Original Article

DOI: 10.7860/JCDR/2016/18593.8229

Dentistry Section

Assesment of Correlation of Herpes Simplex Virus-1 with Oral Cancer and Precancer- A Comparative Study

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ABSTRACT

Introduction: Most common malignant neoplasm in the oral cavity is squamous cell carcinoma. Herpes simplex virus (HSV) may enhance the development of oral carcinoma in individuals who are already at increased risk of the disease because of tobacco consumption and cigarette smoking and so must be considered as a possible etiologic agent in oral cancer and precancer.

Aim: To assess and compare the correlation of HSV-1 in oral cancer and precancerous lesions/conditions with healthy subjects.

Materials and Methods: The study comprised of 150 subjects who were divided into three groups as oral cancer, precancer and control group. Their blood samples were collected and were tested for HSV-1 IgG antibody level, using 'Herpe Select-1' ELISA kit.

Results: There was statistically insignificant difference between the HSV-1 IgG level in cancer and precancer but statistically significant difference was found between the HSV-1 IgG level among control group and cancer/precancer.

Conclusion: The present study clearly indicates that quantitative estimation of IgG antibody against HSV-1 in cancer/precancer patients will give the clue in the etiology of cancer or precancer. However, further studies with a large sample size should be carried out to determine the role of HSV-1 in etiology of oral cancer and precancer.

Keywords: Immunoglobulin, Neoplasm, Potentially malignant lesion

INTRODUCTION

Oral cancer is usually defined as a neoplastic disorder in the oral cavity. It ranks 12th among all cancers and continues to be the most prevalent cancer related to the consumption of tobacco, alcohol and other carcinogenic products [1]. Histologically, over 95% of oral cancers are squamous cell carcinomas [2]. Over 30%-80% of oral squamous cell carcinoma are preceded by precancer. There is a positive relationship between prevalence of oral cancer with leukoplakia and Oral Submucous Fibrosis (OSMF) [3]. Leukoplakia is the most common precancerous lesion. Likewise, OSMF is a well recognized precancerous condition. In various studies it has been found that there is a strong positive correlation between various viruses, oral cancer and precancer. Presence of these viruses like (HSV, HPV and EBV) along with other premalignant and carcinogenic conditions may lead to oral cancer [4]. The role of HSV in oral carcinoma has been studied and its prevalence in both malignant and potentially malignant lesions in the oral cavity was found to be approximately 30% [5]. HSV participates in activation of chromosomal mutation, gene amplification and over expression of preexisting oncogenes with neoplastic tissue. This suggests that, it may contribute to the incidences of head and neck cancer [6]. HSV- 1 is a large, double-stranded DNA virus that primarily affects the oral cavity and causes "cold sores" or "blisters" [7]. It has the ability to remain latent in host neurons for life, and can reactivate to cause lesions at or near the site of initial infection. HSV-1 has been suggested as a risk factor in the development of human malignancies in association with tobacco and alcohol. The perseverance of the virus in the oral mucosa and its capability to encourage host DNA synthesis and repairs during reactivation suggest that it may contribute to progression of oral carcinoma [8]. The aim of this study was to correlate the presence of HSV-1 with oral cancer patients and precancerous lesion/conditions.

MATERIALS AND METHODS

The present case control study was conducted in Maharana Pratap College of Dentistry & Research Centre and Cancer hospital & Research Centre Gwalior (M.P). Ethical approval for the study was obtained from both the Institute. The time period of the study was 1 year. The study comprised of 150 patients who were subdivided as: Group I: 50 patients of squamous cell carcinoma, Group II: 50 patients of precancerous lesion/conditions (Leukoplakia or OSMF) and Group III (control group): 50 normal individuals without any oral mucosal lesions.

Patients suffering from any systemic diseases or history of any venereal disease, patients with previous history of Herpes simplex virus (HSV-1) infection were excluded. For control group, in addition to above criteria, subjects with any habit of tobacco, betel nut and alcohol were excluded.

Under aseptic conditions 5ml venous blood was withdrawn from each individual using sterile disposable syringe, blood was usually collected in tubes with anticoagulant or preservatives. The serum was separated by centrifugation and transferred to another vial. Each specimen was diluted as, control and calibrator 1:101. Herpe Select-1 ELISA kit was used for the estimation of serum IgG value. In the Herpe Select-1 ELISA IgG assay, the polystyrene microwells are coated with recombinant IgG-1 antigen. Diluted serum samples and controls were incubated in the wells to allow specific antibody present in the samples to react with the antigen. Nonspecific reactants were removed by washing and peroxidase-conjugated anti-human IgG was added. Excess conjugate was removed by washing. Enzyme substrate and chromogen were added and the color was allowed to develop. After adding the Stop Reagent, the resultant color change was quantified by a spectrophotometric reading of Optical Density (OD). Sample optical density readings were compared with reference cut-off OD readings to determine results.

Interpretation of Test Results [Table/Fig-1]: The patient's results were reported as index values relative to the Cut-off Calibrator. For the calculation of index values, specimen Optical Density (OD) values were divided by the mean of the Cut-off Calibrator absorbance values.

Positive	
>1.10	An index value of > 1.10 is presumptive for the presence of IgG antibodies to HSV-1.
Equivocal	
≥0.90 and ≤1.10	An index value of ≥0.90 but ≤1.10 is considered an equivocal result.
Negative	
<0.90	An index value of < 0.90 indicates no IgG antibodies to HSV-1.

[Table/Fig-1]: Interpretation of test results from the Herpe Select-1ELISA IgG assay.

STATISTICAL ANALYSIS

The obtained data was analyzed by using statistical software – Statistical package for social science version-18 (SPSS-18). Statistical analysis was done using Chi Square, Kruskal Wallis and Mann Whitney test.

RESULTS

A total of 150 subjects were studied. In the present study gender distribution in the cancer group was 1 (2%) female and 49 (98%) were males. In the precancer group, there were 4 (8%) females and 46 (92%) males. In the control group, there were 14 (28%) females and 36 (72%) males. Chi square test was used and there was a statistically significant difference (p<0.001) in gender distribution between the groups.

The mean age [Table/Fig-2] distribution in the cancer group was 40.94 ± 11.78 year. In the precancer group, the mean age was 45.20 ±13.87 year. In control group the mean age in the present study was 26.92 ± 7.73 year. Kruskal Wallis test was used to check the difference in mean between the three groups. There was a statistically significant difference (p<0.001) between the mean ages of three groups.

The mean Herpes Simplex IgG level [Table/Fig-3a] of subjects in the cancer group was 1.39 ± 1.20. Precancer group was 1.28 ± 1.33. In control group was 0.51 ± 0.74. Kruskal Wallis test was used to check the difference in mean between the three groups. There was a statistically significant difference between the mean Herpes Simplex IgG levels of three groups.

Age	N	Mean	(±)SD	Std. Error	95% Confidence Interval		Minimum	Maximum
Cancer (Group I)	50	40.94	11.78	1.67	37.59	44.29	20.00	82.00
Precancer (Group II)	50	45.20	13.87	1.96	41.26	49.14	23.00	73.00
Control (Group III)	50	26.92	7.73	1.09	24.72	29.12	18.00	60.00
Total	150	37.69	13.78	1.13	35.46	39.91	18.00	82.00

[Table/Fig-2]: Age distribution of study subjects.
Kruskal Wallis ; p < 0.001 ; Sig

Herpes Simplex IgG	N	Mean	(±)SD	Std. Error	95% Confidence Interval		Minimum	Maximum
Cancer (Group I)	50	1.39	1.20	0.17	1.05	1.73	.05	4.15
Precancer (Group II)	50	1.28	1.33	0.19	0.90	1.66	.05	4.19
Control (Group III)	50	0.51	0.74	0.10	0.30	0.72	.05	2.77
Total	150	1.06	1.18	0.10	0.87	1.25	.05	4.19

[Table/Fig-3a]: Herpes simplex IgG levels.
Kruskal Wallis; p = 0.001; Sig

Mann Whitney Test		p-value	Significance
Precancer	Cancer	p = 0.629	N S
precancer	Control	p = 0.012	S
Cancer	Control	p < 0.001	S

[Table/Fig-3b]: Mean age comparison.

Herpes Simplex IgG		Negative	Positive
Cancer (Group I)	N	22	28
	%	44.00%	56.00%
Precancer (Group II)	N	28	22
	%	56.00%	44.00%
Control (Group III)	N	41	9
	%	82.00%	18.00%
Total	N	91	59
	%	60.67%	39.33%

[Table/Fig-4]: Different group response to herpes simplex IgG test.
Chi Square Test; p < 0.001, Sig

Mann Whitney test was done [Table/Fig-3b] to compare the mean age between two groups. Difference between precancer and cancer was statistically not significant (p = 0.629). Difference between precancer and control and cancer and control were statistically significant (p = 0.012) and p <0.001 respectively.

[Table/Fig-4] shows different group response to Herpes Simplex IgG test. In the cancer group, 28 (56%) of them tested positive. In precancer group, 22 (44%) of them tested positive. In the control group, 9 (18%) patients tested positive. Statistically there was a significant difference between the groups and Herpes Simplex IgG test result as tested by chi square test (p <0.001).

DISCUSSION

Oral cancer includes a diverse group of tumors of oral cavity which includes the following areas: lip, buccal mucosa, retromolar area, gingiva, floor of the mouth, upper and lower alveolar ridges, oropharynx, hard palate, and the anterior two thirds of the tongue. The use of tobacco products in any form not only is strongly associated with the development of oral cancer but it also affects course of the disease and leads to a poor prognosis [9]. These environmental insults apparently increase DNA damage, reduce Murine Double Minute (MDM2) increase p53 expression and thereby activate clusters of genes associated with cell growth, and/or cell death [10]. The development of oral cancer is a multistep process arising from precancer lesions like leukoplakia and conditions such as OSMF [11]. It is of interest that Herpes simplex virus-1 has been implicated in the development of head and neck carcinoma. Herpes Simplex Virus (HSV) is a common human pathogen found worldwide, and produces a wide variety of diseases.

Transmission of virus can result from direct contact with infected secretions from a symptomatic or an asymptomatic host, with infections ranging in severity from subclinical to life threatening conditions. When primary HSV-1 infection is symptomatic, it is most often characterized by infection of the gums, mouth, tongue, lip, face and/or pharynx (Herpetic Gingivostomatitis). Following primary infection HSV establishes life-long latency in nerve cells in the brain or spinal cord and is present for life. Recurrence of lesions thus reflects reactivation of the latent virus, rather than re-infection and manifesting as 'cold sores' or 'fever blisters' or 'ocular herpes' in the form of herpes labialis. The presence of HSV antigens and RNA complementary to HSV- DNA in tumor tissue of oral carcinoma but not from normal mucosa from the same patients is the strongest evidence for an involvement of HSV with oral carcinoma [12,13]. Evidence has also supported that HSV may act oncogenically by a "Hit and Run Mutagenic Effect" and the oncogenic potential of HSV has been reaffirmed by representing that HSV-1 can act synergistically with tobacco products to

produce oral carcinoma [14]. Lehner et al., in (1973) their study found that there is an increased cell-mediated immune response to HSV-1 in patients having oral leukoplakia with epithelial atypia as compared with patients having oral leukoplakia without atypia or control subjects (without any adverse habit) [15]. Serological studies have shown that HSV-1 has been associated with oral cancer, animal models and in vitro studies have also demonstrated that the virus is capable of inducing oral cancer. Shillitoe EJ and Silverman S Jr (1979) suggested that individuals having oral cancer show increased immune response to herpes simplex virus and concluded that the virus can be carcinogenic or co-carcinogenic in certain conditions, so the virus must be considered as a risk marker for the development of oral cancer [16]. Fragmentary evidence has accumulated to suggest a connection between the HSV-1 and oral cancer and precancer. A later study by Shillitoe EJ et al., (1982) [17] detected neutralizing antibody to HSV-1, 2 and measles virus in serum of oral cancer, oral leukoplakia and control subjects. They found significantly higher titers to HSV-1 among the untreated oral cancer patients and in controls who smoked, than in controls who did not smoke. This study was followed by another study by the same authors (1983) where they detected IgG, IgA and IgM antibodies against HSV-1 in sera of oral cancer patients, patients of acute and recurrent herpetic infection and age matched control subjects [18]. Their results were consistent with the hypothesis that cancer of the mouth is associated with expression of HSV-1 antigens that stimulate IgG, IgM and IgA antibody responses. In 1984, the same authors conducted another study where they detected antibody to early and late antigens of HSV-1 in patients with oral cancer. Their results indicate the existence of at least two different HSV-1 antigens associated with oral cancer. Both are late antigens, one is recognized by IgA, and other is recognized by IgM antibody [19]. In 2001 Jacqueline R. Starr et al., did a population-based study to find the serological evidence of HSV-1in oral carcinoma patients and suggested that HSV1 may enhance the development of OSCC in individuals who already are at an increased risk of the disease because of adverse oral habits or in presence of HPV infection [8]. In vitro studies have elucidated specific mechanisms through which HSV-1 may encourage the transformation of human cells. In human cells, the virus induces DNA synthesis, is mutagenic and inhibits apoptosis; all of which may contribute to carcinogenesis [20]. The involvement of HSV-1 in oral cancer is based mainly on the finding of raised titers of serum antibodies to HSV in patients with carcinoma. Therefore, the current study was intended to determine if oral cancer and precancer patients have higher levels of IgG antibodies to HSV-1 than the matched control subjects by quantitative estimation of IgG antibody by ELISA. Individuals with HSV infection produce virus specific IgG antibodies that are usually apparent for life even during the periods of latency thus, if HSV IgG antibodies are detected in a particular person it indicates that the person is infected with HSV and are capable of transmitting the virus to others. The presence of IgM antibodies in HSV infections is difficult to interpret and may be confusing because unlike IgG, the IgM tests are not type-specific [21]. The previous studies have been conducted using Western blot, polymerase chain reaction, ELISA, Immunoperoxidase antibody membrane antigen techniques for detection of antibodies against HSV-1 [4]. But ELISA has been gaining favor since its introduction by Engvall and Parlmann and is now in use in a wide variety of applications [22]. ELISA is more accurate and a useful technique. It works on native protein and can determine the temperature sensitivity of a given protein (denatured or not denatured). ELISA has the following advantages over western blot method i.e., ELISA can do competition studies (peptides etc.) and interaction studies (proteins et.), it takes less time and consumes less antibodies, Epitope mapping can be performed with ELISA.

This study has taken the advantage of the ability of the ELISA to measure antibody of specific immunoglobulin classes without the need for fractionation of sera. It has been observed in the present study that IgG levels were increased in patients with cancer and precancer as compared to control. Although there was insignificant difference between the HSV-1 IgG level in cancer and precancer patients, there was significant difference between the HSV- 1 IgG antibody in cancer and control group, and in precancer and control group. In the control group nine patients were detected positive of HSV- 1 IgG antibody. The presence of IgG antibodies to HSV- 1 in control group indicates asymptomatic carrier state. Higher IgG levels in cancer and precancer group suggested that HSV-1 is one of the probable etiological factors and might be a synergistic effect for their development.

LIMITATION

There were few limitations to our study like: small sample size, ratio of male and female was not equal, other precancerous lesion like oral lichen planus, erythroplakia were not included in the study and there is no distribution of subjects by the age.

CONCLUSION

Thus it can be concluded that HSV-1 is a possible etiological factor in causation of cancer and precancer. The results are consistent with the hypothesis that cancer of oral cavity is associated with expression of HSV-1 antigen that stimulates IgG antibody response. Quantitative estimation of IgG antibody against HSV-1 in cancer/precancer patients will give the clue in etiology of cancer or precancer in those where genetic mutation will occur. However, further studies with large sample size and equal distribution of specimen by age and gender should be conducted to validate the same.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Dec 28, 2015**
Date of Peer Review: **Feb 19, 2016**
Date of Acceptance: **Apr 14, 2016**
Date of Publishing: **Aug 01, 2016**