International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

Relationship between High Risk Human Papilloma Virus and Immune Expression of Mutated Retinoblastoma (Rb) Protein in Oral Cancer

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Abstract: ***Background: The Retinoblastoma (Rb) is mutated in many types of cancer, since it can be expression even in advanced stages of the disease, HPV has been linked to etiology of oral cancer (OC). The abilities of high-risk HPV (HRHPV) E7 proteins to relationship with the pRB, has been suggested as a mechanism by which these viral proteins induce tumor. The aim of this study was to find out the relationship between mutated Rb protien and this virus. Methodology: in this study 100 patients with (OC) were investigated retrospectively. Mutarted Rb was demonstrated by immunohistochemistry using mutated Rb antibody. HRHPV was identified by polymerase chain Reaction (PCR). Results: Out of the 200 patients, 12/100(12%) were found with HR-HPV. Rb was identified in 25/100(25%) patients. Rb and HPV correlation was identified in 2/12 (17%) with P value >0.05 . Conclusion: In OC, there is no significant correlation between mutated Rb protien and HRHPV***.

Keywords: Retinoblastoma, HPV, Oral cancer

1. Introduction

Oral cancer (OC) accounts for approximately 3% of all malignancies diagnosed annually in 270,000 patients world-wide (Little, et al. 2013). In 2014 an estimated 42,440 American new cases of cancer of the oral cavity with approximately 8,390 deaths (American Cancer Society, 2014).

OC has the highest rates of incidence in Western Europe, India, South Africa and Australia. There is a particularly high incidence of oral cavity cancer in males in France whereas in females (Ferlay, et al. 2010). In the countries of the European Union (EU), each year an estimated 43,847 new oral cancer cases were diagnosed. India has the world's highest incidence of oral cancer, with 77,003 new cases a year (IARC, 2012). Increases incidence of oral cavity and pharynx cancer have been reported in Germany, Denmark, Scotland, Central and Eastern Europe (Stewardt and Kleiheus, 2010), which is thought to be due to an increase in alcohol consumption (Swerdlow, et al. 2010).

It is also more common in developing countries with estimated cases of 130,933 in men (3.1% of all cancers in men) and 68,617 in women (1.8% of all cancers in women). It represents the seventh most common malignancy for men in developing countries with an estimated mortality rate of 2.5% (IARC, 2012).

Many risk factors were well established in etiology of OC, most of which related to lifestyle and environmental factors. Smoking and other tobacco use age are associated with about 75 % of oral cancer. Another potent risk factor is Human Papilloma Viruses (HPVs), particularly; high risk (HR) types 16 and 18. Many studies have strongly proved the link between HPV and oral cancer (Boyle, et al. 2008; Kreimer, et al. 2005).

The retinoblastoma (Rb) tumor suppressor gene plays a key role in the regulation of cell cycle and differentiation, HPV E7 binds a protein termed Rb; and, imilarly, cell cycle regulation is troubled (Boyer, et al. 1996; Bouda, et al. 2000).

Therefore, the aim of this study was to assess the role of mutation of Rb and presence of these High risk HPV virus as possible etiological agents in OC.

2. Materials and Methods

In this study 100 tissue blocks that were previously diagnosed as having OC and their related data were retrieved form Histopathology Laboratories in Khartoum State, Sudan. Five micron tissue sections were obtained from each sample and subsequently immune-stained using mutated Rb antibodies adopting polymer method. Also small tissue section was obtained for DNA extraction and subsequently screened for the presence of HRHPV (16, 18, 31, 33, 35, 52, 58, 59, 68, 73, and 82) using conventional PCR.

Data analysis: Data management was done using Statistical Package for Social Sciences (SPSS version 16). SPSS was used for analysis and to perform Pearson Chi-square test for statistical significance (P value). The 95% confidence level and confidence intervals were used and P <0.05 was considered statistically significant.

Ethical Consent: The study was approved by Faculty Research Board, Faculty of Medical Laboratory Science, Sudan University for Science and Technology. This in

addition to the fact that, the authors followed the tenants of the Declaration of Helsinki.

3. Result

In this study 100 tissue samples from patients with OC were studied for immune-expression of mutated Rb portion and molecular identification of HRHPV correlation. Out of the 100 samples, 25 were Rb positive. HRHPV was detected in 12/100 (12%) of OC samples. HRHPV was identified in 2/25(8%) of mutated Rb positive samples, all of them were HPV16 ($P < 0.05$).as indicated in Table1 and 2, Fig1

Table 1: Distribution of the studied samples by Rb and HPV

Virus	*Rb*		*Total*
	Positive	Negative	
HPV			
Positive	2	10	12
Negative	23	65	88

Table 2: Distribution of the studied samples by Rb and HRHPV

Rb	*HPV genotyping*				*Total*
	16	*18*	*31*	*33*	
Positive	2	0	0	0	2
Negative	5	3	1	1	10
Total	7	3	1	1	12

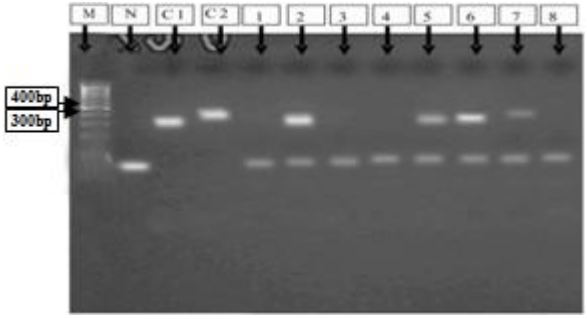

Figure 1: PCR amplification of HPV in oral cancer samples.
Lane M:1000bp ladder, (Arrows shows 300 and 400 band).
Lane N negative control.
Lane C1 positive control for HPV16.
Lane C2 positive control for HPV18.
Lane 2,5,6,7. positive tumor samples.
Lane 2,5,6 HPV 16 positive tumor samples.
Lane 3,4,8 negative samples.
Lane 7 HPV 18. positive tumor samples.

4. Discussion

Although 95% of oral cancers occur in peoples older than 40 (Silverman and Gorsky, 1990), there has been an increase in the incidence of oral cancer in individuals younger than 40 years from 3% in 1973 to 6% in 1993 (Llewellyn et al.2001). This finding may be related to popularity of high-risk habits interacting with genetic predisposition and other environmental factors in young individuals.

In the present study we evaluated the relationship between mutated retinoblastoma protein as factors that are involved in the management of OC and HRHPV as a major causes involved in the development of OC. Several studies have shown that abnormalities of the retinoblastoma are a common mechanism of oral carcinogenesis (Sartor, et al. 1999).

However, the relationship between mutated Rb protein and High risk human papiloma virus was found to be statistically significant. To the best of our knowledge there is no study correlated the relationship between this virus and mutated Rb protein in OC.

The retinoblastoma (Rb) tumor suppressor gene plays a key role in the regulation of cell cycle and differentiation. Its protein product Retinoblastoma tumor suppressor protein (pRb) acts as a regulator at the G1-S restriction point, capable of arresting the growth in mid G1-S phase. Critical to pRb function is the regulation of its phosphorylation state throughout the cell cycle and its ability to interact with other proteins (Pande, et al.1998). Mutations lead to functional pRb inactivation and failure of pRb mediated growth and tumor suppression (Kim, et al. 1993).

Although the alteration in Rb protein expression in tumorigenesis has not been completely elucidated, there is increasing evidence that Rb protein pathway is rendered dysfunctional in oral cancer (de Oliveira, et al. 2012).

The epidemiological association of HRHPV with OC, was well established (Anaya , et al. 2008; Oliveira, et al.20009). The prevalence of oral carcinomas reported to be associated with HPV has varied widely. This is due to differences both in the population studied and in the sensitivity of the assay used for HRHPV detection (Liang, et al. 2008; Lee, et al. 2010).

HPV must adhere to a specific receptor protein on the keratinocytes membrane. Once the virus entered into the cell, it transforms itself of its protein coat and the viral DNA may then utilize host cell themselves. These viruses elaborate early gene proteins (E) that are able to regulate the host cell cycle, or mitotic capabilities. The E6 and E7 proteins are most important in this respect; they bind two host proteins that are regulators of the keratinocytes at the time of cell division. E6 binds to a protein designated p53, a molecule that arrests cell division. However, once bound, it is degraded and this inhibition of keratinocytes mitosis is abrogated. Likewise, E7 binds a protein termed Rb; and, imilarly, cell cycle regulation is troubled (Boyer, et al. 1996; Bouda, et al. 2000).

Future prospect: Further studies on the exact relationship between HRHPV and mutated Rb protiens is needed, which may help in patients management.

In conclusion: There is strong correlation between HRHPV viruses and OC. There is no correlation between HRHPV and mutation of retinoblastoma protein in OC. Knowledge of the exact interaction between Rb and HRHPV viruses may stimulate new ideas that help in prognosis, treatment and overall management of patients with OC.

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