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ORIGINAL ARTICLE

Expression Of P53, Ck7, Ck20 And Rb Proteins in Oesophageal Squamous Cell Carcinoma In Iran

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Background:
Cancer of the oesophagous is one of the most malignant tumours and has a poor prognosis. The p53 and retinoblastoma (Rb) genes are involved in the regulation of the cell population by the suppression of cell proliferation. Our aim was to show whether the expression of the p53, Ck7, Ck20 and Rb genes could be used as a prognostic indicator in oesophageal squamous cell carcinoma.

Methods
Samples obtained from 49 patients undergoing subtotal or total oesophagectomy were mmunohistochemically stained for studying the expression of the p53, Ck7, Ck20 and Rb genes and then the clinicopathological characteristics of those patients were investigated.

Results
P53 was positive in 40.4% of the patients (more than 20% of the tumour cells were stained), 12.8% were partially positive and in others, it was negative. Immunohistochemical staining for the expression of the Ck7, Ck20 and Rb genes in our samples was negative. P53 seemed to have significance in the prognosis of the cancer but Ck7, Ck20 and Rb expression did not have prognostic significance in the surgical treatment of oesophageal cancer.

Key Words: p53, Ck7 (Cytokeratin 7), Ck20 (Cytokeratin 20), Rb, Oesophageal Squamous Cell Carcinoma (ESCC)

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Introduction

Cancer of oesophagus is the 10th most common malignancy in the world, the second ranking after heart infarction according to the last report of the EMRO's regional office for the Eastern Mediterranean Organization [1]. Cancer of the oesophagus shows a great variation in geographical distribution. It is the fifth most common cancer in developing countries and about 300,000 new cases are diagnosed every year. Epidemiological studies have identified several-high incidence areas in China, Singapore, Iran, Russia, Puerto Rico, Chile, Brazil, Switzerland, France and South Africa, but the causes for striking geographical variations in the incidence of human oesophageal cancer remain obscure. One of the features of squamous cell carcinoma of the oesophagus is the fragmentation of its incidence into low risk and high risk areas, based on geographical location. Some of the low risk areas include North America, countries in Western Asia and Northern and Southern Europe, where the incidence rates range from 1.5 to 6.0/100,000 and well defined high risk areas such as South Africa, China, Iran and countries in Eastern Africa, where the incidence rates range from 10 to 25/100,000

[2],[3],[4],[5] Iran, one of the areas with the highest rate of Oesophageal Squamous cell carcinoma (ESCC) cases in the north of Iran, especially the Guilan province, is a region with high incidence. ESCC most commonly show morphological to squamous cell carcinoma of other regions of the body like cervix and oral cavity. It is generally agreed that tobacco, alcohol, hot black tea (bad habit in some regions in the north east of Iran) and alcohol consumption in Europe are the major environmental risk factors for the development of ESCC [6,7,8]. However, some patients developed ESCC even without these 4 risk factors. Hence, this fact suggests that additional causes such as genetic predisposition, diet or oncogenic viruses may also help cells to override or escape the physiological mechanism of proliferation control. p53 protein is a 53-kd nuclear phosphoprotein encoded by the gene that locates on the short arm of chromosome 17 (17p13.1).Wild- type p53 is known to suppress cell proliferating activity in normal tissues .In tumour tissues, however, this function disappears because of mutation of the genes, as observed in tumours originating from the colon, lung, breast, ovary brain, liverand the haematopoietic tissues. p53 mutation has been identified with a frequency of 8% to 85% in studies of oesophageal cancer. Retinoblastoma (Rb) also is linked to a tumour suppressor gene located on the long arm of chromosome 13 (13q14.2)..Rb protein, a 110- to 114 –kd nuclear phosphoprotein, is expressed in most normal cells. The function of this protein depends on its phosphorylation status. An underphosphosphorylated form of the Rb protein is identified mainly in resting cells, whereas the hyperphorylated form is present in proliferating cells. Cellular level Rb protein may vary during the cell cycle, but the inhibitory effect of Rb protein on cell cycle progression can be abrogated during tumour development.Complete absence of Rb protein immunoreactivity indicates Rb gene alteration. [9,10] The use of cytokeratins 20 (CK20) and 7 (CK7) was proposed to identify the primary sites in this situation. Ductal breast carcinomas and lung and non-mucinous ovarian adenocarcinomas showed significant differences in CK7 expression when primary and metastatic tumours were compared. CK20 positivity alone indicates the metastatic spread of adenocarcinoma in several organs. CK7 negativity is consistent with metastases of adenocarcinomas in the lungs, ovaries, liver or serous membranes. CK20/7 phenotyping of adenocarcinomas is a useful diagnostic tool if based on algorithmic and probabilistic [11]

Method and Materials

Sample collection

A total of forty- nine formalin –fixed paraffin embedded samples of ESCC were collected from the 2005 – 2007 surgical pathology archives of Rasht Razi Hospital, Pour Sina Hospital, Golsar Hospital, Dr.Satari (Sina) Pathobiology lab and Dr.Saffari (Fouman) Pathobiology lab. The patients visited the Rasht Razi Hospital from various parts of the Guilan province, which is a major center of Gastro Intestinal diseases. The selection was made according to the availability of fixed tumour tissues and clinico-pathological data. The paraffin-embedded blocks were obtained from surgical (total or sub total oesophagotomy) or biopsy specimens of oesophageal cancer patients. First, Hematoxylin and Eosin –stained slides were prepared by expert histopathologists to confirm ESCC and tumour stage. The carcinomas were further subclassified into 3 categories according to standard histological criteria: well differentiated, moderately differentiated and poorly differentiated. Then, the remaining samples were re-examined for Immunohistochemical studies. Information of age and gender was recorded.

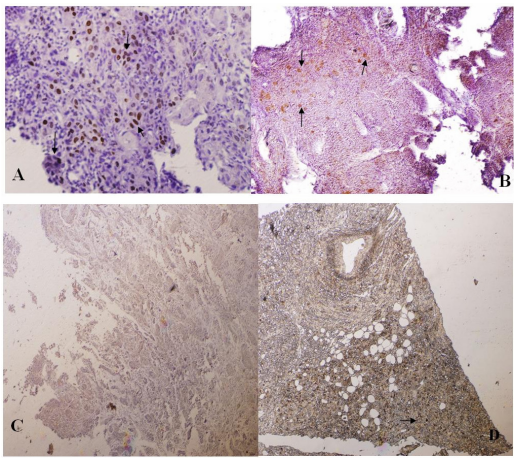
Immunohistochemistry

Immunohistochemical studies were performed on 4 to 5-µm tissue sections which were mounted on silanized slides using commercially available monoclonal antibodies: anti-p16INK4A (clone 6H12) and anti-Rb (clone IF8) obtained from

Novocastra (Newcastle-upon-Tyne, UK) and anti-p53 (Clone DO-7) from DAKO (Glostrup, Denmark). Immunohistochemical staining was conducted using the DAKO system (Envision +dual link system – HRP) according to the manufacturer's instructions. Briefly, dewaxed sections were heated in a microwave oven for 2X for 5 minutes in 10 mM citrate-Na (pH 6.0). After incubation with blocking serum for 20 minutes, the sections were incubatedwith primary antibodies which are described above for 1 hour at room temperature with an antibody dilution of CK7 and CK20 and anti-p53 and anti-Rb. After further incubation with biotynilated link antibody and peroxidase-labeled streptavidin, the staining was developed by reaction with DAB+ chromogenic solution diluted 50:1(DAKO, Glostrup, Denmark) under microscopic control. In each experiment, a negative control in which the primary antibodies were replaced by preimmune mouse immunoglobulin G (IgG), and positive control slides were included. Nuclear staining was considered to be positive for p53, Rb, Ck7 andCk20. A tumour was recorded to be positive if more than 10% of the tumour cells showed immunoreactivity. The staining characteristics were compared with adjacent nonneoplastic squamous epithelium. In normal epithelium, pRb is expressed in the nucleus of epithelial cells which are located in the parabasal and suprabasal layers. Tumour samples were scored as positive when staining was observed in more than 30% of the tumour cell nuclei. p53 expression was rarely detectable in the normal epithelium. We used a 10% cut off for nuclear p53 staining

Results

The mean age at diagnosis was 64 (range 35 to 81) years and there were 22 female patients and 27 male patients. p53 expression was completely positive in 19 patients (40.4%), partially positive in 6 patients (12.8%) and negative in 22 patients (22%).



(Table/Fig. 1. Representative example of a positive ESCC with strong nuclear p53 immunostaining A and B (Arrows show nuclear staining) and negative Rb staining C .D is control positive (breast cancer with p53 positive). All sections shown are 100 × magnifications, with the exception of panel A, 200× magnification

Discussion

Expression of the markers of both SCC of the oesophagus and their associated pre-malignant lesions can be influenced by several factors. These factors could be related to different processes involved in the initiation and progression of the cancer. Factors implicated in the defense mechanisms against toxic intermediates as well as parameters related to inflammatory reactions are often analyzed as markers in the early events of cancer development. Moreover, the inactivation of the protein products of certain genes which are responsible for cell cycle regulation and cellular differentiation are important biomarkers in the development of cancer [12]. In this study, we analyzed P53 mutation patterns in the ESCC samples of 49 patients living in the North of Iran (Guilan ,Rasht).The prevalence of mutations was 20 to 70 % (the overexpression of p53 showed the characteristic nuclear location with variations in the staining intensity and in the number of positive cells). 19 of the 49 tumours were more than 70% p53 positive and 6 of the 49 tumours were less than 20%

positive; the majority of p53 –positive tumours showed definite p53 immunostaining in 20% to 70% of the tumour cells, which is similar to other studies in Iran (50 to 65%) and France (over 80%) or in Eastern countries, such as China (42–70%) . These findings were reported from southern Brazil also [13].

Squamous cell carcinoma of the oesophgous is one of the most malignant diseases and has a dismal prognosis .This poor prognosis is believed to be associated with the structural characteristics of the oesophagus ie; lack of serosa and abundant lymphatic channels. Patients in whom the p53 expression was high had greater tumour diameter, deeper tumour invasion and worse prognosis as compared to patients in whom the p53 expression was low. Metastatic adenocarcinoma from an unknown primary site is a common clinical problem. The use of cytokeratins 20 (CK20) and 7 (CK7) was proposed to identify the primary sites in this situation. Most tumours retained the CK20 phenotype during metastasis. CK20 positivity alone indicates the metastatic spread of adenocarcinoma in several organs. CK7 negativity was consistent with metastases of adenocarcinomas in the lungs andovaries. Ck7 and Ck20 expression was not seen in our samples may be because it was negative in all our patients.

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Conflict Of Interest: Non declare

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