. In each case, when the factor loadings were rotated (using the geomin oblique criterion) a pattern of loadings greater than 0.3 emerged consistent across raters. Therefore, the variables across all three raters were merged by summing. Rotated loadings for the 3-factor solution obtained from the data summed across raters are shown in Table 3. The three factors are consistent with those extracted by Guinot et al. [8]: factor 1 was defined mostly by loadings from pigmented spots, factor 2 mostly by wrinkles and factor 3 mostly by what Guinot et al. [8] labelled as sagging, features considered by them to relate to tissue slackening; milia variables failed to reach acceptable inter-rater reliability so this factor cannot be commented on. Factors 1 and 2 correlated $r = 0.27$, factors 2 and 3 correlated $r = 0.70$ and factors 1 and 3 correlated $r = 0.13$. Hence, although distinct factor emerged, wrinkles and sagging were fairly closely related, consistent with Guinot et al.'s findings that these factors were those most strongly associated with overall skin ageing [8]. This factorial structure was used in a confirmatory factor analysis to derive factor scores for subsequent modelling. The three-factor model was fitted to the variable scores summed across all three raters (RMSEA = 0.06, CFI = 0.91).

Table 2
Inter-rater reliability (Kendall's w) for 16 skin ageing scale items scored by three independent raters in the LBC1921 cohort at age 83 years.

Skin ageing scale item	Kendall's w
Milia (cheek)	0.45
Milia (forehead)	0.44
Pigmented spots (cheek)	**0.73**
Pigmented spots (forehead)	**0.79**
Fine lines (cheek)	0.56
Fine lines (forehead)	**0.62**
Lines on lips	0.53
Wrinkles (cheek)	**0.76**
Wrinkles (under eyes)	**0.65**
Wrinkles (upper lip)	**0.86**
Furrows between eyebrows	**0.61**
Nasolabial folds	**0.68**
Crows feet	**0.72**
Facial tissue slackening	0.50
Bags under eyes	**0.80**
Drooping eyelids	0.59

Items reaching acceptable level of inter-rater reliability (> 0.6) marked in bold.

Table 3
Rotated factor loadings for the skin ageing variables in the LBC1921 cohort at age 83.

Skin ageing scale item	Factor 1	Factor 2	Factor 3
Pigmented spots (cheek)	**0.68**	**0.36**	–0.002
Pigmented spots (forehead)	**0.99**	–0.01	0.006
Fine lines (forehead)	–0.004	**0.32**	0.14
Wrinkles (cheek)	–0.009	**0.57**	**0.30**
Wrinkles (under eyes)	–0.11	0.08	**0.44**
Wrinkles (upper lip)	0.09	**0.84**	0.002
Furrows between eyebrows	0.10	–0.14	**0.43**
Nasolabial folds	–0.16	–0.03	**0.30**
Crows feet	0.02	0.02	**0.72**
Bags under eyes	–0.005	–0.27	0.08

Loadings greater or equal to 0.3 shown in bold.

3.3. Predictors and correlates of skin ageing

Entering Danish twin study variables [10] as factors and covariates in the multivariate general linear model with all three skin ageing factors as dependent variables, sex ($P < 0.001$, partial $\eta^2 = 0.605$), BMI ($P = 0.004$, partial $\eta^2 = 0.063$) and social class ($P = 0.005$, partial $\eta^2 = 0.061$) had significant effects: neither smoking ($P = 0.11$) nor outdoor occupation ($P = 0.81$) contributed significantly and so were removed from the model at this stage. Secondly, oxidative stress measures at age 87 were entered as covariates for those participants with these available ($n = 111$, mean 8-OHdg 22.9 ng/mL, mean TEAC 1.09 mM): 8-OHdG was significant ($P < 0.001$, partial $\eta^2 = 0.152$), but not TEAC ($P = 0.13$). The model, which included 8-OHdG, resulted in both BMI ($P = 0.25$) and social class ($P = 0.44$) no longer being significant. The final model thus comprised sex ($P < 0.001$, partial $\eta^2 = 0.605$) and 8-OHdG ($P = 0.003$, partial $\eta^2 = 0.124$).

Univariate effects of the factors and covariates in the baseline model showed women had more pigmented spots ($P = 0.012$), wrinkles ($P < 0.001$) and sagging ($P < 0.001$). Higher BMI was significantly associated with fewer wrinkles ($P = 0.001$), but not with pigmented spots ($P = 0.77$) or sagging ($P = 0.13$). Lower social class was also associated with more wrinkles ($P = 0.005$), but had no significant effect on either pigmented spots ($P = 0.48$) or sagging ($P = 0.51$). For the final model with oxidative stress, there was no significant difference in pigmented spots between men and women ($P = 0.29$), but women had more wrinkles ($P < 0.001$) and sagging ($P < .001$). Higher levels of 8-OHdG were associated with more pigmented spots ($P = 0.004$) and sagging ($P = 0.032$), but not wrinkles ($P = 0.20$).

4. Discussion

The data are consistent with those of Guinot et al. [8] and the Danish twin study [10]. Like Guinot et al., we found that facial skin ageing comprises several factors. The close similarity between their factor analysis solution and ours indicates that facial skin ageing can be estimated from a relatively small number of variables. For older adults, the major factors are pigmented spots, wrinkles and sagging. Like the Danish twin study, we found facial skin ageing correlated positively with low BMI and lower social class, but we did not find a significant association with sun exposure as indicated by an outdoor occupation: probably we had inadequate power to detect any effect given the low proportion of outdoor workers and the geographical latitude. Similarly, there was only a small proportion of current smokers at age 83, limiting

power to detect effects of smoking, and smokers surviving to this age may not be representative of all smokers: on average, ex-smokers had quit more than thirty years before the facial photographs were taken [18]. Unlike the Danish twin study, we were able to identify that both BMI and social class differentially influence wrinkles rather than pigmented spots or sagging. Secondly, in the LBC1921 cohort, women had significantly more pigmented spots, wrinkles and sagging. Finally, skin ageing, and in particular features related to the pigmented spots and sagging factors, was associated with higher oxidative stress levels as measured by 8-OHdG. No significant association was found with TEAC, but 8-OHdG is a more direct and sensitive assay of oxidative stress and less likely to be affected by factors which may influence TEAC, such as the levels of antioxidants in plasma like albumin or diet. Furthermore, 8-OHdg measures oxidative DNA damage, which is likely to be causally much closer to ageing changes in an organ like skin, which has a high cell replication rate.

There are three possible explanations of the association between skin ageing and increased oxidative stress. First, skin-ageing changes could cause elevated levels of oxidative stress. There is some support for this hypothesis from the observation that collagen fragmentation increases levels of reactive oxygen species [4]. Secondly, increased oxidative stress may cause skin ageing. Evidence supporting this hypothesis has already been cited [1–6]. Finally, skin ageing and elevated oxidative stress levels may both reflect some underlying ‘‘common cause’’. Here one conventional candidate is ‘‘biological ageing’’. Skin wrinkling correlates with biological age as indicated by health status [19] and given the very limited variation in chronological age in the LBC1921 cohort and also the likely limited variation in photoageing, residual variance might be attributed to biological age. However, this fails to advance an understanding of what are the biological pathways that lead to skin ageing, one of which may well be oxidative stress. We have previously proposed oxidative stress, sirtuins and metal ion toxicity as key biological pathways influencing age-related variables in the LBC1921 cohort [20].

There are several limitations of the study. First, the cohort is specifically located in time and space. Although this has important advantages already mentioned and results are consistent with those from France and Denmark, they are less likely to be generalisable to populations with different skin characteristics where skin ageing follows a different pattern [21]. Similarly, the findings are not immediately generalisable to younger populations, but the similarity of the results to those of Guinot et al. [8], who studied a sample aged 18 to 80 years, would support a consistent trend in skin ageing changes throughout adulthood. Secondly, we only studied facial skin ageing. This is probably the anatomical region of greatest interest, but is exposed to ultraviolet light, which is not possible to measure accurately over the preceding 83 years; instead we controlled for this as far as possible by sampling from a limited location in a narrow-age cohort and adjusting for likely occupational exposure differences. Thirdly, the association between skin ageing and oxidative stress levels was not contemporaneous. However, it is unlikely that major between-subjects changes in skin ageing would have occurred in the intervening period. In the Danish twin study, a significant association was found between perceived age estimated from facial photographs and leucocyte telomere length measured three to four years apart [22]. Since telomere length relates to oxidative stress markers in older adults [21], this provides some indirect support that the lack of contemporaneous measures is likely to have a major effect on our findings. Nevertheless, future studies should try to obtain oxidative stress measures at the same time as measuring skin ageing. Fourthly, the study was underpowered to detect anything but relatively large effects of smoking given the small proportion of current smokers at age 83. Fifthly, we have not linked either skin ageing or oxidative stress to other indices of biological ageing. This is a task that requires careful hypothesis generation to keep it focused. Nevertheless, there are good measures of biological ageing for other organs besides the skin (e.g. cognitive change for the brain) that could be tested for associations. Future studies that acquire skin biopsies in addition to photographs may also be helpful here. Researchers conducting such studies would need to be mindful that skin ageing is not a monolithic entity and other organs may have several dimensions of ageing also.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

We are indebted to Alison Pattie and Alan Gow who tested participants and took the photographs.

Funding: This study was supported by grants from the BBSRC, Research Into Ageing, British Academy and the Chief Scientist Office, Scotland. LP is funded by the UK Medical Research Council (Grant Nº 82800), which is part of the Age UK-funded Disconnected Mind research programme. The work was undertaken by The University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology, part of the cross council Lifelong Health and Wellbeing Initiative (award number G0700704/84698). Funding from the BBSRC, EPSRC, ESRC and MRC is gratefully acknowledged.

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