A Study of the Solar Effect on Actinic Keratoses by Quantification of Elastic Fibres Using an Image Analysis System

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It is widely accepted that elastotic changes of the skin are primarily an indicator of cumulative sun exposure of the dermis and are a characteristic finding of actinic keratoses. To date, there have been few reports that measure the amount of elastic tissue objectively and quantitatively, especially in actinic keratoses. The computerized image analysis method has proved useful recently in determining the area of elastic fibres. Using this method, we objectively quantified the elastotic tissue in actinic keratoses and evaluated the relationship between the degree of dermal elastosis, epidermal atypia and histological types of actinic keratoses. Of the 28 actinic keratoses studied, the average percentage area of the elastic fibre was 40.48 ± 14.48 (mean $\pm$ SD) percentile. There was a 3.65-fold increase in the amount of elastic fibre in actinic keratoses compared with that of seborrhoeic keratoses occurring on the face ($p < 0.00001$). In addition, the more severe the atypia, the greater the area of elastic fibres in a representative section of the dermis. In conclusion, we observed that on quantitative assessment of elastic tissue in actinic keratoses, the percentage area of the elastic fibres in a representative section of the dermis ranges from 34.86 to 46.11%. This result may provide information for use in histological diagnosis of actinic keratoses and evidence for the possible role of sunlight in the pathogenesis of actinic keratosis. *Key words: actinic keratosis; elastic fibre; image analysis.*

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Actinic keratoses (AKs) represent *in situ* carcinoma of the epidermis and are extremely common in fair-skinned and elderly people who have had extensive exposure to the sun. Although ultraviolet radiation is believed to be a contributing factor in AK, its specific contribution is not fully determined. Furthermore, the study of the causal relationship of cumulative and intermittent sun exposure to AK has been impaired by the lack of an adequate investigational tool to measure these parameters objectively. Sunlight radiation is a well-known cause of dermal elastosis in exposed areas of the human skin (1). Elastotic changes in the skin are thought primarily to be an indicator of cumulative sun exposure of the dermis (2, 3) and are invariably present in the underlying superficial dermis. A concept of varying degrees of epidermal atypia or dysplasia exists in AK. Histologically AKs are well-demarcated islands of abnormal keratinocytes with overlying parakeratosis. Because of the wide spectrum of atypia in AK, it is thought that some degree of atypia plays a role in the pathogenesis of AK.

The technique of computerized image analysis has recently proved useful in determining the area of elastic fibres and has been taken as the validity and reliability test by several authorities (1, 4). Using this method, we studied the quantification of the elastotic tissue in AKs and evaluated the relationship between the amount of elastotic tissue, epidermal atypia and histological types of AKs.

The purpose of this study was to quantify dermal elastosis objectively and to assess the degree of solar elastosis in the diagnostic criteria of various types of AK and the degree of epidermal atypia of AK.

PATIENTS AND METHODS

Clinical materials

Twenty-eight Korean patients (4 males and 24 females; mean age 68.5 years; range 42–85 years) with histologically proven AK from September 1992 to September 1997 were included in the study. The normal control group comprised 24 patients with facial seborrhoeic keratosis (12 males and 12 females; mean age 55.4 years; range, 41–79 years). The age and sex of each patient were recorded. Only materials containing a sufficient amount of normal tissue near lesions necessary for measurement of elastic fibre were accepted. Sections from biopsy specimens were stained with haematoxylin-eosin stain and Verhoeff-van Gieson stain

Computerized image analysis. The images were monitored with a video camera (Pulnix, IM440) and were displayed on an RGB monitor (Sony Inc. PVM1342). The analogue video signal was digitized and analysed with an IBM PC image processing system (AIC, Ca, USA). Four parameters were measured directly using computer-assisted image analysis: the percentage area of elastic fibres in the total dermal area; epidermal thickness; stratum corneum thickness; and stratum corneum looseness. All parameters were quantified using a $100 \times$ objective and 4-μm sections stained with Verhoeff-van Gieson stain. We chose three rectangular areas (155×116 μm^2) always starting at the dermo-epidermal junction beneath the AKs and in the adjacent site of the seborrhoeic keratoses (SKs) in the interfollicular area. In each unit, we measured the area of elastic fibres by outlining the entire dermis as seen on the video monitor and calculated the area fraction of the elastic fibres. The black and white image, sensing various grey levels were converted to binary images by selecting the threshold levels that provided a binary image of the fibre corresponding as closely as possible to black and white images of the elastic fibre. The average of the three units was considered the true value. The epidermal area was determined by tracing the boundaries of the epidermis from the top of the stratum granulosum to the dermo-epidermal junction within a given length of the biopsy specimen and calculating the enclosed area; the stratum corneum was determined by tracing its boundaries from the upper margin of the stratum granulosum to the uppermost layer of the stratum corneum. For both parameters, the average thickness was calculated by dividing the area by the horizontal length.

We assessed three parameters using conventional light microscopy in AK: epidermal (keratinocytic) atypia, histological types, and dermal inflammation (Fig. 1). All parameters were assessed using a $100 \times$ objective and 4-μm sections stained with haematoxylin-eosin stain. Epidermal atypia of AK was graded histologically using criteria simi-
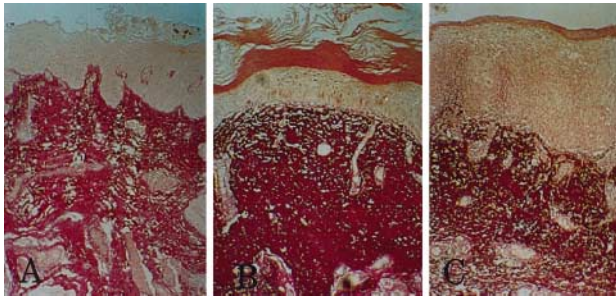
Fig. 1. Histological representation of varying degree of atypia in actinic keratosis. (A) Mild atypia involving lower one-third, bowenoid type. (B) Moderate atypia of the lower two-thirds, atrophic type. (C) Severe atypia involving most of the epidermis, bowenoid type. (Verhoeff-van Gieson stain, ×100).

lar to those reported by Bhawan (5). The criteria for each grade of AK are as follows: grade I, normal; grade II, mild atypia involving the lower one-third of the epidermis; grade III, moderate atypia of the lower two-thirds of the epidermis; grade IV, severe atypia involving most of the epidermis. Four types of AK can be recognized histologically: hypertrophic, atrophic, bowenoid and pigmented. Dermal inflammation was graded as none, mild, moderate or severe, based on the number of inflammatory cells through the entire dermis.

Statistical analysis

Student's t-test was used to compare quantitative variables. Summary statistics are expressed as mean±SD. The analysis of variance (ANOVA) was used to evaluate the relation between the amount of elastic fibre, each histological type and dermal inflammation. The Spearman rank order correlation and Pearson's ratio were used to evaluate the relationship between the amount of elastic fibres, epidermal thickness and stratum corneum looseness. The results were considered significant when the p-value was less than 0.05.

RESULTS

The number of patients with AK included in the present study was 28. The age and sex distributions of these lesions are shown in Table I. Of the 28 AKs studied, the average percentage area of the elastic fibres was 40.48±14.48 (mean±SD) and the 95% confidence interval for the mean change was from 34.86 to 46.11 percentile. There was an appreciable difference in the
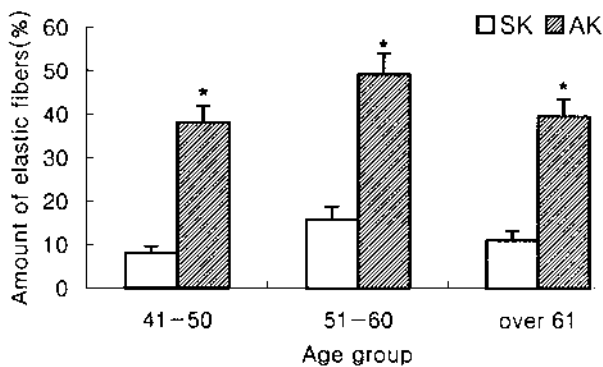

Fig. 2. Comparison of percentage area of elastic fibres between SK and AK in the age groups over the age of 41 years. Comparison in all age groups reveal that elastic fibres increased in the patients with AKs to levels greater than those found in the patients with SKs. These results showed a statistically significant difference between AKs and SKs (p=0.0007). *Error bars*, SD. *p<0.05.
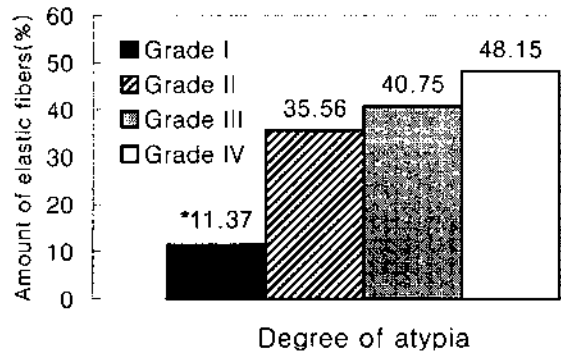

Fig. 3. Percentage area of elastic fibres according to degree of epidermal atypia. These results showed a statistically significant difference between grade I and grades II, III and IV, respectively. ANOVA: p<0.01, student's t-test: grade I–II, grade I–III, grade I–IV; p<0.01.

average percentage area of elastic fibres with increasing age intervals. In the normal controls (24 SKs), the average area fraction of the elastic fibres was 11.37±7.31 (mean±SD) and the 95% confidence interval for the mean change was from 8.28 to 14.46 percentile.

We compared these mean values of AKs with the value of percentage area of the elastic fibres in the SKs occurring on the face in the normal controls (11.37±7.31%) because most AKs were noted on the facial area. There was a 3.65-fold increase in the amounts of elastic fibres in AKs compared with that in the facial area of SKs. The differences in the percentage area of elastic fibres between AKs and SKs are statistically significant (p<0.00001). In addition to the increase with age in the amount of elastic fibres in the patients with AKs, comparison in all the age groups revealed that elastic fibres increased in the patients with AKs to levels greater than those found in the patients with SKs (Fig. 2). These results were statistically significant for differences between AKs and SKs after the age of 40 years.

Fig. 3 describes the range of epidermal atypia and the average percentage area of the elastic fibre of each grade identified in the specimens. As shown in these results, the more severe the atypia, the greater the area of elastic fibres in a representative section of dermis.

There was no significant correlation between the amount of elastic fibres and the histological types of AK. The average percentage area of the elastic fibres in the hypertrophic type (mean value 47.1%, n=7), atrophic type (mean value 38.6%, n=9), bowenoid type (mean value 36.7%, n=8) and pigmented type of AKs (mean value 40.7%, n=4) did not differ significantly on analysis of variance (p=0.572).

Other parameters measured, including epidermal thickness and stratum corneum looseness, showed a statistically poor correlation with the amount of elastic tissue (data not shown). For dermal inflammation the average percentage area of the elastic fibres were none (mean value 40.1%, n=4), mild (mean value 43.7%, n=11), moderate (mean value 28.8%, n=6) and severe (mean value 41.5%, n=7) (data not shown). There was no significant relationship between the amount of elastic fibres and grade of dermal inflammation of AK. Analysis of variance (ANOVA) performed on the data revealed no significance (p=0.365)

Age (years)	Actinic keratosis	Seborrhoeic keratosis
41 – 50	2 (38.10)	5 (8.09)
51 – 60	4 (49.35)	6 (15.79)
Over 61	22 (39.65)	13 (11.12)
Mean age	68.5	55.4
Number (men : women)	4 : 24	12 : 12
Total number	28	24

Figures in brackets = mean percentage area of elastic fibres.

DISCUSSION

It is widely accepted that chronic exposure to the ultraviolet (UV) component of solar radiation is the major environmental cause of AKs. Cumulative repeated exposure to natural sunlight contributes to the development of degenerative alterations in the connective tissue of the skin, especially elastic fibres (6, 7). Solar elastosis may progress proportionally with the amount of exposure to sunlight (8). In AK, elastotic material can be focally distributed within the dermis and is generally located in the superficial dermis. The nature of elastotic material has not been clarified. Many studies to identify the pathogenesis of this elastotic material were carried out by using human and experimental animals. The experimental production of elastosis in animals using artificial ultraviolet sources have been reported (2, 9 – 11). Previous studies have suggested that chronic UV radiation may affect the metabolism of collagen (12, 13). Some investigators have suggested that this elastotic material is primarily derived from elastic fibres and not from pre-existing or newly synthesized collagens (14 – 16). Bouissou et al. (17) have reported that the elastotic material may be the consequence of excessive production of normal or abnormal elastic fibres due to actinic stimulation of fibroblasts. In our study, there was a 3.65-fold increase in the amounts of elastic fibres in AK compared with that in the facial area of SK. The average percentage area of the elastic fibres of the 28 AKs studied was 40.48 ± 14.48 (mean $\pm$ SD) percentile and the 95% confidence interval for the mean change was from 34.86 to 46.11 percentile. There was an appreciable difference in the average area fraction of elastic fibre with increasing age intervals. The amount of elastic fibres and the percentage area showed a significant difference in all age groups between AK and SK. These results are difficult to compare with others in the literature, since we did not find any reports evaluating quantification of elastic fibres of AK. Therefore, for histopathological diagnosis of AK with light microscopy, we propose that at least the percentage area of the elastic fibres in a representative section of dermis should range within the 95% confidence interval for the mean change.

In actinic keratoses, there is a gradation of change from a very mild form of atypia to the very severe form (carcinoma *in situ*). This explains the differences in the definition of actinic keratosis given by different investigators. With this background, difficulties encountered in the histological evaluation of actinic keratoses are discussed. In this study, we found that the more severe the epidermal atypia, the greater the area of elastic fibres in a representative section of dermis. These results implicated elastosis to be correlated with epidermal atypia.

In conclusion, we observed that on quantitative assessment of elastic tissue in A Ks, the percentage area of the elastic fibres in a representative section of dermis ranges from the 34.86 to 46.11 percentile. This result provides information for histological diagnostic criteria of AKs and evidence for the role of sunlight in the pathogenesis of AKs. Furthermore, we have shown a close relationship between the degree of epidermal atypia and the degree of dermal elastosis of AKs. Our present study is a first step towards objective quantification of elastic tissue in AKs and provides a basis for future studies to quantify solar elastosis in the pathogenesis of AKs.

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