Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/1983
DOI URL: http://dx.doi.org/10.21474/IJAR01/1983
ISSN NO. 2320-5407

RESEARCH ARTICLE

EVALUATION OF IMMUNOHISTOCHEMISTRY OF TP53 GENE WITH RISK OF SQUAMOUS CELL CARCINOMA HEAD AND NECK (HNSCC) IN KASHMIRI POPULATION.

Dr Shakeel Mohmad Wani[1], Dr Shahnaz Sheikh[1], Dr Parth Jigishbhai Amin[2], Professor(Dr) Ruby Reshi[3] and Dr Umeek Jeelani[2].

1. Resident ENT HNS, SMHS Hospital, Srinagar, Kashmir, India.
2. Resident ENT, MCH SKIMS, Srinagar, Kashmir, India.
3. Head of Department Pathology Govt. Medical College, Srinagar, Kashmir, India.

Manuscript Info

Manuscript History

Received: 12 August 2016
Final Accepted: 26 September 2016
Published: October 2016

Key words:-
Squamous cell carcinoma Head and neck, Tp53 immunochemistry, Immunohistochemistry.

Abstract

Objective:- To find relationship between varied Immunohistochemistry of TP53 and squamous cell carcinoma head and neck (SCCHN) .
Study design:- Case control study.
Setting:- Tertiary care hospital (SMHS associated Medical College ,Srinagar, Kashmir, India)
Participants: 50 cases and 50 controls of squamous cell carcinoma head and neck reported our hospital from 2013-2016.
Conclusion:-

Background:-

The incidence of squamous-cell carcinoma continues to rise around the world. A recent study estimated that there are between 180,000 to 400,000 cases of SCC in the United States in 2013.[1] Risk factors for squamous-cell carcinoma varies with age, gender, race, geography, and genetics. The incidence of SCC increases with age and the peak incidence is usually around 60 years old. Males are affected with SCC at a ratio of 2:1 in comparison to females. Caucasians are more likely to be affected, especially those with fair Celtic skin and chronically exposed to UV radiation. Squamous-cell carcinoma of the skin is the most common among all sites of the body. Solid organ transplant recipients (heart, lung, liver, pancreas, among others) are also at a heightened risk of developing aggressive, high-risk SCC. There are also a few rare congenital diseases predisposed to cutaneous malignancy. In certain geographic locations, exposure to arsenic in well water or from industrial sources may significantly increase the risk of SCC.

Ninety percent of cases of head and neck cancer (cancer of the mouth, nasal cavity, nasopharynx, throat and associated structures) are due to squamous cell carcinoma. Symptoms may include a poorly healing mouth ulcer, a hoarse voice or other persistent problems in the area. Treatment is usually with surgery (which may be extensive) and radiotherapy. Risk factors include smoking, alcohol consumption and hematopoietic stem cell transplantation[2]

Aim of the study:-

To find relationship between squamous cell carcinoma head and neck (SCCHN) and its impact on p53 protein expression.

Corresponding Author:-Dr Shakeel Mohmad Wani.
Address:-Resident ENT HNS, SMHS Hospital , Srinagar, kashmir, India.

Materials and Methods:-

Study subjects:-

- This study was done in tertiary health center of Kashmir in Department of ENT and Head & Neck surgery SMHS and included patients with histologically confirmed squamous cell carcinoma Head and neck (SCCHN) over a period of 18 months. Patients with squamous cell carcinoma of oral cavity, oropharynx, nasal cavity, nasopharynx, hypo pharynx and larynx, identified at the Department of ENT and head&neck surgery SMHS Hospital were included. The patients with secondary SCCHN, Thyroid malignancies, primaries outside the upper aero digestive tract, cervical metastases of unknown origin or histopathologic diagnoses other than squamous cell carcinoma were excluded. All cases were from Kashmir and had not received any treatment at the time of recruitment. Controls were also taken from Kashmiri population who were admitted to our hospital for some other non-neoplastic disease.

Immuno-histochemistry for tp53 protein expression:-

Procedure:-

The wax blocks of cases and controls were de-paraffinized with xylene 2 changes lasting 5 minutes each. Hydration was done using 100% ethanol 2 changes for 3 minutes each followed by 95% and 80% ethanol for 1 minute each. Then rinsing was done with distilled water. Pre-heating was done using Pre-heat steamer or water bath with staining dish containing Sodium Citrate Buffer or Citrate until temperature reaches 95-100OC .The slides were immersed in the staining dish and incubated for 20-40 minutes. After Turning off steamer slides were removed and allowed to cool for 20 minutes. Sections were rinsed with PBS Tween 20 for 2x2 min. Sections were incubated with primary antibody at appropriate dilution in primary antibody dilution buffer for 1 hour at room temperature or overnight .Then sections were rinsed with PBS Tween 20 for 2x2 min and blocking was done with peroxidase blocking solution for 10 minutes. Again rinsing was done with PBS Tween 20 for 3x2 min.

1 drop of freshly prepared liquid DAB Chromogen in 1 ml stable DAB buffer was added to the tissue and incubated for 5 minutes at room temp(20-25^{O}C). Hematoxylin counter staining was done and incubated for 3 min at room temp ((20-25^{0}C).The slides were mounted and studied for TP53 positivity.

Results and Observations:-

Cases and controls were also analysed by immunohistochemistry, in positive cases and controls the immunoreactive protein was prominent and localized in cell nuclei. Out of 50 HNSCC,30 (60%) showed a positive staining for the p53 protein.In 10 (33.34%) of the cases staining was extensive (+++),15 out of the 30 (50%) showed moderate positivity (++) and 5 out of 30 (16.67) cases showed weak positivity (+).In 6 (27.28%) of the controls staining was extensive (+++),10 out of the 22 (45.46) showed moderate positivity (++) and 6 out of 22 (27.28%) cases showed weak positivity (+).

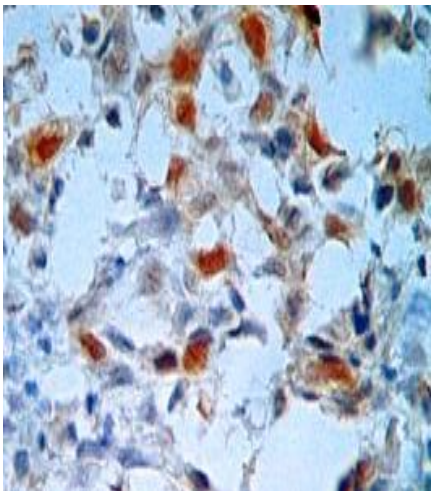
STAINING	CASES	CONTROLS
-VE IHC	20	28
+VE IHC	30	22

Statistical analysis was done using Fichers Exact test and results were found statistically insignificant p value>o.o5 (p value 0.169, RR 1.379, OR 1.379 and 95% CI 0.9266-2.052)

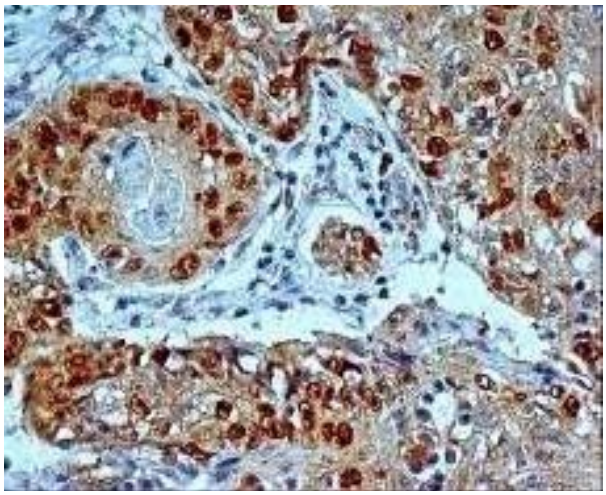
Subjects	Total	Grading of staining intensity	No
Cases	50	_	20
		+	5
		++	15
		+++	10
Controls	50	_	28
		+	6
		++	10
		+++	6

All patients	n	P53 IHC +vity
Cases	50	30

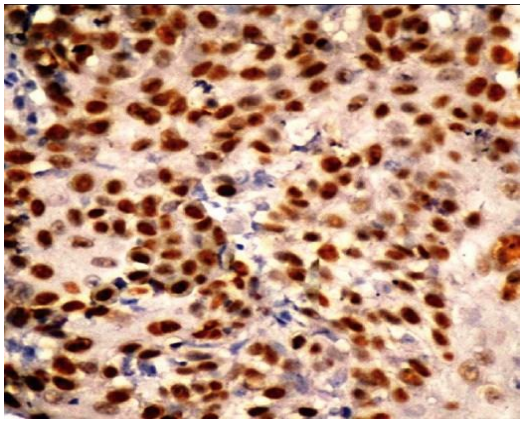
Controls	50	22
SEX		
Cases		
Male	41	24
Female	9	6
Controls		
Males	38	14
Females	12	8
Age		
Cases		
<10	0	0
10-39	1	0
40-60	27	20
>60	22	10
Controls		
<10	2	1
10-39	3	1
40-60	25	8
>60	20	12
Diagnosis		
Cases		
Oral cavity	7	4
Oropharynx	1	0
Larynx	25	16
Hypo pharynx	8	3
Sino-nasal	9	7
Controls		
Vocal polps	10	4
Vocal nodules	3	2
Nasal polyposis	10	3
Lichen planus	2	1
DNS	13	5
Adenotonsillitis	12	7
TNM		
T1-2	38	21
T3-T4	12	9
N0	26	14
N+	24	16
M0	50	30
M1	0	-



WEAK +VE



More than 10% of nuclei stained ++



More than 30% cells stained with dark brown staining +++

Conclusion:-

From our study it is evident that histopathologically diagnosed Squamous cell carcinoma head & neck are not always IHC +ve on immunohistochemistry.

Ethical Clearance:-

Sought from ethical committee GMC Srinagar.

Conflict of interest:-

Nil

Source of funding:-

Hospital

Acknowledgement:-

Nil

Bibliography:-

1. Kari, PS (2012). "Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012". Journal of American Academy of Dermatology 68: 957–66
2. Elad S, Zadik Y, Zeevi I, et al. (December 2010). "Oral cancer in patients after hematopoietic stem-cell transplantation: long-term follow-up suggests an increased risk for recurrence". Transplantation 90 (11): 1243–4