Adhesion molecules expression: Beta 1 integrin and ICAM-1 as potential markers of cutaneous radiation injuries induced by ionizing radiation overexposures

Lendoiro N[1], de la Vega S[2], Portas M[2], Dubner D[1],
Rossini A.[1]

[1]*Nuclear Regulatory Authority, Av. del Libertador 8250, Ciudad Autónoma de Buenos Aires, C1429BNP, Argentina*
[2]*Hospital de Quemados "Dr. Arturo Umberto Illia", Av. Pedro Goyena 369, Ciudad Autónoma de Buenos Aires, C1424BSD, Argentina*
Corresponding author's e-mail: nlendoiro@arn.gob.ar

Abstract: During the inflammatory response, there is a process of leukocyte extravasation that involves the migration of these cells from the bloodstream towards target tissues through their adherence to vascular endothelium. Leukocyte extravasation is coordinated and regulated by the expression of a variety of glycoproteins implicated in cell-cell interactions. Cell adhesion molecules (CAMs) mediate interactions between blood cells and endothelial cells that can occur in all segments of the microvasculature as a response to inflammation under certain conditions such as overexposure to ionizing radiation. On the other hand, the beta integrin family of proteins interacts with the associated ligand (intercellular adhesion molecules) in the vascular endothelium. This transient binding results in further leukocyte activation and subsequent firm adhesion and transendothelial migration into sites of inflammation. The present study examines the expression of two of these proteins: Beta 1 integrin and ICAM-1, using flow cytometry and immunohistochemical techniques, in blood samples and biopsies from patients overexposed to ionizing radiation. This work shows the correlation between the expression of Beta 1 integrin in lymphocytes from blood samples and the expression of the associated ligand in endothelium ICAM-1 and the possible role of this interaction between these molecules in the initial phases of infiltration into the tissue affected by radiation exposure.

KEYWORDS*: Integrin, ICAM, overexposure*

1. INTRODUCTION

Up to now there are no established parameters for the follow-up of delayed radiation injuries [1]. Late toxicity is generally irreversible and can have devastating effects on quality of life of people exposed either accidentally or during therapeutic radiation treatments. These effects are the consequences of both, an imperfect tissue remodeling and persistent radiation induced injuries [2].

The main feature of severe radiation burns is the occurrence of unpredictable successive inflammatory waves leading to the extension, in surface and in depth, of the necrotic process [3]. Histologically, late manifestations of radiation damage include fibrosis, necrosis, atrophy and vascular lesions [4].

Delayed reactions are considered those that occur beyond 90 days from the start of the radiotherapy. They are categorized according to the criteria for registration of late radiotoxicity in skin and subcutaneous tissue proposed by the Toxicity Criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) [5]. In the case of interventional practices, the same criteria are adopted.

Radiotoxicity criteria - EORTC / RTOG - Tissue: skin –
Late toxicity
Grade 1: Skin slight atrophy; pigmentation change; some hair loss.
Grade 2: Patch atrophy; moderate telangiectasia; total hair loss.
Grade 3: Marked atrophy; gross telangiectasia.
Grade4: Ulceration.

A fundamental event in the inflammatory response is the recruitment of blood leukocytes to a site of injury or infection, often resulting in tissue dysfunction and damage [6]. The recruitment of leukocytes

from circulating blood is decisive in the inflammatory reaction. This innate immunity response consists of a well-defined and regulated multi-step cascade involving consecutive steps of adhesive interactions between the leukocytes and the endothelium [7]. All the steps in the recruitment cascade are orchestrated by cell-adhesion molecules (CAMs) on endothelial cells and different subsets of CAMs are responsible for different steps in extravasation. However, long term alterations of CAMs expression in irradiated tissues remain unclear [4]. On the other hand, integrins are adhesion molecules expressed on leucocytes that interact with CAMs in the endothelium playing a main role in transendothelial migration towards the inflammatory foci. Adhesion receptors regulate numerous processes such as cell activation, migration, growth, differentiation, and death [8-10].

Cell-cell interactions are essential for regulating the inflammatory response [8,11,12]. The coordinated functioning of adhesion receptors, the cytoskeleton and signalling molecules is crucial for leukocyte extravasation, a key process in immune response [11,13]. During the initial contact with the activated endothelium leukocytes roll along the endothelium. Subsequently, leukocytes are activated by chemokines presented on the luminal endothelial surface, which results in the activation of leukocyte integrins and the firm leukocyte arrest on the endothelium. After their firm adhesion, leukocytes perform a transmigration process to pass the endothelial barrier [7]. Blood leukocyte trans-endothelial migration involves a sequential, multistep adhesion cascade between leukocyte and endothelial cell adhesion molecules [6]. The steps involved in adhesion cascade are leukocyte tethering and rolling (Step 1), activation (step 2), firm adhesion (step 3), crawling (Step 4) and transmigration across the endothelium (Step 5).

Step 1: Leukocyte tethering and rolling

In the initial phase of an acute inflammatory response, circulating leukocytes respond to locally produced proinflammatory factors or inflammatory mediators, leading to their tethering and rolling along the surface of endothelial cells lining postcapillary venules. This phase involves endothelial E- and P-selectin, leukocyte L-selectin; integrin α4β1/α4β7 [6]. To initiate the inflammatory response, circulating leukocytes in the bloodstream have to establish contact (tethering) with the vascular wall and adhere to it. Tethering and rolling of the leukocytes over the activated endothelium are the first steps in the sequential process of extravasation. The initial contact or tethering is largely mediated by selectins and their ligands α4β1-and α4β7-integrins [8,15]. Tethering slows the speed of travel of the leukocytes and allows them to roll over the endothelial surface, favouring subsequent interactions mediated by integrins and their ligands and increasing leukocyte adherence. As a result, the leukocytes finally come to a halt on the vascular wall [8,16]. On the other hand, the interaction between lymphocyte function-associated antigen LFA-1 and cell-cell adhesion molecule ICAM-1 collaborates with the function of L-selectin, thereby stabilizing the transient contact phase and reducing the rolling velocity [8, 17,18].

Step 2: Activation

Further stimulation by endothelial cell-bound chemokines leads to rapid activation of leukocyte β1 and β2 integrins [6].

Step 3: Firm adhesion

The activation of β1 and β2 integrins results in arrest of leukocyte on the endothelium surface [6]. Integrins are fundamental molecules in cell migration. They control the cell-cell and cell-extracellular matrix interactions during recirculation and inflammation. Thus, circulating leukocytes in blood maintain their integrins in an inactive conformation to avoid nonspecific contact with uninflamed vascular walls, but when they arrive at the inflammatory focus, a rapid in situ activation of the integrins occurs [8,19].

Step 4: Leukocyte crawling

The β2 integrins seem to play an important role in crawling [20], leukocytes initially crawl on endothelial cells before arresting and transmigrating and there is an interaction between the molecule integrin LFA-1/αLβ2 and ICAM-1[21].

Step 5: transmigration across the endothelium (or diapedesis)

Once the leukocytes have reached an appropriate site for transmigration (preferably the intercellular junctions), they deploy exploratory pseudopods between 2 adjacent endothelial cells. During this process, the LFA-1 (CD11a/β2) molecule is the integrin with the predominant role. This molecule is quickly relocalized to form a ring-shaped cluster at the contact interface between the leukocyte and endothelium, where it interacts with ICAM-1 [8,22].

Previous own studies have shown that there is a significant increase of β1 Integrin expression on gated lymphocytes of patients with late cutaneous reactions graded 4 according to the RTOG / EORTC score and this increase showed good correlation with the patient evolution [4].

The objective of the present study is to examine the correlation between the expression of two of these proteins: Beta 1 integrin in lymphocytes and ICAM-1 on endothelial cells in patients overexposed to ionizing radiation in the initial phases of infiltration into the tissue affected, as a useful tool in an emergency.

2. MATERIALS AND METHODS

Patients

Patients referred to the Radiopathology Committee of Hospital de Quemados del Gobierno de la Ciudad de Buenos Aires (Burn Hospital) for the diagnosis and therapy of Cutaneous Radiation Syndrome, presenting radiation lesions classified grade 4 RTOG/EORTC were selected for this study due to the severity of the symptoms and therapeutic challenges.

In this study 3 representative cases, grade 4 late toxicities, were chosen as flow cytometry analysis was complemented by histological analysis of biopsies obtained the same day of blood sampling. Late effect was considered three months from the date of the radiation procedure. The study was approved by the Research and Ethics Committee of Burn Hospital. Informed consent was obtained from all patients.

Sample Collection

A total of 3ml of blood was collected into EDTA venous blood collection tubes (Vacutainer, BD) and maintained at room temperature until processed within 24h.

Flow cytometry

The assessment of B1 integrin (CD29) was performed by staining 50 µl of whole blood with Ab anti CD29 labelled FITC. Samples were lysed with a lysing solution and samples were analyzed in a flow cytometer (BD FACSCalibur) using CellQuest Pro Software.

Histological analysis

Histological examination of 0.3 cm x 0.2 cm tissue sections of skin was performed after fixation and staining with hematoxylin-eosin (H&E).

Immunohistochemical techniques

Tissue preparation: Biopsies were fixed using formaldehyde fixation and embedded with paraffin.
Staining: Tissue sections from biopsies were stained using a FITC-conjugated monoclonal antibody mouse anti- human ICAM-1(CD54). To assess the overexpression of this adhesion molecule, a FITC-conjugated monoclonal antibody mouse anti-human ICAM-1 was diluted at a ratio 1:50 using PBS and applied on histological section of skin biopsies. Normal skin from the thoracic region was used as a control.

3. RESULTS

In order to identify the exposed patients, all samples were named as PQ followed by a number. In our study, β1 integrin normal Mean Fluorescence Index value is 8.75 ± 3.77 obtained from 25 control individuals.

β1 Integrin analysis

The analysis of adhesion molecules expression revealed a higher expression of β1 Integrin on gated lymphocytes of grade 4 patients compared to non-exposed controls.

CAMs analysis

There was a positive staining for ICAM-1 on endothelial cells and lymphocytic infiltrations surrounding the vessels in biopsies from grade 4 patients who expressed high levels of β1 Integrin on blood cells.

Representative cases

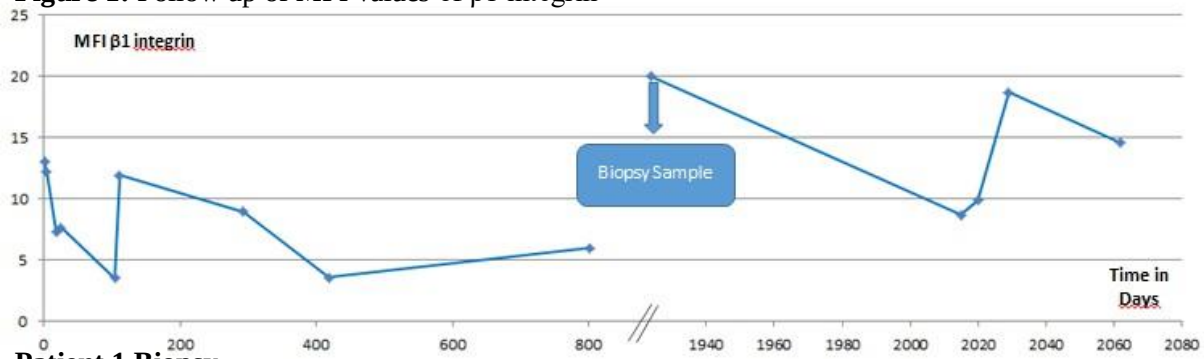
Patient 1:

69-year-old male patient heavy smoker and with high blood pressure who was treated with radiotherapy at his 36 years due to a right leg angioma. According to the equipment and protocol applied at the time of treatment, it can be inferred that the dose delivered to the leg angioma was around 50–60 Gy (2.0 Gy/day given 5 days/week). Initially, ulcers appeared approximately every 10 years. Over time, latency period shortened. In addition, the severity and frequency of the ulcers increased. This patient received two regenerative medicine treatments with mesenchymal stem cells in the Burn Hospital of Argentina: in 2011, an allogenic bone marrow mesenchymal stem cells treatment and in 2018, with autologous mesenchymal stem cells derived from stromal vascular fraction of adipose tissue. Figure 1 shows the expression of β1 integrin over time. It is interesting to point out that the MFI values in 2017 crisis are higher respecting to the MFI values in 2011 crisis, in agreement with clinical symptoms (Table 1).

Table 1. β1 Integrin (MFI values) of the patient over time. Normal MFI value 8.75± 3.77

DATE	B1 INTEGRIN VALUE
11-25-2011	13.08
11-29-2011	12.20
12-14-2011	7.35
12-20-2011	7.65
03-09-2012	3.52
03-15-2012	11.90
09-13-2012	8.95
01-17-2013	3.57
02-04-2014	5.96
10-29-2015	9.97
03-02-2017	19.98
06-01-2017	8.70
06-06-2017	9.91
06-15-2017	**18.70**
07-18-2017	14.59

Figure 1: Follow up of MFI values of β1 integrin



Patient 1 Biopsy

Figure 2-H&E stain- Shows cutaneous tissue with ulceration of the surface and extensive collagenous fibrosis of the dermis and hypodermis, absence of cutaneous annexes, vessels with collapse of their lumen due to fibrosis compression of the surrounding tissues and perivascular lymphocytic infiltrates. Figure 3-H&E stain-Magnification of figure 2 shows the collapse of the vessels lumen due to fibrosis and perivascular lymphocytic infiltrates. Figures 4 and 5 show the appearance of the vessels in healthy dermis. ICAM-1 brown stain is not observed in these controls. Figures 6 and 7 show intense mark for ICAM-1 on endothelial cells. Lymphocytes are also observed within the wall of some vessels with fibrinoid deposits. Overexpression of ICAM-1 is observed on endothelial cells, even in vessels collapsed by peripheral fibrosis.

Figure 2:H&E-Cutaneous tissue with ulceration

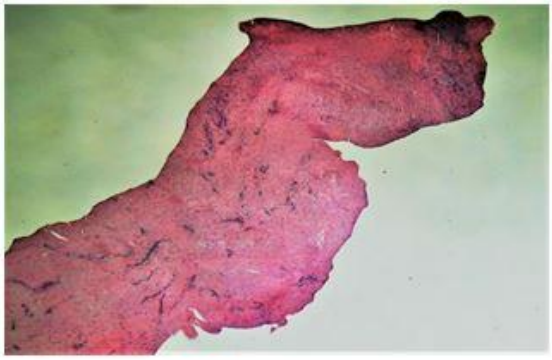


Figure 3: Magnification of figure 2

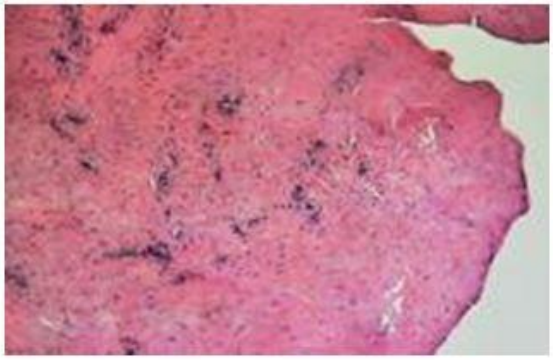


Figure 4: ICAM-1 negative mark -Control 1

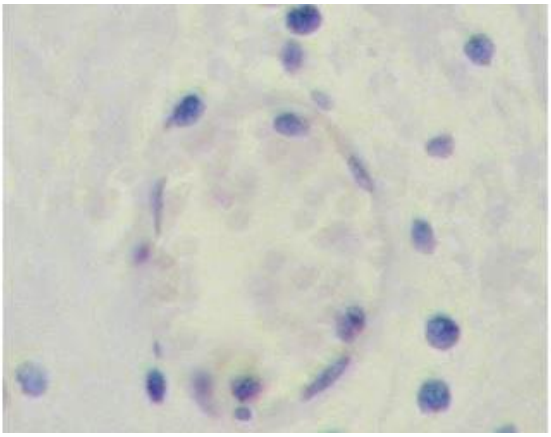


Figure 5: ICAM-1 negative mark -Control 2

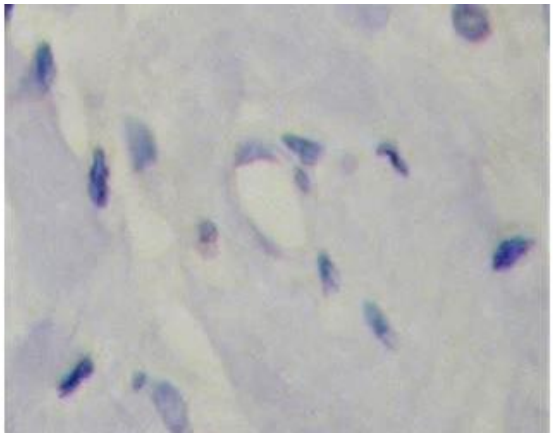


Figure 6: Positive mark for ICAM-1

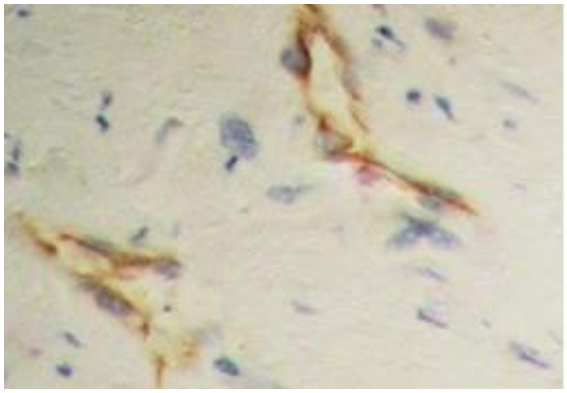
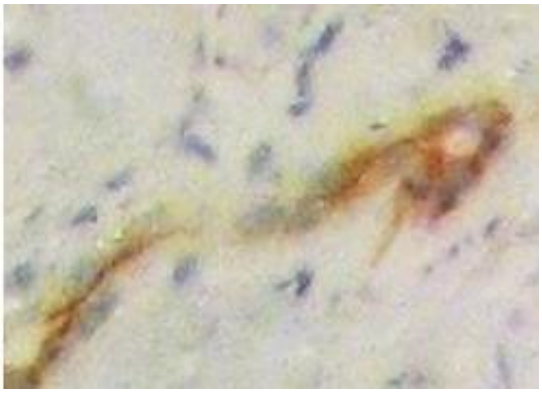


Figure 7: Positive mark for ICAM-1



Patient 2:

66-year-old male patient with paroxysmal refractory atrial flutter, had a fluoroscopy procedure 23 years ago and presented an ulcer on his back. The lesion was treated as a conventional burn, the skin was removed and the patient received an autologous graft that did not succeed. As a consequence of this treatment the ulcer intensified. In this crisis, it was obtained a high β1 Integrin value evaluated by flow cytometry: MFI 19.54.

Patient 2 Biopsy

Figures 8 and 9-H&E stain –show cutaneous tissue with perivascular lymphocytic infiltrates. Figure 10 and 11 show positive mark for ICAM-1 on endothelial cells of vessel walls and perivascular lymphocytic infiltrates.

Figure 8: Lymphocytic infiltrates

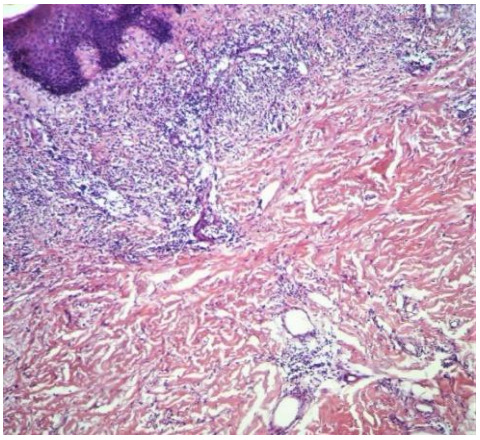


Figure 9: perivascular lymphocytic infiltrates

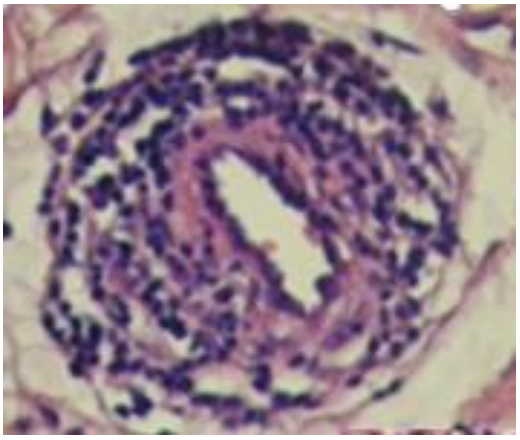


Figure 10: Positive mark for ICAM-1

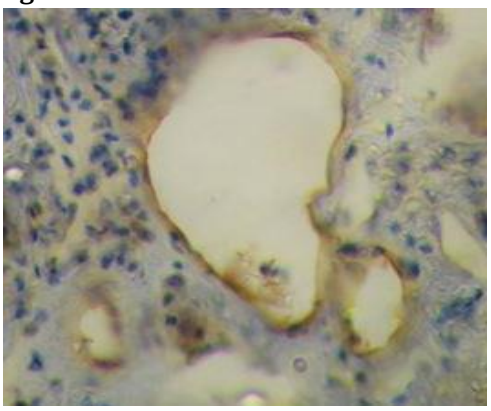
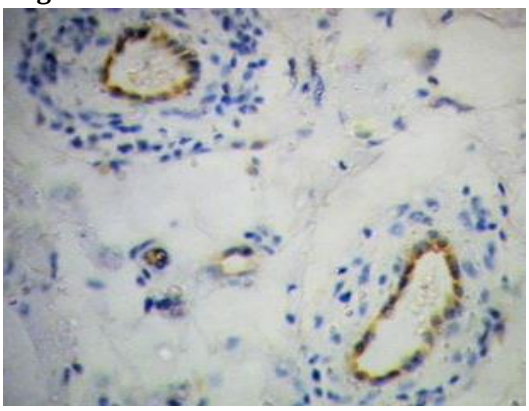


Figure 11: Positive mark for ICAM-1



Patient 3:

61-year-old male patient who had undergone a fluoroscopy procedure, developing a necrotic ulcer grade 4 RTOG/EORTC four months after exposure in the dorsal area in 2003. The follow up was performed until 2010, the patient discontinued the treatment and returned in 2018 showing a radiation induced malignancy with bleeding and severe pain. The MFI of β1 Integrin for the year 2018 is 18.30. This value indicates an overexpression of β1 Integrin in lymphocytes which correlates with the clinical symptoms (inflammatory response).

Patient 3 Biopsy

Figure 12 and 13-H&E- show cutaneous tissue with perivascular lymphocytic infiltrates. Figure 14 shows positive mark for ICAM-1 on endothelial cells of collapsed vessels. Figure 15 shows positive mark for ICAM-1 on endothelial cells, lymphocytes are observed surrounding the vessels.

Figure 12: Lymphocytic infiltrates

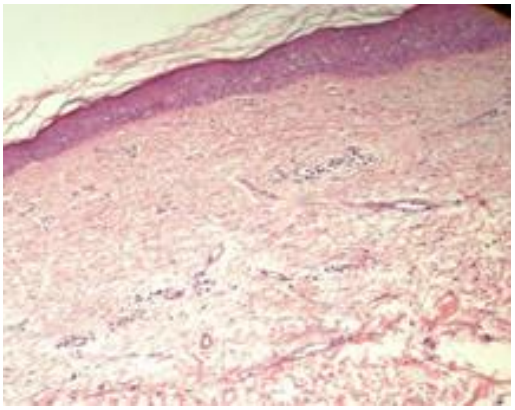


Figure 13: Lymphocytic infiltrates

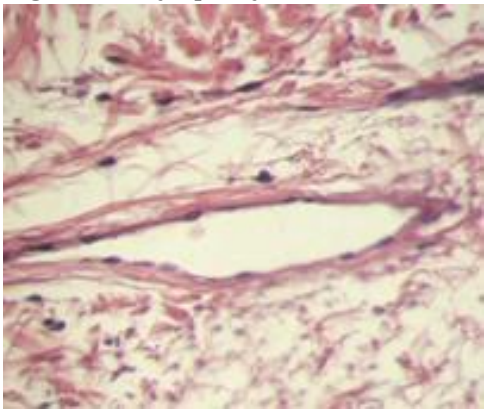


Figure 14: ICAM-1 mark on collapsed vessels

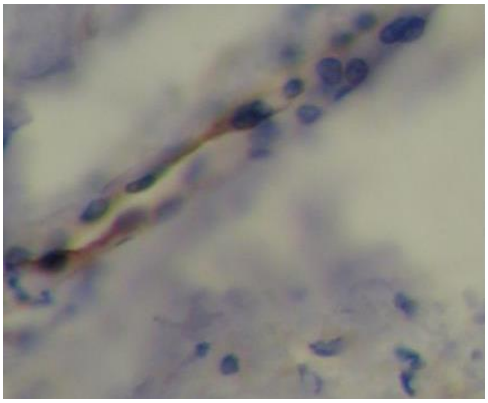
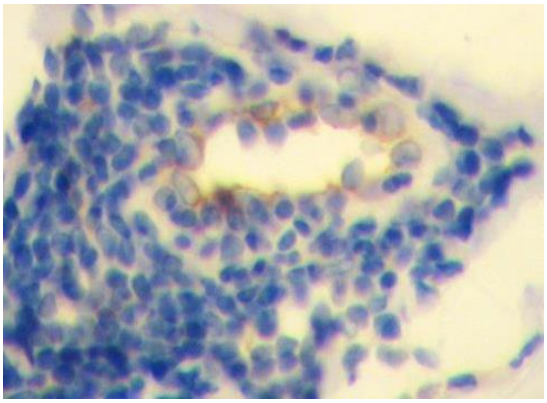


Figure 15: ICAM-1 positive, lymphocytic infiltrate



4. DISCUSSION:

Scientific evidences are supporting the view that integrins and endothelial cell-associated CAMs play a critical role in the vascular dysfunction and tissue injury associated with a wide variety of inflammatory diseases. The coordinated recruitment of leukocytes to sites of inflammation is largely governed by the expression of adhesion molecules. The β1 Integrin is the major integrin expressed on T and B lymphocytes whereas ICAM-1 mediates both lymphocyte and monocyte adhesion but its expression is regulated on endothelial cells.

In this study we observed a correlation between high expression of β1 Integrin on lymphocytes and the expression of ICAM-1 on endothelial cells and the infiltration of lymphocytes on the affected tissue. β1 integrin expression on lymphocytes shows higher values in patients with late cutaneous reactions graded 4 RTOG/EORTC score respect to controls and would correlate with the patient evolution.

5. CONCLUSIONS

This study contributes to understanding the role of this adhesion molecules on irradiated tissue. The analysis of these markers is useful to physicians to predict inflammatory waves and improve the treatment. The β1 integrin values in patients were significantly greater than control values. In addition, ICAM-1 staining on endothelial cells from the vessels of these patients was positive. The same ICAM-1 staining was not observed in healthy tissue. This shows an association between high levels of β1 integrin on blood cells and the expression of ICAM-1 on endothelial cells of grade 4 RTOG / EORTC patients when contrasted with control value.

Flow cytometry techniques are of great importance during an emergency situation due to the high speed of the results. They can be used together with other techniques to guide personalized treatments of victims. This work adds new evidence that supports the use of β1 integrin, in combination with other inflammatory indicators, as a follow-up marker of chronic radio-induced inflammation process just as its response to therapeutic treatments.

6. ACKNOWLEDGEMENTS:

We thank Histotechnician Rosana V. Vargas for technical advice and for performing immunohistochemical techniques in biopsies.

7. REFERENCES:

[1] Scherthan H, Abend M., Müller K. et al (2007) Rad Res 67 :615)
[2] Gallet P., Bérengère P.,Merlin JL. et al(2011) PLos ONE Vol 6 Issue12 e29399
[3] Lataillade J.J., Doucet C., Bey E., et al., (2007). New approach to radiation burn treatment by dosimetry- guided surgery combined with autologous mesenchymal stem cell therapy.Regen Med. 2(5):785-94.)

[4] Portas M., et al (2012). Inflammatory response in radiation induced late effects The 13th International Congress of the International Radiation Protection Association (IRPA) Glasgow, 14-18 May 2012.
[5] Cox JD, Stetz J, Pajak TF. (1995) Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC). Int J Radiat Oncol Biol Phys. Mar 30;31(5):1341-6.
[6] Yuan Liu, Sunil K. Shaw, Shuo Ma, Lin Yang, Francis W. Luscinskas and Charles A. Parkos (2004). Regulation of Leukocyte Transmigration: Cell Surface Interactions and Signaling Events. Immunol 172 (1) 7-13.
[7] H. and Chavakis T., (2009) Leukocyte–endothelial interactions in inflammation. J Cell Mol Med. 13(7):1211-1220.
[8] Barreiro O., Sánchez-Madrid F., (2009) Molecular Basis of Leukocyte-Endothelium Interactions During the Inflammatory Response. Revista Española de cardiología. Vol. 62. Núm. 5. 552-562
[9] Frenette PS, Wagner DD., (1996) Adhesion molecules Part I. N Engl J Med, 334 pp. 1526-9
[10] Frenette PS, Wagner DD., (1996) Adhesion molecules Part II: Blood vessels and blood cells. N Engl J Med, 335 pp. 43-5.
[11] Butcher EC. (1991), Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell, 67 pp. 1033-6.
[12] Butcher EC, Picker LJ., (1996) Lymphocyte homing and homeostasis. Science, 272 pp. 60-6
[13] Springer TA., (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multiple paradigm. Cell, 76 pp. 301-14.
[14] Cerutti C and Ridley AJ.,(2017) Endothelial cell-cell adhesion and signalling. Exp Cell Res. 358(1) pp.31-38.
[15] Alon R, Ley K., (2008), Cells on the run: shear-regulated integrin activation in leukocyte rolling and arrest on endothelial cells. Curr Opin Cell Biol, 20 pp. 525-32.
[16] Evans EA, Calderwood DA., (2007) Forces and bond dynamics in cell adhesion. Science, 316 pp. 1148-53.
[17] Henderson RB, Lim LH, Tessier PA, Gavins FN, Mathies M, Perretti M, et al. (2001). The use of lymphocyte function-associated antigen (LFA)-1-deficient mice to determine the role of LFA-1, Mac-1, and alpha4 integrin in the inflammatory response of neutrophils. J Exp Med, 194pp. 219-26.
[18] Kadono T, Venturi GM, Steeber DA, Tedder TF. (2002) Leukocyte rolling velocities and migration are optimized by cooperative L-selectin and intercellular adhesion molecule-1 functions. J Immunol, 169 pp.4542-50.
[19] Campbell JJ, Hedrick J, Zlotnik A, Siani MA, Thompson DA, Butcher EC. Chemokines and the arrest of lymphocytes rolling under flow conditions. Science, 279 (1998), pp. 381-4.
[20] Schenkel AR, Mamdouh Z, Muller WA. (2004), Locomotion of monocytes on endothelium is a critical step during extravasation. Nat Immunol, 5 pp. 393-400.
[21] Shulman Z1, Shinder V, Klein E, Grabovsky V, Yeger O, Geron E, Montresor A, Bolomini-Vittori M, Feigelson SW, Kirchhausen T, Laudanna C, Shakhar G, Alon R., (2009) Lymphocyte Crawling and Transendothelial Migration Require Chemokine Triggering of High-Affinity LFA-1 Integrin. Immunity. 30(3):384-96.
[22] Shaw SK, Ma S, Kim MB, Rao RM, Hartman CU, Froio RM, et al., (2004) Coordinated redistribution of leukocyte LFA-1 and endothelial cell ICAM-1 accompany neutrophil transmigration. J Exp Med, 200 pp.1571-80.