GAVIN PUBLISHERS

Clinical & Experimental Dermatology and Therapies

Rodrigues MM, et al. Clin Exp Dermatol Ther: CEDT-139.

Research Article

DOI: 10.29011/2575-8268/100039

Ultraviolet B susceptibility and Beta-HPV as Risk Factors for Squamous Cell Carcinoma on Photo-exposed Skin in Adults and Older People

Mecciene Mendes Rodrigues[1,2,3,6*], Julia Smith Cavalcante[4], Joana D'arc Rozendo dos Santos[4], Sandra Elizabeth Barbosa da Silva[4], Danyelly Bruneska Gondim Martins[4], Ricardo Arraes de Alencar Ximenes[1,5]

[1]Postgraduate Program in Tropical Medicine, Center for Health Sciences (CCS), Universidade Federal de Pernambuco (UFPE), Recife, Pernambuco, Brazil

[2]Hospital de Câncer de Pernambuco - Sociedade Pernambucana de Combate ao Câncer, (HCP), Recife, Brazil

[3]Professor of Dermatology at the Agreste Academic Center - Nucleus of Life Sciences - Universidade Federal de Pernambuco, Caruaru, Brazil

[4]Keizo Asami Immunopathology Laboratory - Universidade Federal de Pernambuco - Laboratório de Imunopatologia Keizo Asami (LIKA), Recife, Brazil

[5]Universidade de Pernambuco, Recife, Pernambuco, Brazil

[6]Professor of the postgraduate course in integral health - Instituto de Medicina Integral Professor Fernando Figueira - IMIP, Brazil

[*]**Corresponding author:** Mecciene Mendes Rodrigues, Agreste Academic Center - Nucleus of Life Sciences - Universidade Federal de Pernambuco, Caruaru, Brazil. Tel: +5581991679416; Email: mecciene@gmail.com

Citation: Rodrigues MM, Cavalcante JS, Santos JDRD, Barbosa Silva SEBD, Martins DBG, et al. (2017) Ultraviolet B susceptibility and Beta-HPV as Risk Factors for Squamous Cell Carcinoma on Photo-exposed Skin in Adults and Older People. Clin Exp Dermatol Ther: CEDT-139. DOI: 10.29011/2575-8268/100039

Received Date: 02 October, 2017; **Accepted Date:** 20 October, 2017; **Published Date:** 27 October, 2017

Abstract

Background: Ultraviolet B susceptibility (UVBs) is a risk factor for Squamous Cell Carcinoma (SCC), and there are reports of beta-HPV (β-HPV) associated with SCC on photo-exposed skin of immunocompetent patients, which need to be clarified.

Objective: To verify UVBs and β-HPV associated with SCC on the photo-exposed skin of adults and older people at a referral service in Recife-Brazil.

Methods: This was a case-control study. We calculated the crude and adjusted odds ratio, a CI of 95% and the p-value for the association of socio-demographic variables, UVBs and β-HPV with SCC on the photo-exposed skin of adults and older people.

esults: Sixty-three patients were diagnosed with SCC on photo-exposed skin (64.6% Stages I and II). After the multivariate analysis, the following items remained in the final model: UVBs ($p<0,015$), positivity for at least one subtype of β-HPV on photo-exposed skin ($p<0,01$), Fitzpatrick skin types I and II ($p<0,001$), burns before the age of 20 ($p<0,001$) and being male ($p<0,001$).

Limitations: identifying neither the β-HPV subtypes, nor the viral activity.

Conclusion: UVBs and positivity for at least one subtype of β-HPV on photo-exposed skin are risk factors for SCC.

Keywords: AK; β-HPV; Fitzpatrick skin types; NMSC; Odds Ratio; SCC; Sunburn; UVB Radiation; UVBs

Introduction

Exposure to ultraviolet radiation B (UVRB) remains the main risk factor for Non-Melanoma Skin Cancers (NMSC) in Caucasians; [1-3] and squamous cell carcinoma (SCC) is the second most prevalent within the group, corresponding to around 20% of NMSCs in Brazil [4]. SCC is increasing worldwide [5], depending on exposure to UVRB [6,7], the geographical location [8], or some occupational categories [9]. However, it has been observed that being Ultraviolet B sensitive (UVBs) is a better indicator for the risk of SCC [10].

The phenotypic trait of UVBs is observed in more than 90% of patients with SCC, against only 40% of the population [11,12]. In UVBs there is a deficiency in the innate and adaptive immune response when exposed to minimum doses of Ultraviolet B radiation (UVBR), twice the Minimum Erythematosus Dose (MED), due to genetic variability. Acute exposures suppress Cell-Mediated Immunity (CMI) and Contact Hypersensitivity (CH) with deficits in the recognition of new antigens and immunological memory, and a predisposition to certain infections by intracellular pathogens, such as relapsing herpes simplex [12-15] and Virchowian leprosy [16]. In the carcinogenesis of UVBs individuals, cells that have suffered damage to their DNA through UVBR express a mutation in the tumor suppressor gene p53, with a loss of apoptosis and the consequent induction of SCC [6,17,18].

The major risk factor for cutaneous SCC is exposure to UVBR, with an association of beta-Human Papillomavirus (β-HPV) with SCC on skin exposed to UVBR, in immunosuppressed patients and in patients with Epidermodysplasia Verruciformis (EV). In non-immunosuppressed and non-EV patients, clarification is required on whether there is an association of β-HPV with SCC and also whether β-HPV is associated with photo-exposed skin. There have been reports of an association between β-HPV and SCC on photo-exposed skin and β-HPV with photo-exposed skin [19] and some studies have not demonstrated a difference in the prevalence of cutaneous HPV in photo-exposed skin, such as Harwood et al. [20]. More recently, one study determined the prevalence of cutaneous HPV on several types of benign skin lesions, Actinic Keratosis (AK), SCC and BCC, and observed that β-HPV was the most frequent on all lesions, and was observed in all cases of AK and SCC, which were positive for HPV; no type-specific association was observed [21].

Non-immunosuppressed and non-EV individuals may require additional factors in order to become predisposed to infection and to the persistence of β-HPV on photo-exposed skin, and therefore need to be clarified. In this study our proposal was to investigate the risk of UVBs and the presence of β-HPV associated with NMSC, of the SCC type, on photo-exposed skin of adults and older people, which have not been reported in the literature.

Methods

Patients

Patients were selected at the dermatology outpatient clinic of the Hospital de Câncer de Pernambuco (HCP), and were divided into two groups:

a) Patient Cases: patients with NMSC, type SCC.

b) Patient Controls: patients without skin cancer, as defined by the exclusion criteria. (Figure 1)

SOCIODEMOGRAPHIC CHARACTERISTICS		
	Definition	**Category**
Age	Age on day of interview according to date of birth.	< 60 years 60 years and over
Sex	Relating to male/female.	Male. Female
	VARIÁBLES RELATED TO PHOTO-EXPOSURE	
Fitzpatrick skin type classification		
Very sensitive	Always burns, never tans	**I** – White-Red hair
Sensitive	Burns easily, tans minimally	**II** - White
Normal	Sometimes burns, slowly tans	**III** – Slightly darker skin
Normal	Burns minimally, tans easily	**IV** – Moderate brown
Minimally sensitive	Rarely burns, tans well	**V** – Dark brown
Insensitive	Never burns, deep pigmented	**VI** - Black
Level of sun exposure according to anatomical site (professional)		

Intense exposure	Head, neck and backs of hands.	Category I
Moderate exposure	Torso and limbs	Category II
Susceptibility to ultraviolet B radiation	These are individuals who, when exposed to small doses of UVBR twice the Minimum Erythematous Dose (MED), before the application of the hapten diphenhydramine, do not develop contact dermatitis, which would be expected after the second exposure, and which occurs in those who are UVB-resistant (UVBR).	UVBs
Resistance to ultraviolet B radiation	These are individuals who, when exposed to small doses of UVBR twice the minimal erythematous dose (MED), before the application of hapten diphenhydramine, develop contact dermatitis which would be expected after the second exposure, and that does not occur in those who are UVB susceptible (UVBs).	UVBR
Exposure classification β-HPV	**Definition**	**Category**
β-HPV	The presence is identified of at least one subtype of β-HPV DNA through polymerase chain reaction (PCR)	Yes No

Figure 1: Socio-demographic characterizations referring to the photo-exposure of the patients in the sample.

Inclusion criteria for cases and controls

Aged 18 years or over

Exclusion criteria for cases and controls

a) Patients presenting with other papillomatosis or verrucous cancers identified through clinical skin and mucosal examination;

b) Patients presenting with immunosuppressive diseases, repeated skin or other infections, on corticosteroid therapy or using other immunosuppressants verified during anamnesis, physical examination, search in medical records and conducting laboratory tests;

c) Pregnant women: verified with anamnesis and Beta-HCG test.

After being selected, patients were informed about the research and invited to participate. Those who agreed to participate signed the Informed Consent Form (ICF).

Dermatoscopy

The dermatoscopic criteria applied by Zalaudek et al. 2012 [22].

Cutaneous and histopathological biopsy

a) SCC in situ

b) Invasive SCC.

Irradiation with those who are UVBs

The protocol for exposure to UVBR and determining the minimal erythematous dose (MED) followed the protocol described by Yoshikawa et al. [12] Figure 2.

(-)	**Negative reaction;**
(+)	Weak reaction = discrete erythema, little infiltration, no blistering;
(++)	Strong reaction = moderate erythema, occasional papules, few vesicles;
(+++)	Very strong reaction = strong edema and many vesicles;
(++++)	Extreme reaction = formation of blisters and ulceration.
Legends: -Negative = non-reactors: considered UVB-susceptible (UVBs); -Positive = reactors: UVB-resistant (UVBR).	

Figure 2: Response to the contact test with DPCP for characterizing individuals in UVBs and UVBR.

Material/Swab for PCR and identification of the β-HPV

A swab was taken from the top of the suspected SCC lesion and the photo-exposed area of the wrist and back of the hand, the latter being for both cases and controls. The material was stored at -80ºC.

Automated extraction of DNA

The collected skin swab samples were maintained in PBS buffer, stored at -20˚C until they were used for DNA extraction. The QIAsymphony DNA Mini Kit was used for automated DNA extraction through the QIASymphony SP automated system (Qiagen), following the manufacturer's instructions. All samples

were submitted to a Proteinase K digestion step before being inserted into the extraction system. The diluted DNA concentration was evaluated on NanoDrop 2000 (Thermo Scientific ™) and afterwards stored at -20˚C.

Identification of HPV

The PM-PCR was performed using HPV specific primers from the beta-papillomavirus group, and was able to identify HPV5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 93, 96, 104, 110, 111 and 145.,[23] The PCR analysis was performed according to the protocol described by Koning et al. [23], with some small modifications. The PM-PCR was prepared for the final volume of 12.5µl containing 6.25µl GoTaq Green Master Mix (Promega), 1µl of eluted DNA (up to 100ng), 10 pmoles PM-A (5'-ACTGACCAAAGCTGGAAATC-3') and 10 pmoles PM-D primer (5'-TCTTGCAGAGCATTGAAACG-3'). The PCR reaction was performed on a MyCycler™ thermal cycler (BioRad), under the following cycling: 95˚C for 1 min, followed by 35 cycles of 95˚C for 30s, 52˚C for 45s, and 72˚C for 45s; followed by a final extension at 72˚C for 5 minutes. The generated fragment of 117bp relative to the amplification of an E1 gene region of HPV was observed in 1% agarose prepared in TB buffer and visualized by the presence of ethidium bromide (10µg/ml).

The FAP-PCR was performed using HPV specific primers from the beta-papillomavirus group, and was able to identify HPV3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 42, 43, 45, 47, 48, 49, 50, 51, 52, 53, 54, 57, 59, 60, 61, 62, 64, 65, 67, 68, 69, 70, 72, 73, 75, 76, 77 and 80 [20]. The PCR analysis was performed according to the protocol described by Koning et al. [24], with some small modifications. The FAP-PCR was prepared for the final volume of 12.5µl containing 6.25µl GoTaq Green Master Mix (Promega), 1µl of the eluted DNA (up to 100ng), 10 pmoles FAP59 (5´-TAACWGTIGGICAYCCWTATT-3´) and 10 pmoles FAP64 (5´-CCWATATCWVHCATITCICCATC-3´). The PCR was performed using Veriti™ Thermal Cycler (Applied Biosystems™), with the following parameters: 5 min at 95˚C; 45 cycles of 60s at 95ºC, 60s at 50ºC and 60s at 72ºC, followed by a final extension for 5 min at 72ºC. The generated fragment of 478bpb relative to the amplification of a region of the HPV E1 gene was observed in 1% agarose prepared in TB buffer and visualized by the presence of ethidium bromide (10 µg/ml).

Data analysis

The crude and adjusted odds ratio, a 95% confidence interval and the p-value were calculated for the association of socio-demographic variables, a history of skin cancer, chemical and photo exposures, Fitzpatrick skin type classifications, UVBs, β-HPV, and SCC on exposed skin areas in adults and older people. The chi-square and Fisher tests were applied. The Student's t-test was applied for the age and BMI variables. Variables were considered associated with a p-value <0.05. A multiple logistic regression analysis model was tested using UVBs, β-HPV and other confounding variables as possible predictors of SCC. Variables that presented a p-value of <0.20 in the univariate analysis were selected for the multivariate analysis, after which the variables that presented a value of p <0.05 remained in the final model. The step-up strategy was used, where the variables were introduced sequentially into the model, according to the value of p. The UVBs, β-HPV and SCC associations were adjusted by the set of variables selected and by the final multivariate.

Results

With the use of dermatoscopic and histopathologic criteria [22] for SCC *in situ* or intraepithelial (Tis), 45.2% of the SCC sample was classified as Stage I, and 19.4% as microinvasive stage II (T1N0), and SCC was not characterized in 35.5%. It was accordingly observed that 64.6% of SCC cases were diagnosed in early Stages I and II (TNM-AJCC). Characterizing the profile of the cases, 51.7% reported a history of NMSC; 93.4% presented with Actinic Keratosis (AK) lesions and 43.9% presented with ten or more AKs. Table 1.

Characteristics	Number (%)
History of skin câncer	
Yes	31 (51.7%)
No	29 (48.3%)
Actinic keratosis	
No	4 (6.6%)
Yes	57 (93.4%)
<10	32 (56.1%)
> 10	25 (43.9%)
BCC	
Yes	16 (26.2%)
No	45 (73.8%)
SCC site	
Category 1	33 (54.1%)
Category 2	28 (45.9%)
Staging	
In situ	28 (45.2%)
Microinvasive	12 (19.4%)
No information on HP	22 (35.5%)

Table 1: Characteristics of patients with SCC on photo-exposed skin in adults and older people.

Among the patients with SCC, the frequency of UVBs was 69.8%, whereas in the controls the frequency was 24% (OR 7.33 95% CI 3.45-15.6). The frequency of β-HPV in the SCC group

was 23.4%, whereas in the controls it was 1.8% (OR17.1 CI 2.12 - 138), when we compared the cases lesions and the back of the hand. However, we considered any site of the skin in the cases and compared them with back of the hands of the controls and the frequency was 36.2% in the cases and 1.8% in the controls (OR 31.4 CI 4.02 - 250). In the group of exposures to UVBR, Fitzpatrick skin type classifications I and II were observed, represented in 85.7% of those with SCC, while in the controls it was 25.3% (OR 17.7 CI 95% 7.36 - 42.5). Photo-exposure in an open environment for 4 or more hours, during 10 or more years was observed in 71% of those with SCC and in 30.6% of the controls (OR 5.07 95% CI 2.31-11.1). Additionally, within the UVBR exposure group in an open environment, 41.3% presented with SCC and worked in agriculture, while in the controls this percentage was 14.75% (OR 4.09 95% CI 1.81 - 9.22). In the group of exposure to chemicals, the chances of being a current or former smoker occurred in 57.1% of the SCC cases and in 38.7% of the controls (OR 2.1 95% CI 1.07 - 4.18). The smoking load of over 35 pack/years occurred in 58.1% of the cases and 38.7% of the controls (OR 2.11 CI 1.07 - 4.18). A MED ≥ 18 KJ/cm² was observed in 50% of the SCC cases and in 72% of the controls (OR 0.39 CI 95% 0.18 - 8.84). A total of 58.1% of those with SCC were male and 26.4% in the controls (OR 3.68 and CI 95% 1.87 - 8). The age range of those with SCC was 67.3 ± 13.1 and 49.1 ± 14.8 in the controls (OR 1.10 95% CI 1.06 - 1.12). The 60-year-old age group was observed in 73.1% of SCC cases and in 24% of the controls (OR 6.33 95% CI 3.01 - 13.3). A total of 68.3% of the SCC cases had attended school ≤ 8 years and 50.7% of the controls (OR 2.09 CI 95% 1.04 - 4.20). Table 2(a,b,c).

Variables	SCC		OR (CI (95%))	p-value
	Yes (n = 63)	No (n = 75)		
UVB				
Resistant	19 (30.2%)	57 (76.0%)	1.0	
Susceptible	44 (69.8%)	18 (24.0%)	7.33 (3.45 - 15.6)	<0.001
HPVs (any site)				
Absence	30 (63.8%)	56 (98.2%)	1.0	
Presence	17 (36.2%)	1 (1.8%)	31.7 (4.02 - 250.2)	0.001
β-HPV (only on the lesion)				
Absence	36 (76.6%)	56 (98.2%)	1.0	
Presence	11 (23.4%)	1 (1.8%)	17.1 (2.12 -138.3)	0.008
Socio-demographic variables				
Sex				
Female	26 (41.9%)	53 (73.6%)	1.0	
Male	36 (58.1%)	19 (26.4%)	3.86 (1.87 - 8.00)	<0.001
Age	67.3 ± 13.1	49.1 ± 14.8	1.10 (1.06 - 1.12)	<0.001
Age Group				
Under 60 years	21 (26.9%)	57 (76.0%)	1.0	
60 years or over	42 (73.1%)	18 (24.0%)	6.33 (3.01 - 13.3)	<0.001
Schooling (in years)				
> 8 years	20 (31.7%)	37 (49.3%)	1.0	
≤ 8 years	43 (68.3%)	38 (50.7%)	2.09 (1.04 - 4.20)	0.038
Family income				
More than 3 MS*	10 (16.4%)	9 (12.0%)	1.0	
Between 1and 3 MS*	36 (59.0%)	42 (56.0%)	0.77 (0.28 - 2.10)	0.613
Up to 1 MS*	15 (24.6%)	24 (32.0%)	0.56 (0.19 - 1.70)	0.309
*Minimum Salary				

Table 2a: Socio-demographic characteristics, background to NMSC, UVBs, β-HPV associated with SCC on photo-exposed skin in adults and older people.

Variable	SCC		OR (CI (95%))	p-value
Background to skin câncer				
Family history of skin cancer				
No	48 (76.2%)	62 (82.7%)	1.0	
Yes	15 (23.8%)	13 (17.3%)	1.49 (0.65 - 3.43)	0.348
Chemical exposure				

Smoker				
Never smoked	26 (41.9%)	46 (61.3%)	1.0	
Current or former smoker	36 (58.1%)	29 (38.7%)	2.11 (1.07 - 4.18)	0.031
Smoking load (years/pack)				
Never smoked	26 (44.8%)	46 (61.3%)	1.0	
Less than 35	26 (44.8%)	25 (33.3%)	1.84 (0.87 - 3.81)	0.102
35 and overs	6 (10.4%)	4 (5.4%)	2.65 (0.69 - 10.3)	0.158
Exposure to arsenic				
No	59 (93.7%)	74 (98.7%)	1.0	
Yes	4 (6.3%)	1 (1.3%)	5.02 (0.55 - 46.1)	0.154
Occupation[a]				
Other occupations	37 (58.7%)	64 (85.3%)	1.0	
Agriculture	26 (41.3%)	11 (14.7%)	4.09 (1.81 - 9.22)	0.001

Table 2b: Socio-demographic characteristics, background to NMSC, UVBs, β-HPV associated with SCC on photo-exposed skin in adults and older people.

Variables	**SCC**		OR (CI(95%))	p-value
	Yes (n = 63)	**No (n = 75)**		
Photo exposure				
Photo-damage				
None or Mild	37 (58.7%)	33 (44.6%)	1.0	
Moderate or Severe	26 (41.3%)	41 (55.4%)	0.57 (0.29 - 1.11)	0.100
Fitzpatrick skin type classification				
III to VI	9 (14.3%)	56 (74.7%)	1.0	
I and II	54 (85.7%)	19 (25.3%)	17.7 (7.36 - 42.5)	<0.001
Previous burns				
No	40 (63.5%)	57 (76.0%)	1.0	
Yes	23 (36.5%)	18 (24.0%)	1.82 (0.87 - 3.81)	0.111
Burns before the age of 20 years				
No burns	41 (65.1%)	61 (81.3%)	1.0	
After the age of 20 years	8 (12.7%)	4 (5.3%)	1.77 (0.72 - 4.33)	0.210
Before the age of 20 years	14 (22.2%)	10 (13.3%)	2.85 (0.83 - 9.79)	0.097
Photo-exposure in the environment				
Closed environment	15 (24.2%)	38 (52.8%)	1.0	
Open environment for less than 10 years for at least 4hr/day	3 (4.8%)	12 (16.7%)	0.63 (0.16 - 2.57)	0.522
Open environment for 10 years or more and 4hr or more per day	44 (71.0%)	22 (30.6%)	5.07 (2.31 - 11.1)	<0.001
Minimal erythematous dose				
Mean ± sd	18.1 ± 8.9	20.1 ± 8.3	0.97 (0.93 - 1.02)	0.218
Categorized[a]				
< 18	23 (50.0%)	21 (28.0%)	1.0	
≥ 18	23 (50.0%)	54 (72.0%)	0.39 (0.18 - 0.84)	0.016

Region				
Metropolitan	42 (66.7%)	52 (69.3%)	1.0	
Zona da Mata (Coastal Forest)	7 (11.1%)	9 (12.0%)	0.96 (0.33 - 2.80)	0.945
Agreste (Hot/sub-humid)	11 (17.5%)	11 (14.7%)	1.23 (0.49 - 3.14)	0.652
Backlands	3 (4.8%)	3 (4.0%)	1.23 (0.23 - 6.45)	0.800
BMI				
Normal	24 (38.1%)	30 (40.0%)	1.0	
Overweight	30 (47.6%)	29 (38.7%)	1.29 (0.62 - 2.71)	0.496
Obese	9 (14.3%)	16 (21.3%)	0.70 (0.26 - 1.87)	0.480

Table 2c: Socio-demographic characteristics, background to NMSC, UVBs, β-HPV associated with SCC on photo-exposed skin in adults and older people. (conclusion)

The variables with a p-value <0.20 were selected for multiple logistic regression and the variables that presented a p-value <0.05 remained in the final model, thus characterizing a risk profile of SCC, UVBs (OR 3.69 CI 1, 01-13.5); at least one β-HPV subtype 9.38 (0.46 - 189.5), a Fitzpatrick skin type classification of I and II (OR 40.2 IC 9.34 - 172.9); sunburn after 20 years of age (OR 7.95 CI 1.34 - 47.1); aged 60 years or over (OR 1.11 CI 1.05 - 1.17) and male (OR 9.35 CI 2.26 - 38.8). Table 3 and Table 4.

Variables	OR (CI(95%))	p-value
UVBs		
Resistant	1.0	
Susceptible	7.12 (1.46 - 34.6)	0.015
β-HPV (any site)		
Absence	1.0	
Presence	42.8 (2.34 - 780.6)	0.011
No information	2.47 (0.48 - 12.8)	0.280
Sex		
Female	1.0	
Male	6.49 (1.27 - 33.2)	0.025
Age	1.13 (1.06 - 1.20)	<0.001
Fitzpatrick skin type classification		
III to VI	1.0	
I and II	99.9 (13.9 - 714.7)	<0.001
Burns before the age of 20		
No burns	1.0	-
Before the age of 20	69.1 (5.76 - 829.2)	0.001
After the age of 20	2.45 (0.29 - 21.0)	0.414

Table 3: Multivariate model - Logistic regression - Risk factors for SCC on photo-exposed skin of adults and older people.

Variables	OR (CI(95%))	p-value
UVB		
Resistant	1.0	
Susceptible	6.11 (1.33 - 28.1)	0.020
β-HPV (only on the lesion)		
Absence	1.0	
Presence	9.38 (0.46 - 189.5)	0.0144
No information	1.94 (0.40 - 9.46)	0.414
Sex		
Female	1.0	
Male	9.29 (1.89 - 45.5)	0.006
Age	1.13 (1.06 - 1.20)	<0.001
Fitzpatrick skin type classification		
III to VI	1.0	
I and II	73.7 (12.3 - 441.1)	<0.001
Burns before the age of 20		
No burns	1.0	-
Before the age of 20	56.5 (5.10 - 625)	0.001
After the age of 20	2.21 (0.26 - 18.4)	0.464

Table 4: Multivariate model - Logistic regression - Risk factors for SCC on photo-exposed skin- β-HPV only on the lesion - of adults and older people.

Discussion

The following factors were associated with SCC in the studied sample: UVBs, positivity of at least one subtype of β-HPV, Fitzpatrick skin type classifications I and II, burns from UVBR before 20 years of age, a mean age of 60 years and male.

In our results, in UVBs, we observed an odds ratio of 7.1 for SCC, in comparison to the controls. In these individuals, therefore, previous exposure to UVBR, to twice the MED, prevented contact hypersensitivity to DPCP by immunosuppression, thus characterizing UVBs. For decades, the differences have been described regarding the cutaneous test reactivity of individuals

who had previously been exposed to small doses of UVB. One of the characteristics is cutaneous immunological tolerance to and the non-recognition of new antigens, classifying 30-40% of individuals without SCC in UVBs and 60% of individuals without SCC in UVBR [12]. Our results demonstrate that 68.9% of individuals with SCC were UVBs, differing from the literature, where UVBs is observed in more than 90% of SCC cases from photo-exposure [12] There are some possible explanations for the 69.8% proportion of UVBs in patients with SCC in our study, and this difference may be attributed to the size of the sample studied. Another possible explanation could be the dose of the Narrowband Ultraviolet B radiation (NB-UVB). Therefore, in order to observe this lower proportion of UVBs than that expected in SCC, we investigated the dose used and observed that the median dose ≥ 18 mJ/cm² was encountered in 72% of the controls, and, to a lesser extent, 50% of the patients with SCC. Therefore, the control patients received the highest dose by more than 70%, and even then, the UVRB was 76%. We thus conclude that there was probably no implication of the UVRB dose used. We hypothesized that the non-use of the maximum spectrum of UVRB may have had repercussions on non-immunosuppression. Given that the dose-response curve presents a maximum action in the spectrum of between 260 and 270 nm, followed by 280-290 nm, and then declines steadily to a maximum level of 320 nm, still at the length of a UVRB wave [25], for irradiation we used the spectrum between 311 and 312 nm (Narrowband-UVB). We attempted, however, to minimize this effect by determining the maximum and minimum dose, which was between 4.5 - 18 Kj/m². Therefore, since an implication of the NB-UVB spectrum is unlikely, there was a repercussion in the lowest proportion of UVBs in the SCC patients of the sample studied. With regard to the dose of UVBR required for oncogenesis, it has been reported by Noonan et al., [25] that there are differences in the required dose, which is 13 times greater than that required to induce CH in 50% of irradiated animals.

Our results demonstrated positivity for β-HPV in SCC patients with an odds ratio of 9.38 in patients with positive β-HPV, when compared to controls. These findings differ from the findings of Asgari et al. [26] who encountered no difference in SCC patients compared to healthy controls. In the present study, it was also observed that, when we consider the presence of β-HPV in all sites of photo-exposed skin, the odds ratio for SCC increases to 42.8. Therefore, it is possible to accept that there is an association between β-HPV and photo-exposed skin and SCC on photo-exposed skin, contrary to the findings of Asgari et al. [26], Koning et al. [27] and Harwood et al. [28], who observed no differences in the prevalence of HPV on UVR-exposed or -unexposed skin. Our results, however, are in agreement with the findings of Forslund et al. [19] who reported an association of β-HPV with SCC on photo-exposed skin and also with the areas of photo-exposed skin with no lesions. In the present study, we only selected patients with the SCC-type of NMSC and for the control group, any individual with lesions or dermatoses suggestive of association with HPV, such as papillomatous, verrucous or squamo-keratotic lesions, such as black papular dermatosis, keratoses and seborrheic warts. Thus, the possibility of selection bias was reduced by including individuals with dermatoses possibly associated with β-HPV in the control group. In our control group, the observed β-HPV frequency was 1.8%.

In our sample, PCR was used to identify β-HPV in SCC *in situ* in 45.2% of the studied sample, and positivity for β-HPV was found in 36.2%. It is therefore suggested that β-HPV may be associated with SCC at induction, although it is not possible to state whether it is in the maintenance of SCC, since our tests (PCRs) were applied to SCC samples during the initial stages, *in situ* or micro invasive. In the literature, it has not been defined as to whether β-HPV is associated with SCC on photo-exposed skin and whether participation takes place during induction or maintenance or in both phases of SCC development. For some authors, the participation of β-HPV in SCC on the skin occurs during induction, but not maintenance [29,30]. In a recent study, with the immune-histochemical analysis of 3,846 specimens from biopsies diagnosed with SCC *in situ*, it was observed that all tumors with a multicentric characteristic presented HPV positivity [31].

Our results demonstrated the presence of Actinic Keratosis (AK) lesions in approximately 93% of patients with SCC, with more than 10 AKs in 43.9% of cases. In the literature, an association between β-HPV and the presence of more than 10 AK lesions has been reported [32]. In another study, persistent β-HPV infection associated with the presence of AK lesions on the face was observed [33]. There are also reports of an association between β-HPV and AKs, pointing to the participation of β-HPV in the induction of SCC [33].

The present study suggests that HPV positivity may be associated with photo-exposed skin and SCC on photo-exposed skin. The incidence of SCC has been increasing worldwide, mainly resulting from determinants of environmental exposure, especially exposure to UVRB, which remains the main risk factor for SCC in Caucasians. The absence of β-HPV on exposed skin of control patients indicates the possibility of protection from the UVBR phenotype, present in 76% in the controls and only 30.2% of the patients with SCC. It is therefore suggested that the non-predisposition to the infection of photo-exposed skin by β-HPV may be explained by UVRB in this group of individuals. To confirm our results, it is necessary to carry out further studies to assess both exposed and unexposed skin to UVRB and to investigate the presence of viral activity apart from HPV DNA.

Our results have demonstrated that skin types I and II were associated with SCC. Skin types I and II are characterized by skin color and light-colored eyes, are more prone to burning and either tan slightly or not at all and in accordance with the literature studied, are associated with SCC [34].

In our study, working in agriculture was associated with SCC in the univariate analysis. However, this association was lost after adjustment for other factors, in the multivariate analysis, and did not remain in the final model. In another study, it was observed that occupational categories with a high socioeconomic status not involving exposure to the sun, and certain other occupations with potent exposure to chemical substances, as well as occupational categories in open environments exposed to UVRB, presented a higher risk of SCC compared to the general population [9] .In our study, we investigated exposure to UVRB in certain occupations, and examined some chemical exposures, such as arsenic and other chemicals applied in agricultural practice, and we encountered no association.

We observed an association between burns before the age of 20 and the occurrence of SCC, thus corroborating the findings of S Wu et al. [8] Ziegler et al. [35] reported that mutation in the p53 tumor suppressor gene encountered in more than 90% of SCC cases is also observed in AK, and that sunburn can select clonal expansion cells with mutated p53 within AK, and may act doubly as primers and as promoters of the tumor. It was observed that burns on the torso are associated with SCC in men (OR 1.48 CI 1.08 - 2.03) [36].

With respect to age, we observed an association between the group aged 60 years and over and SCC. Our results are in accordance with the studied literature, which demonstrates a higher incidence of SCC from the age of 40, with higher indices from 60 years [8,10,37]. However, there is a risk of increased SCC in earlier ages (15 to 30 years) depending on the geographical area of high or medium incidences of UVRB [38].

In the present study, we observed a higher occurrence of SCC in males. As possible explanatory factors, Chen et al. [39] observed differences in the form of sun exposure between the sexes, observing that exposure during the whole life is more related to the risk of SCC in women, whereas, initiating early exposure seems to be more relevant for the risk of SCC in males. In our study we encountered no difference in behavior between the sexes, and we would suggest further exploration in this area. For Rudolph et al., [5] there has been an increase in the incidence of skin cancer in both sexes worldwide, however, this is predominately in females. Welsh et al. [40] reported that this growth trend predominantly in females may be explained by genetic risk determinants of different NMSCs by gender, in UBRV-induced immune suppression, with IL10 functional variants associated with increased odds ratios of NMSC, which occur largely in females.

Conclusion

The presence of β-HPV on exposed skin is a risk factor for SCC in the population of patients treated at the skin cancer outpatient clinic at the Hospital de Câncer de PE - Brazil. The risk factors indicated by the research for SCC in our population were: UVBs, presence of β-HPV, older people, light-colored skin, hair and eyes, and being male.

In Brazil, which is a tropical country with medium to high UVRB rates, there has been an increased incidence of SCC and further research is needed in order to verify factors associated with the infection and persistence of β-HPV in SCC on photo-exposed skin, such as immune deficiency due to susceptibility to UVRB in our population, and β-HPV, which was the object of our study. We recommend that patients with more than 10 AKs should be observed in referral services and skin cancer outpatient clinics in order to search for suspected SCC lesions with dermatoscopy and histopathology.

Conflict of interest statement

The authors hereby declare they have no conflict of interest.

Acknowledgements

We would like to express our sincerest thanks to the patients and families who have contributed by believing in new scientific knowledge related to skin cancer, and in improving the health of people and their quality of life.

References

1. Armstrong BK, Kricker A (2001) The epidemiology of UV induced skin cancer. J Photochem Photobiol B 63: 8-18.
2. Leiter U, Eigentler T, Garbe C (2014) Epidemiology of skin cancer. Adv Exp Med Biol 810: 120-140.
3. Rigel DS (2008) Cutaneous Ultraviolet Exposure and Its Relationship to the Development of Skin Cancer. J Am Acad Dermatol 58(5 Suppl 2): S129-132.
4. INCA. Pele não melanoma.
5. Rudolph C, Schnoor M, Eisemann N, Katalinic A (2015) Incidence trends of nonmelanoma skin cancer in Germany from 1998 to 2010. J Dtsch Dermatol Ges 13: 788-797.
6. Rebel HG, Bodmann CA, van de Glind GC, de Gruijl FR (2012) UV-induced ablation of the epidermal basal layer including p53-mutant clones resets UV carcinogenesis showing squamous cell carcinomas to originate from interfollicular epidermis. Carcinogenesis 33: 714-720.
7. Thompson EA, Zhu S, Hall JR, House JS, Ranjan R, et al. (2011) C/EBPα expression is downregulated in human nonmelanoma skin cancers and inactivation of C/EBPα confers susceptibility to UVB-induced skin squamous cell carcinomas. J Invest Dermatol 131: 1339-1346.
8. WU S, Han J, Vleugels RA, Puett R, Laden F, et al. (2014) Cumulative ultraviolet radiation flux in adulthood and risk of incident skin cancers in women. Br J Cancer 110: 1855-1861.
9. Alfonso JH, Martinsen JI, Pukkala E, et al. (2016) Occupation and relative risk cutaneous skin carcinoma (cSCC): A 45-year follow-up study in 4 Nordic countries. J Am Acad Dermatol 75: 548-555.

10. Schmieder GJ, Yoshikawa T, Mata SM, Streilein JW, Taylor JR (1992) Cumulative sunlight exposure and the risk of developing skin cancer in Florida. J Dermatol Surg Oncol 18: 517-522.

11. Kurimoto I, Streilein JW (1994) Characterization of the immunogenetic basis of ultraviolet-B light effects on contact hypersensitivity induction. Immunology 81: 352-358.

12. Yoshikawa T, Era V, Bruins-Slot W, van den Berg JW, Taylor JR, et al. (1990) Susceptibility to Effects of UVB Radiation on Induction of Contact Hypersensitivity as a Risk Factor for Skin Cancer in Humans. The Journal of Investigative Dermatology. J Invest Dermatol 95: 530-536.

13. Taylor JR, Schmieder GJ, Shimizu T, Tie C, Streilein JW (1994) Interrelationship between ultraviolet light and recurrent herpes simplex infections in man. J Dermatol Sci 8: 224-232.

14. Vermeer M, Streilein JW (1990) Ultraviolet B ligth-induced alterations in epidermal Langerhans cells are mediated in part by tumor necrosis factor-alpha. Photodermatol Photoimmunol Photomed 7: 258-265.

15. Yoshikawa T, Kurimoto I, Streilein JW (1992) Tumour necrosis factor-alpha mediates ultraviolet light B-enhanced expression of contact hypersensitivity. Immunology 76: 264-271.

16. Rodrigues MM, Ximenes RAA, Dantas MMS, Batista TD, Freire AL (2010) A UVB-Suscetibilidade como fator de risco para o desenvolvimento da hanseníase virchowiana. An Bras Dermatol 85: 835-842.

17. Barzilai A, Lyakhovitsky A, Trau H, Fogel M, Huszar M (2007) Expression of p53 in the evolution of squamous cell carcinoma: correlation with the histology of the lesion. J Am Acad Dermatol 57: 669-676.

18. Marinescu, A, Stepan AE, Margaritescu C, Marinescu AM, Zăvoi RE, et al. (2016) P53, p16 and immunossupression in cutaneous squamous cell carcinoma and its precursos lesions. Rom J Morphol Embriol 57(2 Suppl): 691-696.

19. Forslund O, Iftner T, Andersson K, Lindelof B, Hradil E, et al. (2007) Cutaneous Human Papillomaviruses Found in Sun-Exposed Skin: Beta-papillomavirus Species 2 Predominates in Squamous Cell Carcinoma. J Infect Dis 196: 876-883.

20. Harwood CA, Surentheran T, Sasieni P, Proby CM, Bordea C, et al. (2004) Increased risk of skin cancer associated with the presence of epidermodysplasia verruciformis human papillomavirus types in normal skin. Br J Dermatol 150: 949-957.

21. Correa RM, Vladimirsky S, Heideman DA, Coringrato M, Abeldaño A, et al. (2017) Cutaneous human papillomavirus genotypes in different kinds of skin lesions in Argentina. J Med Virol 89: 352-357.

22. Zalaudek I, Gilacomel J, Schmid K, Bondino S, Rosendahl C, et al. (2012) Dermatoscopy of facial actinic keratosis, intraepidermal carcinoma, and invasive squamous cell carcinoma: A progression model. J Am Acad Dermatol 66: 589-597.

23. Koning M, Quint W, Struijk L, Kleter B, Wanningen P, et al. (2006) Evaluation of a Novel Highly Sensitive, Broad-Spectrum PCR-Reverse Hybridization Assay for Detection and Identification of Beta-Papillomavirus DNA. J Clin Microbiol 44: 1792-1800.

24. Michael KM, Forslund O, Bacevskij O, Waterboer T, Bravo IG, et al. (2011) Bead-Based Multiplex Genotyping of 58 Cutaneous Human Papillomavirus Types. J Clin Microbiol 49: 3560-3567.

25. Noonan FP, De Fabo EC, Kripke ML (1981) Suppression of contact hypersensitivity by UV radiation and its relationship to UV-induced suppression of tumor immunity. Photochem Photobiol 34: 683-689.

26. Asgari MM, Kiviat NB, Critchlow CW, Stern JE, Argenyi ZB, et al. (2008) Detection of human papillomavirus DNA in cutaneous squamous cell carcinoma among immunocompetent individuals. J Invest Dermatol 128: 1409-1417.

27. de Koning MN1, Weissenborn SJ, Abeni D, Bouwes Bavinck JN, Euvrard S, et al. (2009) Prevalence and associated factors of betapapillomavirus infections in individuals without cutaneous squamous cell carcinoma. J Gen Virol 90(Pt 7): 1611-1621.

28. Harwood CA, Surentheran T, Sasieni P, Proby CM, Bordea C, et al. (2004) Increased risk of skin cancer associated with the presence of epidermodysplasia verruciformis human papillomavirus types in normal skin. Br J Dermatol 150: 949-957.

29. Aldabagh B, Angeles JGC, Cardones AR, et al. (2013) Cutaneous Squamous Cell Carcinoma and Human Papillomavirus: Is There Na Association? Dermatol Surg 39(1 Pt 1): 1-23.

30. Schiller JT, Buck CB (2011) Cutaneous squamous cell carcinoma: a smoking gun but still no suspects. J Invest Dermatol 131: 1595-1596.

31. Gross JA, Perniciaro C (2015) Histopathologic Variants of Cutaneous Squamous Cell Carcinoma *in situ* With Analysis of Multicentric Subtypes: Possible Relationship to Human Papillomavirus. Am J Dermatopathol 37: 680-685.

32. McBride P, Neale R, Pandeya N, Green A (2007) Sun-related factors, betapapillomavirus, and actinic keratoses: a prospective study. Arch Dermatol 143: 862-868.

33. Plasmeijer EI1, Neale RE, de Koning MN, Quint WG, McBride P, et al. (2009) Persistence of betapapillomavirus infections as a risk factor for actinic keratoses, precursor to cutaneous squamous cell carcinoma. Cancer Res 69: 8926-8931.

34. MacKie RM (2006) Long-term health risk to the skin of ultra-violet radiation. Prog Biophys Mol Biol 92: 92-96.

35. Ziegler A, Jonason AS, Leffell DJ, Simon JA, Sharma HW, et al. (1994) Sunburn and p53 in the onset of skin cancer. Nature 372: 773-776.

36. Wu S, Cho E, Li WQ, Weinstock MA, Han J, et al. (2016) History of Severe Sunburn and Risk of Skin Cancer Among Women and Men in 2 Prospective Cohort Studies. Am J Epidemiol 183: 824-833.

37. Videnović G, Miljuš D, Ilić D, Krasić D, Živković S (2015) Nonmelanoma Skin Cancer in the Population of the City of Belgrade in the Period 1999-2011. Srp Arh Celok Lek 143: 290-295.

38. Wei-Passanese EX, Han J, Lin W, Li T, Laden F, et al. (2012) Geographical variation in residence and risk of multiple nonmelanoma skin cancers in US women and men. Photochem Photobiol 88: 483-489.

39. Chen YC1, Christiani DC, Su HJ, Hsueh YM, Smith TJ, et al. (2010) Early-life or lifetime sun exposure, sun reaction, and the risk of squamous cell carcinoma in an Asian population. Cancer Causes Control 21: 771-776.

40. Welsh MM, Karagas MR, Kuriger JK, Houseman A, Spencer SK, et al. (2011) Genetic Determinants of UV-Susceptibility in Non-Melanoma Skin Cancer. PLoS One 6: e20019.