Journal of Biosciences and Medicines, 2023, 11, 150-161
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Role of Genetic Ancestry in Oropharyngeal Squamous-Cell Carcinoma: A Cross-Sectional Study in Brazil

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How to cite this paper: Ferreira, C.C., Gama, R.R., De Carvalho, A.C., Santana, I., Carvalho, R.S., e Silva, D.S.D.A., De Jesus, L.M., Reis, R.M. and Dufloth, R. (2023) Role of Genetic Ancestry in Oropharyngeal Squamous-Cell Carcinoma: A Cross-Sectional Study in Brazil. *Journal of Biosciences and Medicines*, **11**, 150-161.
https://doi.org/10.4236/jbm.2023.111015

Received: December 18, 2022
Accepted: January 25, 2023
Published: January 28, 2023

Abstract

Background: HPV infection represents an important etiologic factor for Oropharyngeal Squamous Cell Carcinoma (OPSCC). The different ethnic backgrounds could be related to different susceptibility to Human Papillomavirus (HPV). The aim of our study was to assess the whole of genetic ancestry in HPV status in OPSCC patients. **Methods:** We conducted a cross-sectional study on patients with OPSCC admitted to the Barretos Cancer Hospital, Brazil from 2014 to 2019. Of these, DNA extraction was performed on 40 patients and genetic ancestry was assessed using a specific panel of 46 informative ancestry markers. **Results:** We observed a predominance of European ancestry (63%), followed by African (18%), Amerindian (9%) and Asian (8%) both in the OPSCC HPV-positive and HPV-negative group. We did not find any statistically significant differences between the HPV-positive and HPV-negative OPSCC groups in relation to European ($p = 0.499$), African ($p = 0.448$), Asian ($p = 0.275$) or Amerindian ($p = 0.836$) ancestry. **Conclusions:** We found a predominance of European ancestry, both in the HPV-positive and HPV-negative groups. In our study, we did not find statistically significant differences between HPV-positive or HPV-negative groups in relation to ancestry.

Keywords

Oropharyngeal Neoplasms, Genetic Ancestry, HPV, Head and Neck Neoplasms, P16

1. Introduction

Oropharyngeal cancer is one of the most common types of cancer in men [1] [2]. According to INCA (2019), the number of new cases of oral cancer expected for Brazil, for each year of the 2020-2022 triennium, will be 11,200 cases in men and 4010 in women [3]. Nowadays, Human Papillomavirus (HPV) infection represents an important etiologic factor for Oropharyngeal Squamous Cell Carcinoma (OPSCC) [4]. Human papillomavirus represents the most frequent viral sexually-transmitted infection [4]. In Brazil, it is estimated that there are 9 to 10 million infected by this virus and every year 700 thousand new cases occur [1].

There is no specific treatment for HPV infection. There are only treatments for injuries that are caused by viruses such as genital warts. The virus is usually eliminated from the body within 2 years after contact, but in a small portion of the population, this virus is not eliminated and the long-term permanence of the virus in the body may cause the appearance of some neoplasms, including oropharyngeal cancer [4].

Several factors can be related to a patient's susceptibility to a particular biological agent [5]. Thus, different genetic ancestry could be related to different susceptibility to infections, including HPV and this makes the correlation of OPSCC with the different genomic, clinical and epidemiological profiles important.

The Brazilian population has a heterogeneous genetic constitution. This admixture is the result of more than 500 years of interethnic crosses between European colonizers, Native Americans and African slaves [6]. Due to this great miscegenation, skin color and self-declared ethnicity are often insufficient to classify or characterize an individual's ethnicity and do not always correspond with their genomic ancestry [7] [8] [9]. In addition, each region of Brazil experienced colonization from different nations and this is reflected in the ethnical differences between these populations [10]. Recent studies have shown a weak correlation between skin color and self-declared ethnicity with genetic ancestry determined by DNA markers [11] [12].

To determine the profile of genetic ancestry, the use of genetic Ancestry Informative Markers (AIMs) is described in the literature [6] [13]. These ancestry markers are made up of Insertion and Deletion Polymorphisms (INDELs) which show frequency differentials of marked alleles between ancestral or geographically distant populations. These are especially useful for inferring the likely ancestral origin of an individual or estimating the breakdown of ancestry components in individuals or populations, namely European, African, Amerindian (indigenous) and Asian backgrounds [13].

In this context, the aim of our study was to assess the hole of genetic ancestry in HPV status in OPSCC patients. For that, we used the technique described by Pereira *et al.* [13] using 46 Ancestry Informative Markers (AIMs). Based on these markers, we estimated the proportion that each patient has of each ancestry component (African, European, Asian and Amerindian).

2. Materials and Methods

2.1. Study Design and Patients

We performed a cross-sectional study from 2014 to 2019 with 40 patients diagnosed with OPSCC treated at Barretos Cancer Hospital which is a tertiary referral institution for cancer treatment in Brazil. Demographic data (sex, age, race/ethnicity, schooling) and clinical-pathological data (TNM clinical stage according to the eighth edition of the American Joint Committee on Cancer (AJCC) TNM staging system) were obtained from the medical records.

In all patients, HPV status was determined by p16 immunohistochemistry and DNA extraction was performed to characterize genetic ancestry.

2.2. HPV Status

The HPV status was determined by p16 immunohistochemistry, which is a well-established surrogate marker to characterize HPV-positive oropharyngeal tumors. The protein p16 is an important regulator of the cell cycle. It is encoded by the CDKN2 gene, also referred to as INKA4, which is a tumor suppressor gene located on human chromosome 9p21 [14] [15].

The presence of the p16 protein has been related to the infection of cells by high-risk HPV. Thus, detection of p16 protein overexpression by immunohistochemistry can be used as a marker, indicating infection by [14] [15]. The advantages of using p16 are a practical, simple and low-cost way to detect HPV [14] [15].

Formalin-fixed, paraffin embedded primary-site tissue blocks were cut to 4 μm sections. p16 Immunohistochemistry (IHC) was performed using the CINtec® p16 Histology kit (Roche MTM Laboratories, Heidelberg, Germany), according to the manufacturer's instructions. The expression of p16 was classified as positive in the presence of strong and diffuse staining in more than 75% of both nuclei and cytoplasms. Any other color pattern was classified as negative. Thus, as reported, the patients with p16 positivity by immunohistochemistry were considered to be HPV-positive [16] [17] [18].

2.3. DNA Extraction

The tumor DNA was extracted through surgical samples of paraffinized oropharyngeal tissues as previously reported by our group [19]. The paraffin blocks of the selected patients were subjected to 4 - 5 cuts of 10 μm to perform DNA isolation, using the Qiagen DNA Microkit, according to manufacturers' specifications.

2.4. Genetic Ancestry Determination

After obtaining the DNA from the selected samples, the ancestry of the patients included in the study was determined using a panel of 46 Ancestry Informative Markers (AIMs), consisting of Insertion and Deletion Polymorphisms (INDELs), as previously described [13] [20].

Following multiplex PCR with 46 primers, the amplified products were subjected to capillary electrophoresis and fragment analysis on an ABI 3500 Genetic Analyzer xL (Applied Biosystems), according to the manufacturer's instructions. PCR conditions and primer sequences were performed according to Pereira *et al.* [13] [20]. The ancestry proportion was obtained from the analysis with GeneMapper v.4.1 (Life Technologies) and Structure v.2.3.4.13.

We considered the four main groups of natives that make up the Brazilian population (African, European, Asian and Amerindian). Based on these 46 markers, we estimated the proportion that each patient has of each ancestry component (African, European, Asian and Amerindian).

The sum of the proportions of all the ancestry components of a patient resulted in the number 1 or 100%. We used the means and medians of the ancestry components of each patient to make comparisons between groups.

2.5. Data Analysis

All statistical analyses were performed using SPSS software version 21 (SPSS, Inc, Chicago, Illinois).

We correlated the mean and median of each component of genetic ancestry (European, African, Asian and Amerindian) with each variable studied.

To verify the relationships between the studied variables and genetic ancestry, we used Kruskal-Wallis and Man-Whitney tests. A p-value < 0.05 was considered statistically significant.

2.6. Ethics

The study was approved by the institutional ethics committee of Barretos Cancer Hospital, number: 1.943.689 and conducted according to the Helsinki declaration. Informed consent was obtained from all subjects or their nominees prior to participationin the study.

3. Results

3.1. Patient Population

A total of 40 patients were included in the study. The clinic and pathologic variables are summarized in **Table 1**. The majority were men (n = 34, 85%), and the most frequent age group was >50 years, (n = 30, 75%). Regarding race, the majority (n = 25, 62.5%) self-declared as nonwhite (brown, black, Asian, indigenous) and 15 (37.5%) of the patients self-declared as white. Regarding HPV status, 16 (40%) were HPV-positive and 24 (60%) were HPV-negative.

Table 1. Clinical-pathological characteristics of oropharyngeal squamous cell carcinoma patients.

Characteristic	n	(%)
Sex		
Male	34	85
Female	6	6.15
Age		
<50 Years	10	25
>50 Years	30	75
Self-declared Race/Ethnicity		
White	15	37.5
Other	25	62.5
Schooling		
Illiterate	10	25
Elementary School	13	33.8
High School	8	20.6
Higher	8	20.6
Clinicalstage[a]		
I and II—Early	11	28.2
III and IV—Advanced	29	71.8
P16 Status		
Negative	24	60
Positive	16	40

Note: a: UICC 8th Edition.

3.2. Genetic Ancestry

The genetic ancestry was performed in all cases. In Table 2, we observe the composition of ancestry in the population studied, where we obtained a predominance of European ancestry, with the following averages of ancestry proportions: European 0.63, African 0.18, Asian 0.08 and Amerindian 0.09 (Table 2).

Next, we analyzed the composition of the ancestry markers in the HPV-positive and HPV-negative groups and observed a predominance of proportion of European ancestry in both groups (Figure 1). No statistical significance was observed when comparing HPV-positive and HPV-negative groups in relation to European ($p = 0.499$), African ($p = 0.448$), Asian ($p = 0.275$) or Amerindian ($p = 0.836$) ancestry (Table 3).

In Figure 1, we observe a graphical representation of the estimated ancestry of HPV-positive and HPV-negative groups. Each vertical bar represents a case (Figure 1).

In order to assess the role of each distinct ethnicity in the epidemiological and

pathological profile, we independently analyzed the markers of the 4 ethnic groups and associated them with some variables (Table 3). We observed that African ancestry is associated with a self-declared black patients (p = 0.040) (Table 3) while European ancestry was significantly associated with self-reported white patients (p = 0.005). Asian and Amerindian ancestry markers did not show a significant association with self-reported color (Table 4, Table 5).

4. Discussion

In this study, we found a predominance of European ancestry, both in HPV-positive and HPV-negative patients. We also found that African ancestry was significantly associated with self-reported black color and the absence of cervical metastases and European ancestry for self-reported white patients.

We chose to identify the hyperexpression of the p16 protein as a substitute marker for HPV, due to the low cost and high correlation with HPV positivity, as has been widely reported in several studies [21] [22] [23].

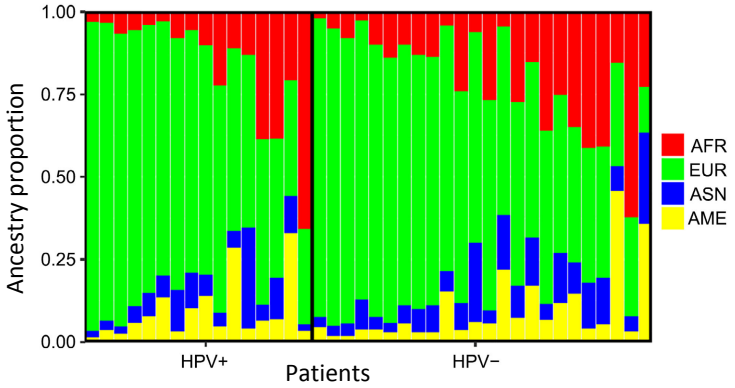

Figure 1. Graphical representation of the estimated ancestry of HPV-positive and HPV-negative groups from the genotyping data of the 46 AIMs panel. Each vertical bar represents a case. The ancestral proportion (percentage) is represented in colors: Red—African; Green—European; Blue—Asian; Yellow—Amerindian.

Table 2. Composition of genetic Ancestry in the studied patients.

Ancestry	N	Mean	Median
African	40	0.18 (0.16)	0.13 (0.02 - 0.66)
European	40	0.63 (0.21)	0.66 (0.14 - 0.94)
Asian	40	0.08 (0.07)	0.07 (0.02 - 0.31)
Amerindian	40	0.09 (0.10)	0.06 (0.0 - 0.46)

Table 3. Distribution of genetic ancestry according to HPV status.

HPV Status	African	European	Asian	Amerindian
HPV-negative (N = 24)	0.19	0.61	0.09	0.10
HPV-positive (N = 16)	0.17	0.67	0.08	0.09

Table 4. Association between African and European ancestry and clinical-pathological variables.

		African Ancestry				European Ancestry		
		N	Mean	Median	p-value	Mean	Median	p-value
Sex[a]	Male	34	0.17	0.13 (0.02 - 0.62)	0.820	0.62	0.64 (0.14 - 0.90)	0.677
	Female	6	0.24	0.16 (0.03 - 0.66)		0.67	0.73 (0.29 - 0.94)	
Age[b]	<50	10	0.2	0.11 (0.04 - 0.62)	0.803	0.60	0.60 (0.30 - 0.90)	0.887
	>50	30	0.22	0.14 (0.03 - 0.66)		0.66	0.69 (0.35 - 0.94)	
Schooling[b]	Illiterate	10	0.19	0.182 (0.03 - 0.62)	0.904	0.58	0.63 (0.13 - 0.84)	0.710
	elementary School	13	0.19	0.13 (0.04 - 0.66)		0.64	0.73 (0.28 - 0.86)	
	High School	8	0.16	0.104 (0.02 - 0.40)		0.67	0.65 (0.39 - 0.90)	
	Higher	8	0.2	0.19 (0.03 - 0.39)		0.69	0.69 (0.42 - 0.94)	
Self-declarated race/ethnicity[b]	White	15	0.13	0.07 (002 - 0.41)		0.75	**0.79 (0.41 - 0.94)**	
	Brown	20	0.18	0.14 (0.03 - 0.41)		0.6	0.60 (0.31 - 0.86)	
	Black	4	0.44	**0.44 (0.23 - 0.66)**	**0.040***	0.34	0.29 (0.14 - 0.86)	**0.005***
	Asian	1	0.13	0.13 (0.13 - 0.13)		0.52	0.52 (0.52 - 0.52)	
	Indigenous	0	-	-		-	-	
Clinical Stage[a]	Early (I/II)	11	0.16	0.13 (0.03 - 0.38)	0.778	0.66	0.68 (0.42 - 0.94)	0.779
	Advanced (III/IV)	29	0.2	0.14 (0.02 - 0.66)		0.62	0.64 (0.14 - 0.90)	
P16 Status[a]	Negative	24	0.19	0.14 (0.02 - 0.62)	0.448	0.61	0.64 (0.14 - 0.90)	0.499
	Positive	16	0.17	0.10 (0.03 - 0.66)		0.67	0.71 (0.29 - 0.94)	

Note: a: Analysis by Mann-Whitney test; b: Analysis by Kruskal-Wallis test. * Statistically significant difference $p \leq 0.05$.

Table 5. Association between Asian and Amerindian ancestry and clinical-pathological variables.

		Asian Ancestry				Amerindian Ancestry		
		N	Mean	Median	p-value	Mean	Median	p-value
Sex[a]	Male	34	0.10	0.08 (0.02 - 0.31)	0.092	0.10	0.05 (0.02 - 0.46)	0.495
	Female	6	0.05	0.06 (0.02 - 0.07)		0.07	0.06 (0.00 - 0.14)	
Age[b]	<50	10	0.11	0.08 (0.03 - 0.31)	0.232	0.08	0.05 (0.02 - 0.22)	0.038
	>50	30	0.08	0.07 (0.02 - 0.15)		0.09	0.05 (0.00 - 0.33)	
Schooling[b]	Illiterate	10	0.10	0.08 (0.04 - 0.27)	0.427	0.12	0.09 (0.03 - 0.36)	0.56
	Elementary School	13	0.09	0.07 (0.02 - 0.24)		0.08	0.06 (0.02 - 0.33)	
	High School	8	0.10	0.05 (0.03 - 0.31)		0.07	0.04 (0.02 - 0.28)	
	Higher	8	0.06	0.05 (0.02 - 0.15)		0.06	0.04 (0.00 - 0.17)	
Self-declarated Race/Ethnicity[b]	White	15	0.07	0.06 (0.02 - 0.15)	0.137	0.06	0.04 (0.01 - 0.17)	0.465
	Brown	20	0.09	0.08 (0.04 - 0.24)		0.12	0.07 (0.00 - 0.46)	
	Black	4	0.09	0.04 (0.02 - 0.27)		0.12	0.04 (0.03 - 0.36)	
	Asian	1	0.31	0.31 (0.31 - 0.31)		0.04	0.04 (0.04 - 0.04)	
	Indigenous	0	-	-		-	-	
Clinical Stage[a]	Early (I/II)	11	0.10	0.06 (0.02 - 0.31)	0.708	0.10	0.10 (0.01 - 0.28)	0.296
	Advanced (III/IV)	29	0.09	0.07 (0.02 - 0.27)		0.09	0.05 (0.00 - 0.46)	
P16 Status[a]	Negative	24	0.09	0.08 (0.03 - 0.27)	0.275	0.10	0.06 (0.02 - 0.46)	0.836
	Positive	16	0.08	0.06 (0.02 - 0.31)		0.09	0.05 (0.00 - 0.33)	

Note: a: Analysis by Mann-Whitney test; b: Analysis by Kruskal-Wallis test. * Statistically significant difference $p \leq 0.05$.

The Brazilian population has great genetic heterogeneity, mainly due to the miscegenation of the native populations of the Americas (Amerindians), the colonizing populations (Europeans) and the slaves brought from Africa (Africans) [12] [24].

In our study, we determined the percentages of African, European, Amerindian and Asian ancestry for patients with OPSCC, using the AIMs panel (Ancestry-informative Markers). We found a predominance of the European component (63%) followed by African (18%), and a balance between the proportions of the Amerindian (9%) and Asian (8%) components.

This predominance of the European component in the ancestry of Brazilians has been evidenced by several studies [12] [24] [25] [26] [27]. In the study by Leite *et al.* [12], 172 patients showed 69% European ancestry, 21% African ancestry and 10% Amerindian ancestry, respectively. In another study [26] with 934 patients from various regions of Brazil, Leite *et al.* identified European ancestry ranging from 60.6% to 77.7%, African from 10.9% to 30.3% and Amerindian from 7.4% to 19.4%. Another study by Lins *et al.* [13], conducted on 189 women, found that European ranged from 69.5% to 87.7%, African from 7% to 18.7% and Amerindian from 5.2% to 11.8%, respectively.

These data show the strong European influence on the genetic composition of the Brazilian population.

We found no relationship between HPV status and African ($p = 0.448$), European ($p = 0.448$) Asian ($p = 0.275$) or Amerindian ($p = 0.836$) genetic ancestry.

We found no studies that compared genetic ancestry with HPV status. We did find, however, a correlation between self-declared white ethnicity and HPV positivity described in the literature as in the study by Settle *et al.* [28]. In this study, a higher prevalence of HPV (+) in white patients was found. A possible explanation for not finding a correlation in our study is due to the great miscegenation of the Brazilian population.

When we analyzed the components of ancestry separately, we found that the African ancestral component is significantly associated with self-declared black patients and the European ancestral component was significantly associated with self-declared white patients. The study by Leite *et al.* [12] analyzed 172 Brazilian patients whose self-declared color correlated with the amount of melanin and the genomic ancestry pattern based on 21 AIM. They observed a low relationship between the self-declared color and the ancestral profile. Patients who Self-reported as white had 72% European ancestry, followed by 19% African and 9% Amerindian. Participants who declared themselves brown had 68% European ancestry followed by 21% African and 11% Amerindian, respectively. In contrast, the self-declared black patient group had 63% European ancestry, 27% African and 10% Amerindian, respectively.

The study of Lins *et al.* [24] analyzed 189 women in the climacteric period and also compared the self-declared color with genomic ancestry based on 13 AIM. He also found a low correlation between the self-declared color and the ancestral profile. Lins *et al.* found 73.8% European ancestry in individuals who declared

themselves to be white, followed by 17.2% African and 9% Amerindian. Participants, who declared themselves brown had 61.5% European ancestry followed by 25.6% African and 14.1% Amerindian, while patients who self-declared as black, had 62.9% European ancestry, 25.4% African and 11.7% Amerindian.

We did not find a correlation between genetic ancestry and clinical staging. The studies found in the literature are controversial, but most studies show that blacks have a tendency to have more advanced clinical stages than white [29] [30] [31]. Black patients also have a tendency to have advanced T and advanced N compared to white patients [31] [32].

Thus, more studies should be carried out on the prevalence of HPV in oropharyngeal carcinomas, to demonstrate the importance of this topic and to understand this pathology.

This study contributes to the understanding of racial disparities in OPSCC. While previous studies have focused specifically on self-declared skin color, our study demonstrates the whole of genetic ancestry in OPSCC.

5. Conclusions

We found a predominance of European ancestry, followed by African ancestry and a balance between the proportions of the Asian and Amerindian components both in the groups HPV-positive and HPV-negative OPSCC. In our study, we did not find any statistically significant differences between HPV-positive or HPV-negative groups in relation to ancestry.

Although we did not find a direct relationship between ancestry and HPV positivity in our study, the use of these ancestry markers may be used in future studies to better understand the relationship between genetic ancestry, head and neck and HPV tumors.

Acknowledgements

We thank the Barretos Cancer Hospital and the Nucleus of Epidemiology and Statistics of Barretos Cancer Hospital for their support in statistical analysis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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