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EPIDEMIOLOGICAL ANALYSIS AND INVESTIGATION OF +114G/T POLYMORPHISM IN THE GENE IL-2 IN PATIENTS WITH BREAST CANCER IN THE STATE OF MATO GROSSO

Análise epidemiológica e investigação do polimorfismo +114G/T no gene IL-2 em pacientes com câncer de mama no estado de Mato Grosso

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ABSTRACT

Objective: This study aimed to determine the relationship between the rs2069763 polymorphism of the Interleukin-2 (IL-2) gene and the development of breast cancer (BC) in women in the state of Mato Grosso. **Methods:** This is an observational epidemiological study of case-control based on a bank of samples with 254 patients and 243 control women. The patients were subjected to anamnesis and collection of peripheral blood after their permission. The collecting of peripheral blood was carried out and was used for DNA extraction, followed by a genotyping process by Polymerase Chain Reaction-Restriction Fragment Length Polymorfism (PCR-RFLP) and polyacrylamide gel electrophoresis 8%. **Results:** The frequency of genotypes between cases and controls was 19.5% for the TT genotype; 44.2% and 32.1% for TG and GG, respectively. It was found, in samples of patients, a frequency of 14.9% for the TT genotype, 77.2% and 31.8% for TG and GG, respectively. In the controls, it was observed frequency of 24.1% for the TT genotype, 41.5% and 30.4% for TG and GG, respectively. **Conclusions:** The present study showed a higher incidence of polymorphism +114G/T in the control sample, suggesting a protective effect for the BC in the sample of women from Mato Grosso.

KEYWORDS: Breast Cancer; Genetic Polymorphism; Interleukin.

RESUMO

Objetivo: O presente estudo teve como objetivo verificar a relação existente entre o polimorfismo rs2069763 do gene interleucina-2 (IL-2) e o desenvolvimento do câncer de mama (CM) em mulheres do estado de Mato Grosso. **Métodos:** Trata-se de um estudo epidemiológico observacional do tipo caso controle tendo como base um banco de amostras com 254 pacientes casos e 243 mulheres controles. As pacientes foram submetidas à anamnese e à coleta de sangue periférico após terem autorizado os procedimentos. Procedeu-se a coleta de sangue periférico e este foi utilizado para a extração do DNA, seguido pelo processo de genotipagem por meio da técnica de *Polymerase Chain Reaction-Restriction Fragment Length Polymorfism* (PCR-RFLP – Reação em Cadeia da Polimerase-Polimorfismo no Comprimento de Fragmentos de Restrição) e eletroforese em gel de poliacrilamida 8%. **Resultados:** A frequência dos genótipos, entre casos e controles, foi de 19,5% para o genótipo TT; 44,2% para TG e 32,1% para GG. Encontrou-se, nas amostras de pacientes, uma frequência de 14,9% para o genótipo TT, 77,2% para TG e 31,8% para GG. Nos controles observou-se uma frequência de 24,1% para o genótipo TT, 41,5% para TG e 30,4% para GG. **Conclusões:** O presente estudo demonstrou maior incidência do polimorfismo +114G/T na amostra controle, sugerindo um efeito protetor para o CM na amostra de mulheres de Mato Grosso.

DESCRITORES: Câncer de mama; Polimorfismo genético; Interleucinas.

Study carried out at Universidade Federal de Mato Grosso – Cuiabá (MT), Brazil.
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INTRODUCTION

Breast cancer (BC) is a malignant tumor of great incidence among women, accounting for approximately 3% of deaths due to neoplasia in this genre[1,2].

BC occurs when mammary cells, especially those located in the mammary ducts, go through mutations and begin to divide and reproduce in a fast and disorderly way. It affects mainly women around the age of 50, being less frequent among those aged below 35 years old[3]. In general, the first sign of the disease is characterized by the presence of a nodule of varying size and location; other less frequent symptoms are breast retraction, breast deformity, presence of enlarged axillary nodes, pain and edema[4].

The etiology of BC may be classified as sporadic or hereditary. About 90% of the cases are sporadic with a multifactorial model, i.e., they result from a strong interaction between genetic and environmental components. The remaining ones represent hereditary cases[3]. Mutations in the BRCA1 and BRCA2 tumor suppressor genes confer an increased risk of developing BC[5].

Given the complex nature of the sporadic cases, it is necessary to investigate other mechanisms which may influence their appearance. Studying the genes involved with the human immune system is a perspective to be considered.

Cytokines are multiple action glycoproteins secreted by different cells of the defense system. The main cytokines sources are T lymphocytes, especially the auxiliary ones. Some cytokines are named interleukins, which are proteins involved in the process of communication between lymphocytes[7].

Interleukin-2 (IL-2) is the cytokine involved in the regulation and proliferation of T cells functional activity. The gene responsible for this protein is mapped in 4q26. IL-2 functions include the ability to stimulate the survival and differentiation of T cells, acting out as a growth factor for immunological cells and increasing cellular cytolytic activity, promoting the production of immunoglobulin by B lymphocytes. Furthermore, IL-2 contributes to the regulation of clonal expansion and the apoptosis process performed between T and infected cells[8,9].

Recent studies emphasized the role of IL-2 as a promising agent for the activation of the immune response against tumors, playing an essential part in antitumor immunity and displaying inhibition effects to the growth and dissemination of tumor metastases[10,11].

Several polymorphisms in IL-2 have been identified, among which the single nucleotide polymorphism (SNP) +114G/T. This polymorphism is located in the first exon of the IL-2 gene, whose alteration in the base, although silent, abolishes a cleavage site for endonuclease restriction. Studies demonstrate this polymorphism influences the production of IL-2, as well as the disease susceptibility[12].

The present study had the objective of investigating +114G/T polymorphism among women from Mato Grosso and their possible association with BC.

METHODS

It is an epidemiological case-control study. The population of the study consisted of 497 adult female subjects, of which 254 cases and 243 controls, living in Mato Grosso. The sample was obtained by the recruitment of patients in reference oncology services in the municipality of Cuiabá, Mato Grosso, Brazil.

The cases consisted of patients diagnosed through positive histopathologic tests for BC, while the control group consisted of women who had a negative mammography examination for this neoplasm. The present study was approved by the Ethics Committee of the *Universidade de Cuiabá* (No. 308. CEP UNIC 0307-308).

The genomic deoxyribonucleic acid (DNA) was obtained from the lymphocytes present in peripheral blood and the DNA extraction technique used followed the protocol described by Lahiri and Nurnberger, with changes[13]. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used in order to identify the +114G/T polymorphism (rs2069763) in the IL-2 gene. The primers used were 5' ATGTACAGGATGCAACTCCT-3' (forward) and 5' TGGTGAGTTTGGGATTCTTG-3'(reverse)[11,14]. The conditions of the Polymerase Chain Reaction (PCR) consisted of an initial denaturation of 94ºC for 5 minutes, annealing temperature of 63ºC for 2 minutes and 72ºC for 1 minute; followed by 30 cycles of 94ºC for 30 seconds, 63ºC for 45 seconds and 72ºC for 45 seconds. The final extension was carried out under a temperature of 72ºC for 8 minutes and 4ºC for 15 minutes. PCR products were digested at 37ºC for 12 hours with restriction MWOI enzyme (New England). The digestion products were visualized through electrophoresis in 8% polyacrylamide. The fragments obtained were sized 262pb, 151pb and 111pb corresponding to the TT, TG and GG genotypes, respectively. The quantitative independent variables were analyzed and evaluated by the χ^2 test, with confidence interval of 95% (95% CI). The value of $p<0.05$ was considered statistically significant.

RESULTS

The characterization of the studied population showed that the mean age of patients was 50.79 (±10.76) years old and controls were aged 47.2 (±9.55) years, showing a balance between groups regarding age. There was no predominance of ethnicity among patients, though there was a predominance of non-white/Caucasian subjects in the control group. As for the variable of menarche age, 43.3% of patients had their first menstrual period before 12 years of age and 49.6% had it after 12 years of age. In the control group, it was observed that 57.2% of subjects had their first menstrual period after 12 years of age and 40.3% had it before that. About 51.6% of patients had already gone through menopause. The control group was more homogeneous for this variable. About 61% of patients did not have family history of BC.

Alcohol intake and smoking habits were more frequent in the control group: 81.9 and 59.7%, respectively. The results regarding the number of pregnancies were balanced among the analyzed groups. Both presented higher frequency for three or more pregnancies. When analyzing the age of the first pregnancy, 58% of control women reported having had their first pregnancy before 20 years of age, followed by 32.5% who had their first pregnancy between 21 and 30 years of age and only 2.5% after 30 years of age. In the patients sample, 37.4% of them had their first pregnancy between 21 and 30 years of age, followed by 36.6% before the age of 20 and 9.1% after 30 years of age. The variable regarding the use of contraceptive presented relevant results, in which 89.3% of the control sample used this medication and 8.6% of it did not do so. The patient group had greater homogeneity for contraceptive use: 53.5% reported its use and 38.6% did not use it. Another difference was found when analyzing the results for hormonal therapy. In the patients group, only 13.4% of the sample reported the use of hormonal therapy at some point in their lives, while 89.3% of the control group sample referred to this use. Only 8.6% of the interviewees in the control group did not use hormonal therapy, while 78.3% of patients did not use it at any point in their lives. There was no difference between groups regarding the breastfeeding (for at least three months) variable. As for the clinical and laboratory aspects of the patients, it was observed that 47.1% of the sample presented positive estrogen receptor and 34.5% a negative one. When observing the progesterone receptor variable, a greater frequency of positive women was observed. Most patients had tumors between 2 and 5 cm. The patients group had a slight variation when comparing the presence or absence of affected lymph nodes: 47.5% of them did not present affected lymph nodes, while 32.5% of them reported their presence. When analyzing the frequency of symptoms, it was noted that about 49.4% of them developed a nodule as the first symptom. Many patients (44.7%) reported having found out about their disease through self-examination, and the remainder through mammography, medical consultations and ultrasonography. As for the immunohistochemical analysis regarding p53, her2 and ki67, it was observed that 45.1% of the sample was negative for p53 and the remaining ones were positive. About 50.2% of the sample was negative for the presence of her2. Most of the sample — about 66.7% — displayed the presence of ki67. When inquired about self-examination 51.8% of the subjects stated they perform it, while 20.4% of them reported not having this habit. (Table 1)

The analysis of the frequency distribution of +114G/T polymorphism showed that, both in the patients and the control group, the most evident genotype for polymorphism was TG, followed by GG and TT frequencies. The control group presented higher numbers of polymorphic TT genotype, when compared to the patients group. About 60.8% of the TT genotype was found in the control group, while only 39.1% was found

Table 1. Epidemiological characterization of cases and controls of breast cancer in women from Mato Grosso, Brazil

Characteristics	Cases	Controls
Age		
Mean	50.79	47.2
SD	10.76	9.55
Ethnicity		
White/Caucasian	120 (47.2%)	64 (26.3%)
Non-White	116 (45.6%)	173 (71.2%)
Did not answer	21 (7.2%)	6 (2.5%)
Menarche age		
Up to 12	110 (43.3%)	98 (40.3%)
More than 12	125 (49.6%)	139 (57.2)
Did not answer	19 (7.4%)	6 (2.5%)
Menopaused		
Yes	131 (51.6%)	116 (47.7%)
No	104 (40.9%)	121 (49.8%)
Did not answer	19 (7.5%)	6 (2.5%)
Family history of breast cancer		
Yes	80 (31.5%)	
No	155 (61.0%)	
Did not answer	19 (7.5%)	243 (100%)
Alcoholism		
Yes	45 (17.7%)	199 (81.9%)
No	189 (74.4%)	36 (14.8%)
Did not answer	20 (7.9%)	8 (3.3%)
Smoking		
Yes	61 (24%)	145 (59.7%)
No	174 (68.5%)	55 (22.6%)
Did not answer	19 (7.5%)	43 (17.7%)
No. of pregnancies		
Did not have children	29 (11.4%)	13 (5.3%)
Up to 2	81 (31.9%)	88 (36.2%)
3 or more	126 (49.6%)	137(56.4%)
Did not answer	18 (7.1%)	5 (2.1%)
Age of the 1st pregnancy		
Up to 20	93 (36.6%)	141 (58.0%)
Between 21 and 30	95 (37.45%)	79 (32.5%)
More than 30	23 (9.1%)	6 (2.5%)
Did not answer	43 (17.0%)	17 (7.0%)
Use of contraceptive		
Yes	136 (53.5%)	217 (89.3%)
No	98 (38.6%)	21 (8.6%)
Did not answer	20 (7.9%)	5 (2.1%)
Use of Hormonal Replacement Therapy		
Yes	34 (13.4%)	217 (89.3%)
No	199 (78.3%)	21 (8.6%)
Did not answer	21 (8.3%)	5 (2.1%)
Breastfeeding (>3 months)		
Yes	165 (64.9%)	204 (83.9%)
No	64 (25.1)	25 (10.2%)
Did not answer	25 (9.8%)	14 (5.7%)
Total	254 (100.0%)	243 (100.0%)

in the patients group. The remaining genotypes, TG and GG, were observed in a more evenly balanced way when comparing the groups (Table 2).

DISCUSSION

According to the Ministry of Health, age remained the main risk factor for BC in 2012. The present study obtained a mean age for women with BC of about 50 years old, corroborating several previous findings[15]. The White/Caucasian ethnicity was more present in the control group, according to a study published by the National Institutes of Health Consensus Development, in 2001, based on the population of the United States of America. It was concluded that White patients had greater risk of developing BC when compared to Black ones, also considering that the case group displayed predominance of the White/Caucasian ethnicity. It should be noted that, in the present study, ethnicity was self-reported, a fact which could be considered a limitation, although several other studies follow this same line.

McPherson et al.[15], when studying English women, observed that the number of pregnancies is a relevant component to increase the risk of BC development. However, the present study observed that both the control and the case group had greater incidence of pregnancies number, an evidence that, for the female population of Mato Grosso, the low number of pregnancies did not influence the development of BC. On the other hand, it was observed that the control group showed greater incidence in pregnancies in early ages, while the case group had greater frequencies observed in late pregnancies. This epidemiological data was obtained from a previous study by Vieira et al. (2012)[3], based on the Brazilian population.

When analyzing the use of alcohol and use of cigarette variables, it was observed that most patients did not consume these drugs, while the control group presented greater incidence of their use. The analysis of this variable agrees with a Brazilian study by Rodrigues et al.[2], in which alcohol consumption and smoking habits were considered risk factor for the development of BC. Most of the sample analyzed was obtained in public institutions, which may have caused this contrary result.

Table 2. Distribution of the frequency of +114G/T polymorphism of the IL-2 gene in women from Mato Grosso, Brazil

Genotype	Patients	Controls	Total	p-value
TT	38	59	97	0.005
TG	119	101	220	
GG	81	79	160	
Not found	16	4	20	
Total	254	243	497	

The use of hormonal and contraceptive therapy is also considered a risk factor for BC. In this work, the use of contraceptive is not in agreement with the literature, considering that most patients reported not to use it. Therefore, the use of hormonal therapy did not behave as a risk factor for the population of Mato Grosso.

According to McPherson et al.[15], the risk of developing BC is increased among women with a family history of the disease. However, most patients in the present study did not report having any kind of BC family history.

The analysis of the age or menarche variable found a greater frequency, both for case and control groups, for the menarche after 12 years of age, i.e., late menarche — contrary to the findings of previous studies, in Brazil by Rodrigues et al.[2] and Silva et al. (2012)[1] and, in the United Kingdom, by McPherson et al. (1994)[15].

The presence of nodules is considered the main and first symptom of BC; Brazilian studies carried out by Trufelli (2008)[6] and Silva et al. (2012)[1] corroborate this information. This research is in agreement with the literature, showing the presence of nodules as the first sign of neoplasia, like the American study carried out by Mavaddat et al. (2010)[5].

This study had the objective of determining the distribution of +114G/T polymorphism in the IL-2 gene of a female population in Mato Grosso. In a sample of 497 women, between case and control groups, a genotype frequency of 19.5% for TT, 44.2% for TG and 32.1% for GG was observed. In a study carried out in China, Hu et al. (2013)[14] found genotype frequency, for the same polymorphism of this study, of 29.0% for TT, 50.1% for TG, and 20.7% for GG.

Several studies have been carried out with the objective of associating +114 G/T polymorphism in the IL-2 gene with carcinomas and autoimmune pathologies, some with positive association results and others with negative results. Therefore, the objective here was to also demonstrate the association of +114 G/T polymorphism in the IL-2 gene with BC.

According to Lin et al.[11], in a study carried out in Taiwan, in 2008, an association between the presence of +114G/T polymorphism in the IL-2 gene and autoimmune diseases such as sclerosis, arthritis and lupus was observed.

In 2010, a case-control study carried out in China, by Wei et al.[16], associated +114G/T polymorphism in the IL-2 gene with nasopharyngeal carcinoma, although no significant difference was found between the groups. On the other hand, in a case-control study carried out in Taiwan, in 2006, Wu et al.[10] observed a greater prevalence of polymorphism among patients with prostate cancer. Another study carried out in China, in 2012, by Hu et al.[14], showed no association between polymorphism and BC.

The present study observed no statistically significant association between polymorphism and BC, however, a statistical difference was observed (p=0.005) for the control group. The polymorphism in the IL-2 gene may have behaved as a protection

factor for BC. Previous studies demonstrated that this polymorphism in the IL-2 gene abolishes a restriction site of the MWOI. It is a silent point mutation. However, researches demonstrate that this polymorphism influence the production of IL-2 and the functions of these cytokines include the ability to stimulate the survival and differentiation of T cells, acting as a growth factor for immunological cells and for the promotion of increase cell cytolytic activity. Also it promotes the production of immunoglobulin by lymphocytes B. IL-2, which contributes to the regulation of clonal expansion and the process of apoptosis performed by the cells and, thus, performs and essential function in antitumor immunity[10,11].

In the present study, the polymorphism was more frequent in the group of control women for BC. This group consisted of, for the most part, women with habits considered as risky for BC in the literature, such as smoking, alcoholism and use of hormonal therapy. The presence of polymorphism in this group exposed to BC risk factors is suggested to work as a strong protector for this neoplasia.

CONCLUSION

The presence of +114G/T polymorphism in the IL-2 gene behaved as a protection factor for BC in women from Mato Grosso.

REFERENCES

1. Silva PA, Riul SS. Câncer de mama : fatores de risco e detecção precoce. Rev Bras Enferm. 2012;64(6):1016-21.
2. Rodrigues V. Epidemiologia do cancro da mama. Permanyer Portugal. 2010;34(Parte 1).
3. Vieira SC, Lustosa AML, Barbosa CNB, Teixeira, JMR, Brito LXE, Soares LFM, et al. Oncologia Básica. Teresina: Fundação Quixote; 2012.
4. Schwanke CHA, Schneider RH. Atualizações em Geriatria e Gerontologia: da pesquisa básica à prática clínica. Porto Alegre: EdiPUCRS; 2008. 175p.
5. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Molecular Oncology [internet]. 2010 [cited on 2016 Apr. 05];4(3):174-91. Available from: http://dx.doi.org/10.1016/j.molonc.2010.04.011
6. Trufelli DC, Miranda VDC, Santos MBB dos, Fraile NMP, Pecoroni PG, Gonzaga SDFR, et al. Análise do atraso no diagnóstico e tratamento do câncer de mama em um hospital público. Rev Assoc Méd Bras. 2008;54(1):72-6.
7. Naoum PC. Citocinas e interleucinas. São José do Rio Preto: Academia de Ciência e Tecnologia de São José do Rio Preto; 2009.
8. Abbas AK, Lichtman AH, Pillai S. Imunologia celular e molecular. 7ª ed. Rio de Janeiro: Elsevier; 2012.
9. Fedetz M, Matesanz F, Caliz R, Ferrer MA, Collado MD, Alcina A, et al. Lack of association between – 384 and 114 IL-2 gene polymorphisms and rheumatoid arthritis. J Rheumatol. 2003;30(3):435-7.
10. Wu H, Chang C, Wan L, Wu C, Tsai F, Chen W. IL-2 Gene C/T Polymorphism is associated with prostate cancer. J Clin Laboratory Analysis. 2006;249(2):245-9.
11. Lin Y-J, Wan L, Sheu JJ-C, Huang C-M, Lin C-W, Lan Y-C, et al. G/T polymorphism in the interleukin-2 exon 1 region among Han Chinese systemic lupus erythematosus patients in Taiwan. Clin Immunol (Orlando, Fla.) [internet]. 2008 Oct. [cited 2013 Apr. 26];129(1):36-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18650128
12. Matesanz F, Delgado C, Fresno M, Alcina A. Allelic selection of human IL-2 gene. Eur J Immunol. 2000;30:3516-21.
13. Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic acids research [internet]. 1991 Oct. 11 [cited on 2016 mês 05];19(19):5444. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=328920&tool=pmcentrez&rendertype=abstract
14. Hu X-B, Ouyang L-Z, Tang L-L. Interleukin-2 gene polymorphisms and prognosis of breast cancer. Genet Test Mol Biomarkers [Internet]. 2013 Jun. [cited 2013 Apr. 26];17(6):453-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23477313
15. McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer--epidemiology, risk factors and genetics. BMJ . 2000;321:624. DOI:10.1136/bmj.321.7261.624.
16. Wei, YS, Lan, Y, Zhang, L, Wang, JC. Association of the interleukin-2 polymorphism with interleukin-2 serum levels and risk of nasopharyngeal carcinoma. DNA and cell biology. 2010;29(7):363-8. DOI: 10.1089/dna.2010.1019.