INVESTIGATION

Effects of long-term chronic exposure to sun radiation in immunological system of commercial fishermen in Recife, Brazil*

Efeitos da radiação solar crônica prolongada sobre o sistema imunológico de pescadores profissionais em Recife (PE), Brasil

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Abstract: BACKGROUND: Among the various occupations which necessarily require long-term and chronic sun exposure is that of a fisherman. However, clinical experience in dermatology earned over several years of medical practice does not seem to confirm this hypothesis.
OBJECTIVE: To evaluate clinical, histological and immunological effects of long-term and chronic exposure to ultraviolet radiation in fishermen.
METHODS: A prospective, cross-sectional and observational study characterized skin lesions, immunological markers and histological alterations in fishermen, as well as lymphocyte subpopulations compared to a control group. Mann-Whitney, Fisher's and Wilcoxon statistical tests were used at a significance level of 0.05.
RESULTS: There were significant differences between the exposed group and the group protected due to elastosis ($p = 0.03$), ectasia of dermal vessels ($p = 0.012$) and number of cells in the epidermal layers between cones ($p = 0.029$). Most common among fishermen were CD45RO, CD68 + and mastocytes in the skin ($p = 0.040$, $p < 0.001$, $p = 0.001$) and CD3CD8CD45RO in the blood ($p = 0.016$).
CONCLUSION: The alterations suggest that long-term and chronic sun exposure promotes tolerance to ultraviolet radiation, which protects against immunosuppression.
Keywords: Allergy and immunology, Antigens; Dermatology; Skin; Ultraviolet Rays

Resumo: FUNDAMENTOS: Existe um consenso de que a exposição à radiação ultravioleta determina alterações no sistema imunológico da pele, o que permite que se avente a hipótese de que a exposição prolongada e crônica ao Sol pode representar uma das maiores agressões ambientais à saúde humana. Entre as várias ocupações que requerem, necessariamente, exposição prolongada e crônica ao Sol está a de pescador. No entanto, a experiência clínica dermatológica, amealhada ao longo de vários anos de exercício da Medicina, não parece confirmar essa hipótese.
OBJETIVO: Avaliar efeitos clínicos, histológicos e imunológicos da exposição crônica e prolongada à radiação ultravioleta em pescadores.
MÉTODOS: Em estudo prospectivo, transversal, observacional, foram caracterizadas lesões dermatológicas, marcadores imunológicos e alterações histológicas de pescadores e subpopulações de linfócitos comparadas a grupo-controle. Empregaram-se testes de Mann-Whitney, exato de Fisher, Wilcoxon em nível de 0,05.
RESULTADOS: Houve diferenças entre os grupos exposto e protegido em elastose ($p = 0,03$), ectasia de vasos dérmicos ($p = 0,012$) e número de células nas camadas epidérmicas entre os cones ($p = 0,029$). Foram mais comuns em pescadores CD45RO, CD68+ e mastócitos na pele ($p = 0,040$, $p < 0,001$ e $p = 0,001$); CD3CD8CD45RO no sangue ($p = 0,016$).
CONCLUSÃO: As alterações sugerem que exposição crônica e prolongada ao sol promove tolerância à radiação ultravioleta, protetora da imunossupressão.
Palavras-chave: Alergia e imunologia; Antígenos; Dermatologia; Pele; Raios ultravioleta

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INTRODUCTION

Although the majority of non-melanoma skin cancers do not start by chronic sun exposure, such cancers can develop quickly as a result of exposure to ultraviolet radiation (UVR), as it has been observed in experimental animal models.[1]

Studies show that ultraviolet radiation on the skin not only produces local, non-specific immunosuppression, but also specific systemic suppression of antigens introduced in the critical phase of exposure.[2,3,4,5] Other authors suggest that the damage induced in the DNA by ultraviolet radiation triggers a cascade of events: systemic immunosuppression mediated by T lymphocytes, decrease of natural killer (NK) cells, improper regulation of cytokines, and changes in the antigen presentation to the Langerhans cells.[6] The key components of this cascade are the epidermal cytokines, which modulate the immune response to antigens introduced into the host subjected to ultraviolet radiation, redirecting the response to a state of immunosuppression.[7,8]

Although it has been speculated that exposure to ultraviolet radiation in low doses (for a few hours) may exert some beneficial effects on people, by reducing the immune response of the skin and the risk of developing autoimmune processes, particularly in atopic individuals9, there is a consensus that ultraviolet radiation causes alterations in the skin's immune system. For this reason, scientific interest has been given to the understanding of quantification of these effects, taking into account the amount of time of sun exposure.[3,10,11]

This consensus allows us to formulate the hypothesis that chronic and prolonged sun exposure may pose a major damage that nature can cause to human health.9 Among various occupations that necessarily require long-term and chronic sun exposure, we can cite that of fishermen. However, dermatological clinical experience earned over many years of medical practice does not confirm this hypothesis.

The objective of this article is to evaluate the clinical, histopathological and immunological effects resulting from solar radiation on fishermen that have been professionals for more than 10 years.

INDIVIDUALS AND METHODS

A prospective, cross-sectional and observational study was conducted on the dermatological characteristics diagnosed by physical examination, with comparison of groups for the analysis of immunological markers in the skin and blood and histological changes in the skin.

The study was conducted in Recife, in the state of Pernambuco, Brazil, in the following geographic coordinates: latitude 8º04´03"S, longitude 34º55´00"W, at an altitude of 4m above sea level, and average annual temperature of 25.2 º C.

The population of the study consisted of 3,000 fishermen living in a neighborhood called Pina, in Recife (PE), coming from a colony of registered professionals. It was assumed that 75% of the fishermen in the population had secondary skin alterations due to prolonged exposure to solar radiation, as a result of their occupation, and that 9% of non-fishermen could present such alterations, adopting a 2:1 ratio for fishermen and non-fishermen, significance level of 0.05, and power of the test of 90%. The minimum sample size was estimated at 19 fishermen to 10 non-fishermen.

Graph 1 illustrates the steps for defining the sample studied, showing that, to complete the estimated sample size in the period from September 2005 to September 2006, 75 male individuals were invited to participate in the study. These 75 men were registered professional fishermen and had been in the job for more than 10 consecutive years. The 20 non-fishermen had also been in the job for more than 10 consecutive years. All of the participating individuals agreed to take part in the study and signed the Free and Informed Consent Form.

The authors excluded the subjects with history of the following diseases, or whose laboratory exams indicated the following: diabetes mellitus, nephropathy, hepatopathy, malnutrition, anemia, infection or allergy. In addition, subjects with these problems were also excluded: depression, vaccination dated three months before data collection, any form of malignant neoplasm, or use of any medication or dietary supplements.

For screening of the subjects for the existence of problems related to alcohol consumption, the authors used the questionnaire Alcohol Use Disorders Identification Test (AUDIT), classifying those who scored a number equal to or greater than eight as having alcohol-related problems.12

For assessment of the general status and investigation of changes that could act as confounding factors that compromise the immunological status of the participants, the following were analyzed: complete blood count, protein electrophoresis, urea, creatinine, glucose, aspartate aminotransferase (AST) and alanine animotransferase (ALT), as well as coproparasitoscopic examinations of three samples from subsequent fecal evacuations.

For the quantification of lymphocyte subsets, 10 male individuals, Pina residents, non-fishermen, to whom the professional activity did not require prolonged solar radiation exposure, were included. The non-fishermen were investigated for compliance with

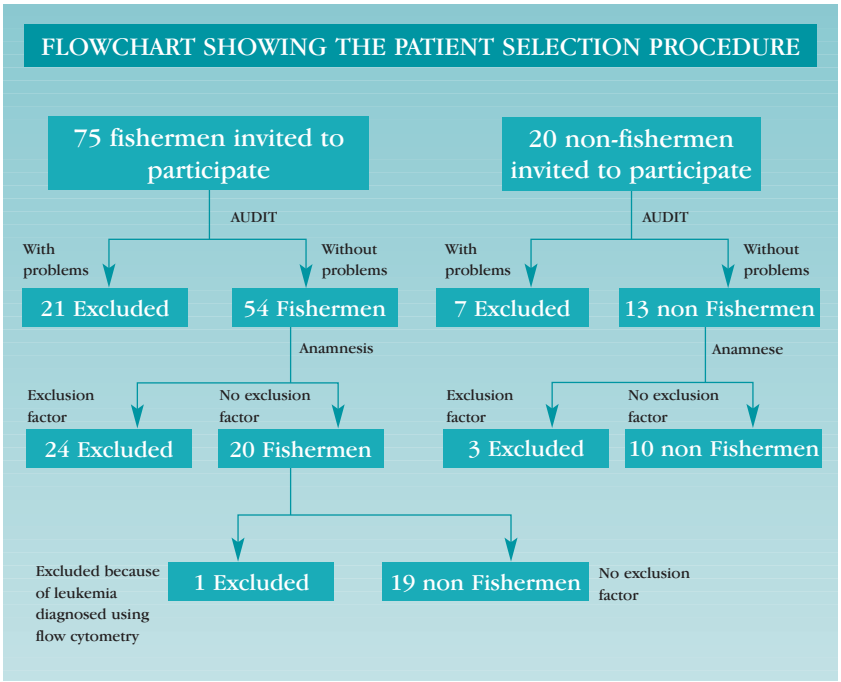


Figure 1: Flow of the sample of the study conducted in Recife, Brazil - September 2005 - September 2006

Audit = Alcohol Use Disorders Identification Test

the same criteria of inclusion and exclusion required from the fishermen.

The fishermen's age average was 46.3 ± 9.54 years old, ranging between 30 and 60 years old, while the non-fishermen's age average was 42.5 ± 11.63, ranging from 26 to 58 years old. The age difference was not statistically significant (p=0.377).

Concerning the distribution of skin type, there was a predominance of type IV, in the Fitzpatrick's classification, in the fishermen's group, while type VI was only present in the non-fishermen's group, without a significant difference between the two groups (p=0.137) (Table 1).

The fishermen claimed to have worked an average amount of time of 29 ± 10.3 years, ranging between 12 and 45 years, and this was significantly different from the non-fishermen, whose average amount of time as professional workers was 13.8 ± 9.3 years, ranging between 5 and 34 years (p=0.001).

The duration of daily exposure to the sun among the fishermen was 12 hours, while the average time of solar exposure was 1.2 ± 0.42 h among the non-fishermen. There was a variation of one to two hours.

In the dermatological clinical examination, any changes in skin, mucous membranes or attachments were considered, regardless of the etiologic factors involved.

Each fisherman underwent two 4-mm punch biopsies of healthy skin, carried out under local anesthesia using lidocaine without epinephrine: one in the midline of the third sector of the cranium, near the cranial medial scapular area, which is the area exposed to the sun, and another, in the midline of the left buttock, corresponding to the area which is not exposed to solar radiation (covered area). As soon as the biopsy samples were collected, they were fixed in 10% buffered formalin (pH 7.4), cut into 4-mm thick sections and covered with adhesive solution of aminopropyltriethoxysilane (Sigma ChemicalTM Brand, code A3648), at a concentration of 3% in PA acetone for subsequent staining using the hematoxylin-eosin stain method.

Microscopic examination of each of the biopsies showed hyperkeratosis, average number of cell layers of the epidermis in the epithelial cones and in the segments of the epidermis between epithelial cones. In the dermis, the following was investigated: presence of elastosis, ectasia of superficial dermal blood vessels, intensity, composition and location of inflammatory infiltrate around superficial or deep vascular structures. Histological findings were classified according to intensity as absent, mild, moderate or intense.

For the quantification of mastocytes, we proceeded to the staining of skin biopsy sections using aqueous solution of 0.5% toluidine blue-HC1. The mastocytes were recognized by optical microscopy by means of presence of purple-stained cytoplasmic granules.

The histological sections were subjected to the immunohistochemical streptavidin-biotin peroxi-

Table 1: Demographic characteristics and skin types in the sample of fishermen and non-fishermen, Recife, Brazil – September 2005-September 2006.

Variables	Groups		Comparison between groups	
	Non-fishermen	Fishermen	p value	Significance
Number of individuals				
total	10 (34·5%)	19 (65·5%)		
Age (years)			**Mann-Whitney Test**	
Average (SD)	42·5 (11·63)	46·3 (9·54)	0·377	Ns
Average IC 95%	34·2; 50·8	41·7; 50·9		
1st q–med.–3rd q	33·8–41·5–56·3	36·0–47·0–55·0		
Minimum; maximum	26·0; 58·0	30·0; 60·0		
Skin types		**Test of independence****		
frequency		0·137	Ns	
type II, % (n)	40·0 (4)	36·8 (7)		
type IV, % (n)	40·0 (4)	63·2 (12)		
type VI, % (n)	20·0 (2)	0·0 (0)		
Cramer's Coef	0·392			

SD = standard deviation; 1st q–med.–3rd q = 1st quartile median–3rd quartile; Cramer's Coef. = Cramer's Coefficient of contingence (value 0 indicates independence up to a maximum value of 1); ns = not significant (p ? 0·05).
**Fisher's Exact Test

dase13 technique for demonstration of CD8+ T lymphocytes, NK cells, CDE45RO and Langerhans cells. CD 4 and CD68 markers were determined by using the detection method called StreptoABComplex/HRP (Kit Duet, Mouse/Rabbit, code nº. K0492, Dako Cytomation®, Carpinteria, California.)

The number of CD8+, CD4+ T lymphocytes, natural killer cells, CD68 monocytes/macrophages, CD45RO and mastocytes was obtained by counting the number of immunostained dermal cells, using a 10x eyepiece graticule and a 40X objective. The area of the grid, at this magnification, corresponded to 0.0625mm2. For each of the fragments, the areas of at least six fields were analyzed (three near the vessels of the superficial dermal plexus, and three in a deeper layer of the dermis), determining the average number of immunostained cells per square millimeter dermal area.

Evaluation of epidermal CD1a+ cells (Langerhans cells) was obtained by fraction of epidermal area with CD1a antigen expression.14 The authors chose this method of measurement because Lengerhans cells exhibit dentrites, which position themselves in between the keratinocytes and may anastomose, making it difficult to individualize the cells for counting. The fraction of the CD1a+ area was obtained by counting the number of points of positive reaction, divided by the total points that focus on the epidermis, excluding the stratum corneum. In order to do this, the authors used the same 10X eyepiece graticule and the 40X objective. The entire epidermis of each skin fragment was evaluated.

Each participant of the investigation (fisherman or not) was submitted to collection of 10mL of whole blood through a puncture in the median cephalic vein, using vacuum tubes containing potassium ethylenediamine-tetra-acetate (EDTA) 1mg/dL. Monoclonal antibody was added to each 50mL of homogenized whole blood (Immunotech®, Marseille, France) and marked with fluorescein isothiocyanate to CD4 and CD8, fluorescein isothiocyanate + phycoerythrin to CD3, and associated to cyan phycoerythrin to IgG1; cyan phycoerythrin to CD3 5CD19 5 and CD25; phycoerythrin Texas Red-X to CD8, CD45RO and CD45RO; phycoerythrin to CD28, HLA-DR and CD69, so that the subsets of cells could be distinguished by means of flow cytometry.

Images were obtained from 5,000 cells in the lymphocyte gate, using a graph with Cartesian coordinates with abscissa for the analysis of frontal dispersion, and ordinates for lateral dispersion (FS x SS). The figures related to the counting of lymphocyte subsets and of HLA were converted into absolute count, based on the total lymphocyte count determined in hematologic cell counter, model STKS (Beckman-Coulter®, Fullerton, CA – USA), around the period of 24h post-collection.

For histopathological and immunohistochemichal analysis of skin continuously and chronically exposed to solar radiation, the authors established the parameters of covered skin as control for each

patient, as this area is not directly exposed to solar radiation.

The study was approved by the Research Ethics Committee, University of Sao Paulo.

For sample description, the parameters used were those of descriptive statistics to characterize variables in interval scale variables, including mean, median, quartiles and standard deviation. For variables in nominal or ordinal scale, the authors used the distribution of absolute and relative frequencies. To test the hypothesis of equality between the group of fishermen and the group of non-fishermen, the Mann-Whitney test was used, as well as Fisher's exact test to the analysis of interdependence between the group and the characteristics analyzed. In both tests, a monocaudal comparison with a 0.05 level of significance (monocaudal to the right) was adopted. For the analysis of interdependence between the group of fishermen and the group of non-fishermen, Cramer's coefficient was used. To compare the histopathological and immunological findings in the covered skin and in the skin exposed to solar radiation, the Wilcoxon test was used, as well as the sign test, both with a monocaudal comparison with a 0.05 significance level (monocauldal to the right).

RESULTS

The dermatological examination of the group of non-fishermen did not show any changes in the head, face, trunk, upper and lower limbs, hands, feet and nails, or changes in the oral cavity. Among the fishermen, regardless of the topographic distribution, the most frequent changes were: elastosis, melanosis and hyperkeratosis. There was one case of actinic cheilitis.

Comparison between covered skin and skin exposed to the sun

The histopathological and immunohistochemical findings in the area exposed to solar radiation compared to those of the area covered are shown in Tables 2 and 3. The histopathological examination of skin showed hyperkeratosis of mild intensity in the skin exposed to solar radiation (23%), when compared to the covered skin (10.5%). However, this difference was not significant. Two fishermen (10.5%) did not show hyperkeratosis in the covered skin.

Variation in the number of cell layers in the cones and segments of the epidermis between the cones was higher in the exposed skin than in the covered skin, but this difference was only significant for the number of cell layers in the segments of the epidermis between the cones. (Figures 1A and 1B) (Table 2)

Elastosis, which was also diagnosed in the physical examination, was significantly related to skin exposure to solar radiation (Figures 1A and 1B), and when it occurred, it was more intense on exposed skin. (Table 2)

The number of ectatic dermal vessels was significantly higher in skin exposed to solar radiation than in covered skin (78.9% versus 31.6%, $p=0.012$) (Figures 1C and 1D). The superficial dermal perivascular lymphohistiocytic inflammatory infiltrate was also more frequent in exposed skin, except in three cases in which this infiltrate was mild. However, these differences were not significant (Table 2).

Comparative analysis of the studied cellular elements allowed the authors to ascertain that there were significant differences when comparing the skin exposed to sunlight and the covered skin: higher number of CD45RO+ lymphocytes ($p=0.04$) (Figures 2A and 2B), higher number of macrophages revealed by CD68 antibody ($p<0.001$) (Figures 2C and 2D), and an increased quantity of mastocytes ($p=0.001$) (Figure 3). In skin exposed to solar radiation, the macrophages predominated in the upper and middle dermal layers, around blood vessels adjacent to elastotic material, unlike what occurred in the covered skin, where the macrophages were found scattered in the upper dermal layer (Figures 2C and 2D). In skin exposed to solar radiation, mastocytes were bigger in size than those in covered skin. The number of NK cells (CD56+) was higher in skin exposed to solar radiation, but without statistical significance ($p = 0.413$). Covered skin resembled skin exposed regarding the other cellular elements related to skin immune response: Langerhans cells (CD1a+), CD4+ and CD8+ lymphocytes (Table 3).

In Graph 2, it is observed that the count of CD45RO and CD68 markers, as well as the number of mastocytes was significantly higher in skin exposed to ultraviolet radiation than in covered skin.

The count of immunological markers of subsets of CD3, CD3CD4, CD3CD8CD19, CD45RA, CD3CD4CD45RA, CD3CD8CD45RA, CD45RO, CD3CD45RO, CD3CD4CD45RO, CD28, CD3CD28, CD3CD4CD28, CD3CD8CD28, CD3CD4CD25 and HLA-DR lymphocytes was higher among the fishermen than among the non-fishermen. On the other hand, the count of subsets of CD3CD56, CD3CD45RA, CD69, CD3CD69, CD3CD4CD69, CD3CD8CD69, CD25, CD3CD25, CD3CD8CD25, CD3HLA-DR, CD3CD4HLA-DR and CD3CD8HLA-DR lymphocytes was lower among the fishermen than among the non-fishermen. Despite the reported differences, only the increase of the CD3CD8CD45RO subset was significant ($p+0.016$) (Table 4).

DISCUSSION

Some relevant aspects concerning the fishermen's own characteristics deserve attention in the dis-

Table 2: Histological findings on biopsy of covered skin and skin exposed to UVR in 19 fishermen in Recife, Brazil - September 2005 - September 2006

Variables	Groups		Comparison between regions	
	covered	exposed	p Value	Significance
Hyperkeratosis			**Sign Test**	
frequency			0·125	Ns
absent, % (n)	10·5 (2)	0·0 (0)		
discrete, % (n)	78·9 (15)	73·7 (14)		
mild, % (n)	10·5 (2)	26·3 (5)		
Cones			**Wilcoxon Test**	
average (SD)	10·1 (1·29)	10·8 (0·96)	0·051	Ns
average IC 95%	9·5; 10·7	10·4; 11·3		
1st q–med.–3rd q	9·0–10·0–11·0	10·0–11·0–12·0		
minimum; maximum	8·0; 13·0	9·0; 12·0		
Epidermal segments between cones			**Wilcoxon Test**	
average (SD)	5·2 (0·42)	5·8 (1·08)		
average IC 95%	5·0; 5·4	5·3; 6·3		
1st q–med.–3rd q	5·0–5·0–5·0	5·0–6·0–6·0		
minimum; maximum	5·0; 6·0	5·0; 9·0		
Elastosis			**Sign test**	
frequency			0·003	S
absent % (n)	73·7 (14)	21·1 (4)		
discrete, % (n)	21·1 (4)	47·4 (9)		
mild, % (n)	0·0 (0)	10·5 (2)		
intense, % (n)	5·3 (1)	21·1 (4)		
Ectasia			**Sign test**	
frequency		0·012	S	
absent, % (n)	68·4 (13)	21·1 (4)		
present, % (n)	31·6 (6)	78·9 (15)		

SD = standard deviation; 1st q–med.–3rd q = 1st quartile–median–3rd quartile; ns = not significant (p ? 0·05); s = significant (p < 0·05).

cussion of the results. These characteristics make them a special group to conduct studies on prolonged and chronic exposure to ultraviolet radiation, and also on matters related to histopathological and immunological changes resulting from sun exposure.

A major methodological difficulty in conducting this study was the lack of reference in the literature regarding the standardization of the number of elements in the lymphocyte subsets involved in the immunology of individuals chronically and continuously exposed to solar radiation, which has become a unique characteristic of this study. The great majority of the studies are restricted to the immunology of the skin that is occasionally or briefly exposed to solar radiation. In this study, the authors adopted as a control group a number of non-fishermen who are not exposed chronically and continuously to solar radiation, but which were proven to have epidemiological and socioeconomic characteristics that were similar to those of the fishermen.

To establish the protocol for analysis of systemic immunological changes, the authors initially sought to compare the group of fishermen and the group of non-fishermen, with respect to demographic variables, identifying a significant difference in relation to education and years of professional activity.

The significant difference observed in the duration of employment, which was longer among the fishermen, may be attributed to the instructional process to work as fishermen. Fishermen start their career in an empirical way by following family traditions and through contact with other fishermen. This process begins early in adolescence and continues throughout the person's life, therefore resulting in a long career. The members of non-fishermen group had occupations tha t required professional training through formal instruction, so they started working

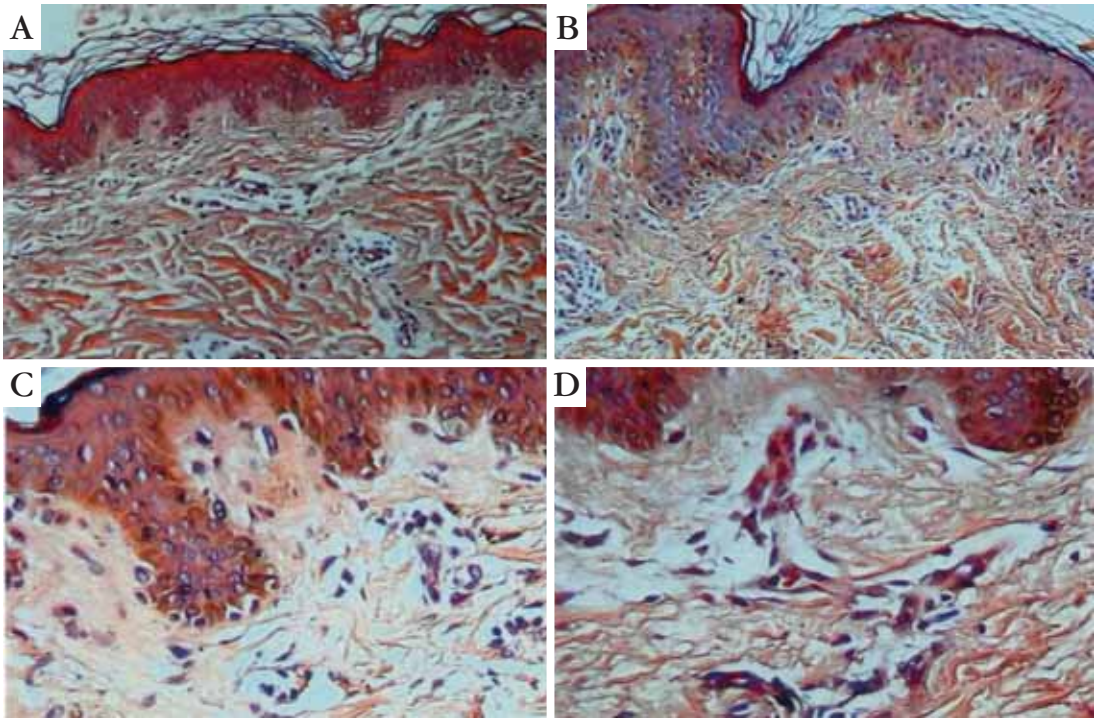


Figure 2: Histological aspect of skin biopsy
Notes: (A) covered skin; (B) presence of elastosis in the dermis and increased number of cell layers between the cones in the exposed skin (H& E, increase x100);
(C) covered skin; (D) presence of ectasis in the dermis of exposed skin (H& E, increase x400).

later in life than the fishermen.

The clinical and dermatological changes diagnosed in fishermen not only derived from chronic and prolonged sun exposure, but also from factors that are typical of their occupation.

Ephelides are small, yellowish-brown stains that are present in photoexposed areas of the skin, resulting from a protective phenomenon originated in increased melanin in the bottom layer of the skin, due to focal hyperactivity of melanocytes. It is the first reaction that the skin develops when it is exposed to the sun and which usually begins in childhood, without any risk of malignant degeneration. It is found in fishermen who, even being intensely exposed to the

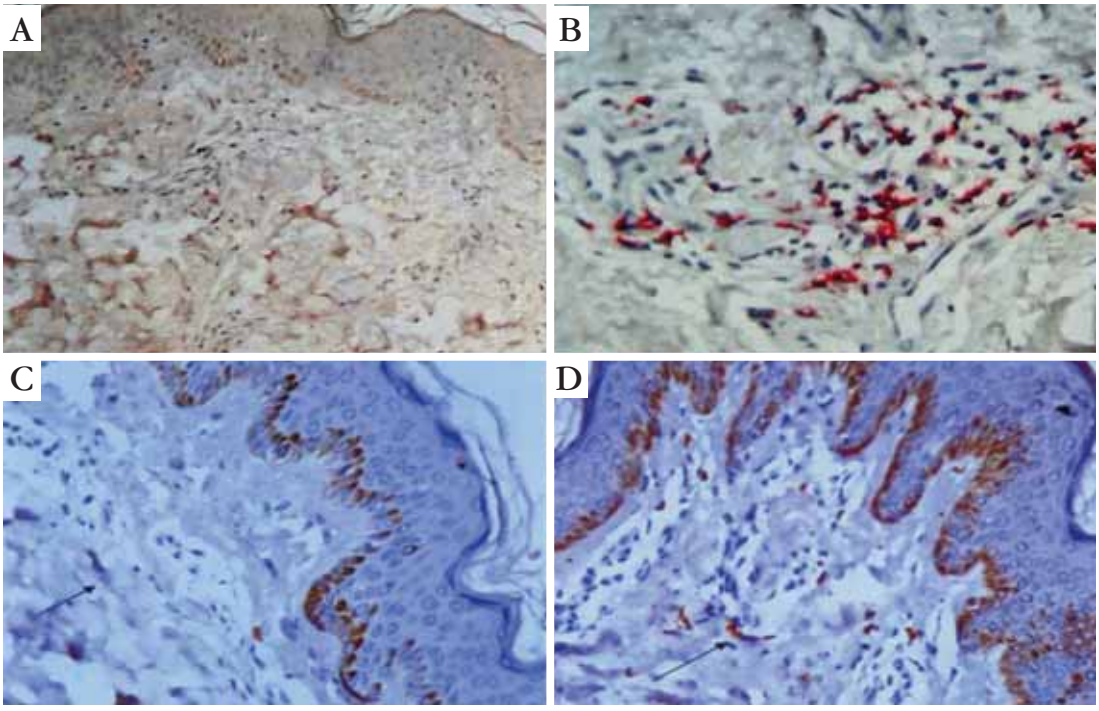


Figure 3: CD45RO and CD 68 T Lymphocytes in skin biopsy
Notes: (A) covered skin – rare immunostained CD45RO T cells around dermal blood vessels (increase x100)
(B) exposed skin – observe numerous immunostained CD45RO T cells around dermal blood vessels (increase x400)
(C) covered skin – rare immunostained CD68 cells around dermal blood vessels (increase x100)
(D) exposed skin – observe numerous immunostained CD68 cells around dermal blood vessels (increase x400).

Table 3: Immunological markers on skin biopsy in 19 fishermen in Recife, Brazil - September 2005 - September 2006

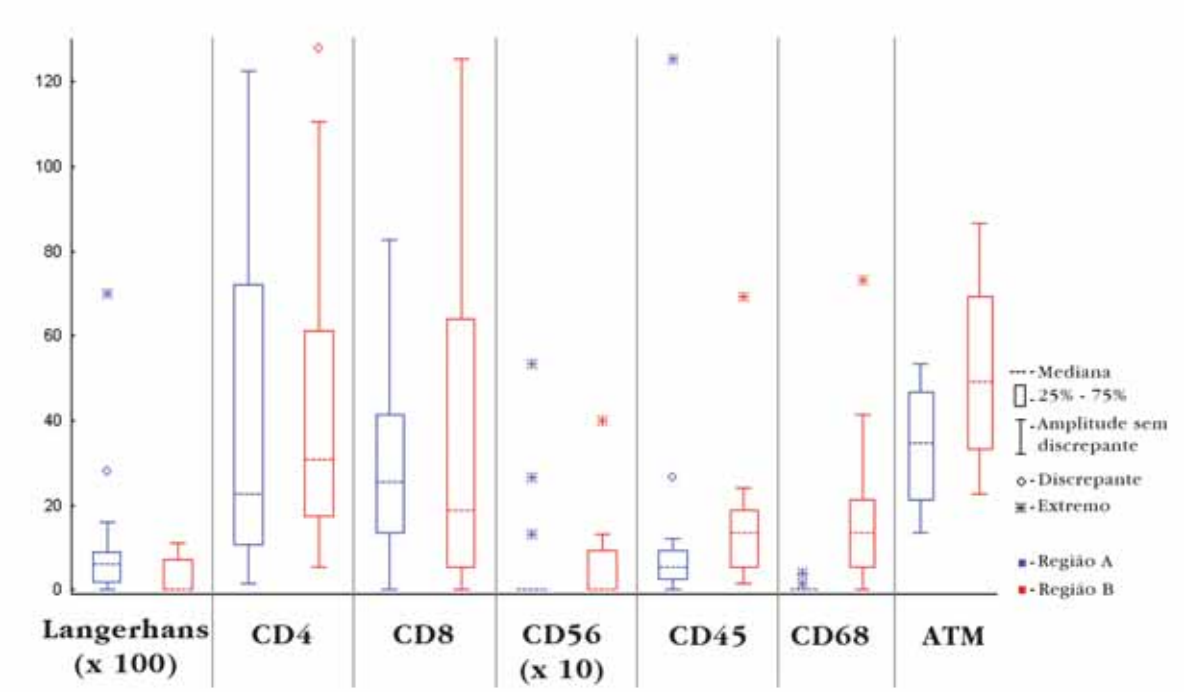
Variables	Region		Comparison between regions	
	covered	exposed	p Value	Significance
CD1a			**Wilcoxon Test**	
average (SD)	0·0089 (0·00775)	0·0085 (0·01539)	0·313	Ns
average IC 95%	0·0052; 0·0126	0·0011; 0·0159		
1st q–med.–3rd q	0·0020–0·0080–0·0150	0·0010–0·0060–0·0090		
minimum; maximum	0·0000: 0·0280	0·0000: 0·0700		
CD4			**Wilcoxon Test**	
average (SD)	38·7 (36·31)	43·0 (35·51)	0·360	Ns
average IC 95%	21·2; 56·2	25·9; 60·1		
1st q–med.–3rd q	10·7–22·7–72·0	17·3–30·7–61·3		
minimum; maximum	1·3; 122·7	5·3; 128·0		
CD8			**Wilcoxon Test**	
average (SD)	29·2 (25·41)	36·9 (39·16)	0·360	Ns
average IC 95%	17·0; 41·5	18·0; 55·7		
1st q–med.–3rd q	13·3–25·3–41·3	5·3–18·7–64·0		
minimum; maximum	0·0; 82·7	0·0; 125·3		
CD56			**Wilcoxon Test**	
average (SD)	0·49 (1·347)	1·05 (2·375)	0·413	Ns
average IC 95%	0·00; 1·14	0·00; 2·20		
1st q–med.–3rd q	0·00–0·00–0·00	0·00–0·00–1·33		
minimum; maximum	0·00; 5·33	0·00; 9·33		
CD45RO			**Wilcoxon Test**	
average (SD)	12·7 (27·92)	14·4 (14·90)	0·040	S
average IC 95%	0·0; 26·2	7·2; 21·6		
1st q–med.–3rd q	2·7–5·3–9·3	5·3–13·3–18·7		
minimum; maximum	0·0; 125·3	1·3; 69·3		
CD68			**Wilcoxon Test**	
average (SD)	0·42 (1·093)	16·77 (17·380)	< 0·001	S
average IC 95%	0·00; 0·95	8·39; 25·15		
1st q–med.–3rd q	0·00–0·00–0·00	5·33–13·33–21·33		
minimum; maximum	0·00; 4·00	0·00; 73·33		
Mastocytes			**Wilcoxon Test**	
average (SD)	33·7 (13·02)	50·8 (18·81)	0·001	S
average IC 95%	27·4; 40·0	41·7; 59·9		
1st q–med.–3rd q	21·3–34·7–46·7	33·3–49·3–69·3		
minimum; maximum	13·3; 53·3	22·7; 86·7		

SD = standard deviation; 1st q–med.–3rd q = 1st quartile–median–3rd quartile; ns = not significant (p ? 0·05); s = significant (p < 0·05).

sun, did not show any malignant neoplasia in the other areas of the dermis.

The absence of premalignant and malignant neoplasia on the skin of fishermen allows us to presuppose the existence of skin adaptation to prolonged and chronic sun exposure. This fact suggests that the body creates protective mechanisms against solar radiation, resulting in a minor damage than that found in individuals that are occasionally exposed to the sun and, thus, do not have a specific defense mechanism from the skin.

In 2005, Schwartz[9] proposed this hypothesis when he studied the effect of ultraviolet radiation on Caucasian individuals, but he concluded that the statement should be confirmed in future studies. However, one cannot abandon the hypothesis that these fishermen show a genetic adaptation of the skin to chronic solar radiation, as they descend from parents and



Graph 2: Dispersion in relation to the median of immunological markers on covered and exposed skin

grandparents that were fishermen as well, as it will be discussed below. To confirm this hypothesis, these aspects, in our opinion, also need to be investigated in their descendents that are not fishermen.

The effect of chronic and prolonged sun exposure can also be identified in the fishermen subject of this study by the frequent presence of elastosis and malanosis in photoexposed areas. Elastosis, also known as peau citriné, is an alteration characterized by coriaceous, thickened skin, having a yellowish color and a grooved surface, as well as by light brown or dark brown spots. Elastosis is an increase in the number and activity of melanocytes, whose function is to prevent solar penetration into the deepest layers of the skin, thus reducing the risk of major damage.

Solar cheilitis, clinically characterized by scaling and crusting at the level of the lower lip, was diagnosed in a fisherman and attibuted to his skin phototype. A similar phenomenon was reported by Nicolini et al.[15] in 1989, when they studied 556 fishermen from Valparaiso, Chile. These fishermen had a similar biotype: blond or red-haired, blue or green-eyed. Solar cheilitis was diagnosed in 43% of the sample, severe or acute in 8%, and chronic in 35% of the cases.

In sum, most of the clinical-dermatological findings diagnosed in the fishermen reflected adaptation

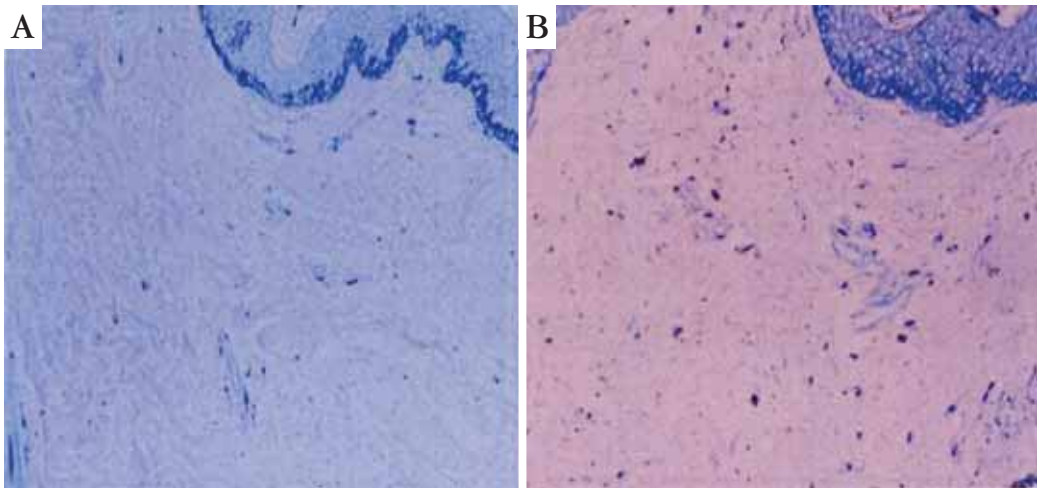


Figure 4: Histological staining with toluidine blue for mastocytes in skin biopsy
Notes: (A) covered skin; (B) exposed skin

Table 4: Lymphocyte subset in peripheral blood of fishermen and non-fishermen in Recife, Brazil - September 2005 - September 2006

Variables	Group		Comparison between groups	
	Non-fishermen	Fishermen	p Value	Significance
N. of individuals				
total	10 (34·5%)	19 (65·5%)		
CD45RO			Mann-Whitney Test	
average (SD)	680·3 (215·41)	974·9 (433·83)	0·056	ns
average IC 95%	526·2; 834·4	765·8; 1184·0		
1st q–med.–3rd q	483·8–656·0–824·5	718·0–925·0–1147·0		
minimum; maximum	370·0; 1091·0	316·0; 1928·0		
CD3CD45RO			Mann-Whitney Test	
average (SD)	651·0 (229·61)	925·6 (435·57)	0·094	ns
average IC 95%	486·7; 815·3	715·6; 1135·5		
1st q–med.–3rd q	441·5–577·0–844·3	579·0–846·0–1101·0		
minimum; maximum	355·0; 1070·0	337·0; 1894·0		
CD3CD4CD45RO			Mann-Whitney Test	
average (SD)	461·7 (150·90)	650·6 (316·77)	0·094	ns
average IC 95%	353·8; 569·6	498·0; 803·3		
1st q–med.–3rd q	292·5–455·5–596·0	423·0–688·0–787·0		
minimum; maximum	261·0; 669·0	150·0; 1508·0		
CD3CD8CD45RO			Mann-Whitney Test	
average (SD)	160·5 (105·39)	315·7 (222·35)	0·016	s
average IC 95%	85·1; 235·9	208·5; 422·9		
1st q–med.–3rd q	91·3–132·0–190·0	148·0–264·0–388·0		
minimum; maximum	71·0; 429·0	66·0; 901·0		

SD = standard deviation; 1st q–med.–3rd q = 1st quartile–median–3rd quartile; ns = not significant (p ? 0·05); s = significant (p < 0·05).

changes related to the fishing activity. These changes were considered appropriate to these professionals, who are empirically trained and uneducated, which, in turn, makes it difficult for them to understand the need to adopt preventive habits against such diseases.

Regarding the changes identified on the histopathological examination of exposed and covered skin biopsy, there were two findings that are worth detailing. The predominance of elastosis in exposed skin provided evidence that chronic exposure to solar radiation leads to collagen degradation and accumulation of abnormal elastin in the dermis.

It is also worth mentioning that it has been stated that UV radiation results in damages to the DNA molecule and to the keratinocyte molecule, but it has also been proved that there is impairment of fibroblasts, whose change in the protein synthesis could explain the presence of abnormal elastin, responsible for solar elastosis. Bosset et al.[16], when observing the concomitant increase in elastosis, ectasia and the number of TCD4+ and CD45RO+ cells in skin chronically exposed to the sun, enunciated the hypothesis that the damage in the DNA cell would lead to the synthesis of abnormal molecules, whose presence triggers the release of inflammatory mediators by mastocytes, directly or indirectly modulating the synthesis of proteinases that would degrade the extracellular matrix or trigger the activation of metalloproteinases.[17,19]

Also, the significant difference of cells immunologically marked in the segments of the epidermis between the cones came to prove the findings of Baba et al.,[20] in 2005. These authors observed that epidermal stimulation by means of solar radiation promotes hyperproliferation of bottom layer cells in the cones, resulting in extension of epidermal projections between the cones, which will form a greater number of cell layers, acting as a defense mechanism against such damage.

This finding seems to confirm the existence of an adaptive protective phenomenon which occurs due to chronic and prolonged skin exposure to the sun. Baba et al.20 showed that cell projections between the cones happen on the third day of UVB exposure, and disappear on the tenth day, when there is no more

exposure. These authors stated that this mechanism is similar to that which occurs during the healing of wounds in the epidermis. It seems plausible to assume that the same mechanism would continue if there were prolonged and chronic exposure to solar radiation, increasing the depth of the cones into the dermis, and, consequently, making the keratinocytes capable of mitosis become more distant in relation to solar radiation. Cell migration between the cones would promote the increase in the number of keratinocytes in postmitotic phase, thus more capable of apoptosis. However, the findings could not prove this hypothesis.

The increase of elastosis, confirmed in this study by the increase in the skin resistance to the punch on the biopsy examination, by the thickening and hardening of the skin identified during the physical examination, as well as by histopathological examination, turns the skin more resistant to tension and pressure. This could have hindered the penetration of the hyperplastic cones, which would cause an even greater cell migration between the cones, increasing the number of cell layers that would act as protectors against the penetration of UV radiation into the deepest region of the skin.

In sum, the histopathological changes related to the proliferation of cell layers in the segments of the epidermis in the cones and between the cones, and also the changes related to elastosis and ectasia, are protective and adaptive processes against chronic and prolonged exposure to solar radiation, resulting from metabolic activities of fibroblasts, and from immunologic activities of mastocytes, associated to cell proliferation.

By means of immunohistochemistry, the absence of immunosuppression in skin exposed to solar radiation could be identified, since CD1a, CD4, CD8 and CD56 markers were similar to those of covered skin, considered as control. A higher number of T CD45RO+ lymphocytes, macrophages revealed by antibody CD68+, and of mastocytes in exposed skin allows us to hypothesize that solar radiation promotes a tolerance effect represented by the increase of the immunological sign, capable of triggering an increase in the influx of leukocytes, macrophages and mastocytes in irradiated skin, consistent with the significant presence of ectasia, connecting prolonged and chronic exposure to chronic inflammation and photoaging of the skin.[8,17,21]

Considering the local mechanism of skin changes attributed to solar radiation referred to in the literature, one can raise the hypothesis that the mechanism of tolerance consisted in triggering the protective barrier against the penetration of solar radiation by means of elastosis, of an increase in the cell layers between cones, and by tanning of the skin, which is the phenotypic expression of melanocytes. Adding to these processes, there was an increase in the vasculature, represented by ectasia, with immunological sign in the skin fostering the leukocyte influx, expressed by an increase of CD45RO+, macrophages and mastocytes.

As for the systemic changes triggered by solar radiation, immunosuppression could not be proved because the immunological markers in the fishermen behaved in a similar way as those in the individuals not subject to prolonged and chronic sun exposure. However, the significant increase in the ratio CD3+CD8+CD45RO+, which occurred exclusively in fishermen, seemed to corroborate the local behavior, signaling an immunological, protective and systemic effect of tolerance.

This means that solar radiation probably triggers immunosuppression in the skin, when exposure is occasional and brief, since other studies prove that the changes in the markers occur in the first post-exposure hours and become normal in 24 to 48 hours. Thus, immunosuppression associated to DNA damage may act as a risk factor for neoplasia, mainly in genetically susceptible individuals.[5]

In the case of prolonged and chronic sun exposure, this inactivation of DNA, both by barrier effect and immunological adaptation, would not occur. This could explain the fact that there is no case of malignant neoplasia in the skin of the fishermen.

Our results complement the publication of Lautenschlager et al.[22] with the chronic and prolonged effects of sun exposure that promote immunoadaptation and photoaging.

This study does not put an end to the subject because we did not find any other investigation in the literature that studied the histological and immunological behavior of skin chronically and continuously exposed to solar radiation. The present study developed a logical line of reasoning from the point of view of physiology. However, further studies are needed to elucidate the thesis defended here.

CONCLUSION

The study of skin exposed and skin not exposed to solar radiation in fishermen who had worked for more than 10 years in the area and been subjected to an average of 12 hours of sun exposure per day indicated that: 1. Chronic sun exposure produces skin changes that represent defense mechanisms, such as an increase in the number of cell layers of the epidermis between the epithelial cones and an increase in melanocytes, but it does not trigger cutaneous immunosuppression, unlike acute exposure to UV, since immunocompetent cells behave identically both in the skin that is exposed and in the skin that is cov-

ered. In the skin that is chronically exposed to the sun, there is an increase in immunologically active cells such as CD+45RO, CD68 and mastocytes; 2. The study of immunologic markers in the blood in individuals chronically exposed to the sun and in individuals from the control group does not indicate signs of immunosuppression. In the individuals exposed to the sun, there is an increase in most lymphocytes subsets, but only the increase in the CD3CD8CD45RO subset is significantly important; and 3.clinically, the absence of actinic keratoses and neoplasia in the fishermen studied emphasizes the absence of immunosuppression. ❑

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