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Role of Human Papillomavirus and its Association with an Indian Oral Squamous Cell Carcinoma Subjects

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Abstract

Oral Cancer; especially Oral Squamous Cell Carcinoma (OSCC) is the eighth most common cancer all over the globe and most common neoplasm in India. Due to the tobacco addiction pattern in Indians specially Guajarati's are well known for high consumption of tobacco in non-smoking form may have the high-risk for developing oral cancer. Apart of this, Human papilloma virus(HPV) might be one of the cause for developing oral cancer hence, the proposed study was carried out for 50 primary OSCC subjects from January 2018 to October 2018. Tumour specimens were HPV-genotyped by type specific PCR method. Statistical analyses were performed by Fisher's exact test. The present study identified 4% HPV infection frequency in advanced staged OSCC Indian subset, where One OSCC stage IV patient observed with HPV 16 infection and one OSCC stage III patient with HPV 18. Our finding differs with rest of the population, as the region have high amount of consumption of tobacco. Current study need to be validated in larger sample size to get the clear view for association of HPV with oral cancer.

Introduction

Oral cancer; the eighth most common neoplasm and blazing dilemma over the globe where 90 % cases are for oral squamous cell carcinoma[1–5]. India has highest incidence and mortality rate throughout the world, mainly males are prominently affected for the oral cancer[6,7]. Average 177,384 mortality occur from 354,864 incidents annually with 913,514 prevalence[8].

High consumption of tobacco in smoking and non-smoking form is the major risk factor for onset of oral cancer end up with 50-fold increase in reactive oxygen species. Gujarat is the hub for tobacco productions leads to high consumption of tobacco in form of Gutkha and mava headed to development of oral cancer[9]. Our previous study has shown most of the patients having genetic alterations found in the patients with the history of tobacco, which support the notion and pointed out the association of tobacco with the development of oral cancer[10]. However, approximately 20% of oral cancers developed in patients lacking these traditional risk factors[11–12]. Apart from tobacco there are several risk factor for developing oral cancer. There is evidence for HPV infected people having the history of tobacco and alcohol have developed oral cancer[13]. Human papilloma virus is characteristic member of papillomaviridae family of small non-enveloped virus that contain double-stranded DNA viral genome of ~ 8 kb[14–17]. Approximately 120 types are entirely sequenced among the 150 different types of HPV[18]. Previous study suggested that more than 10% of human cancers are developed by HPV infection[19]. There are 24 different types of HPV have been categorised as high risk types that associated with benign lesions of OSCC including 1, 2, 3, 4, 6, 7, 10, 11, 13, 16, 18, 30, 31, 32, 33, 35, 45, 52, 55, 57, 59, 69, 72, and 73, whereas 12 types with malignant OSCC lesions including 2, 3, 6, 11, 13, 16, 18, 31, 33, 35, 52, and 57[20]. The HPV type 16 if the most leading type that detected in OSCC, followed by HPV 18[21–23]. The younger patients are affected three times more with the high-risk HPV types than elders, due to viral transmission by direct physical contact[24]. In India, there are no more data which evaluating the prevalence of HPV in OSCC. HPV infection status can become an ordinary element in the diagnosis of

oral cancer. The aim of this study was to determine the association between HPV and OSCC in Saurashtra region of Gujarat.

Results

The current study recruited 50 oral cancer tumor tissues for HPV detection. The age of the OSCC patients was ranged from 20 to 80 year (mean age 47.82). Among fifty OSCC patients thirty-eight (76%) were male and twelve (24%) were female. Twenty-four (48%) patients had a history of chewing tobacco and five (10%) patients had a history of alcohol consumption while only two (4%) patient had a history of smoking. Among fifty patients six (12%) patients were habituated with tobacco and smoking while only two (4%) patient was habituated with tobacco and alcohol. Different frequency of OSCC patients as described in (Table 1).

Table 1
Clinicopathological parameters of the OSCC patients

Characteristics	Oral Cancer Patients (n = 50), no (%)		
	Male (n = 38) (76%)	Female (n = 12) (24%)	Both (n = 50) (100%)
Age Range (years)	10 (26.3%)	1 (8.3%)	11 (22%)
Mean age – 47.82	18 (47.3%)	9 (75%)	27 (54%)
20–39	10 (26.3%)	2 (16.6%)	12 (24%)
40–59			
60–79			
Site of primary tumor	28 (73.6%)	6 (50.0%)	34 (68%)
Buccal mucosa	3 (7.8%)	4 (33.3%)	7 (14%)
Tongue	1 (2.6%)	1 (8.3%)	2 (4%)
Lip	3 (7.8%)	0 (0%)	3 (6%)
GB complex	1 (2.6%)	1 (8.3%)	2 (4%)
Alveolus	2 (5.2%)	0 (0%)	2 (4%)
Hard palate			
Risk habit	19 (50.0%)	5 (41.6%)	24 (48%)
Tobacco chewing	4 (10.5%)	1 (8.3%)	5 (10%)
Alcohol consumption	2 (5.2%)	0 (0%)	2 (4%)
Smoking	6 (15.7%)	0 (0%)	6 (12%)
Tobacco + Smoking	2 (5.2%)	0 (0%)	2 (4%)
Tobacco + Alcohol	5 (13.1%)	6 (50.0%)	11 (22%)
Non-habited			
HPV genotype	1 (2.6%)	0 (0%)	1 (2%)
HPV-16	0 (0%)	1 (8.3%)	1 (2%)
HPV-18			

HPV infection was detected by type specific PCR method. The DNA used for the PCR amplification was checked quantitatively and qualitatively by UV spectrophotometer and 0.5% agarose gel electrophoresis, respectively. The DNA samples have 260nm/280nm ratio > 1.8 were used for the type specific PCR for HPV 16 and HPV 18. The primers used for the type specific PCR have shown in (Table 2). B-globin gene

of 268 bp was used as a positive internal control. Bands of 217 bp and 100 bp of HPV-16 and HPV-18 respectively were checked on 2% agarose gel electrophoresis.

Table 2
Co-relation of HPV and clinicopathological characteristics of the OSCC patients

Characteristics		HPV infection	NO HPV infection	p value
Total number (%)		2 (4%)	48 (96%)	
Age (years) [mean]		50 (40–60)	47.82 (27–70)	
Gender	Male	1	37	**p < 0.5**
	Female	1	11	
Tumor site	BM	1	33	**p < 0.5**
	Others	1	15	
Disease stage	I-III	1	28	**p < 0.5**
	IV	1	22	

The present study identified two sample with HPV infection. One patients identified with HPV-16 history was at advanced stage of oral carcinoma. Another patient was observed with HPV − 18 was also at advanced stage of carcinoma. Both the patients neither shown tobacco history nor genetic history in their lifetime, it clearly indicates HPV infection might be the reason for the development of oral carcinogenesis. Both samples were replicated using type specific PCR and confirmed on 2% agarose gel electrophoresis (Fig. 1). The current study observed 4% HPV infection frequency in the Gujarati population.

The current study was performed for statistical analysis using fisher exact test to check whether HPV infection has any significant association with any of the clinicopathological characteristics of the OSCC patients or not. The study was not found any statistical significance with Age, Gender, tumor site and disease stage (Table 3).

Table 3
Sequences of Primers used for PCR amplification

HPV genotype	Primers	Tm [°C]
HPV-16	Forward primer – 5’-AAGGCCAACTAAATGTCAC-3’	52.4
	Reverse primer – 5’-CTGCTTTTATACTAACCGG-3’	
HPV-18	Forward primer – 5’-ACCTTAATGAAAAACCACGA-3’	54.3
	Reverse primer – 5’-CGTCGTTTAGAGTCGTTCCTG-3’	
β-globin	Forward primer – 5’-CAACTTCATCCACGTTCACC-3’	48–58
	Reverse primer – 5’-GAAGAGCCAAGGACAGGTAC-3’	

Discussion

The current study identified 4% HPV infection in the Gujarati oral squamous cell carcinoma subset. We screened total of 50 patients using type specific PCR. Out of fifty patients one patient with advanced stage carcinoma, without any carcinogenic history found infected with HPV-16 and another patient with stage IV, without any personal, genetic or tobacco history observed infected with HPV-18.

The same study has been done in same population recently; they found 2% HPV frequency in oral cancer subset[25]. They have enrolled 200 cancer samples and found 2 HPV infected patients. They did this study in year 2010, so it may be the difference in frequency which support the notion of HPV infection, as this is the highly tobacco consumed region, the development of OSCC was observed due to tobacco, a rare history of HPV found in both the studies. Another previous study reported in Gujarati population stated absence of HPV infection[12] (Fig. 2). It seems, as this is the tobacco growing belt and tobacco consuming region, the frequency of HPV infection is very low compared to another population of India. The main reason of onset of oral cancer is tobacco. The previous study reported 80% of the OSCC patients had history of tobacco in form of chewing and smoking[7]. Hence, it might be the reason of lower frequency of the HPV infection.

Same study in Eastern India[26,27] reported highest (51.3%) prevalence of HPV infection as compared to south India (48.63%) [14,28–32], Followed by North India (35.2%)[33–36]. West India[12,25,37–39] accounted 15.54% frequency of HPV infection and present study in Western India stated only 6.6% prevalence of HPV infection (Fig. 3). The western Indian population is showing lower HPV frequency compare to rest of the Indian region, tobacco history might be the main factor for the OSCC carcinogenesis over Guajarati population.

The frequency of HPV infection varies from ethnicity to ethnicity. When we have compared the HPV infection frequency of Western part of the India with other parts of the globe, it is showing contrast result (Fig. 4). The difference in results may be due to race, ethnicity, etiological factor, dietary patterns, environmental factors, personal history such as genetic history and mostly tobacco history do affect the results. HPV infection found more frequent in Japan (93.89%)[40–46] then Greece (65.67%)[16,47]. The similar study performed in Venezuela[48–50] and Netherland[51,52] demonstrated 62.93% and 31.27% prevalence of HPV infection respectively. The study in USA[24,53–59], China[60–66] and Brazil[67–69] has shown 22.95%, 22.83% and 23.8% HPV prevalence respectively. Total HPV mutation frequency of Indian population is showing 36.04%[12,14,25–39] which seems similar to Netherland population.

The HPV screening must require before any genetic analysis of the cancer patient. This is the personalized medicine era, based on the patient's genomic profile; the prognosis of the patients can be improved. The patients with HPV infection has better prognosis than any other personal history. Thus, HPV studies must have been carried out in line with all the basic test of the cancer patients. The limitation of the study is lower sample size. The further study with larger cohort should be needed to validate the findings and to establish HPV as a prognostic biomarker.

The present study identified 4% HPV infection frequency in advanced staged OSCC Indian subset. Both the patients were not observed with any personal history. Our finding differs with rest of the population, as the region have high amount of consumption of tobacco, so that might be the reason for developing oral cancer. Another reason race, ethnicity, environmental factor, etiology, dietary factor may affect the results. The future studies with large cohort must be carried out to establish HPV infection has a better prognosis then patients have history of tobacco. The further study would be needed to establish the findings that will open the new vista in cancer biology.

Within the limitation of study, the Saurashtra region of Gujarat is not HPV prone region for onset of OSCC. Tobacco is the prime factor responsible for the development of OSCC. HPV infected patients have shown better prognosis, compared with non-HPV oral cancer patients. Thus, before medication, HPV screening must be done as it shows promising prognostic biomarker.

Methods

Study design and population

The current study was approved from the Institutional Ethics committee (Human) of P.D.U. Medical College, Rajkot, Gujarat. **All the experiments and methods were performed in accordance with the relevant guidelines and regulations.** Each and every patient provided written informed consent and were personally interviewed in proportion for the structure questionnaire. 50 primary OSCC tumors were obtained from January 2018 to October 2018 during the surgery at Shree Nathalal Parekh Cancer Institute and Shree Gulabchand Talakchand Sheth Cancer Hospital, Rajkot. All the samples were histopathologically confirmed and collected at the time of surgery from the primary site of tumor. All the tissue samples were washed thrice with 1X phosphate-buffered saline (pH 7.2) to remove contamination of blood cells from the samples. The washed samples were stored in RNA later® (Qiagen) at -20 ℃ for further downstream analysis.

Dna Isolation

Each tissue sample were micro dissected, and the genomic DNA was isolated from the tumor tissue using a DNeasy® Blood and tissue mini kit (Qiagen) by following the manufacturer's tissue protocol. The quantity of DNA was analysed by measuring the UV absorption at 260nm and 280nm and quality were analysed by 0.5% agarose gel electrophoresis.

Detection Of Hpv-16 And Hpv-18

The present study was performed by Type-specific PCR (TSPCR) using HPV-16 and 18 primers (Table 1) and amplification was performed on thermal cycler (Biorad T100™). Total volume of 25 µl of PCR mixture containing 1X PCR Master Mix (Thermoscientific #K0171), 100 ng DNA, 10 pmole of each forward and

reverse primers and nuclease free water. The PCR condition of HPV-16 amplification was an initial denaturation at 94°C for 3 minutes, followed by 34 cycles each at 94°C for 1 minute, annealing at 52.1°C for 30 seconds and extension at 72°C for 30 seconds which was extended for 5 min at the final cycle. HPV-18 detection was performed with initial denaturation at 94°C for 3 minutes, followed by 34 cycles each at 94°C for 1 minute, annealing at 47.5°C for 1 minute and extension at 72°C for 1 minute. At the final cycle extension was extended for 10 minutes. β-globin gene was used as internal positive control.

Statistical analysis

The presence of reported HPV is indicated as a percentage of the total number of samples screened. Fisher's exact test was used to check correlations between HPV infection and clinicopathological characteristics of the patients. The p value less than 0.05 were defined as being statistically significant. Two tailed fisher exact test was performed to check association with OSCC risk.

Declarations

Acknowledgement

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Author contributions

The conception and design of study, manuscript editing and polishing by SS, acquisition of data, analysis, interpretation and manuscript drafting by AP and VG has provided oral cancer tumor-control tissues, biopsy reports and all the necessary histopathological analysis.

Competing interest: The authors declare no competing interest.

Ethical approval

The present study is ethically approved by the Institutional Ethics committee (Human) of P.D.U. Medical College, Rajkot, India.

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Figures

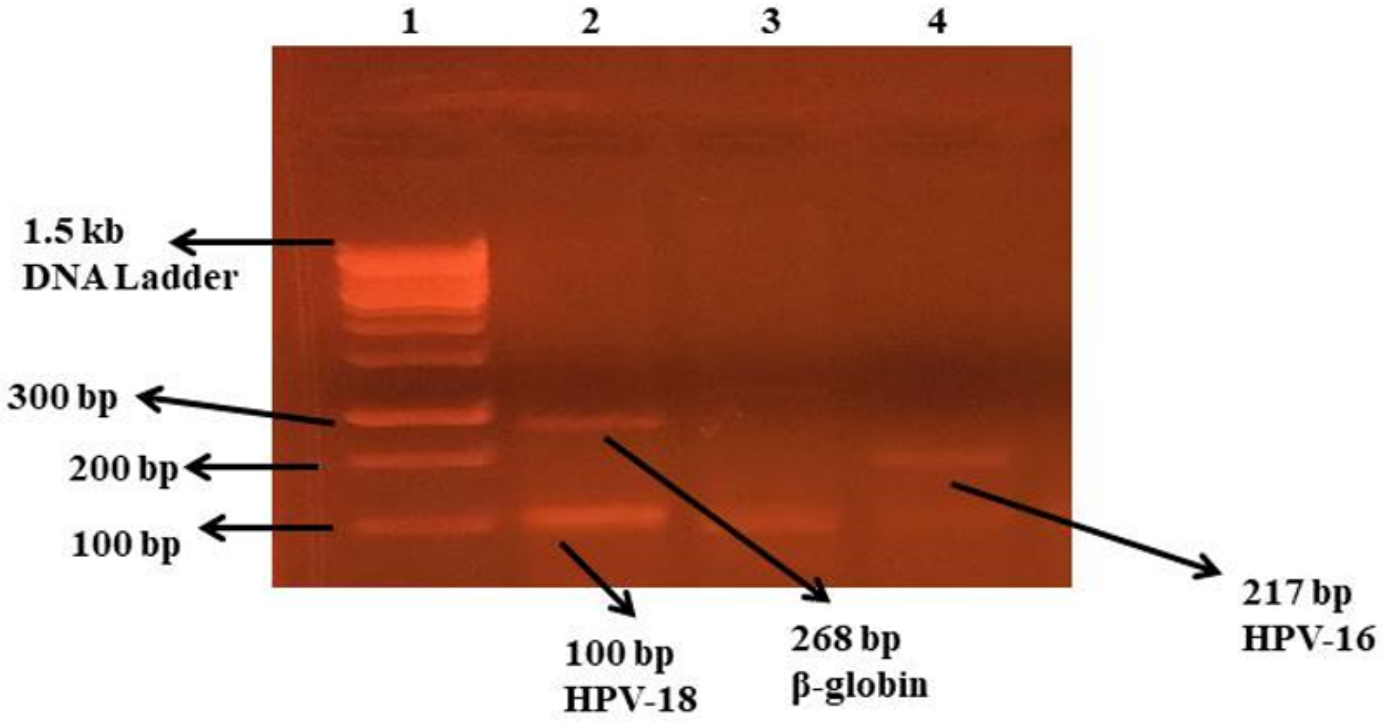

Figure 1

The confirmation of observed HPV 16, and HPV 18 infection using type specific PCR

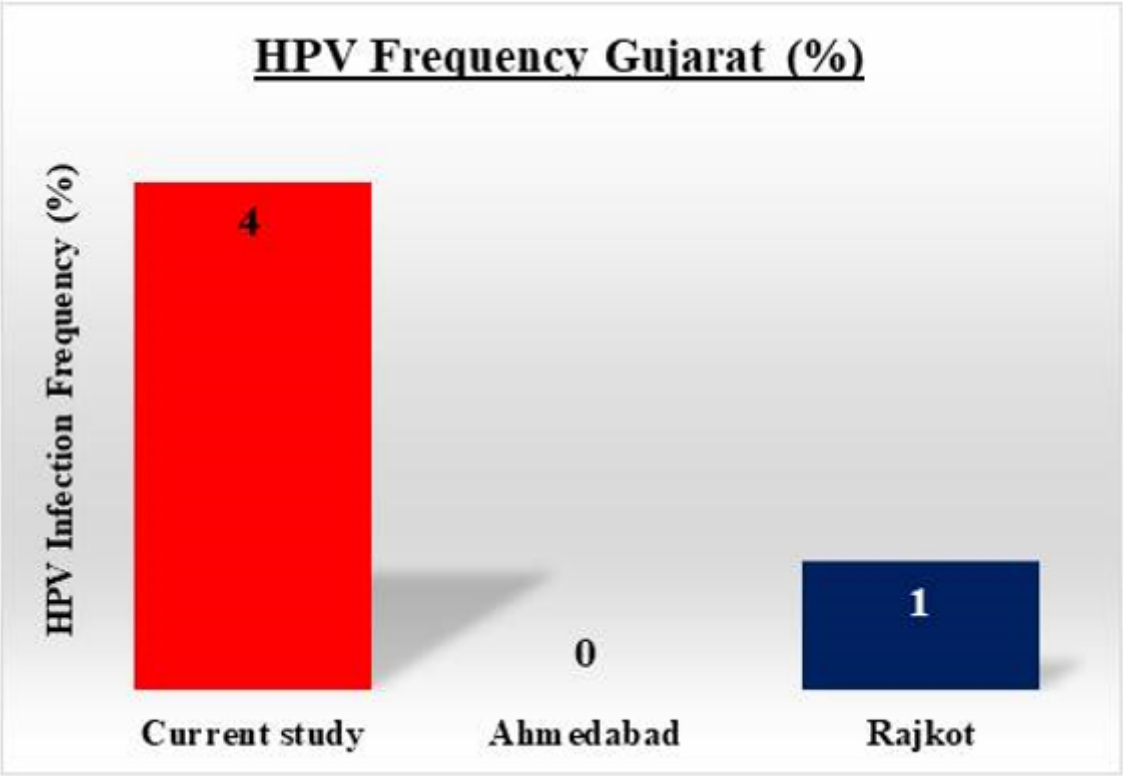

Figure 2

HPV infection frequency in Gujarati population

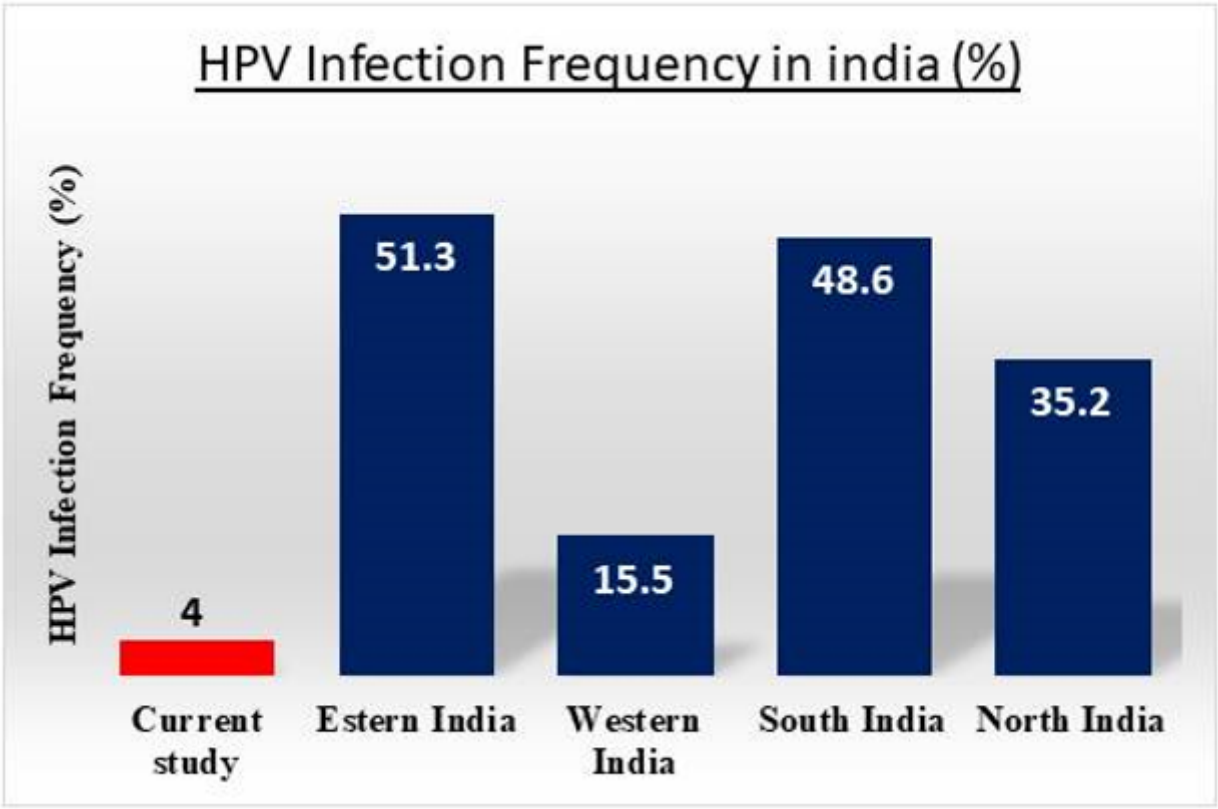

Figure 3

Frequency of HPV infection in an Indian population

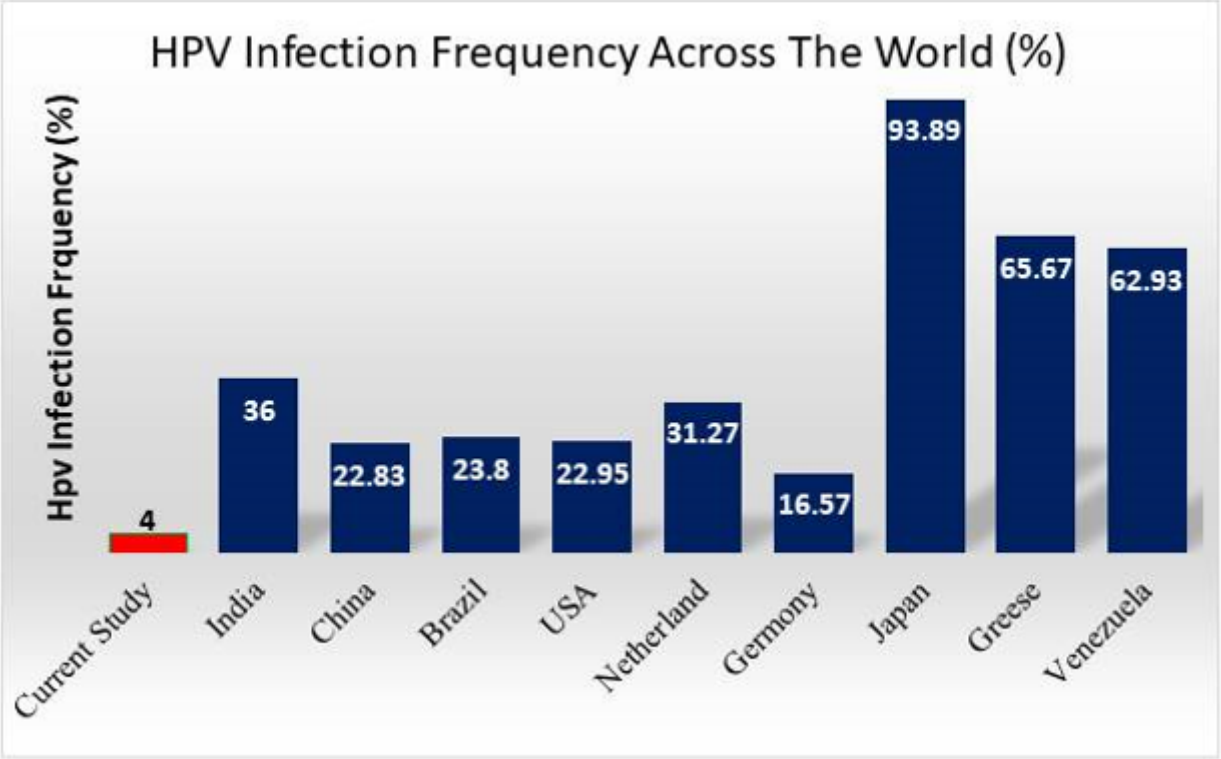

Figure 4

Global HPV infection frequency