

IMMUNOHISTOCHEMICAL EXPRESSION OF P53, EBV-LMPI AND CYCLIN D1 PROTEINS IN OESOPHAGEAL CARCINOMA: A PILOT RETROSPECTIVE STUDY OF 26 CASES.

T.L.Shiramba¹ H.ALodeny², A.K.Gachii³, J.M Kabanga², J.K Kuria²,

¹King Faisal Hospital, Kigali, Rwanda.

²Kenya Medical Research Institute.

³Kenyatta National Hospital, Nairobi, Kenya

ABSTRACT

Introduction: The development of oesophageal carcinoma has been attributed to various environmental factors and its incidence varies regionally. The development of this disease is known to occur in recognized histological stages from normal through dysplasia to the malignant stage. Like other cancers, the diagnosis of oesophageal cancer in its premalignant stage would improve the survival. The diagnosis of this cancer on cytomorphology alone is usually done in the late stage of the disease. To be able to diagnose this disease in its early stage, specific tumour markers must be found. The objective of this study was to evaluate p53 tumour suppressor gene protein expression, Epstein-Barr virus latent membrane protein expression and cyclin DI cell cyase protein expression in malignant and normal oesophageal tissues to see whether any variation in their expression in these tissues could be of diagnostic or prognostic value.

Methods: 26 archival formalin-fixed paraffin wax embedded tissue blocks of oesophageal carcinoma and 6 of normal oesophagus obtained by endoscopy were studied. 5µm thick tissue sections were cut onto poly-L-Lysine coated microscope slides and dried at 60°C for 60 minutes. p53 gene protein expression, EBV-LMPI protein expression and cyclin DI expression were studied immunohistochemically in these tissue sections. Sections were dewaxed and hydrated to Tris-buffered saline, pH 7.6. Appropriately diluted primary antibodies to p53, EBV-LMPI and Cyclin DI were applied to different sections and incubated overnight at 40°C in a humidity chamber. Sequential applications of other reagents in a three-stage peroxidase anti-peroxidase method were applied for chromogen immunoreaction for light microscope visualization. The sections from normal oesophageal tissues were processed with carcinomatous tissues.

Results: p53 gene protein was overexpressed in 17 of 26 cases of carcinoma; EBV-LMPI was expressed in 12 of 26 cases of carcinoma; cyclin DI protein was expressed in 14 of the 26 cases of carcinoma; 10 cases of p53 expression were also associated with EBV-LMPI protein expression; 7 cases of p53 protein overexpression did not express EBV-LMPI; 2 cases of EBV-LMPI protein expression did not express p53 protein and 7 cases did not express both p53 and EBV-LMPI proteins.

Conclusion: Overexpression of p53 tumour suppressor gene protein in tumour cells of 17 of 26 cases of oesophageal carcinoma while no such expression was demonstrated in normal oesophagus, may have diagnostic and prognostic value. EBV-LMPI expression in tumour cells of 10 of 26 cases of oesophageal carcinoma may also be of value in diagnosis and pathogenesis. Cyclin DI was overexpressed in 14 of the 26 cases and may have diagnostic and prognostic value.

Keywords: p53 :Tumour suppressor gene - EBV-LMPI : Epstein-Barr virus latent membrane protein 1 - Cyclin DI

INTRODUCTION

Carcinoma of the oesophagus is a common malignant tumor, but the least studied, and is among cancers with the poorest prognosis. Environmental factors may contribute to the development of the disease that is demonstrated by the striking geographic variations in its incidence. Regions of the world where oesophageal

carcinoma is quite common include parts of Africa and Asia. In some parts of China, the mortality rates from oesophageal carcinoma in men has been reported to be about 10 times the rates reported in the USA, where it accounts for 7% of all gastrointestinal cancers [1].

Although the etiology of esophageal carcinoma is not clear, some studies have suggested that carcinogenesis may be initiated by oxidative damage caused by cigarette smoking or gastro-oesophageal reflux (GERD) which cause

*Correspondence to: Dr. Titus L. Shiramba
King Faisal Hospital, Kigali
P.O. Box 2534 Kigali-Rwanda
E-mail: tshiramba@yahoo.com

inflammation and increase the cell turnover, thereby potentially increasing chances of cell mutations. Other studies have suggested that consumption of alcoholic beverages and ingestion of caustic fluids may predispose to squamous cell carcinoma of the oesophagus. Yet other studies have incriminated food contamination by fungal toxins as contributing factors [1,2,3,4,5].

The development of oesophageal carcinoma is known to go through stages of mild, moderate and severe dysplasia before the malignant stage. It has been reported that the genetic lesions, which determine the destiny to malignancy, can be found in these premalignant and malignant lesions. It is also known that some dysplastic lesions do not progress to malignancy. A factor common to both the premalignant and malignant lesions, which could be demonstrated in the tissue sections of these lesions, would be for the diagnostic and prognostic value and even be targeted in treatment modalities. This study aims at the immunohistochemical expression of p53 gene, Epstein-Barr latent membrane protein (EBV-LMP1) and cyclin D1 in esophageal carcinoma, and examines their diagnostic and prognostic potential [6,7,8,9,11-14].

p53 is a tumour suppressor gene whose name is derived from the protein product it encodes: p53, a polypeptide having a molecular mass of 53.000 daltons. p53 acts as a transcription factor that activates the expression of protein p21 that inhibits the cyclin-dependent kinase moving a cell through the cell cycle [3-10].

The primary function of p53 is to arrest cell cycle progression or initiate apoptosis in response to cell injury. Accumulation of p53 gene product, which is amenable to demonstration by immunostaining, is believed to be due to point mutations with conformational change and increased half-life, complex formation with viral and cellular oncogenes, and aberrant expression by cellular transcriptional regulators. The gene lies on the chromosome 17p and has been studied in many inherited and a wide variety of human primary tumors, and cell lines derived from tumours. p53 overexpression has been described in several studies as occurring with increased frequency as lesions progress from normal to dysplastic and to carcinomatous [2-6].

It has been suggested that p53 alterations in the oesophagus may precede the morphologic changes seen during tumour development. If these alterations in p53 could be detected earlier by immunostaining, it may raise the possibility of earlier diagnosis before morphologic changes on which histologic diagnosis is based [6-9].

Epstein-Barr Virus (EBV) is one of the eight herpes viruses. The virus is so widely disseminated that 95% of adults in the world expresses its antibodies. It has been shown

that EBV gains direct access to the squamous epithelial cells of the nasopharynx by forming an IgA-EBV complex, which is then taken up by endocytosis. Once infected with EBV, individuals remain asymptotically infected for life [10,11,12,13].

EBV has been implicated in the pathogenesis of Burkitt's lymphoma and nasopharyngeal carcinoma. EBV is known to immortalize and induce proliferation in infected cells. It is thought to be an initiator in the process of carcinogenesis, with other factors acting as promoters [12].

The pathogenesis of nasopharyngeal carcinoma is related to the infection with EBV in early childhood, with the reactivation later in life and the subsequent appearance of tumours [12].

Due to the close association of EBV in the development of nasopharyngeal carcinoma, EBV antigen was studied in benign, dysplastic and carcinomatous lesions of the oesophagus to evaluate the stage of correlation of EBV with pathogenesis. In nasopharyngeal carcinomas, EBV antigen has been demonstrated in tumour cells [12].

Cyclins are proteins that activate crucial protein kinases and help control progression through the cell cycle. 15,18 Cyclin D1 (bc1-1/PRAD) gene is a GI-specific cyclin located on chromosome 11p13, within a region that has been reported to be amplified in about 20% of carcinomas of head and neck, breast, oesophagus and lungs [15-18]. The immunohistochemical demonstration of the overexpression of cyclin D1 would be of value in the investigations of the tumorigenesis, the diagnosis and the prognosis [15].

MATERIALS & METHODS

26 archival formalin-fixed paraffin wax embedded tissue blocks of oesophageal carcinoma and 6 of normal oesophagus obtained by endoscopy were studied. 5µm thick tissue sections were cut onto poly-L-Lysine coated microscope slides and dried at 60°C for 60 minutes. p53 gene protein expression, EBV-LMP1 protein expression and cyclin D1 oncoprotein expression were studied immunohistochemically in tissue sections. Sections were dewaxed and hydrated to Tris-buffered saline, pH=7.6. Appropriately diluted primary antibodies to p53, EBV-LMP1 and cyclin D1 were applied to the different sections and incubated overnight at 40°C in a humidity chamber. Sequential applications of other reagents in a three-stage anti-peroxidase method were applied for chromogen immunoreaction for light microscope visualization. The sections from normal oesophageal tissues were processed with carcinomatous tissues. Sections were brought to room temperature for 30 minutes. After washing in 3 changes of 2 minutes in washing buffer, biotinylated secondary antibody

was applied to the sections for 30 minutes at room temperature in a humidity chamber. After washing the secondary antibody, sections were sequentially incubated in streptavidine-peroxidase for 30 minutes, reaction with 3,3-diaminobenzidine and hydrogen peroxide in TBS-pH 7.6, for 10 minutes. Sections were lightly counterstained with haematoxylin, dehydrated, cleared in xylene and mounted in DPX mountant. For negative controls, sections were incubated with antibody diluent buffer without primary antibody. Evaluation of the immunoreactivity was done on the light microscope. The immunoreactivity was scored on a scale of 0 to 3 based on the intensity of the positive cells. Negative immunoreactivity was scored as minus (-), weakly positive cells scored as 1+, moderately-positive cells scored as 2+, strongly-positive cells scored as 3+.

RESULTS

P53 gene protein was overexpressed in 17 of 26 cases of carcinoma; EBV-LMPI was expressed in 12; cyclin DI protein was expressed in 14; 10 cases of p53 expression were also associated with EBV-LMPI protein expression; 7 cases of p53 protein overexpression did not express EBV-LMPI; 2 cases of EBV-LMPI protein expression did not express p53 protein and 7 cases did not express both p53 and EBV-LMPI proteins. [Table 1, Appendix figures 1, 2, 3, 4, 5, 6].

Twenty six primary oesophageal carcinoma cases were assessed immunohistochemically for p53, EBV-LMPI and Cyclin DI.

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Table1: Immunoreactions for P53, EBV-LMPI and Cyclin DI in 26 cases

Antibodies	Positive	Negative	Total
P53	17	9	26
EBV-LMPI	12	14	26
Cyclin D ₁	14	12	26

P53 immunostaining positivity was found in 17 (65.3%) of all oesophageal carcinomas, using the monoclonal primary antibody.

The results of this study are consistent with those of other studies which reported p53 positive staining in squamous cell carcinomas of oesophagus using monoclonal antibodies. A larger study on a larger number of cases is planned to follow this study.

EBV-LMPI immunohistochemical and haematoxylin/eosin staining in adjacent cells revealed that carcinoma cells expressed EBV-LMPI in their nuclei. These results suggest an association between the carcinoma cells and EBV. The EBV-LMPI positive carcinoma cells were p53 positive in 10 (38.5%) cases. Cyclin DI showed positive immunostaining in 14 cases.

CONCLUSION

Overexpression of p53 tumour suppressor gene protein in tumour cells of 17 cases - while no such expression was demonstrated in normal oesophagus - may have diagnostic and prognostic value. EBV-LMPI expression in tumour cells of 10 cases may also be of value in diagnosis and pathogenesis. The 10 EBV-LMPI positive cases were also p53 positive. Cyclin DI was over expressed in 14 cases and may have also diagnostic and prognostic value.