Involvement of HPV Infection in the Release of Macrophage Migration Inhibitory Factor in Head and Neck Squamous Cell Carcinoma

Dr. Basavaraj T Bhagawati[1], Dr. Anuj Maheshwari[2], Dr. Neeta Mishra[3]

[1]MDS, Professor and Head Oral Medicine and Radiology SDCRI Sriganganagar, Rajasthan, India
[2]MD, Professor and Head, Department of General Medicine, BBDCODS, Lucknow, U.P., India
[3]MDS, Professor and Head, Department of Oral Medicine and Radiology, BBDCODS Lucknow, U.P., India

Corresponding Author: Dr. Basavaraj T Bhagawati, Email: drbasavrajb@yahoo.com

Abstract

Background: To study the involvement of HPV infection in the release of macrophage migratory inhibitory factor in head and neck squamous cell carcinoma.**Materials & methods:** A total of 100 subjects were enrolled. 50 were oral cavity cancer patients and 50 were oropharyngeal carcinomas. In orophayngeal cancer patients 30 were male and 30 were female whereas in oral cavity cancer patients 33 were male and 17 were female. . Statistical analysis was done using SPSS software and results were obtained. A $p \leq 0.05$ was considered to indicate a statistically significant difference.**Results:** There is no statistical correlations between MIF expression and gender, histological grade recurrence rate and risk factors including HPV status . A staining intensity analysis demonstrated that oropharyngeal and oral cavity cancer tissues infected with transcriptionally active HPV (p16+) showed a decrease in MIF expression compared to oropharyngeal and oral cavity cancer tissues not infected by HPV (n = 25 and n = 30 respectively) p = 0.001 and p = 0.004 respectively. Under hypoxia, HPV-positive cell lines did not secrete significantly more MIF than they did under normoxia.**Conclusion:** There is decrease in MIF expression as comparing both the cases infected with HPV and not infected with HPV.

Keywords: MIF, HPV, cancer, head and neck.
DOI Number: 10.14704/NQ.2022.20.12.NQ77207 **NeuroQuantology2022;20(12): 2295-2306**

Introduction

Head and neck squamous cell carcinomas (HNSCC) have been intensively studied regarding their high incidence worldwide, ranking fifth among most frequent cancers in men and eleventh in women. [1] The majority of HNSCCs are traditionally related to tobacco and alcohol exposure, but the high-risk human papilloma virus infection is now recognized as a causal agent responsible for the development of a subset of oropharyngeal carcinomas. [2,3] Although some differences in terms of prevalence are reported between Europe, US and Asia, the trends of HPV-related HNSCC continue to rise significantly, unlike to the non-HPV related HNSCC which has declined for 30 years. [4] HPV infection is predominantly assigned to types HPV-16 and HPV-18, but geographical heterogeneities of 60% in the US and 31% in Europe have been reported in recent studies. [5] HNSCC can be classified into two groups, depending to their risk factors: firstly, HNSCC occurring in young people (≤40 years old) who never smoke/drink, which is usually associated with HPV infection; and, secondly, HNSCC associated with smoking and drinking habits occurring in non-infected older people (>40 years old). [6,7] This classification makes sense according to the HNSCC prognosis.

Macrophage migration inhibitory factor (MIF) is an ubiquitous pro-inflammatory cytokine discovered in 1966 by Bloom and Bennett. [8] This cytokine has been investigated in many clinical and experimental studies in both inflammatory diseases and cancer. Indeed, MIF is involved in cancer progression through different pathways leading to cell proliferation, cell invasion, angiogenesis and tumor immune escape. [9] Implications for MIF in HNSCC have already been reported in several studies, which have established that MIF leads to cancer progression and poorer prognosis, notably in laryngeal carcinoma. [10]

Human papilloma virus (HPV) was identified as an additional contributor leading to the

eISSN 1303-5150

development of a new subgroup of HNSCC, mainly associated with HPV-16 and HPV-18 types. [11] The impact of HPV infection remains controversial concerning the prognostic values of HNSCC, but recent studies put to light a new blood test that contributes to the detection of circulating tumor HPV DNA (notably HPV-16) and that can predict cancer recurrence. [12] Hence, this study is conducted to study the involvement of HPV infection in the release of macrophage migratory inhibitory factor in head and neck squamous cell carcinoma.

Materials & methods

A total of 100 subjects were enrolled in the Department of Oral Medicine and radiology at Saraswati Dhanwantari Dental College and Hospital, Parbhani. 50 were oral cavity cancer patients and 50 were oropharyngeal carcinomas. In orophayngeal cancer patients 30 were male and 30 were female whereas in oral cavity cancer patients 33 were male and 17 were female. All the detailed information, medical history was collected. Investigations were done. Statistical analysis was done using SPSS software and results were obtained. A $p \leq 0.05$ was considered to indicate a statistically significant difference.

Results

The immunohistochemical staining of MIF was examined in two series of 50 cases of oral cavity, and 50 cases of oropharyngeal carcinomas. There is no statistical correlations between MIF expression and gender, histological grade recurrence rate and risk factors including HPV status . A staining intensity analysis demonstrated that oropharyngeal and oral cavity cancer tissues infected with transcriptionally active HPV (p16+) showed a decrease in MIF expression compared to oropharyngeal and oral cavity cancer t issues not infected by HPV (n = 25 and n = 30 respectively) p = 0.001 and p = 0.004 respectively.

Table 1: characteristics of oropharyngeal and oral cancer patients

Characteristics	Oraoharyngeal cancer patients	Oral cavity cancer patients
Gender		
Male	30	33
Female	20	17
Histological grade		
Well differentiated	26	10
Moderately differentiated	12	28
Poorly differentiated	5	9
Unknown	7	3
Recurrence		
Yes	24	14
None	18	28
Unknown	8	8
Risk factor HPV status		
HPV -ve	25	30
HPV +ve/p16+	2	2
HPV+ve/p16-	15	16
HPV+ve/ p16 unknown	8	2

Under hypoxic conditions, which stimulate HIF-1α expression and activity, HPV-negative cell lines) released more MIF in the culture medium ($p < 0.001$) than did the HPV-positive ones, thus demonstrating that the increase in HIF-1α led to the rise in MIF secretion. Under hypoxia, HPV-positive cell lines did not secrete significantly more MIF than they did under normoxia; this effect was potentially due to a high baseline level of HIF-1α expression and MIF secretion in these cells.

Discussion

HPVs are small circular double-stranded DNA viruses (±8000 base pairs), non-enveloped, that can infect epithelial cells especially those present in the upper-aerodigestive tract. Those viruses can be classified into cutaneous

(responsible for genital warts) or mucosal types, mucosal HPV types being mostly found in potentially malignant and cancerous lesions of the epithelium, leading to their classification as "high-risk HPVs" (HPV-16, -18, -31, -33, -34, -35, - 39, -45, -51, -52, -56, -58, -59, -66, -68, -70). [13,14] To date, there is no specific antiviral treatment against HPV infections, with most of these infections (up to 90%) being asymptomatic and resorbing spontaneously within 2 years. For this reason, priority is given to screening and, in the case of lesions in the cervix, to the treatment of pre-invasive cervical lesions, particularly via burn ablation, cryotherapy or surgical excision. [15]

Vaccination is the only way to prevent HPV infection, because it is done at the time of puberty (from the age of 9), so before the beginning of sexual life and the exposure to the virus. Three prophylactic vaccines are currently available worldwilde: Gardasil®, Cervarix® and Garadasil-9®, both using L1 virus-like particles (VLP), which will generate neutralizing antibodies against HPV major capsid protein L1. Gardasil® (Merck & Co., Whitehouse Station, NJ, USA), the first L1 VLP recombinant vaccine approved by the US Food and Drug Administration (FDA) in 2006, protects against two low-risk HPV subtypes -6 and -11, and against two high-risk HPV types -16 and -18. This vaccine is produced by using a yeast substrate and amorphous aluminium hydroxyphosphate sulfate (AAHS) as adjuvant. Each dose of Gardasil® vaccine contains 20 µg, 40 µg, 40 µg and 20 µg of L1 protein of HPV-6, -11, -16 and -18 respectively, adsorbed on 225 µg of AAHS. [16]

In our study, the immunohistochemical staining of MIF was examined in two series of 50 cases of oral cavity, and 50 cases of oropharyngeal carcinomas. There is no statistical correlations between MIF expression and gender, histological grade recurrence rate and risk factors including HPV status.

According to the study conducted by lechien JR et al, head and neck squamous cell carcinomas (HNSCC) are one of the most prevalent cancers worldwide. Active human papillomavirus (HPV) infection has been identified as an important additional risk factor and seems to be associated with a better prognosis in non-drinker and non-smoker young patients with oropharyngeal SCC. The better response of the immune system against the HPV-induced HNSCC is suspected as a potential explanation for the better prognosis of young patients. They review the preventive (HPV vaccines) and therapeutic (checkpoint inhibitors) strategies against HPV-related HNSCC, stressing the use of anti-CTLA4, PD-L1, PD-L2 antibodies alone and in combination with other agents able to modulate immune responses. [17] In our study, a staining intensity analysis demonstrated that oropharyngeal and oral cavity cancer tissues infected with transcriptionally active HPV (p16+) showed a decrease in MIF expression compared to oropharyngeal and oral cavity cancer tissues not infected by HPV (n = 25 and n = 30 respectively) p = 0.001 and p = 0.004 respectively.

Other researchers have demonstrated that the E6 and E7 oncoproteins also take part in a perturbation of cellular metabolism termed the Warburg effect, which is a hallmark of cancer cells. Indeed, it was demonstrated that E6 interacts with the transcription factor HIF-1α to induce glycolysis under hypoxia. [18] Moreover, previous studies have shown that the E6 oncoprotein promotes the activity of the mammalian target of rapamycin (mTOR) signaling pathway, thus leading to the accumulation of HIF-1α, pyruvate kinase, lactate dehydrogenase, and pyruvate dehydrogenase kinase 1. [19]

Conclusion

There is decrease in MIF expression as comparing both the cases infected with HPV and not infected with HPV and there is increase in lactate production and hypoxia inducible factor 1α (HIF-1α) expression, which finally induces MIF secretion.

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eISSN 1303-5150