Faculty and PostDoc,
Medical, Biomedical and Health Sciences

Co-incidence of HPV and EBV is Associated with Advanced Tumor Stage: A Tissue Microarray of Head and Neck Cancers

Ishita Gupta[1], Hamda Al-Thawadi[1], Faruk Skenderi[2], Mohammed I. Malki[1] Semir Vranic[1], & Ala-Eddin Al Moustafa[1,3]

[1]College of Medicine, Qatar University, Doha, Qatar; [2]Pathology Department, Clinical Center, University of Sarajevo, Bosnia;

[3] Biomedical Research Centre, Qatar University, Doha, Qatar

Background

Viral infections by high-risk human papillomaviruses (HPVs) and Epstein-Barr virus (EBV) are associated with the development and/or progression of head and neck (HN) carcinomas.

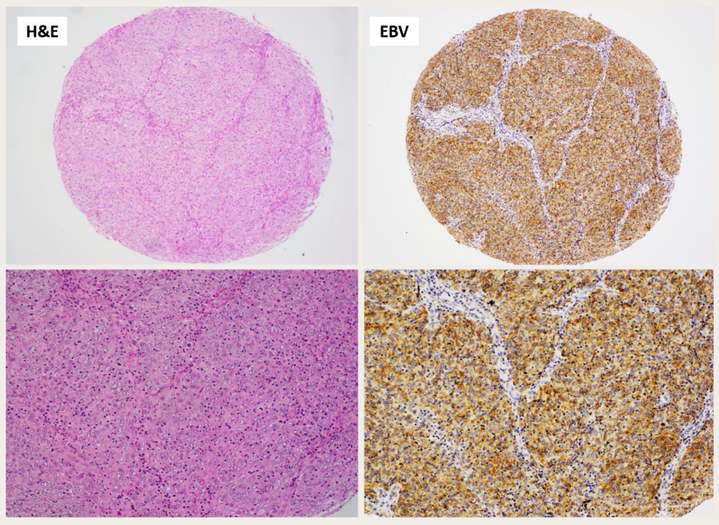
We have reported the prevalence of both HPVs and EBV in cervical and breast cancers. We herein explore for the first time the co-prevalence of high-risk HPVs and EBV in 98 head and neck (HN) squamous cell carcinoma (SCC) tissues from Bosnian patients.

Materials and Methods

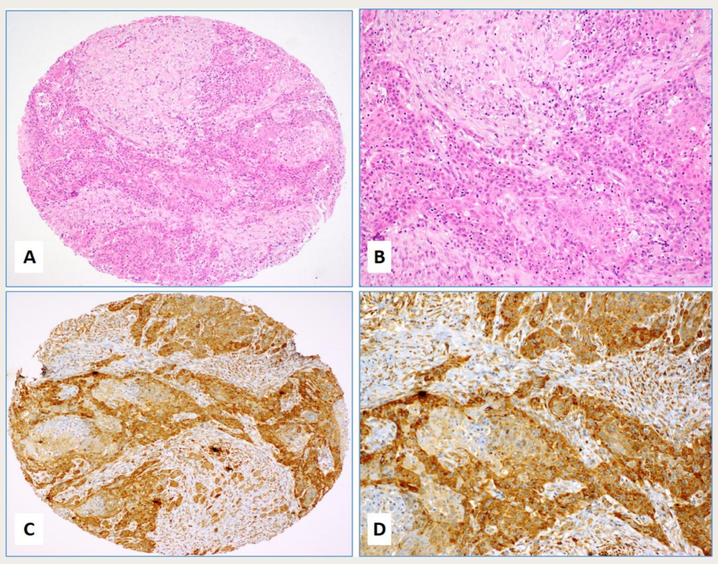
Nighty-eight samples of oral SCC were explored using tissue microarray for LMP1 (clone1–4, DAKO Agilent) and HPV-E6 (clone C1P5, Calbiochem, Canada) using IHC analysis. The tumors were considered positive for LMP1 and E6 onco-proteins if cancer cells exhibited positivity ≥1%.

Five micrograms of purified genomic DNA (Qiagen) from each sample was analyzed for EBV and HPV by PCR using specific primers for LMP1 as well as E6/E7 of HPV while, primers for GAPDH gene were used as an internal control.

Results



Figures 1A-D. The case of moderately differentiated (G2) oral squamous cell carcinoma (A-B; Hematoxylin and Eosin staining) with a diffuse and strong expression of E6 protein of HPV by immunohistochemistry (A and C figures, 4x; B and D figures 20x).



Figures 2A-D. The case of poorly differentiated (G3) oral squamous cell carcinoma (A-B; Hematoxylin and Eosin staining) that was diffusely and strongly positive for LMP1 protein of EBV; This cancer also strongly co-expressed E6 protein of HPV (the image in the right upper corner of B) (A-C, 4x; B-D 20x).

Stages	HPV+/-/EBV+/- (%)	HPV+/EBV+ (%)	*p-value*
pT1	16 (29)	2 (3.6)	***p=0.035****
pT2	14 (25.4)	5 (9)	
pT3	4 (7.2)	5 (9)	
pT4	4 (7.2)	5 (9)	
Total	**38 (69)**	**17 (30.9)**	

Table 1. Co-expression of LMP1 of EBV and E6 of high-risk HPVs (EBV+/HPV+) is associated with advanced tumor stage (*p=0.035*)

Tumor Grade			
Degree of Differentiation	**HPV (E6) Positive (%)**	**HPV (E6) Negative (%)**	***p-value***
Grade 1	6 (8.6)	9 (13)	***p=0.02****
Grade 2	23 (33.3)	20 (28.9)	
Grade 3	10 (14.4)	1 (1.4)	
Total	**39 (56.5)**	**30 (43.4)**	
Tumor Stage (pT)			
Stages	**HPV (E6) Positive (%)**	**HPV (E6) Negative (%)**	***p-value***
pT1	3 (4.4)	14 (20.8)	***p<0.001****
pT2	10 (14.9)	12 (17.9)	
pT3	13 (19.4)	2 (2.9)	
pT4	10 (14.9)	3 (4.4)	
Total	**36 (53.7)**	**31 (46.2)**	
pN Stage			
Stages	**HPV (E6) Positive (%)**	**HPV (E6) Negative (%)**	***p-value***
pN0	11 (29.7)	8 (21.6)	***p=0.045****
pN1	8 (21.6)	2 (5.4)	
pN2	7 (18.9)	1 (2.7)	
Total	**26 (70.2)**	**11 (29.7)**	

Table 2. HPV positivity correlated strongly with tumor grade (*p=0.02*), tumor stage (pT) (*p<0.001*) and advanced pN stage (*p=0.045*)

Conclusion

- HPV and EBV oncoviruses are co-present in HNSCC (35%); with a significant correlation between the various HPV types and EBV co-incidence.
- We report the co-presence of HPV and EBV is strongly correlated with advanced tumor stage.
- Co-presence of these viruses may be involved in the initiation and/or progression of HNSCC.