

# EXPRESSION OF HYPOXIA INDUCIBLE FACTOR AND VASCULAR ENDOTHELIUM GROWTH FACTOR IN IRRADIATED BLADDERS

RICARDO CASTELLANI DE MATTOS, ISABELLA DOS SANTOS GUIMARÃES, ELIZÂNGELA MARQUES RODRIGUESLEANDRO DE SOUZA THIAGO, ANDRÉIA CRISTINA DE MELO.

Instituto Nacional de Câncer José Alencar Gomes da Silva

## Introduction

Pelvic radiotherapy is indicated as a primary treatment for cervical cancer or part of its treatment at all stages from IA2. Radiotherapy can have significant acute and chronic adverse effects that can be related to the development of hypoxia. The main presentation symptom of chronic radiation-induced cystitis is hematuria, which can be a result of the development of new vasculature in the bladder mucosa. Under low oxygen tensions, Hypoxia Inducible Factor (HIF) can activate genes that promote angiogenesis. The primary objective is to analyze the expression of HIF and Vascular Endothelial Growth Factor (VEGF) in fragments of tissues of patients with cervical cancer submitted to radiotherapy treatment versus normal tissue.

## Materials and Methods

The study is case-control design. The expression of HIF1a and VEGFR mRNA expression levels will be evaluated on tissue fragments collected by endoscopic bladder biopsy, comparing patients submitted to pelvic radiotherapy for treatment of cervical cancer with patients without previous pelvic radiation.

### Inclusion criteria

- Patients who signed the Informed Consent Form.
- Patients treated for cervical cancer, submitted to external radiotherapy at the Cancer Hospital I / INCA from January 2010 to June 2016. As a control group, we will evaluate patients with cervical cancer before treatment, submitted to endoscopic urological evaluation at the time of its staging.
- Histology of epidermoid carcinoma or adenocarcinoma.
- Available records for data collection (clinical and laboratorial findings and treatment strategies).

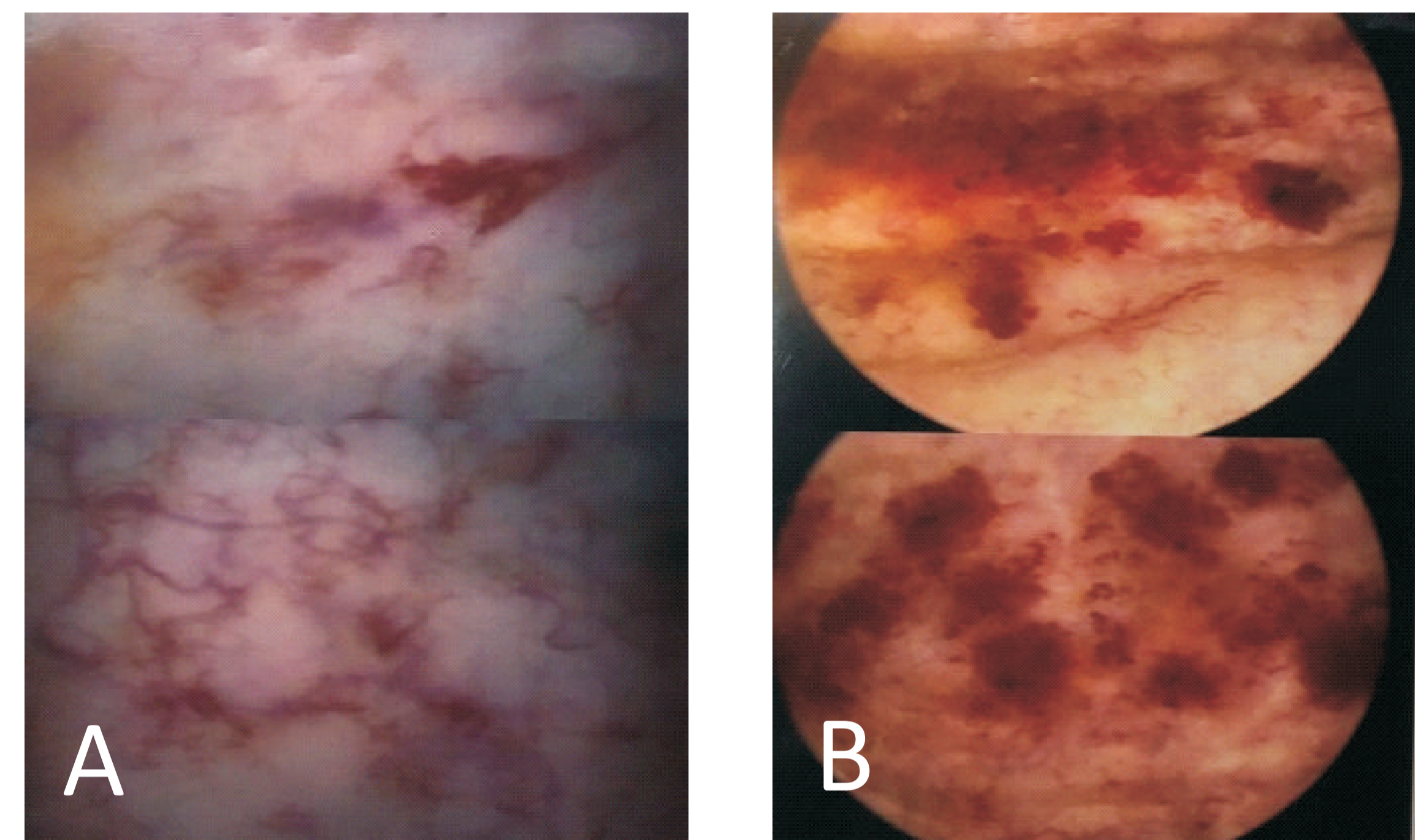
**Table 1.** Clinical and demographic characteristics of patients with cervical cancer included in the study.

Variables	Categories	Irradiated patients (n=14)	Non treated patients (n=10)
Age (median. years)		48 (31-66)	46,5 (33-68)
Race/color of skin	white	7	4
	brown	5	3
	black	1	3
FIGO <sup>1</sup> Staging	Ib1	2	1
	Ib2	1	1
	IIa	1	1
	IIb	9	7
	IIIb	1	0
Histology	Squamous cell carcinoma	12	10
	adenocarcinoma	2	0
EBRT <sup>2</sup> dosage (mean, cGy)		4577.14	
Brachytherapy dosage (mean, cGy)		2461.53	
Total radiation dosage (mean, cGy)		7044.61	
Time between radiation and cystoscopy (mean, months)		46	
RTOG/EORTC <sup>3</sup> Scoring	0	0	
	1	8	
	2	3	
	3	3	
	4	0	
	5	0	
Radiation Proctitis		7	

1- International Federation of Gynecology and Obstetrics;

2- External Beam Radiation Therapy;

3- Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer.



**Figure 1.** Endoscopic findings among irradiated patients. A – RTOG/EORTC grade 1; B – grade 3

## Expected Results

Samples from 14 patients staged Ib1 to IIIb, that received 6900 to 7840 cGy (external radiotherapy and brachytherapy) were collected between 24 to 69 months after treatment.

RNA was extracted from samples using QIAGEN® RNeasy Mini Kit following manufacturer's instructions. RNA quantification was determined by Nanodrop-model ND-1000 spectrophotometer (Thermo Fisher Scientific, MA, USA). Subsequently, the sample was stored at -80° C for cDNA synthesis. The SuperScript™ II RT kit (Bio-Rad, CA, USA) was used for the synthesis of complementary DNA (cDNA) from 1µg of previously extracted RNA. HIF1a and VEGFR mRNA levels will be investigated by droplet digital PCR (ddPCR). They will be compared to 8 control patients also Ib1 to IIb without previous treatment.

Since under low oxygen tensions HIF alfa escapes hydroxylation and interacts with transcriptional coactivators to regulate the cellular adaptative response to hypoxia, including glycolysis, angiogenesis and erythropoiesis, and since hypoxia is a well-known result of tecidual radiation, it can be expected that the increase in vascular proliferation in the bladder of irradiated patients be at least in part explained by the