

Effect of smoking on skin elastic fibers: a morphometric and immunohistochemical analysis

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Background/aim

Tobacco smoking is one of the major health problems in the world. This study was conducted to investigate the morphological changes of the dermal elastic tissue of sun-protected skin induced by smoking.

Participants and methods

Punch biopsies were obtained from the nonexposed skin of 15 smokers and 15 nonsmokers to evaluate the dermal elastic fibers using morphometric analysis and immunohistochemical techniques.

Results

Elastic fibers of the reticular dermis were more numerous, thickened and fragmented in smokers than in nonsmokers. Correlations between the cumulative tobacco dose and the morphology of the elastic fibers were highly significant. Immunohistochemical analysis for α -1-antitrypsin showed negative cytoplasmic staining for all samples of both smokers and nonsmokers.

Conclusion

This study indicates that smoking is one of the risk factors that lead to an increase in elastic fibers of the reticular dermis. This increase is due to elastic tissue degradation rather than by new synthesis, as in solar elastosis. Furthermore, smoking has multiplicative effects on facial ageing and could share, at least in part, a pathophysiological pathway.

Keywords:

elastic fibers, immunohistochemistry, morphometry, skin, smoking

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Introduction

Tobacco smoking is one of the major health problems in the world and is causally related to many chronic and malignant diseases [1]. Human skin is exposed to smoke directly through its irritant components on the epidermis and indirectly on the dermis through the bloodstream. It is not surprising therefore that smoking has many effects on the skin and is associated with significant morbidity of this organ [2].

In the past three decades, several studies have shown that smoking is an independent risk factor for the development of premature facial wrinkling and skin aging [3,4]. To establish the pathophysiological mechanisms underlying this premature wrinkling and on the basis of the finding that elastin is the major target in smoke-induced emphysema [5,6], several reports have used morphometry to analyze the elastic fibers of the skin in smokers and nonsmokers [7,8].

The results showed an increase in the number, the thickness, and the total area occupied by dermal elastic fibers in smokers compared with nonsmokers. Another study, however, shows contradictory findings that question the effects of smoking on elastic fibers of the skin [9].

There is a hypothesis that elastin could also be the main target in smoke-induced wrinkles, as in emphysema. Lung function impairment with an obstructive pattern is associated with morphologic abnormalities in the reticular dermis appearing in the histological section as an increase in the percentage of the field filled by elastic fibers. This relationship depends on cumulative smoking and suggests a common effect of smoking on the elastic fibers in both the lung and the skin [10].

This study was conducted to investigate the morphological changes of the dermal elastic tissue of sun-protected skin induced by smoking, and the possible mechanisms responsible for them.

Participants and methods

This study included 15 cigarette smokers, selected from the Outpatient Clinic of Dermatology, Ain Shams University, during the period of February 2009 to August 2009. The study also included 15 nonsmokers as the control group. Only men were included in the study to avoid sex-related confounding factors. Patients in the age group of 30–50 years were included in our study. Patients were categorized as nonsmokers and current

smokers who smoke more than 10 cigarettes per day for more than 10 years. The lifetime cumulative tobacco dose was expressed as pack-years (the average number of packs smoked per day multiplied by years smoked).

Patients reporting a history of connective tissue disease, treatment with D-penicillamine, colchicine, artificial ultraviolet radiation, and oral corticosteroids for more than months in their lifetime, BMI greater than 40 kgm² and pipe or cigar smokers were excluded from our study.

The study was approved by the ethical committee of the National Research Center and fulfilled all the ethical aspects required in human research. All participants gave an informed consent to participate in this study and the steps of the study were explained to all the participants.

Morphometric analysis

Skin biopsies were obtained from the upper inner arm of 15 cigarette smokers and 15 nonsmokers. These biopsies were fixed in 10% formalin and processed into paraffin sections of 3- μ m thickness. They were stained with hematoxylin and eosin for the demonstration of the histological structure and orcein stain for the detection of elastic fibers.

Orcein-stained sections were examined using an image analysis system. This station included a Leica Q500 IW (Leica DM LB, Cambridge, UK) photo microscope with position captors and a CCD video camera module N 50 (JVC TK-C 1380, Victor Company of Japan LT, Japan). Images were captured with a $\times 20$ magnification objective, digitized after interactive light intensity equilibration, and analyzed as RGB 24-bit images. Five fields of papillary and reticular dermis were selected randomly from those that did not include large nonconnective tissue elements (i.e. hair follicles and sebaceous glands). From every section, an image of gray levels was obtained and converted into a binary image showing brown elastic fibers. This image was processed and the number of elastic fibers in every field and the area filled by them were determined, with the results expressed as elastic fibers mm² and the percentage of the field containing elastic fibers. Morphometric values were obtained in the five examined fields of the papillary and reticular dermis to determine the values for the elastic fiber content for each participant.

Immunohistochemistry for α -1-antitrypsin Ab-1

Immunohistochemical analysis was performed on routinely processed, formalin-fixed, paraffin-embedded tissues. Tissue sections were cut at 5 μ m and mounted on poly-L-lysine-coated slides. After routine

deparaffinization in xylene, the sections were hydrated through a series of graded alcohols, distilled water and PBS at pH 7.2–7.4. Antigen retrieval was performed using Tris-EDTA (pH 9) in a pressure cooker. The slides were incubated in a Dako autostainer S 3400, Dako North America Inc., Carpinteria, California, USA with monoclonal rabbit anti-human α -1-antitrypsin Ab-1 (RB-367-R7; NeoMarkers, Fremont, California, USA) (ready-to-use). The slides in the autostainer were removed and hematoxylin counterstaining was performed. Slides were dehydrated in ascending grades of alcohol and were cleared in xylene for three changes, and cover slips were placed. Histological assessment of the intensity of the staining was performed using a visual scale ranging from 0 to 2 (0, positive; 1, weakly positive; and 2, strongly positive).

Statistical analysis

All data were introduced in a database and analyzed using the SPSS statistical software package, version 11.5 (SPSS Inc., Chicago, Illinois, USA). Results for categorical variables were expressed as absolute and relative frequencies and results for continuous variables as mean and SD, or as the median and the interquartile range when the distribution was not normal.

Skin parameters in nonsmokers and smokers were compared (Student's *t*-test), and the correlation between skin morphology and the cumulative tobacco dosage was examined to determine as to which skin parameters were affected by the lifetime amount of tobacco consumed (Pearson's correlation coefficient). All statistical tests were two sided, and *P* value less than 0.05 was reported as statistically significant.

Results

This study was carried out on 30 participants: 15 of them were active smokers and the remaining 15 patients were nonsmokers and taken as a control group. The age of all patients ranged from 30 to 50 years, with a mean $\pm$ SD of 37.8 ± 5.7 in smokers and 36.6 ± 6.6 in the control group. On comparing the smokers' group and the nonsmokers' group with regard to the age, there were no statistically significant differences (*P* < 0.05). The criterion of wrinkles in the face were detected in two of 15 smokers (Fig. 1). These criteria include prominent lines, wrinkles emanating from the corner of the eyes (crow's feet) extending on the cheeks, the cheeks appear sunken with prominence of the underlying bone, the skin has a dry and tough appearance and appear more atrophied than nonsmokers' skin (Fig. 2). The correlation between the morphological characteristics of dermal elastic fibers (including the number of

fibers and the mean area filled by them) and age in the studied sample was not statistically significant ($P < 0.05$), confirming the homogeneity of the sample with respect to this parameter (Table 1).

Morphometric analysis

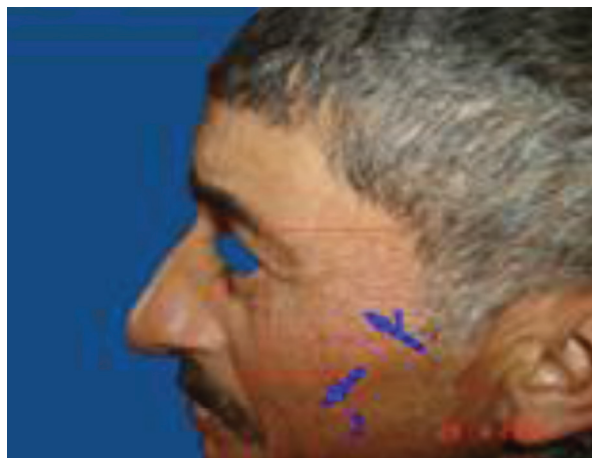
The elastic fibers of the reticular dermis were numerous, thickened and more fragmented in smokers than in nonsmokers (Fig. 3), whereas there was no morphological difference in the papillary dermis between smokers and

Table 1 Correlation between the morphological characteristics of dermal elastic fibers with age

	Age of participants	
	Pearson's correlation	P value
Mean area of elastic fibers	-0.231	0.084
Number of elastic fibers (μm^2)	-0.312	0.093

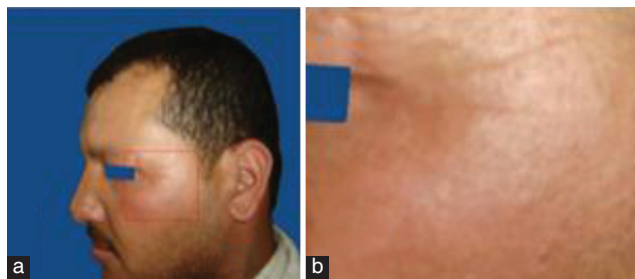
Correlation is insignificant at $P > 0.05$.

Figure 1



A photograph of a smoker (39 years old) shows prominent wrinkles emanating from the corner of the eyes (crow's feet) (1), lines extending on the cheeks and around the mouth (2), with noticeable dryness and decreased elasticity.

Figure 3



(a) A photograph of a nonsmoker (39 years old) shows a healthier appearance of the skin as compared with smokers, (b) a closer view of the highlighted area.

nonsmokers (Fig. 4). The total area of elastic fibers in smokers showed a significant increase as compared with nonsmokers ($P < 0.05$) (Table 2).

Correlations between the cumulative tobacco dose and the morphology of the elastic fibers was highly significant ($P < 0.01$) with regard to the number and the mean of the area occupied by elastic fibers (Table 3 and Figs 5 and 6).

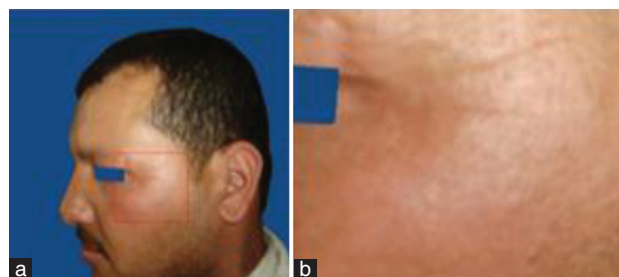
Immunohistochemical analysis for α -1-antitrypsin

All samples of both smokers and nonsmokers showed negative cytoplasmic staining for α -1-antitrypsin (Figs. 7-a and 7b). There was no difference in the epidermal thickness between smokers and nonsmokers (Fig. 8).

Discussion

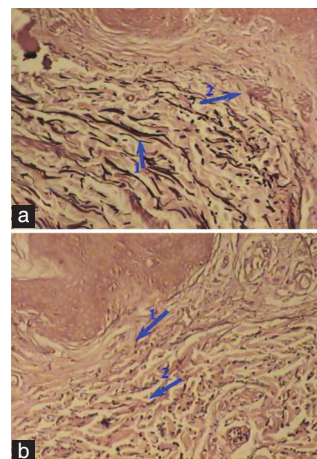
Our results support the concept that smoking increases the number, the thickness, and the total area occupied by

Figure 2



(a) A closer view of area 1; (b) a closer view of area 2.

Figure 4



(a) An increase in the number and thickness of elastic fibers in the reticular dermis (1), with few and thin fibers the papillary dermis (2) in a smoker's skin. (b) Few and thin elastic fibers both in the papillary (1) and the reticular dermis (2) in a nonsmoker's skin were noticed (orcein, $\times 200$).

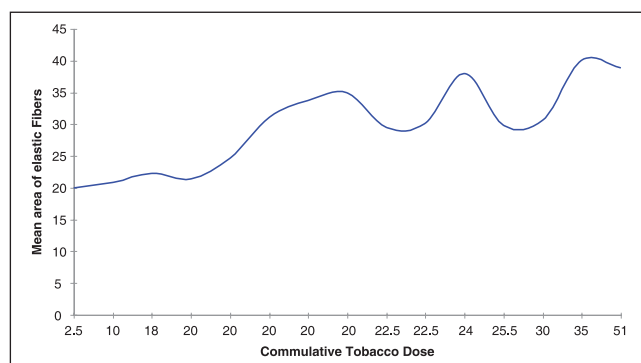
Table 2 Comparison between the total area of elastic fibers in smokers and nonsmokers

Parameters	Groups		t-Test	P value
	Smokers	Nonsmokers		
Total area of elastic fibers	891.95±306.14	653.47±194.62	3.430	< 0.05
Number of participants	15	15		

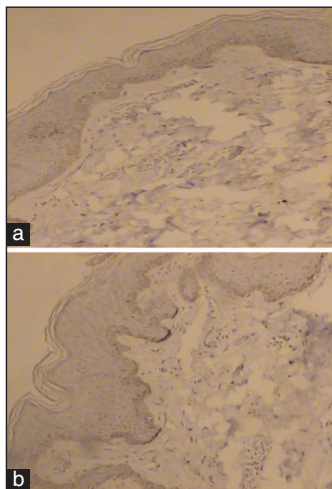
Table 3 Correlations between the cumulative tobacco dose and the morphology of elastic fibers

	Cumulative tobacco dose	
	Pearson's correlation	P value
Mean area of elastic fibers	0.733**	0.0 02
Number of elastic fibers (μm ²)	0.712**	0.001

**Correlation is significant at $P < 0.01$.

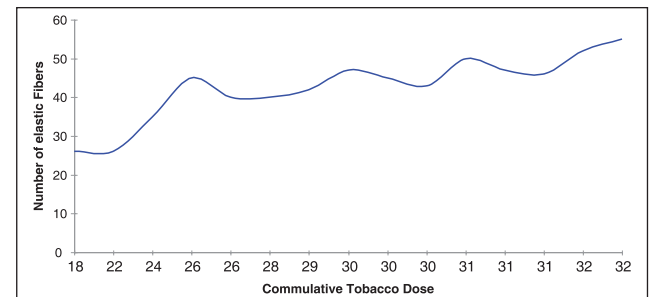
Figure 5

The relation between the mean area of elastic fibers and the cumulative tobacco dose.

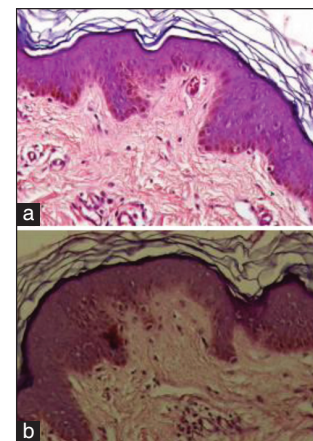
Figure 7

Negative cytoplasmic staining of epidermal keratinocytes and the dermal matrix in (a) smokers and (b) nonsmokers (immunohistochemistry for α -1-antitrypsin, $\times 200$).

elastic fibers in the reticular dermis of nonexposed skin, but not the papillary dermis in smokers compared with nonsmokers. This association is in agreement with earlier

Figure 6

The relation between the number of elastic fibers and the cumulative tobacco dose.

Figure 8

No difference in the epidermal thickness between smokers (a) and nonsmokers (b) (H&E, $\times 200$).

studies [7,8,11], but not with the results reported by Knuutinen *et al.* [9] who showed contradictory findings.

The morphological changes of elastic fibers including the number and the mean area showed a significant correlation with the cumulative tobacco dose; these findings indicate that the morphological effects are dose related ($P < 0.01$; $r = 0.733$).

Similar findings are reported by Just *et al.* [11] who lend support to the fact that smoking is a risk factor for the development of facial wrinkling.

Smoking-induced changes in skin elastic fibers occur deeper in the dermis and are less intense than those associated with sun exposure, and must be considered

as being related to cigarette smoke components that reach the reticular dermis through the bloodstream [12]. Hence, it is not surprising that smoking by itself is unable to change the macroscopic appearance of the sun-protected skin of smokers [13,14].

However, when the exposed skin was analyzed, a significant increase in the elastic tissue was found in both the papillary and the reticular dermis of smokers compared with nonsmokers [15].

This explains, at least in part, the increase in facial wrinkling of smokers, which depends on the cumulative dose of tobacco consumed. This relationship is probably modulated by a genetic predisposition, because only one quarter of heavy smokers show abnormally severe wrinkling in their exposed skin [12,16,17].

The increase in the number seems to be due to fiber fragmentation, as reported previously [8,12]. The increase in the area occupied by elastic fibers in the reticular dermis could result from the synthesis of new normal or abnormal elastic tissue, from degradation of elastic fibers, from a decrease in dermal thickness or from external substance deposition.

This study showed that there is no difference in the epidermal or the dermal thickness between smokers and nonsmokers or external substance deposition in dermal elastic fibers of smokers, which is in agreement with other previous studies [8,12,18].

Using immunohistochemistry, we found that all samples showed negative cytoplasmic staining for α -1-antitrypsin. Our result support the result found by Just *et al.* [10] who detected lack of immunostaining for plasma protease inhibitor reactivity in all the samples, which suggests the hypothesis that the increase in the area of elastic fibers in the reticular dermis in smokers is not due to newly synthesized elastic material, but due to their degradation, as in solar elastosis [19].

The vascular structure of the skin may play a role in these elastotic changes. The cutaneous microvasculature is constricted by acute and long-term smoking. The decreased capillary and arteriolar blood flow may induce local ischemia and focal shunt of toxic substances to these tissues [20].

Smoker's elastosis may be partially due to biochemical effects on the elastic tissue. Previous studies have shown that cigarette smoke can increase the plasma neutrophils' elastase activity and the release of elastase from neutrophils [21].

The presence of the criterion of wrinkles on the face in two of 15 smokers is an indicator that other factors

contribute to its occurrence such as sun exposure and age of the participants. In addition, we cannot ignore the genetic factors.

Acknowledgements

Conflicts of interest

None

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