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LACK OF ASSOCIATION OF HIGH-RISK HUMAN PAPILLOMAVIRUSES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Introduction

Head and neck cancer (HNC) are reportedly the sixth most occuring common cancer diagnosed worldwide. Yearly it was noted that around 633,000 new cases were diagnosed with Head and Neck cancer in which around 355,000 cases resulted in mortality [1].

It encompases several types of malignant tumors of the upper aerodigestive tract, which includes from the vermilion border of the lips and extends upto the beginning of the esophagus. Majority of the malignanat tumors of the head and neck are squamous cell carcinomas (SCCs), while adenocarcinomas were known to appear in less cases. [2][3] The incidence rate varies according to geographic region and associated risk factors of differing severity. Recent literature survey showed the declining incidence of head and neck asquamous cell carcinomas (HNSCC) has been due to reduced tobacco consumption in industrialized countries.

Along with the cofacotrs such as tobacco and alcohol consumption, which are the leading factors for the genesis of HNSCC, seveal studies have pointed out the presence of oncogenic Humanpailloma virus as a etiological factor for HNSCC. Studies showed that in USA and European coutries has shown rapid increase in the incidence rate of oropharyngeal cancers, particularly of those of the tonsils and base of the tongue to be associated with human papilloma virus (HPV) [5-8]. In Asia, paritucllary in Indian subcontinent studies has shwon highest HNC incidence (6.1/100,000 women and 20.9/100,000 men) which accounts for one-third of the total world HNC burden.[10][3]. A research review of the studies done from India has pointed out the occurence of HPV in squamous cell carcinomas (SCC) of the oral cavity in India has been varied ranging from 33.6% in the Eastern region,[11]. 48% in South India,[12] 15% in West India,[13] .The prevalance of the high risk HPV in the genesis of the HNSCC is not well known as per our knowledge at the Southern region of India.

In an attempt to understand the trends prevailing of HPV, the study was conducted at our tertiary care hospital in Hyderabad, Telangana. The present pilot study of retrospective and prospective non comparative case series has been conducted to evalute the presence of high risk HPV16 and HPV18 in the histologically proven cases of HNSCC.

He biopsies taken from various sites of head and neck cancers were taken from tissue embedded blocks preserved by the pathology department in our hospital and subjected to DNA extraction and gel electrophoresis for detection of HPV DNA. The squamous cell carcinoma biopsies of cervix which occurred due to HPV etiology were taken as controls for HPV 16 and 18 subtypes. This exercise attempts to evaluate HPV's role as one of the possible infectious causes of tumor in HNSCCs in the populace under study. It also helps us in regarding the selection of the therapeutic approach in the future is whether treatment that is less intense will be sufficient for the HPV associated tumors.

Materials and Methods

The study protocol has been approved by the Institutinal Ethical Committee of Kamineni Academy of Medical Sciences and Research Centre and it is a approved project from the STS ICMR, Govt of India. In this retropspective and prospective study, the Laboratory processing of the preserved blocks involved fixation with 10% formalin (neutralized) and embedding in paraffin, with storage at room temperature. Blocks with a large amount of tissue were sectioned and selected for DNA extraction. The duration of the study was of 4 months, a total of 40 cases of Formalin fixed paraffin embedded (FFPE) HNSCC cases

have been evaluated along with 20 controls (normal tissue taken for other purpose not tumours). A total of 20 FFPE histologically confirmed cervical tumor cases were used as positive controls for presence of high risk HPV. Histologically confirmed cases from the Department of Pathology, Kamineni Hospitals (FFPE) blocks were obtained and sectioned while maintaining utmost precautions to avoid inter-block contamination of DNA. This was achieved by pre-chilling and moistening each block in a separate ice container before sectioning, thorough cleaning of the microtome, single use of brush and forceps, changing gloves in between each block and changing the water bath for each block and using a new blade for each block.

DNA extraction and PCR

Genomic DNA was extracted from a 10 µM thick section, using Nuclieospin DNA FFPE (Germany). The HPV 16 and 18 gene are amplified using specific primers. The PCR reaction mix has specific primers along with readymade PCR mix (Takara Emerald PCR Mix) and a final volume of 20 µl was prepared using deionized water. The cervical tumors were screened for the presence of HPV, later the HNCC were screened. DNA amplification was carried out in an automated thermal cycler (Takara Bio Inc., Japan). The PCR cycle parameters were 95 °C for 15 minutes (initial denaturation), followed by 10 cycles at 94 °C for 30 seconds, followed by 65 °C for 90 seconds and at 72°C for 90 seconds. This followed by 30 cycles with temperatures of 94 °C for 30 seconds, 63°C for 90 seconds, 72°C for 90 seconds and finally 72°C for 10 minutes. The product size for HPV16 207bp and 274bp for HPV 18. . Negative controls for PCR assays were set up using deionized water instead of the DNA template. Amplification products were subjected to electrophoresis using 1.5% of the agarose gel. [14]

Results

The median age of the patients is 49.5 years (range Min-21 yrs- Max-85yrs) and female-13 years and male-27 years. The site wise distriubtion of HNSCC were oral cavity- 26 cases, Esophagus- 6 cases, Pharynx-4 cases and Larynx-6 cases. Histologically all HNSCC and cervical tumor are invasive in nature. In cervical specimens, the HPV16 has been detected in 4 cervical tumors and HPV18 has been found 4 cervical tumors (figure 1). A total of 40 specimens of HNSCC which were screened for the presence of high risk HPV16 and HPV18 showing absence of both high-risk HPV (figure 2).

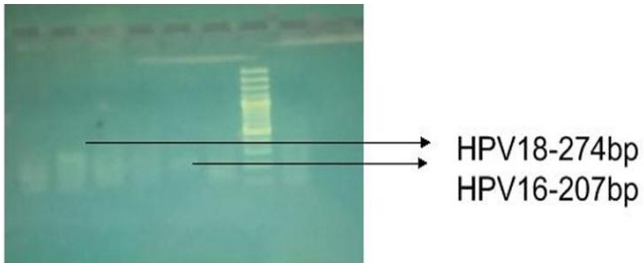

Figure 1: Detection of HPV 16 and HPV 18 in cervical tumor

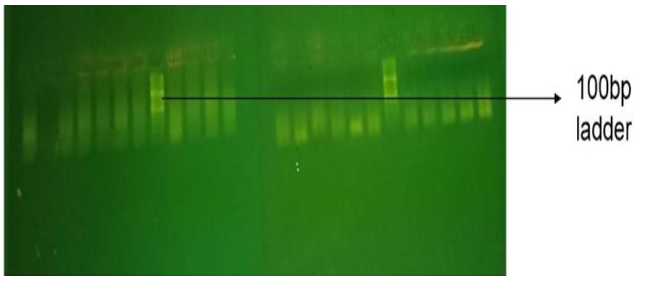

Figure 2: PCR for HPV 16 and HPV18 in HNC – No amplification has been noticed for high risk HPV

Discussion

In the recent history there is an increase in the incidence of HNSCC and overall constitute 4% of cancers worldwide. High risk person are those who are involved in smoking, tobacco chewing and extensive alcohol consumption. Along with these cofactors, studies supported and negated the role of high risk HPV as etiological factor for the gensis of HNSCC.

In the present study we found the absence of high risk HPV in HNSCC cases, similar to the research work conducted by Patel KR suggested that HPV are not associated with HNSCC. [15]. There is a lot of discripency in the detection of HPV as due to the application of different technques used for its detection. Analysis of the several Indian studies have pointed out that the detection of high risk HPV has been found from a range of 0-74% , indicating the role of HPV acts a cofactor in HNSCC formation.

The study, being a pilot study and having a relatively small sample size, use of the FFPE tumor sections and screening only high risk HPV, cannot state the prevalence rates in the region by itself. Further studies are warranted with high sample size along with more than one technique for the detection of HPV. We therefore conclude that HPV subtypes, including types 16 and 18, are unlikely to play a significant role in the genesis of HNSCCin patients at our teritary care centre.

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