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ATM Polymorphisms and their Relationship to Radiation Toxicity in Breast Cancer Patients

Polimorfismos ATM e sua relação com a toxicidade por radiação em pacientes com câncer de mama

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ABSTRACT

Aims: The breast cancer is one of the most common types and it treatment brings complications such as skin, dermis and subcutaneous toxicity. Studies about genetic variations of patients are those that enable the identification of prognostic factors for treatment, generally based on greater risk of injury to healthy tissue. **Study design:** This study examined the association between single nucleotide polymorphisms (SNPs) of ATM gene in patients with breast cancer with adverse reactions presented in normal tissues as result of radiotherapy. **Place and Duration of Study:** The study was conduct at Pontifícia Universidade Católica de Goiás, and the patients were recruited at Hospital Araújo Jorge, Associação de Combate ao Câncer em Goiás, Radiotherapy Service. **Methodology**: We evaluated 76 patients, through a retrospective study, based on data contained in records and teletherapy records of patients with this cancer who underwent radiotherapy for at least 5 years. Polymorphisms of the ATM gene were analyzed by microarray technique. **Results:** The mean age of patients was 50 years and the total dose of radiation was an average of 50,21 Gy ranging from 45Gy to 50.4Gy. Regarding the late toxicities, patients analyzed showed a higher frequency of low-grade morbidities when compared to high grade. Nineteen patients interrupted the radiation therapy for any reason. Patients who have studied polymorphisms have no increased risk of developing acute toxicity changes of the skin. (P>.05). Patients presenting polymorphisms AX-8315255 (TTT insertion) (RR=11.0, 1.08 - 111.97, p=0.045) and rs56128736 (RR=11.0, 1.08 - 111.97, p=0.045) had an increased risk for developing late skin toxicity, but not at subcutaneous tract. Conclusion: ATM is a large gene with many variants documented. Association studies of these SNPs will be needed in larger sample groups to establish whether the single base variants or haplotypes of this gene may indeed contribute to the toxicity of normal tissue. Thus, the personalized treatment with ionizing radiation can be prescribed for patients decreasing complications and improving the effectiveness of treatment and quality of life of patients.

Keywords: Breast cancer, ATM, adverse effects, radiotherapy, radiosensitivity

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RESUMO

Objetivos: O câncer de mama é um dos tipos mais comuns e seu tratamento traz complicações como pele, derme e toxicidade subcutânea. Os estudos sobre as variações genéticas dos pacientes são aqueles que possibilitam a identificação de fatores prognósticos para o tratamento, geralmente baseados no maior risco de lesão ao tecido saudável. **Desenho do estudo:** Este estudo examinou a associação entre polimorfismos de nucleotídeo único (SNPs) do gene ATM em pacientes com câncer de mama com reações adversas apresentadas em tecidos normais como resultado da radioterapia. **Local e Duração do Estudo:** O estudo foi realizado na Pontifícia Universidade Católica de Goiás, e os pacientes foram recrutados no Hospital Araújo Jorge da Associação de Combate ao Câncer em Goiás, Serviço de Radioterapia. **Metodologia:** Foram avaliados 76 pacientes, por meio de um estudo retrospectivo, com base em dados contidos em prontuários e fichas de teleterapia de pacientes com esse câncer submetidos à radioterapia há pelo menos 5 anos. Os polimorfismos do gene ATM foram analisados pela técnica de microarray. **Resultados:** A idade média dos pacientes foi de 50 anos e a dose total de radiação foi em média 50,21 Gy variando de 45Gy a 50,4Gy. Em relação às toxicidades tardias, os pacientes analisados apresentaram maior frequência de morbidades de baixo grau quando comparados aos de alto grau. Dezenove pacientes interromperam a radioterapia por qualquer motivo. Os pacientes que estudaram polimorfismos não têm risco aumentado de desenvolver alterações de toxicidade aguda da pele. (P> 0,05). Pacientes com polimorfismos AX-8315255 (inserção TTT) (RR = 11,0, 1,08 - 111,97, p = 0,045) e rs56128736 (RR = 11,0, 1,08 - 111,97, p = 0,045) tiveram um risco aumentado de desenvolver toxicidade cutânea tardia, mas não no trato subcutâneo. Conclusão: ATM é um grande gene com muitas variantes documentadas. Estudos de associação desses SNPs serão necessários em grupos de amostra maiores para estabelecer se as variantes de base única ou haplótipos desse gene podem de fato contribuir para a toxicidade do tecido normal. Assim, o tratamento personalizado com radiação ionizante pode ser prescrito para os pacientes diminuindo complicações e melhorando a eficácia do tratamento e a qualidade de vida dos pacientes.

Palavras-chave: Câncer de mama, ATM, efeitos adversos, radioterapia, radiossensibilidade

1 INTRODUCTION

Breast cancer is the most common type of cancer among women, in both Brazil and the world after non-melanoma skin cancer, with one in nine women diagnosed throughout life in developed countries. It is still the second leading cause of cancer death and the main cause of death in women at ages of 45 to 55. In the last year, an incidence of approximately 57,960 new cases was registered in Brazil, accounting for about 28% of new cases each year, according to the Ministry of Health [1] [2] [3].

The Globocan / IARC project conducted a global estimate in 2012, pointing out that of the more than 14 million new cases of breast cancer in the world, more than 60% occurred in developing countries. Around the world, the estimate is approximately 20 million new cases for the year 2025 [4].

Family breast cancer accounts for about 10% to 20% of all breast cancers diagnosed. In addition to the mutations in the germ line of tumor suppressors, such as BRCA1 and BRCA2, the great genetic heterogeneity also presents non-BRCA types, also called BRCAx. Analyzes of mammary tumors in several families where mutations in the germ line of the ATM gene were identified, revealed a high frequency of loss of wild type alleles in this gene. These results strongly reinforce the association between mutations in the ATM gene, which is a tumor suppressor, and familial BRCAx breast cancer [1].

The ATM gene encodes a protein kinase important checkpoint of the cell cycle. ATM and other enzyme of the same family ATR are known as key controllers of checkpoints during the cellular response to DNA damage and to maintain the integrity of the human genome. DNA damage leads to activation of ATM which phosphorylates and activates SMC1, which is crucial in the control of cell replication and repair after DNA damage. More than 80 polymorphisms were identified in the ATM gene, which makes it one of the most variable genes in the entire human genome [5]. The most radiosensitive human cells come from homozygous carriers of mutations in ATM or LIG4. Homozygous mutations in ATM protein are associated with hyperradiosensitivity causing fatal reactions following radiotherapy and the increased risk of leukemia and lymphoma. However, cell death and genomic instability do not necessarily depend on the same molecular pathway, even though mutations in proteins such as BRCA or ATM are associated with intrinsic radiosensitivity or cancer susceptibility [6] [7].

Advances in high-throughput genotyping and embracing catalogs of genetic variants have allowed so-called wide genome association studies (GWASs). These studies have addressed a variety of biomedical differences, including traits from a range of different diseases. Interestingly, GWAS provided a dramatic increase in the number of convincing associations already reported [8] [9] [10]. Today, radiogenomics and radioproteomics are emerging fields of research focused on the study of genetic variation as an explanation of individuality in response to exposure to accidental and therapeutic radiation. These studies have as main focus the identification of a probable association among polymorphisms of candidate genes to immediate or late specific biological reactions and exposure to radiation [11] [12].

Several studies have recently applied microarray analyzes in cells of cancer patients submitted to radiotherapy, in order to compare the expression profiles of patients with normal or severe normal tissue injury after radiotherapy [13] [14] [15] [16].

The single nucleotide polymorphisms (SNPs) can cause functional alterations of the proteins encoded by the affected genes, altering their expression and cellular location, modifying their affinity for ligands [17] [18], when located in a non-coding region, for example,

as well as changes in their functional sites and kinetics [19], or causing disorders in flexibility, folding, stability and protein aggregation [20], due to perturbations in the three-dimensional structure of the protein caused by the exchange of residues in its primary structure.

Most of the identified genes in these studies belongs to the apoptosis pathway and stress signaling networks. Protein expression of these pathways may be a proper approach for the identification of individuals with genetic predisposition for the development of radiation-induced secondary adverse effects. Genes related to DNA repair mechanisms, cell cycle and apoptosis pathways have been studied worldwide, however, some of these target genes have recently been shown to be related to the response to treatment and have not been studied in relation to the radiotoxicity of patients [21] [22].

Other studies show that cytogenetic tests can be developed to predict markers for possible individualized radiotherapy to improve tumor control and minimize undesirable effects in other tissues. These reports evaluate the presence of single base polymorphisms as predictive biomarkers before the start of radiotherapy [23] [24].

The aim of the present study was to analyze the ATM gene polymorphisms in breast cancer patients treated with radiotherapy in the period 2008-2012. A total of 32 SNPs of the ATM gene were studied using the microarray technique, and compared with data from the patients submitted to radiotherapy.

2 EXPERIMENTAL DETAILS

Clinical information of patients undergoing radiotherapy were collected from records of radiotherapy / teletherapy and records of the Medical Records Department, Hospital Araújo Jorge (HAJ) of the Associação de Combate ao Câncer em Goiás (ACCG) of patients with breast cancer treated with radiotherapy. It was selected 76 patients with histopathologic diagnosis of non-metastatic breast cancer, with no other diagnosis of cancer or prior radiotherapy, which started treatment at Radiotherapy Sector of HAJ, in the ACCG. Adverse reactions caused by radiotherapy were analyzed and sorted acute morbidity scoring criteria of the RTOG and late morbidity of RTOG / EORTC. Inclusion criteria were: patients with histological diagnosis of breast cancer referred to the Radiotherapy Department of the HAJ to perform adjuvant radiotherapy; patients with no other diagnosis of cancer or prior radiotherapy and patients who agreed to sign the Instrument of Consent Form (ICF) to participate in the study. The exclusion criteria in the study were patients who developed previous cancers elsewhere; patients who evolved to death during treatment; patients referred for radiotherapy services external to HAJ and patients who did not agree to sign the ICF.

Thus, all patients included in the study signed the informed consent before obtaining the biological sample. Peripheral blood was collected and all the material was stored in appropriately labeled tubes and stored at -80 °C for later DNA extraction, DNA integrity and quantification to analyze the selected polymorphisms. Genomic DNA was quantified using the NanoDrop bioanalisador DNA (ThermoScientific, California, USA). The DNA integrity was analyzed on 0.8% agarose gel and photodocumentation Molecular Imager Gel Doc XR System (Bio-Rad Laboratories, USA).

Polymorphisms of the ATM gene were analyzed by microarray technique, and the following SNPs analyzed: rs145847315, rs1800056, rs3092856, rs2235002, rs2234997, rs2229020, rs1800054, rs3218707, rs2235000, rs2227924, rs3218695, rs3218673, rs3092857, rs1800057, rs4988111, rs201773026, rs147187700, rs56815840, rs2234995, rs139552233, rs148432863, rs28904919, rs1800061, rs200381392, AX-83152555 (without rs), rs35963548, rs56128736, rs1800059, rs148590073, rs34231402, AX-83572589 (without rs), rs147934285, as present in the panel Axiom®Exome319 (Affymetrix, Inc California, USA). Genotyping was conducted on the Affymetrix GeneTitan system according to the procedure described by Affymetrix (Axiom® 2.0 Assay Manual Workflow User Guide Rev3). Allele calling was carried out using a modified version of the Affymetrix proprietary software packages Affymetrix Power Tools (APT) and SNPolisher™

To analyze the data, all the information provided on the forms of teletherapy and the medical records of patients diagnosed with breast cancer were analyzed using qui square and exact Fisher test using the software SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA), for Windows®. The data generated by the microarrays were translated using the *Genotyping Console Software version 4.2* (Axiom®Exome, Affymetrix, Inc California, USA). Univariate analysis between allele frequencies of SNPs and the degree of acute and chronic effects were measured by relative risk (RR) and 95% confidence interval. A p-value of 0.05 was considered statistically significant for the study.

3 RESULTS

It was evaluated 76 records, reviewed on different days to be avoided selection biases. The classification RTOG was performed by an experienced radiation oncologist. The mean age of the selected patients was 50 years; 13.2% (10) were under 40 years of age and 23.7% (18) were over 60 years old. Eight patients presented breast cancer history in the family, representing 10.53% of the sample; 23 patients presented arterial hypertension (30.2%); four with diabetes mellitus (5.26%), two with collagenosis (2.6%).About the carcinoma characteristics, two

patients presented intraductal carcinoma (2.6%), 68 presented ductal carcinoma infiltrative (89.4%), two presented lobular carcinoma infiltrative (2.6%) and four with lobular and ductal carcinoma infiltrative (5.26%) The patients also presented different degrees of anaplasia; 15 of them with degree I (19.74%), 35 presented degree II (46.05%), 17 with degree III (22.3%), one with degree IV (1.3%), and eight with non-specified degree (10.5%). Among the sample and concerning staging, two patients presented stage Tis (2.6%), 14 presented stage I (18.6%), 21 presented stage IIa (28%), 16 patients presented IIb (21.3%), nine presented IIIa (12%), 12 presented IIIb (16%), one presented IIIc (1.3%).

Among the sample studied, different approaches were used to treat the breast cancer: 75% of the patients were submitted to the breast conservative surgery and 25% to a radical mastectomy.

In total, 41 patients used Anthracyclines or Taxanes before radiotherapy (53.9%). 18 patients were submitted to both hormone therapy and radiotherapy (25%) and 19 patients did not make use of hormone therapy (25.3%). 43 patients used Tamoxifen (57.3%); 1 used Aromasin (1.3%); 11 used Tamoxifen after using Aromasin (14.6%) and 1 patient used Aromasin after using Tamoxifen (1.3 %). (**Table 01)**

Table 01. Distribution of patients according to epidemiological, clinical and morphological variables.

Variable	Number of patients (N=76)	
	n	%
Age (years)		
30 |— 40	10	13.2
40 |— 50	32	42.1
50 |— 60	16	21.1
≥ 60	18	23.7
HAS		
No	52	68.4
Yes	23	30.2
No information	1	1.3
Diabetes Mellittus		
No	70	92.1
Yes	4	5.2
No information	2	2.6
Collagenosis		
No	71	93,4
Yes	2	2,6

No information	3	3,9
Histology		
Ductal carcinoma	2	2,6
Ductal carcinoma infiltrative	68	89,4
Lobular Carcinoma infiltrative	2	2,6
Ductal and lobular Carcinoma infiltrative	4	5,2
Differentiation		
NOS	8	10.5
GI	15	19.7
GII	35	46.0
GIII	17	22.3
GIV	1	1.3
Family history of câncer		
Absent	68	89.4
Present (breast cancer)	7	9.2
Other tumor sites	1	1.3
Staging		
0	2	2.6
I	14	18.6
IIA	21	28.0
IIB	16	21.3
IIIA	9	12.0
IIIB	12	16.0
IIIC	1	1.3
SurgeryType		
Breast conserving surgery	57	75.0
Radical Mastectomy	19	25.0
Concomitant chemotherapy and radiation therapy		
No	44	57.8
Yes	28	36.8
No Chemotherapy	4	5.2
Anthracyclines and Taxanes before Radiation Therapy?		
No	32	42.1
Yes	41	53.9
No chemotherapy	3	3.9
Hormone Therapy Concomitant to Radiation Therapy		
No	57	76.0
Yes	18	24.0
Type of HormoneTherapy		

No used	19	25.3
Tamoxifen	43	57.3
Anastrozole (Arimidex[@])	1	1.3
Letrozole (Femara[@])	11	14.6
Tamoxifen after Arimidex	1	1.3

Legend: n: number of patients; %: Percentage of the total; SD: standard deviation; NOS: not otherwise specified.
* Count patients was excluded due to lack of information in some records

Moreover, 28 patients were submitted to chemotherapy concomitantly with radiotherapy (36.84%) – 12 patients were submitted to post-operative radiotherapy (15.79%); 36 had post-operative radiotherapy and concomitantly to chemotherapy (47.37%); 12 patients had radiotherapy post-operative (15.79%). four of them did not attend chemotherapy (5.26%). (**Table 2)**

Concerning the radiation doses, 2 patients received 4500 cGy, (2.63%); 72 received 5040cGy (94.74%); 2 patients received other doses (2.63%); 19 patients interrupted radiotherapy (25%); and 30 patients received a radio boost (39.47%). The total dose of radiation was an average of 50.21Gy, ranging between 45Gy to 50.4Gy. The medium treatment duration was 61.5 months, ranging between 49Gy to 86Gy. The medium follow-up was 48 months, varying from 2 to 92 months, 64 (86.49%) had no evidence of disease (NED).

There were 7 patients which had distant metastasis (9.46%); 2 patients had a second primary tumor (2.7%), 1 patient developed a contralateral breast tumor (1.35%), and 3 patients died (3.95%) (**Table 2**).

Radiotherapy was interrupted in 19 patients (25%). The interruption occurred before 12 days in 9 patients (4.4%) and in the other 10 patients (52.6%) there was interruption with 12 or more days. The duration of radiotherapy was 1 month for 80.3% of patients and 2 months for 19.7%. (**Table 2**)

Table 02. Frequency of therapeutic modalities.

Variable	Patients (N=76)	
	n	%
Radiotherapy		
After surgery and after chemotherapy	36	47.3
Before surgery	28	36.8
After surgery	12	15.7
Follow up (months) after radiotherapy		
1	13	17.3
2	31	41.3

3	23	30.7
4	8	10.7
Evolution		
NED	64	86.4
Metastasis	7	9.4
Second Primary Tumor	2	2.7
Contralateral breast tumor	1	1.3
Last medical appointment status		
NED	68	89.4
Distant Metastasis	5	6.5
Death	3	3.9
RT dose (cGy)		
4500	2	2.6
4860	2	2.6
5040	72	94.7
RT dose category (cGy)		
4500	2	2.6
5040	72	94.7
Other	2	2.6
Boost		
No	46	60.5
Yes	30	39.4
Boostdosis (cGy)		
900	4	13.3
1000	2	6.6
1080	6	20.0
1200	1	3.3
1260	1	3.3
1360	1	3.3
1400	4	13.3
1440	11	36.6
RT interruption		
No	57	75.0
Yes	19	25.0
Interruption (days)		
< 12	9	47.4
≥ 12	10	52.6
RT duration (months)		
1	61	80.3

2	15	19.7

The patients were classified in high grade (RTOG ≥ 2) and low grade (RTOG< 2). From the total of patients with acute skin reaction, 38 were high grade (50%) and 38 were low grade (50%). There was a decrease in the number of patients when observing the incidence of late symptoms of high degree in the skin and subcutaneous tissue, respectively 10 (12,8%) and 14 (17,9%) (**Table 3**).

Table 3 Distribution of acute morbidity in low and high RTOG score.

RTOG	Patients (N=76)	
	n	%
RTOG acuteskinreaction		
G0	11	14.4
GI	27	35.5
GII	28	36.8
GIII	9	11.8
GIV	1	1.3
RTOG late skinreaction		
G0	47	63.5
GI	17	22.9
GII	9	12.1
GIII	1	1.3
RTOG late subcutaneousreactions		
G0	57	77.0
GI	3	4.0
GII	2	2.7
GIII	5	6.7
GIV	7	9.4

The quantitative variables were associated with the presence of acute skin effects of high-grade (HG) and low grade (LG), and these associations are shown in Table **4 and 6.**

As expected, no comorbidity showed statistically significant correlation with the presence of high-grade acute effects of RT on the skin. The family history of cancer, performing mastectomy, chemotherapy or hormone therapy, either neoadjuvant or concurrent with RT showed no association with these effects. Only the realization of a RT booster dose and RT suspension was statistically associated with the presence of acute effects of high-grade (OR=3.05% CI 1.16-7.93, *p*= 0.035) (OR=3.81 95% CI 1.21-11.96, *p*= 0.033). The SNPs evaluated for the presence of gene ATM were not statistically associated with a higher incidence of acute effects of high-grade skin and subcutaneous tissue in the sample studied. Age is not shown to be associated with the presence of high grade radiosensitivity of the skin for patients in this study. The realization of the booster RT was shown to have positive effects associated with high grade, however, this dose did not affect the enhanced presence of the same effects. The number of days that lasted the RT was associated with high-grade effects, but the total dose of RT did not show this association. Patients who had discontinued treatment due to the development of side effects have extended the duration of treatment (data not shown).

Table 4. Association between acute side effects of skin and ATM polymorphisms.

***Radiation Therapy Oncology Group* – RTOG**									
	Acute Skin RTOG								
SNPs ATM	LG		HG		*RR*	CI 95%		*P*	HWE
	N	(%)	n	(%)		Min	Max		
rs1800056									
Major (TT)	37	48.68	38	50	2,0	0.05	20.64	0.500	1.00
Heterozig (TC)	1	1.32	0	0					1.00
Total	38	50	38	50					
rs3218707									
Major (GG)	37	48.68	38	50	2.0	0.05	20.64	0.500	1.00
Heterozig (GC)	1	1.32	0	0					1.00
Total	38	50	38	50					
rs3092857									
Major (AA)	36	47.37	38	50	3.0	0.33	27.63	0.304	1.00
Heterozig (AG)	2	2.63	0	0					1.00
Total	38	50	38	50					

AX-83152555 (no rs)									
Major (---)	38	50	37	48.68	0.5	0.08	13.75	0.500	1.00
Heterozig (TTT)	0	0	1	1.32					1.00
Total	38	50	38	50					
rs3092856									
Major (CC)	35	46.05	37	48.68	3.0	0.33	27.63	0.304	0.99
Heterozig (TC)	3	3.95	1	1.32					1.00
Total	38	50	38	50					
rs2235000									
Major (GG)	35	46.05	38	50	4.0	0.47	34.24	0.178	0.99
Heterozig (AG)	3	3.95	0	0					1.00
Total	38	50	38	50					
rs1800057									
Major (CC)	37	48.68	37	48.68	1.00	0.13	7.64	0.237	1.00
Heterozig (CG)	1	1.32	1	1.32					1.00
Total	38	50	38	50					
rs56128736									
Major (TT)	38	50	36	47.37	0.3	0.04	3.07	0.304	1.00
Heterozig (TC)	0	0	2	2.63					1.00
Total	38	50	38	50					
rs2235002									
Major (GG)	36	47.37	36	47.37	1.0	0.20	4.97	0.304	1.00
Heterozig (TG)	2	2.63	2	2.63					1.00
Total	38	50	38	50					
rs2227924									
Major (CC)	37	48.68	37	48.68	1.0	0.13	6.64	0.237	1.00
Heterozig (CG)	1	1.32	1	1.32					1.00
Total	38	50	38	50					
rs4988111									
Major (TT)	37	48.68	37	48.68	1.0	0.13	6.64	0.237	1.00

Heterozig (TC)	1	1.32	1	1.32					1.00
Total	38	50	38	50					
rs1800059									
Major (AA)	37	48.68	38	50	2.0	0.05	20.64	0.500	1.00
Heterozig (AC)	1	1.32	0	0					1.00
Total	38	50	38	50					
rs2234997									
Major (TT)	34	44.74	32	42.11	0.7	0.20	2.18	0.367	0.99
Heterozig (AT)	4	4.26	6	7.89					0.98
Total	38	50	38	50					
rs3218695									
Major (CC)	36	47.37	38	50	3.0	0.33	27.63	0.304	1.00
Heterozig (AC)	2	2.63	0	0					1.00
Total	38	50	38	50					
rs147187700									
Major (GG)	37	48.68	38	50	2.0	0.05	20.64	0.500	1.00
Heterozig (CG)	1	1.32	0	0					1.00
Total	38	50	38	50					
rs148590073									
Major (AA)	38	50	37	48.68	0.5	0.08	13.75	0.500	1.00
Heterozig (AG)	0	0	1	1.32					1.00
Total	38	50	38	50					
rs1800054									
Major (CC)	37	48.68	36	47.37	0.5	0.18	5.96	0.500	1.00
Heterozig (CT)	1	1.32	2	2.63					1.00
Total	38	50	38	50					
Rs3218673									
Major (CC)	37	48.68	37	48.68	1.0	0.13	7.64		1.00
Heterozig (CT)	1	1.32	1	1.32					1.00
Total	38	50	38	50					

rs2234995								
Major (--)	36	47.37	38	50	3.0	0.33	27.63	1.00
Heterozig (-A)	2	2.63	0	0				1.00
Total	38	50	38	50				
rs34231402								
Major (TT)	36	47.37	38	50	3.0	0.33	27.63	1.00
Heterozig (AT)	2	2.63	0	0				1.00
Total	38	50	38	50				

Abbreviations: RTOG = *Radiation Therapy Oncology Group*. HG = High grade RTOG≥2. LG = Low grade RTOG<2. RR = Relative Risk. IC = Confidence Interval. * *P*= .05, HWE=Hardy-Weinberg Equilibrium.

The PolyPhen-2 (Polymorphism Phenotyping v2) is a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations. One of the selected polymorphisms was Val410Ala (rs56128736). The result for prediction of possible impact in the change Val410Ala is that this mutation is predicted to be Possible Damaging with a score of 0.726 - sensitivity: 0.86 and specificity 0.92 in the analysis on the PolyPhen-2 [25]

Only the SNPs rs56128736 (RR=11, 95% CI 1.08-111.97, *p*= 0.045) and AX-83152555 (RR=11, 95% CI 1.08-111.97, *p*= 0.045) has shown an increased risk for developing late toxicity of the skin (**Table 5**).

Table 5. Association between late side effects of skin and ATM polymorphisms.

***Radiation Therapy Oncology Group* – RTOG**									
	Late Skin RTOG								
SNPs ATM	LG		HG		*RR*	CI 95%		*P*	HWE
	n	(%)	n	(%)		Min	Max		
rs1800056					0.4	0.04	3.70	0.475	1.00
Major (TT)	63	85.16	10	13.51					1.00
Heterozig (TC)	1	1.35	0	0					
Total	64	86.51	10	13.49					
rs3218707									
Major (GG)	63	85.16	10	13.51	0.4	0.04	3.70	0.475	1.00
Heterozig (GC)	1	1.35	0	0					1.00

Total	64	86.51	10	13.49					
rs3092857									
Major (AA)	63	85.16	9	12.14	0.2	0.01	2.30	0.315	1.00
Heterozig (AG)	1	1.35	1	1.35					0.99
Total	64	86.51	10	13.49					
AX-83152555 (no rs)									
Major (---)	64	86.51	9	12.14	11.0	1.08	111.97	***0.045***	1.00
Heterozig (TTT)	0	0	1	1.35					0.99
Total	64	86.51	10	13.49					
rs3092856									
Major (CC)	60	81.08	10	13.51	0.2	0.01	2.63	0.345	1.00
Heterozig (TC)	4	5.42	0	0					1.00
Total	64	86.51	10	13.49					
rs2235000									
Major (GG)	61	82.43	10	13.51	0.7	0.09	5.96	0.365	1.00
Heterozig (AG)	3	4.07	0	0					1.00
Total	64	86.51	10	13.49					
rs1800057									
Major (CC)	62	83.78	10	13.51	0.6	0.06	4.82	0.435	1.00
Heterozig (CG)	2	2.72	0	0					1.00
Total	64	86.51	10	13.49					
rs56128736									
Major (TT)	64	86.51	9	12.14	11.0	1.08	111.97	***0.045***	1.00
Heterozig (TC)	0	0	1	1.35					1.00
Total	64	86.51	10	13.49					
rs2235002									
Major (GG)	60	81.08	10	13.51	0.9	0.12	7.11	0.309	1.00
Heterozig (TG)	4	5.42	0	0					1.00
Total	64	86.51	10	13.49					

rs2227924									
Major (CC)	63	85.16	9	12.14	0.2	0.01	2.30	0.315	1.00
Heterozig (CG)	1	1.35	1	1.35					0.99
Total	64	86.51	10	13.49					
rs4988111									
Major (TT)	63	85.16	9	12.14	0.2	0.01	2.30	0.315	1.00
Heterozig (TC)	1	1.35	1	1.35					0.99
Total	64	86.51	10	13.49					
rs1800059									
Major (AA)	63	85.16	10	13.51	0.4	0.04	3.70	0.475	1.00
Heterozig (AC)	1	1.35	0	0					1.00
Total	64	86.51	10	13.49					
rs2234997									
Major (TT)	54	72.97	10	13.51	2.0	0.28	14.09	0.382	0.98
Heterozig (AT)	10	13.53	0	0					1.00
Total	64	86.51	10	13.49					
rs3218695									
Major (CC)	62	83.78	10	13.51	0.6	0.06	4.82	0.435	1.00
Heterozig (AC)	2	2.72	0	0					1.00
Total	64	86.51	10	13.49					
rs147187700									
Major (GG)	63	85.16	10	13.51	0.4	0.04	3.70	0.475	1.00
Heterozig (CG)	1	1.35	0	0					1.00
Total	64	86.51	10	13.49					
rs148590073									
Major (AA)	63	85.16	10	13.51	0.4	0.04	3.70	0.475	1.00
Heterozig (AG)	1	1.35	0	0					1.00
Total	64	86.51	10	13.49					
rs1800054									
Major (CC)	62	83.78	9	12.14	0.3	0.03	3.14	0.475	1.00

Heterozig (CT)	2	2.72	1	1.35					0.99
Total	64	86.51	10	13.49					
Rs3218673									
Major (CC)	63	85.16	9	12.14	0.2	0.01	2.30	0.315	1.00
Heterozig (CT)	1	1.35	1	1.35					0.99
Total	64	86.51	10	13.49					
rs2234995									
Major (--)	62	83.78	10	13.51	0.6	0.06	4.82	0.435	1.00
Heterozig (-A)	2	2.72	0	0					1.00
Total	64	86.51	10	13.49					
rs34231402									
Major (TT)	62	83.78	10	13.51	0.6	0.06	4.82	0.435	1.00
Heterozig (AT)	2	2.72	0	0					1.00
Total	64	86.51	10	13.49					

Abbreviations: RTOG = *Radiation Therapy Oncology Group*. HG = High grade RTOG≥2. LG = Low grade RTOG<2. RR = Relative Risk. IC = Confidence Interval. * *P*= .05, HWE=Hardy-Weinberg Equilibrium.

Of the 14 patients who had subcutaneous lesions classified as high grade, this finding did not show any statistical significant association when compared to the effect of all analysed SNPs in the group of patients who developed low-grade lesions. Only the SNP rs1800054 show an increased risk for developing subcutaneous toxicity (RR=8.6, 95% CI 0.83-88.00, *p*= 0.080). (**Table 6)**.

Table 6. Association between late side effects of subcutaneous and ATM polymorphisms.

***Radiation Therapy Oncology Group* – RTOG**									
	Late Subcutaneous RTOG								
SNPs ATM	LG		HG		*RR*	CI 95%		*P*	HWE
	n	(%)	N	(%)		Min	Max		
rs1800056									
Major (TT)	59	79.73	14	18.92	0.5	0.77	2.76	0.433	1.00
Heterozig (TC)	1	1.35	0	0					1.00
Total	60	81.08	14	18.92					
rs3218707									

Major (GG)	60	81.08	13	17.57	0.1	0.01	1.37	0.099	1.00
Heterozig (GC)	0	0	1	1.35					0.99
Total	60	81.08	14	18.92					
rs3092857									
Major (AA)	58	78.38	14	18.92	0.8	0.12	3.35	0.342	1.00
Heterozig (AG)	2	2.7	0	0					1.00
Total	60	81.08	14	18.92					
AX-83152555 (no rs)									
Major (---)	60	81.08	13	17.57	0.1	0.01	1.37	0.099	1.00
Heterozig (TTT)	0	0	1	1.35					0.99
Total	60	81.08	14	18.92					
rs3092856									
Major (CC)	57	77.03	13	17.57	0.7	0.11	3.10	0.368	1.00
Heterozig (TC)	3	4.05	1	1.35					0.99
Total	60	81.08	14	18.92					
rs2235000									
Major (GG)	58	78.38	13	17.57	0.5	0.07	2.56	0.460	1.00
Heterozig (AG)	2	2.7	1	1.35					0.99
Total	60	81.08	14	18.92					
rs1800057									
Major (CC)	58	78.38	14	18.92	0.8	0.12	3.35	0.342	1.00
Heterozig (CG)	2	2.7	0	0					1.00
Total	60	81.08	14	18.92					
rs56128736									
Major (TT)	59	79.73	14	18.92	0.5	0.77	2.76	0.433	1.00
Heterozig (TC)	1	1.35	0	0					1.00
Total	60	81.08	14	18.92					
rs2235002									
Major (GG)	57	77.03	13	17.57	0.7	0.11	3.10	0.368	1.00
Heterozig (TG)	3	4.05	1	1.35					0.99

Total	60	81.08	14	18.92					
rs2227924									
Major (CC)	59	79.73	13	17.57	0.2	0.03	2.04	0.412	1.00
Heterozig (CG)	1	1.35	1	1.35					0.99
Total	60	81.08	14	18.92					
rs4988111									
Major (TT)	59	79.73	13	17.57	0.2	0.03	2.04	0.412	1.00
Heterozig (TC)	1	1.35	1	1.35					0.99
Total	60	81.08	14	18.92					
rs1800059									
Major (AA)	59	79.73	14	18.92	0.5	0.77	2.76	0.433	1.00
Heterozig (AC)	1	1.35	0	0					1.00
Total	60	81.08	14	18.92					
rs2234997									
Major (TT)	53	71.62	11	14.86	0.5	0.16	1.85	0.299	0.99
Heterozig (AT)	7	9.46	3	4.06					0.95
Total	60	81.08	14	18.92					
rs3218695									
Major (CC)	58	78.38	14	18.92	0.8	0.12	3.35	0.342	1.00
Heterozig (AC)	2	2.7	0	0					1.00
Total	60	81.08	14	18.92					
rs147187700									
Major (GG)	59	79.73	14	18.92	0.5	0.77	2.76	0.433	1.00
Heterozig (CG)	1	1.35	0	0					1.00
Total	60	81.08	14	18.92					
rs148590073									
Major (AA)	59	79.73	14	18.92	0.5	0.77	2.76	0.433	1.00
Heterozig (AG)	1	1.35	0	0					1.00
Total	60	81.08	14	18.92					

rs1800054									
Major (CC)	59	79.73	12	16.22	8.6	0.83	88.00	0.080	1.00
Heterozig (CT)	1	1.35	2	2.70					1.00
Total	60	81.08	14	18.92					
rs3218673									
Major (CC)	59	79.73	13	17.57	0.2	0.03	2.04	0.412	1.00
Heterozig (CT)	1	1.35	1	1.35					1.00
Total	60	81.08	14	18.92					
rs2234995									
Major (--)	58	78.38	14	18.92	0.8	0.12	3.35	0.342	1.00
Heterozig (-A)	2	2.7	0	0					1.00
Total	60	81.08	14	18.92					
rs34231402									
Major (TT)	58	78.38	14	18.92	0.8	0.12	3.35	0.342	1.00
Heterozig (AT)	2	2.7	0	0					1.00
Total	60	81.08	14	18.92					

Abbreviations: RTOG = *Radiation Therapy Oncology Group*. HG = High grade RTOG≥2. LG = Low grade RTOG<2. RR = Relative Risk. IC = Confidence Interval. * *P*= .05, HWE=Hardy-Weinberg Equilibrium.

4 DISCUSSION

Several studies have been published showing the association between polymorphisms of ATM and radiosensitive phenotype for patients with breast cancer treated with radiotherapy. The ATM plays a central role in the activation of DNA damage response following DNA double-strand in checkpoint control of a cell cycle. The mutations in ATM (heterozygous germline) occur in approximately 1% of the population, associated with breast cancer [26]. According to Foray *et al*[6], the most radiosensitive human cells are from homozygous carriers of ATM mutations, showing that patients with mutations on that gene usually succumb to radiation therapy [27].

Our findings showed that polymorphisms analyzed were not associated with the development of any acute but to chronic adverse effect of high degree in patients with breast cancer treated with radiotherapy. While radiation therapy is often successful in the local eradication of tumor cells, late normal tissue effects may significantly reduce organ function and health-related quality of life in a proportion of long term cancer survivors [28].

The SNPs rs56128736 (RR=11, 95% CI 1.08-111.97, *p*= 0.045) comprehends a Val410Ala mutation. Both aminoacids of this change are nonpolar. Despite this, our results show that this mutation is probably damaging for DNA. The result for prediction of possible impact in the Harvard tool Polyphen v2, showed that the mutation in the rs568936 is predicted to be Possible Damaging as showed before. This prediction result was corroborated in our study for this SNP, which demonstrated an increased risk for developing late toxicity of the skin for this SNP. That strong association, including a bioinformatics tool and the analysis of patients data, takes the relevance of the present mutation to another level of importance.

The SNP AX-83152555 with a TTT insertion (RR=11.0, 1.08 - 111.97, *p*=0.045) presents a mutation in an intron. Because there are no data in the NCBI files about this mutation, it was not possible to generate the FASTA file to be used in the prediction of possible impact in the Polyphen v2 Harvard tool. There are no studies in the literature about this SNP. As showed in our results, it has also an increased risk for developing late skin toxicity in normal tissue.

ATM is a large gene with many variants documented. Association studies of these SNPs will be needed in larger sample groups to establish whether the single base variants or haplotypes of this gene may indeed contribute to the toxicity of normal tissue.

In our study, a major limitation was to associate the radiation effects in normal tissue only with the genetic profile of the patient, since the service radiotherapy during the treatment of selected patients used conventional radiotherapy and not the 3D conformal radiation therapy that is associated with lower rates of side effects. This is definitely an important parameter in comparison with studies in other laboratories and using more advanced types of treatments.

Another important limitation was the size of the sample group. Seventy-six patients were selected for the study, which in statistical terms is considered a small number. Therefore, the findings of this study require further validation by subsequent studies. Nonetheless, our findings might provide relevant information about the importance of polymorphism of ATM gene, which remain uncertain as determinants of radiosensitivity phenotypes.

Our study was conducted retrospectively on the collection of samples and data from the files after radiotherapy. A prospective study could generate more information about patients and their symptoms before and after radiotherapy, as well as being possible to establish the relative risk associated with the presence of polymorphisms.

5 CONCLUSION

The present study showed that the patients who had polymorphism AX-8315255 (TTT insertion) (RR=11.0, 1.08 - 111.97, *p*=0.045) and rs56128736 (RR=11.0, 1.08 - 111.97,

p=0.045) had an increased risk for developing late skin toxicity, while the other evaluated polymorphisms showed no significant relevance to the development of other late events analyzed. Moreover, none of the polymorphisms showed statistically significant correlation to the increased risk of developing acute changes of skin.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

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