Pathology – Research and Practice xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Short Communication

Are HLA-E*0103 alleles predictive markers for nasopharyngeal cancer risk?

Hayet Douik[a,*], Neila Attia Romdhane[b], Fethi Guemira[a]

[a] Clinical Biology Department, Salah Azaiz Institute of Cancer, 1006 Bab Saadoun, Tunis, Tunisia
[b] Statistic and Epidemiology Department, Faculty of Medicine, 1007, Djebel Lakhdar, La Rabta, Tunis, Tunisia

ARTICLE INFO

Article history:
Received 19 June 2015
Accepted 26 January 2016

Keywords:
HLA-E
Polymorphism
Nasopharyngeal cancer
Prognosis

ABSTRACT

Background: Nasopharyngeal carcinoma (NPC) is a particular entity of head neck cancer, tightly related to Epstein–Barr virus infection and thus to HLA genes. In this study, we aimed to analyze HLA-E polymorphism in NPC advent and prognosis.

130 unrelated patients with CNP and 180 unrelated and healthy controls were included in our study. HLA-E genotyping was performed by PCR/RFLP method; SPSS (13.0) was used for statistical analysis, and survival curbs were established with the "Kaplan–Meier" method (Log Rank < 0.05).

Results: We found a significant difference within HLA-E*103 variants between patients and controls: E*1031 and E*1032 were associated with CNP (OR = 1.613, $p = 0.013$ and OR = 1.0809, $p = 0.055$), and E*1033 with controls (OR = 0.254, $p < 10^{-4}$).

Conclusion: Our study reveals that HLA-E polymorphism is associated with nasopharyngeal cancer. HLA-E expression studies could be used to understand the implication of E*103 variants.

1. Background

Nasopharyngeal carcinoma (NPC) is one of the most common head and neck cancers in most of the countries of the Mediterranean Area and North Africa, with an incidence varying from 1 to 50/100,000 [1]. In Tunisia, NPC is the first ORL cancer in women and the 2nd, after larynx, in men. It represents 34% of the upper airway cancers, with a frequency of 3–4/100,000 [2].

Another particularity of this cancer, in Magrebian population, is the bimodal profile of the age of diagnosis with a first little peak at 20 years and a second, most important, around 55 years.

The original distribution of NPC around the world could be explained by the tight imbrication of its etiological factors, mostly the Epstein–Barr virus (EBV) infection, the dietary nitrosamines implication and diverse genes interactions [3–6]. In fact, EBV is consistently detected in patients with nasopharyngeal carcinoma from regions of high and low incidence, and the detection of nuclear antigen associated with Epstein–Barr virus (EBNA) and viral DNA in NPC type 2 and 3 (undifferentiated carcinoma) has revealed that EBV can infect epithelial cells and is associated with their transformation [3,7,8]. Because EBV infection is common in NPC, human leukocyte antigens (HLA) have been proposed to be important, given their central role in the presentation of viral antigens to the immune system, and recently, completed genome-wide association studies (GWAS) confirmed the strong evidence for NPC association within the Major Histocompatibility Complex (MHC), particularly for HLA-A and HLA-B alleles [11–16]. These molecules, with HLA-C, belong to the classical HLA class I family and are involved in the presentation of foreign peptide antigens including EBV-derived peptides [12]. In the same MHC region, on 6p21.3, are located the non-classical HLA I molecules: the MHC class Ib members include HLA-E, -F, -G and HFE (HLA-H); these molecules are best known to regulate innate immune responses, but it has been demonstrated that HLA-E and -G present antigenic peptides to T cells, playing a role in acquired immune responses to bacteria and viruses [17,18]. Unlike HLA-class I molecules, these molecules are less polymorphic, and HLA-E is the least polymorphic of the MHC class I with only one functional polymorphism in codon 107. Thus, an arginine at position 107 in HLA-E*0101 ($HLA\text{-}E^{107R}$) is replaced by a glycine in HLA-E*0103 ($HLA\text{-}E^{107G}$) conferring a significantly higher expression of $HLA\text{-}E^{107G}$ on normal cells, a higher affinity for available peptides, and thus a higher stability than $HLA\text{-}E^{107R}$ [17,19]. Recent Tunisian observations found an association between non synonymous HLA-G polymorphisms and NPC risk but none with HLA-E functional polymorphism [20,21]. In this case-controls study, we analyzed HLA-E alleles role in nasopharyngeal cancer risk and prognosis.

* Corresponding author.
E-mail address: htdouik@yahoo.fr (H. Douik).

http://dx.doi.org/10.1016/j.prp.2016.01.010
0344-0338/

2. Methods

2.1. Study population

130 unrelated patients with histologically confirmed Undifferentiated Carcinoma of Nasopharyngeal Type (UCNT) were enrolled at the Institute of cancer Salah Azaiz. There were 80 men (61.5%) and 50 women (38.5%), with a mean age of 43 years (range 11–80 years).

Patients were compared to 180 healthy and unrelated subjects taken as controls: 102 women (56.7%) and 78 men (43.3%) with a mean age of 54 years (range 38–70 years).

For all the subjects, a form of consent was given.

2.2. Molecular study

Genomic DNA was isolated from peripheral blood leukocytes by phenol/chloroform method.

HLA-E variants were spotted by PCR/RFLP technique: exons 2 and 3 of HLA-E gene were amplified by PCR using 3 primers pair (ex2F: 5′gAA ACg gCC TCT ACC ggg AgT Ag 3′; ex2R: 5′gTT CCg CAg CCT Tgg ggT gAA TC 3′; ex3F: 5′Cgg gAC TgA CTA Agg ggC 3′; ex3R: 5′ AgC CCT gTg gAC CCT CTT 3′; ex3MF: 5′ggC TgC gAg CTg ggg CCC gCC 3′ with ex3R). Then, PCR products were digested overnight at 37 °C by 5 different restriction enzymes (HaeII, HinfI, BglI, NlaIV and HpaII) to detect the different HLA-E variants on 4% agarose gel.

2.3. Statistical analysis

SPSS (13.0) was used to compare allele frequencies in patients and controls, and to analyze clinical data ($p < 0.05$). The Chi-Square test was used.

The Kaplan–Meier method was used to establish survival curbs (Log Rank < 0.05).

3. Results

3.1. Patients' clinical data

Patients have a mean age of 43 years, with a characteristic Mediterranean bimodal curve of age: a first peak around 20 years, and a second peak at 45 (Fig. 1). The majority of patients consult 11 months after the discovery of the primary symptoms (range: 1 month–20 years), and young patients (<40 years) consult within 6 months after primary symptoms. The reasons of medical advice are numerous but not isolated: headache (44%), earache (33%), epistaxis (40%), nasal obstruction (46%), tinnitus or hearing loss (20%) and adenopathies (56%). Using the TNM classification according to UICC/AJCCC [20], 112 patients (86.2%) were classified as stage IV, 8 (6.2%) were stage III, 4 (3.1%) stage II, 1 (0.8%) stage I, and 5 patients (3.7%) could not be classified.

The global survive without relapse was 57% at 10 years (Fig. 2), and young patients have significant, best survival at 10 years, possibly because they consult earlier (Fig. 3).

3.2. Distribution of HLA-E variants in patients and controls

HLA-E genotyping reveals four distinct alleles in our study population: 2 functional variants HLA-E*0101 and HLA-E*01031 and 2 silent variants HLA-E*01032 and HLA-E*01033. Genotypes distribution did not show any difference between the two groups regarding E*0101 and E*0103 combinations, but HLA-E alleles are unequally distributed in patients and controls: in fact HLA-E*01031 and HLA-E*01032 are more frequent in patients and could be considered as risk factors for NPC with, respectively, Odds Ratio = 1.613 (95% CI 51.10–2.36)) for HLA-E*01031 and 1.809

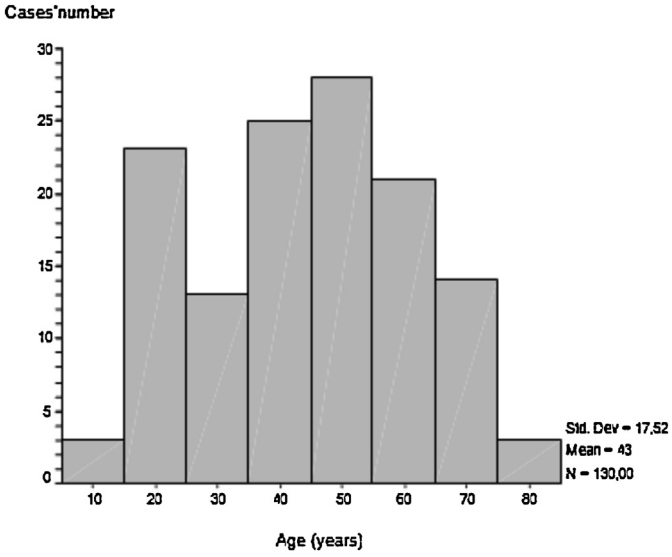

Fig. 1. Patients' age distribution. In this histogram, we can note the two picks in patients' age distribution (a first pick at 20 years, the second at 45 years) characteristic of the Maghrebin bimodal age's distribution for NPC.

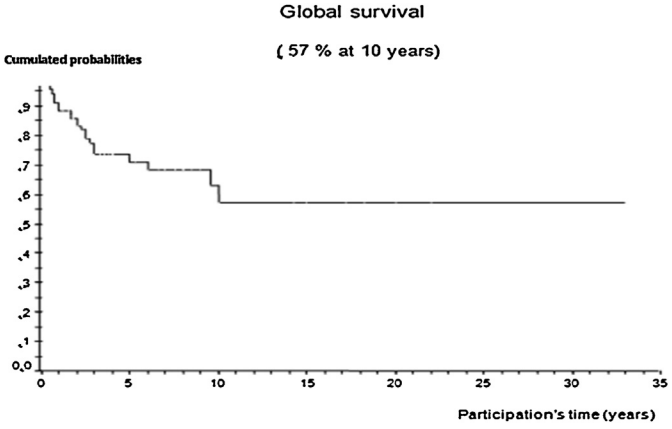

Fig. 2. Global survival. The curb reveals a global survival of 57% at 10 years.

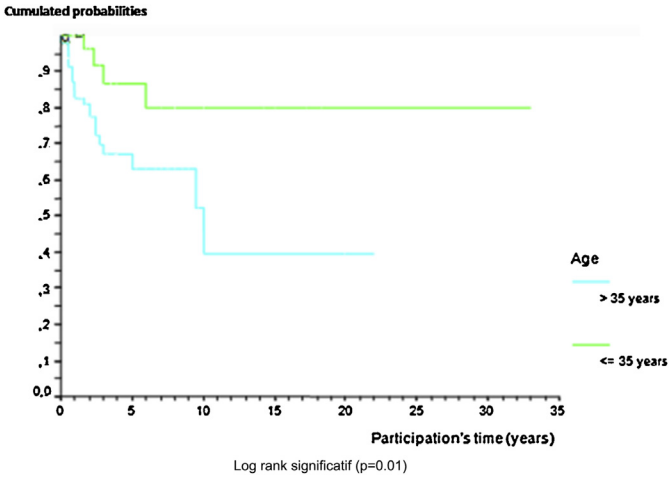

Fig. 3. Survival correlated to patients' age. Survival keeping with patients' age clearly shows that young patients (<35 years) have a twice better survival at 10 years than old patients (80% versus 40%).

Please cite this article in press as: H. Douik, et al., Are HLA-E*0103 alleles predictive markers for nasopharyngeal cancer risk? Pathol. – Res. Pract (2016), http://dx.doi.org/10.1016/j.prp.2016.01.010