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COMPARATIVE EVALUATION OF HYBRID CAPTURE 2 AND GENEXPERT FOR DETECTION OF HIGH RISK HUMAN PAPILLOMAVIRUS IN PATIENTS WITH OROPHARYNGEAL SQUAMOUS CELL CARCINOMA AT SMS HOSPITAL, JAIPUR.

Dr. Rahul Agarwal Resident, Department of Microbiology, SMS medical college and attached group of Hospitals, Jaipur, India

Dr. Jitendra Tiwari Research Scientist, Department of Microbiology, SMS medical college and attached group of Hospitals, Jaipur, India

Dr. Bharti Malhotra* Senior Professor, Department of Microbiology, SMS medical college and attached group of Hospitals, Jaipur, India *Corresponding Author

ABSTRACT

BACKGROUND: Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common cancer and the eighth most common cause of death in cancer worldwide. Its important to carry out regular HPV testing on such patients as HNSCC which are HPV (Human papillomavirus) positive respond better to radiotherapy.

METHODS: A total of 50 oral brushing samples were collected in specimen transport medium (STM) from patients with oropharyngeal squamous cell carcinoma. The HR-HPV (High risk Human papillomavirus) was detected by Hybrid Caputre 2 (HC 2) HPV system (Qiagen) which is a nucleic acid hybridization based method and GeneXpert system (Cepheid) which is a point of care real-time Polymerase Chain Reaction (PCR) assay.

RESULTS: Out of 50 oral brushing samples, 08/50 (16.00%) samples were found positive for HR-HPV by HC 2 and 05/50 (10%) by GeneXpert. Among 05 samples positive by GeneXpert, 4 were HPV 16 positive and 1 sample was other than type 16,18 and 45. However HC2 could not differentiate between the types.

CONCLUSION: Qiagen Hybrid capture 2 is a well established method but is cumbersome and time consuming, newer methods like GeneXpert are easy to perform and give results in 1 hour but needs to be compared with established methods.

KEYWORDS : Human Papillomavirus, Hybrid capture 2, GeneXpert

INTRODUCTION:

Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth most common cancer and the eighth most common cause of death due to cancer worldwide. Its incidence varies with different regions. In North America and the Eastern Europe, HNSCC accounts for 3 to 4 % of all cancer diagnoses but in India, it accounts for 30 % of all cancers in males and 11–16 % in females.[1]

Most of the cases of head and neck cancer are considered to be associated with various common risk factors such as smoking, tobacco chewing and alcohol abuse. Diets lacking in essential micronutrients, exposure to agents like radiation and poor oral hygiene, have also been associated with an increased risk of cancer.[2] However approximately 20% cases show different etiology and HPV is a leading cause of these cases.[3-4] HPV is a DNA virus belongs to the papillomaviridae family. It is a non-enveloped virus containing a small, circular double-stranded deoxyribonucleic acid (DNA) genome and an icosahedral (20 sided) protein coat. At present, over 200 different genotypes of papillomaviridiae have been identified by various techniques. These can be classified according to similarities in their DNA sequences. They can also be classified into low and high risk types based on their capacity to promote malignant transformation in host cells. Of these, HPV 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82 are examples of those classified as high risk viruses while HPV 6, 11, 40, 42, 43, 44, 54, 61, 72, 81, and 89 can be considered as viruses with low oncogenic risk.

This is very important to do routine HPV testing in oropharyngeal carcinoma patients because HPV status is a powerful indicator of patient prognosis. HPV-positivity is associated with a lower risk of tumour progression and death due to enhanced sensitivity to ionizing radiation with or without chemotherapy.[5-6] Numerous methods for detection of high risk Human papillomavirus (HR-HPV) in tissue samples are available including In situ hybridization (ISH), Polymerase Chain Reaction (PCR), Hybrid Capture 2, GeneXpert and Immunohistochemical testing for P16 expression. Preferential use of one method over another must consider not just test sensitivity and specificity but also depends on balance of a variety of practical concerns related to cost, ease of specimen acquisition and processing, turnaround time, automation and standardization. In this study , we compared Hybrid Capture 2 and GeneXpert for detection of HPV in patients with oropharyngeal carcinoma.

MATERIAL AND METHODS:

Study Area and Site: The study was conducted in SMS Medical College, Jaipur from May 2015 to May 2016. Samples were obtained from histo-pathologically confirmed case of oropharyngeal squamous cell carcinoma patients at SMS hospital, Jaipur.

Study Design: This was a laboratory based descriptive type of observational study.

Sample size: The calculated sample size was 50 cases at 10% allowable error with 95% confidence level.

Inclusion Criteria: Oral brushing samples collected from recently diagnosed oropharyngeal SSC patients.

Exclusion Criteria: Patients who had undergone surgery or radiation for treatment of lesion.

Sample Collection: Informed consent was taken for taking samples and personal data was collected using a specific form. An oral brushing sample was obtained by brushing the visible lesion with a brush-tipped swab stick. The brushing material then transferred to a vial of STM (Specimen Transport Medium) for testing.

Methodology: The specimens were tested for HR-HPV by Hybrid Capture 2 and GeneXpert.

HC2 is a nucleic acid hybridization microplate assay for the chemiluminescent detection of HPV types of low risk (6/11/42/43/44) and high risk (16/ 18/ 31/ 33/ 35/ 39/ 45/ 51/ 52/

56/58/59/68). Specimens containing the target DNA hybridize with a specific HPV RNA probe. The resultant RNA: DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids.

Immobilized hybrids are then reacted with alkaline phosphatase conjugated antibodies specific for the RNA:DNA hybrids and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted which is measured as relative light units (RLUs) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen. The specimens with an RLU/cutoff value ratio of 1 or greater were considered positive.

The GeneXpert HPV Assay is a point of care real-time Polymerase Chain Reaction (PCR) assay, each assay is in a disposable cartridge, that detects 14 types of high-risk HPV DNA (types 16, 18-45 and others which include type 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68). GeneXpert instrument system automate and integrate sample processing, cell lysis, purification, nucleic acid amplification and detection of the target sequences in clinical samples by using real-time PCR.

RESULTS:

In our study, most of the study subjects were male (84%) and only 16% of the subjects were female (Table I)

Most of the patients had lesion at base of tongue (40%), followed by tonsil (28%), soft palate (22%) and buccal mucosa (10%) (Table II).

Out of 50 samples, 8 samples were found positive for HR-HPV by Hybrid capture 2 and only 5 samples were found positive by GeneXpert (Table III). 3 cases which was found positive by Hybrid Capture 2, was found negative by GeneExpert. So sensitivity of Genexpert was very low (25%), though specificity was good (>90%) (Table IV).

Out of 5 samples which was found positive by GeneExpert, 4 samples were HPV-16 positive and 1 sample was other than HPV-16,18 and 45 postive (Table V).

Table I Sex distribution of study subjects

Sex	N	Percentage
Female	8	16%
Male	42	84%
Total	**50**	100%

Table II Distribution of study subjects according to the site of lesion

Site	N	Percentage
Base of tongue	20	40%
Soft palate	11	22%
Buccal mucosa	5	10%
Tonsil	14	28%
Grand Total	**50**	100%

Table III Comparison of GeneXpert result with HC 2 test results

	By Hybrid Capture 2	By GeneXpert
Positive cases for HR-HPV	8	5
Negative cases for HR-HPV	42	45
Grand Total	50	50

Table IV Diagnostic parameters of GeneXpert in relation to Hc2

DIAGNOSTIC PARAMETERS	Percentage
Sensitivity	**25%**
Specificity	**90.62%**
Positive Predictive Value (PPV)	**40%**
Negative Predictive Value (NPV)	**82.86%**
Positive Likelihood Ratio	**2.67**
Negative Likelihood Ratio	**0.83**
Diagnostic Accuracy	**77.5%**

Table V HPV genotype detected by GeneXpert

HPV genotype Detected by Genexpert	No.	Percentage
HPV 16	4	80%
HPV 18 and 45	0	0
Others(from type 31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68)	1	20%
Total	5	100%

DISCUSSION:

HPV status is a powerful prognostic indicator for patients with oropharyngeal squamous cell carcinoma and it is being used as an essential parameter in the recruitment of patients into various clinical trials. It is important to carry out regular HPV testing on such patients as HPV positive oropharyngeal squamous cell carcinoma patients respond better to radiotherapy. Sample collection can be a difficult task in patients with oropharyngeal squamous cell carcinoma. In this study, we used oral brushing samples for detection of HR-HPV in patients with OSCC by Hybrid Capture 2 and GeneXpert. Specimens could be easily collected by oral brushing without the need for tumour microdissection, formalin-fixation or specimen processing of any kind.

In our study 8 samples were found positive by HC 2 while only 5 samples were found positive by GeneXpert. This data demonstrated that sensitivity of GeneXpert was very low, though specificity was good (>90%). A study was conducted in London,on 3408 patients with cervical carcinoma by Jack Cuzick et al showed Positivity for Xpert was 19.6%, and HC2 19.9% with high concordance (kappa=81.55 vs HC2). Xpert, and HC 2 showed similar sensitivity (98.7%, 98.7%) but HC 2 was more specific than Genexpert.[8]

In our study, HPV-16 was the predominant type which was present in approximately 80% of HR-HPV positive patients. Most of other Indian and foreign studies also reported that HPV-16 was the most common type causing HR-HPV associated carcinoma. An Indian study from Rohtak, Haryana in 2016 by Sanjeev Parshad et al reported that HPV 16 was the major subtype as it was present in 95.2% of positive cases and HPV 18 was detected in 4.8% patients.[9]

Hybrid capture 2 was a well established method but was cumbersome and time consuming (app. 5 hours), samples had to be put in batches. GeneXpert offers simplicity of testing, flexibility with non-batching of individual samples and rapid turn-around time but needs to be compared with established methods. Both systems have their advantages but discrepant samples need to be further tested by sequencing.

REFERENCES

1. Chin D, Boyle GM, Porceddu S, Theile DR, Parsons PG, Coman WB (2006) Head and neck cancer: past, present and future. Expert Rev Anticancer Ther 6:1111–111.
2. Paz IB, Cook N, Odom-Maryon T, Xie Y, Wilczynski SP. Human papillomavirus (HPV) in head and neck cancer: An association of HPV-16 with squamous cell carcinoma of Waldeyer's tonsillar ring. Cancer 1997;9:595-604.
3. Klussmann JP, Gültekin E, Weissenborn SJ, Wieland U, Dries V, Dienes HP et al. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. Am J Pathol 2003;162:747-53.
4. Venuti A, Badaracco G, Rizzo C, Mafera B, Rahimi S, Vigili M. Presence of HPV in head and neck tumours: High prevalence in tonsillar localization. J Exp Clin

Cancer Res 2004;23:561-6.
5. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, et al. Improved Survival of Patients With Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma in a Prospective Clinical Trial. J Natl Cancer Inst. 2008
6. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med. 2007 356:1944–56.
7. Kulmala SM, Syrjänen S, Shabalova I, PetrovichevN, Kozachenko V, Podistov J, Ivanchenko O, Zakharenko S, Nerovjna R, Kljukina L, Branovskaja M. Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools. Journal of clinical microbiology. 2004 Jun 1;42(6):2470-5.
8. Jack Cuzick, K. Cuschieri, K.Denton, M.Hopkins, M.A. Thorat et al. Performance of the Xpert HPV assay in women attending for cervical screening. Papillomavirus Research1 (2015) 32–37.
9. Sanjeev Parshad, Sourabh Nandi, Nisha Marwah, Promod Mehta, Mayank Tripathi et al. Human papillomavirus 16 and 18 in squamous cell carcinoma of oral cavity and sexual practices: A pilot study at a Tertiary Care Hospital of North India. National Journal of Maxillofacial Surgery | Vol 6 | Issue 2 | Jul-Dec 2015 |185-189.