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ASSESSMENT OF P53 AT CODON 72 POLYMORPHISM IN BREAST CANCER PATIENTS FROM KING GEORGE HOSPITAL, VISAKHAPATNAM

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ABSTRACT The p53 is a tumor suppressor gene and plays an important role in the etiology of breast cancer. p53 tumor suppressor gene frequently is mutated in many forms of human carcinomas. Studies have produced conflicting results concerning the role of p53 codon 72 polymorphism (G>C) on the risk of breast cancer; therefore, a meta-analysis was performed to estimate the association between the p53 codon 72 polymorphism and breast cancer. Breast cancer is among cancers with high prevalence and is the second most common cancer in women around the world. Thirty percent of all cancers and 15% of cancers- caused deaths among women are due to this cancer. According to the report of World Health Organization in February 2009, the annual breast cancer death rate is 519 thousand people worldwide. We tested the hypothesis that the Pro/Pro genotype is associated with increased breast cancer risk in a case-control study of breast cancer that included 98 cases and 100 controls. Samples were obtained from Department of Gynaecology and Obstetrics , King George Hospital and Padmasri Clinic, Visakhapatnam from January 2016 to November 2017. The DNA was isolated by a simple, rapid, non-enzymatic method. In the current study of 98 breast cancer patients, we found that the patients with p53Arg/Pro heterozygotes or the combined p53 Arg/Pro heterozygotes (58%) with Pro/Pro (17%) homozygotes had a significantly increased risk of malignancy. It is likely that the different functions of Arg allele and Pro allele affect DNA repair capacity, apoptosis, and chromosome sensitivity to mutagens, and consequently influence risk of cancer. The p53 gene may modulate the response to environment carcinogens and thereby affect the risk of developing breast cancer.

KEYWORDS : p53, tumor suppressor gene, heterozygous, homozygous

I. Introduction

Breast cancer is the most common cancer in women, accounting for 20% of all new cases of cancer (1). While numerous risk factors for breast cancer have been identified, including genetic predisposition and estrogen level, the molecular mechanisms related to breast carcinogenesis remain under analysis (2,3). Previous studies have shown alterations in cell cycle regulatory proteins in breast carcinoma, including the over expression and increase of the cyclin genes, inactivation and deletions of the Rb gene and alterations of the p53 gene (4–6). Therefore, this disease is a result of collective alterations of oncogenes and tumor suppressor genes. It is well-known that p53, the guardian of the genome, is a stress response protein. p53 functions mainly as a tetramer transcription factor that regulates a large number of genes in response to various stresses, including ontogeny activation and DNA damage (7). p53 is involved in the pro-survival response of cell cycle arrest and DNA damage repair, as well as the pro-death response of apoptosis (8). In the case of a mutation occurring in the p53 gene, p53 may not only lose its normal functions, but also gain new abilities that promote tumorigenesis (9). p53 is the most frequently mutated gene in human tumors; >50% of tumors harbor mutations in the p53 gene (10). Besides its role as a tumor suppressor gene, aberrant p53 expression may play a significant role in regulating angiogenesis (11,12). Chromosomal aberrations and p53 protein abnormalities may be involved in malignant transformation of endometriosis in the ovary (13).

The p53 tumor suppressor gene contains 11 exons, located on chromosome 17p13. The codon 72 polymorphism (rs1042522) is located in exon 4 with a CGC to CCC transition, leading to an arginine to proline amino acid substitution in amino acid position 72 (Arg72Pro). Studies have reported that the codon 72 polymorphism is associated with a risk for the development of cancer (14). The two polymorphic variants have been shown to have not only structural differences, as reflected by distinct electrophoresis patterns of migration, but also different biological properties (15,16). A number of case-control studies have been conducted to explore the correlation between the p53 codon 72 polymorphism and breast cancer risk in humans(16). However, the results are inconsistent. Another problem is that these published studies have only modest sample sizes, which limits their significance. By performing a meta-analysis, a prevailing method for the quantitative summary of different results, the data may be assessed and the sample size increased to a reasonable level. In the present study, a meta-analysis was conducted to quantitatively assess the effect of the p53 codon 72 polymorphism on the risk of breast cancer (17).

II. Methodology

II. 1. Samples and DNA Isolation:

Samples were obtained from Department of Gynaecology and Obstetrics, King George Hospital and Padmasri Clinic, Visakhapatnam from January 2016 to November 2017. Study conducted 98 Breast cancer patients who were histopathologically diagnosed and 100 controls. The DNA was isolated by a simple, rapid, non-enzymatic method.

II. 2. Amplification:

A polymerase chain reaction (PCR) was then carried out to amplify the DNA and RNA sequences of interest by denaturing the DNA molecule and replicating it by utilizing primers, free nucleotides, and a polymerase designed to help the DNA withstand the high temperatures involved in PCR. Because PCR can only be applied when the nucleotide sequence of at least one DNA segment is known, allele-specific primers were used. The proline forward primer was 5'-GCC AGA GGG TGC TCC CC-3' and its reverse primer was 5'-CGT GCA AGT CAC AGA CTT T-3'. Conversely, the arginine forward primer was 5'-TCC CCC TTG CCG TCC CAA-3' and its reverse primer was 5'-CTG GTG CAG GGG CCA CG-3'.

II. 3. Electrophoresis and Analysis:

Agarose gel electrophoresis was then performed to analyze the DNA samples using a 2% agarose gel at 100 V for 30 minutes. Ethedium bromide was used to color each band under ultraviolet light. Band sizes were subsequently compared to the molecular weight band markers for 100-1000 base pairs for confirmation.

III. Results

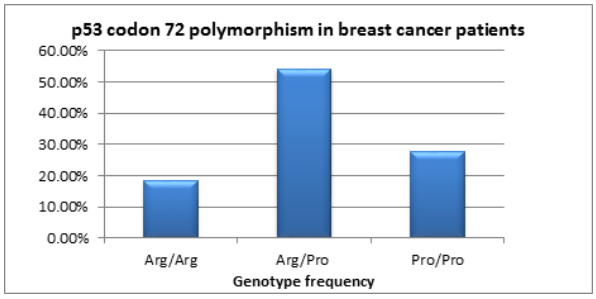
In an attempt to understand the association of tumor suppressor gene p53 polymorphisms with breast cancer, the polymorphic status of the p53 codon 72 was analyzed by allele-specific PCR in 98 breast cancer patients. The present study is to evaluate the rate of polymorphism of p53 gene codon 72 and its relationship to breast cancer risk in women. This has been carried out by collecting, documenting, employing case control studies that have been done in this area, and meta-analyzing the existing findings. The genotype frequencies were determined as 18.4% for the arginine/arginine allele, 54.1% for the heterozygous arginine/proline allele, and 27.6% for the proline/proline allele. The genotype and frequency percentages are given in Table 1. Statistical analysis was performed by means of the x2 test.

P53 codon 72 polymorphic status

Polymorphic status of the P53 codon 72 was analyzed by allele-

specific PCR in 98 breast cancer patients to determine the genotype. The genotype frequencies were determined as 18.4% for the arginine /arginine allele, 54.1% for the heterozygous arginine/proline status, and 27.6% for the proline/proline allele respectively.

Graph : Genotype Distrubution between Patients



The p53 codon 72 genotype distribution for the patients in this study is in agreement with the Hardy-Weinberg equilibrium. Table- 2 shows the expected and observed values for the Hardy-Weinberg equilibrium ($X^2 = 0.622$ and $P > 0.05$).

IV. Discussion

Given the important roles of p53 in multiple cellular functions, including gene transcription, DNA repair and apoptosis, it is biologically plausible that p53 polymorphisms may be associated with a risk of breast cancer (14- 16). Human breast cancer is a disease with significant clinical consequences. The mechanism of breast cancer remains relatively unknown.

Although a number of previous studies have reported a significant association between the p53 codon 72 polymorphism and breast cancer risk, others have identified no such association. In order to resolve this conflict, in the current study, a meta-analysis was conducted to examine the association between a commonly studied p53 polymorphism (codon 72 G<C, Arg72Pro) and breast cancer risk (18). In the present study the genotype frequency were determined as 18.4% for the arginine /arginine allele, 54.1% for the heterozygous arginine/proline status, and 27.6% for the proline/proline allele respectively. The p53 codon 72 genotype distribution for the patients in this study is in agreement with the Hardy-Weinberg equilibrium. Table- 2 shows the expected and observed values for the Hardy-Weinberg equilibrium $X^2 = 0.622$ and the $P > 0.05$ (19). The controls in the studies were not homogenously defined, such that the control subjects in the different studies have varying risks of evolving breast cancer (20). It has been established that p53 plays a pivotal role in cell cycle regulation, DNA repair, and apoptosis, thereby influencing tumor development, progression, and response to DNA damage (21, 22). The p53 codon 72 polymorphism results in a substitution of proline (Pro) for arginine (Arg) in the p53 protein sequence and thus may be functional at the cellular level, which may influence apoptotic potential and cellular arrest in G1 of the cell cycle. In the current study of 98 patients with breast cancer, we found that the patients with p53Arg/Pro heterozygotes (54.1%) combined with Pro/Pro homozygotes (27.6%) had a significantly increased risk of malignancy (23). It is likely that the different functions of the arginine allele and proline alleles affect DNA repair capacity, apoptosis, and chromosome sensitivity to mutagens, and consequently influence the risk of breast cancer (24).

V. Conclusion

In conclusion, we could show a significantly enhanced breast cancer risk associated with the Pro-allele, a significantly later age at breast cancer onset for Pro/Pro patients**.** A more accurate analysis could be conducted if more detailed individual data were available to allow it to be adjusted according to other covariates, including premenopause, postmenopause, family history and environmental factors.

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