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Association between XRCC3 Thr241Met Polymorphism and the Risk of Cancer in Northern Brazil

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Cancer is a genetic disease characterized by an unbalance between cell growth and regulatory factors. The gene XRCC3 encodes a protein that contributes to the integrity of the genome and XRCC3 Thr241Met variants have their capacity of repair altered.
Study Design: Our goal was to evaluate XRCC3 241Met polymorphism in a sample of cancer patients in the city of Macapá.
Place and Duration of Study: Laboratory of Molecular Biology (Biological Sciences Program of the Federal University of Amapá), Dr. Alberto Lima Clinical Hospital (Hcal) and Institute of Hematology and Hemotherapy of Amapá between June 2009 and July 2010.
Methodology: We analyzed 100 DNA samples of patients (50 cases diagnosed with cancer and 50 controls). DNA samples were amplified and analyzed by PCR-RFLP with the enzyme *NLaIII*.

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Results: The molecular analysis revealed that 58% of cases and 12% of controls had the Thr/Met genotype, while 82% of controls and 36% of cases had the Thr/Thr genotype.
Conclusion: Non-invasive independent predictors for screening esophageal varices may decrease medical as well as financial burden, hence improving the management of cirrhotic patients. These predictors, however, need further work to validate reliability. The frequency of the Thr/Met genotype was higher among cancer patients when compared to the control group. Our findings suggest that XRCC3 241Met polymorphism may be associated with the risk cancer in the study population.

Keywords: Cancer; gene polymorphism; XRCC3 gene; Macapá.

1. INTRODUCTION

Cancer is a genetic disease characterized by a disruption of the complex balance between cell growth and the factors that participate in this process. Cell growth may be initiated and continued as the result of mutations in the genes responsible for the maintenance of homeostatic cell proliferation [1,2]. These alterations might be caused by exposure to carcinogens, random replication errors, and defective DNA repair mechanisms [1,3].

Repair pathways maintain the integrity of the genome against environmental aggressors and replication errors through four main mechanisms: Nucleotide excision repair (NER), base excision repair (BER), double strand break repair (DSBR), and mismatch repair (MMR). Associated to these pathways, more than 100 proteins have been identified in human cells [4-6].

The X-ray repair cross-complementing group 3 gene (XRCC3), located at 14q32.3 in the human chromosome, encodes a protein with the same name. This protein consists of 346 amino acids and participates in the maintenance of chromosome stability and the formation of heteroduplex DNA, by repairing double strand breaks (DSBs) through homologous recombination (HR) associated to RAD-51. To perform its function in the initial phase of this process, XRCC3 carries out homology searches in the intact molecule, invading the chain for the synthesis of DNA [6-13]. The most frequent polymorphism of this gene involves the substitution of Threonine (Thr) with Methionine (Met) in the codon 241 of exon 7. Variants of Thr241Met have impaired enzymatic function, altering their DNA repair capacity [6,13-15].

Several studies have associated these variants with some types of cancer, such as breast [16], gastric [17,18], lung [19-21], melanomas [22], oral cavity [23] and bladder [19,24] cancers. This study was aimed at examining a possible association between XRCC3 Thr241Met polymorphism and the risk of cancer among individuals in a city in the Amazon region.

2. MATERIALS AND METHODS

We analyzed 100 samples of peripheral blood from 50 cancer patients from the Dr. Alberto Lima Clinical Hospital (Hcal) from 30 samples are from patients with gastric cancer and the remaining patients with breast and prostate cancer, in the city of Macapá and 50 blood donors of the Institute of Hematology and Hemotherapy of Amapá (Instituto de Hematologia e Hemoterapia do Amapá – HEMOAP) after signing an Informed Consent Form.

Blood samples were analyzed at the Laboratory of Molecular Biology of the Biological Sciences Program of the Federal University of Amapá (Universidade Federal do Amapá – UNIFAP) for the identification of genetic polymorphism in the gene XRCC3.

The procedure to isolate the DNA from blood samples followed the protocol recommended by Invitrogen, the manufacturer of Genomic DNA Mini Kit. XRCC3 241Thr/Thr homozygotes have a 208-bp fragment; Thr/Met heterozygotes have 208, 120 and 88-bp fragment; and Met/Met homozygotes 120 and 88-bp fragments. The samples were amplified and analyzed by PCR-RFLP. The PCR chain reaction for the XRCC3 gene was carried out according to the method described by Shen. et.al (2004). The primers used to amplify the 208 pb fragment were 241F: 5-GCTGTCTCGGGGCATGGCTC-3 and 241R: 5-ACGAGCTCAGGGGTGCAACC-3. The enzyme used was NlaIII (New England Biolabs, Beverly, MA). The conditions for 23 µl of PCR were: 1.0 µl of each primer, 2.0 µl of genomic DNA, 0.5 µl of Taq DNA polymerase, 11.5 µl of H_2O, 5 ul of buffer solution, and 2 ul of dNTP.

The amplification cycle was carried out at 94℃ for 5 minutes, 30 cycles at 94℃ for 30 seconds, 59℃ for 30 seconds, and 72℃ for 1 minute, final extension for 10 minute at 72℃ and 4℃. After the amplification reaction, 10 µl of the PCR product were digested with 1 µl of the enzyme NlaIII (New England Biolabs, Beverly, MA), 1,0 µl of buffer, 2 µl of sterile water at 37℃ for one night and later subjected to electrophoresis for the identification of fragments.

The results were analyzed by statistical tests x^2 using BioEstat 5.3 program to compare the distribution of genotype frequencies between the cases and controls. The association between XRCC3 genotype and gastric cancer was estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from multivariate logistic regression analyses.

3. RESULTS AND DISCUSSION

Fifty DNA samples of cancer patients and 50 samples of the control group were analyzed and our findings demonstrated that 58% (0.091 (0.032 - 0.257) of patients had the Thr/Met genotype, while in the control group, the frequency of this genotype was only 12%. The frequency of the genotype Thr/Thr was 36% and 82% in case and controls, respectively. For the genotype Met/Met, the frequency observed was 6% in both groups (Table 1).

The gene XRCC3 plays an important role in the repair mechanism by homologous recombination of double strand breaks (DSBs) caused by ionizing irradiation and reactive oxygen species. These are some of the most DNA-damaging agents and frequently lead to apoptosis and loss of genetic material [6,25,26]. Given the importance of this DNA repair mechanism, it is biologically reasonable that a genetic polymorphism might modulate the risk of development of many types of cancer [6,17,25].
In the present study, we investigated the association between XRCC3 Thr241Met polymorphism and the risk of cancer. The study of this polymorphism in specific populations is justified by the wide regional differences in its effect on the risk of developing cancer. In a meta-analysis, Lee et al. [27] found a low association between this polymorphism and susceptibility to breast cancer among Korean women. However, Qureshi et al. [28] reported an increased risk associated with this polymorphism in Pakistani women.

Regarding the frequency of Thr/Thr, our results revealed that 36% of cancer patients had this genotype, compared to 82% of the control group. This demonstrates that the frequency of this wild genotype is higher in healthy individuals than in those diagnosed with cancer (Table 1) [6,17,29,30].

Among patients diagnosed with malignant neoplasms, only 6% had the homozygous genotype Met/Met. This was also observed by He et al. [6] in a meta-analysis, suggesting that the allele met has a low association with risk of cancer (except breast and bladder cancers), contrary to the expected based on the biochemical properties associated with XRCC3 Thr241Met polymorphism. When an association with susceptibility to gastric cancer was examined, a strong relationship was found between this genotype and non-cardia gastric cancer but not with cardia cancer [18].

Regarding Thr/Met, 58% of cancer patients and 12% of individuals of the control group had this heterozygous genotype (0.91 (0.32 – 2.05). This is in agreement with Bastos et al. [31] that found a significant association between XRCC3 Thr/Met polymorphism and the risk of developing thyroid cancer in the Chinese population, mainly among smokers and alcohol consumers [32]. Fang et al. [33] carried out a meta-analysis and found a similar pattern in all six studies examined, with heterozygosis with the strongest association with susceptibility to gastric cancer.

Table 1. Frequency of the XRCC3 Thr241Met variants in cases and controls

Genotype	Case (n = 50)		Control (n = 50)		OR (95% CI)
	Nº.	(%)	Nº.	(%)	Crude
XRCC3 Thr241Met					
Thr/Thr	18	36	41	82	1.00
Thr/Met	29	58	6	12	0.91 (0.32 – 2.05)
Met/Met	3	6	3	6	1.0

χ^2 = 24.080, p < 0.001 (Genotype: Thr/Thr vs Thr/Met); χ^2 = 0.946, p = 0.331 (Genotype: Thr/Thr vs Met/Met); χ^2 = 3.228, p < 0.072 (Genotype Thr/Met vs Met/Met);

4. CONCLUSION

The present study found evidences that support the reported in other studies on the association between polymorphism in the repair gene XRCC3 and the risk of cancer. Our results revealed that the frequency of the genotype Thr/Met was higher in cancer patients when compare to the control group and the time interval analysis set forth that the polymorphism likely to occur within 1 year in our population is 52,35%. Given the sample size of our study, further molecular studies on the genotype 241Met and the risk of cancer in other populations are needed to confirm this association. Despite this our results were statistically significant to elucidate the role of these polymorphisms in carcinogenesis.

CONSENT

All authors declare that written informed consent was obtained from all the patient.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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