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Distribution of TYMS, MTHFR, p53 and MDR1 gene polymorphisms in patients with breast cancer treated with neoadjuvant chemotherapy

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ABSTRACT

Purpose To investigate the role of TSER (TYMS), C677T (MTHFR), Arg72Pro (p53) and C3435T (MDR1) gene polymorphisms in breast cancer patients treated with 5-fluorouracil and cyclophosphamide-based neoadjuvant chemotherapy. *Results* Observed allelic frequencies were: TSER, (2) 0.54 and (3) 0.46; MTHFR C677T, (C) 0.59 and (T) 0.41; p53 Arg72Pro, (Arg) 0.73 and (Pro) 0.27; MDR1 C3435T, (C) 0.52 and (T) 0.48. MTHFR allele T and p53 allele Pro were strongly associated with toxicity due to chemotherapy (odds ratio, 7.1 (95% confidence interval, 1.4–36.1; $p = 0.018$) and 7.0 (95% confidence interval, 1.2–40.5; $p = 0.029$), respectively). *Conclusion* We introduced new data related to the contribution of p53 codon 72 to toxicity due to 5-fluorouracil and cyclophosphamide-based neoadjuvant chemotherapy in patients with breast cancer.

1. Introduction

Breast cancer (BC) is the most common cancer in women [1]. Gran Canaria Island (Canary Islands, Spain) has one of the highest rates of the disease compared with the rest of the Spanish territory (http://www.gobiernodecanarias.org/sanidad/scs/3/3_6/cancer/ppal.jsp). Primary systemic therapy (PST) or neoadjuvant therapy, is used in nonmetastatic breast cancer to treat systemic disease earlier, decrease tumour bulk ideally to a complete pathological response, and reduce the extent of surgery [2,3]. Neoadjuvant chemotherapy allows observing the response of primary tumours to the treatment, providing an ideal platform to identify predictive markers. Thymidylate synthase (TYMS) catalyses the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) in the process of DNA synthesis [4]. It is an important target for drugs such as 5-fluorouracil (5-FU), methotrexate (MTX), oral 5-FU prodrugs and other novel folate-based drugs [5]. A tandem repeat polymorphism has been identified in the 5′-UTR enhancer region of the TYMS promoter (TSER), which contains triple (TSER 3R) or double (TSER 2R) repeats of a 28-bp sequence as well as several rare alleles containing 4, 5 or 9 repeats [6]. In vitro and in vivo studies showed that TYMS expression was TSER genotype-dependent and that 3R allele was associated with a higher TYMS expression level [6]. The role of TSER in the response to fluoropyrimidine-based chemotherapy has been reported and accepted [7]. TSER was included in genotype-guided clinical trials for patients with rectal cancer [8] but less is known related to PST in BC. Methylene-tetrahydrofolate reductase (MTHFR) is a key enzyme that forms the reduced folate cofactor essential for TYMS inhibition by 5-FU. Two linked no synonymous SNPs, C677T and A1298C, have been shown to alter enzyme activity and possibly 5-FU sensitivity [9,10]. MTHFR C677T has been implicated in different adverse reactions [11]. Severe toxicity to MTX has been reported with the implication of those polymorphisms [12]. MTHFR gene variants seem to play a critical role in non-Hodgkiń lymphoma outcome, possibly by interfering with the action of MTX with significant effects on toxicity and survival [13]. Mutations in the p53 gene are the most common genetic alterations in human

Abbreviations: 5-FU, 5-fluorouracil; BC, breast cancer; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; MDR1, multidrug resistance gene 1; MTHFR, methylene-tetrahydrofolate reductase; Pgp, product P-glycoprotein; TYMS, thymidylate synthase.

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cancer [14]. Some polymorphisms in p53 gene have been suggested to play a role in BC [15], and as such may influence the response to chemotherapy. Codon 72 is a common polymorphism in p53 gene encodes either an arginine or a proline at the codon position 72 in the proline rich domain, which has been shown to be an important component in the apoptotic function of p53 [16]. Few studies have investigated the predictive value of this polymorphism for response to neoadjuvant chemotherapy in cancer patients. In that way, the Pro/Pro variant has seen to be less sensitive to cyclophosphamide-based PST in BC patients [17]. The multidrug resistance gene 1 (MDR1) product P-glycoprotein (Pgp) represents the most widely studied membrane protein of the large mammalian ABC transporter family [18]. MDR1 gene is polymorphic and more than 40 SNPs have been identified so far. The C3435T polymorphism in exon 26 of the MDR1 gene affects the expression and function of the Pgp in many ways [19,20]. C3435T polymorphism in the MDR1 gene may limit the local detoxification activity in breast tissue and be a risk factor for cancer development and behaviour [21]. Because these gene polymorphisms have been proposed as important factors in chemotherapy response, toxicity and outcome; and considering all these background and observations, we studied the distribution of TSER, MTHFR C677T, p53 codon 72 and MDR1 C3435T gene polymorphisms in BC patients from Gran Canaria treated with neoadjuvant chemotherapy.

2. Patients and methods

2.1. Patients

Between March 2005 and March 2008, 50 consecutive and unselected patients, diagnosed with primary breast cancer at our Service, were treated with 5-FU and cyclophosphamide-based neoadjuvant treatment. Patients in clinical stages I–IV were included in the study (TNM staging, American Joint Committee on Cancer). The diagnosis of the disease was confirmed by histological examinations. All participants were Caucasians of Canary origin. The Ethics Committee of our Hospital approved the present study and written informed consent was obtained from each participant before biological samples were obtained. Blood samples were collected at the first visit. All women in this trial filled out a structured questionnaire designed by the "CDC de Canarias" project [22]. This questionnaire included patient history and risk factors related to BC development. Data were collected in all cases.ImmunohistochemistryEstrogen receptor, progesterone receptor and erbB2 were determined in the tumour tissue by immunohistochemistry. Immunostaining was done as described elsewhere [23]. For estrogen receptor and progesterone receptor staining, cells were considered positive when $\geq$10% of tumour cells showed positive staining. For erbB2 staining, only the membrane staining was considered as positive. The score of erbB2 staining was graded as follows: No staining or membrane staining observed in <10% of tumour cells was given a score 0; faint/barely perceptible membrane staining detected in >10% of tumour cells was scored as 1+; a moderate or strong complete membrane staining observed in >10% of tumour cells was graded 2+ or 3+, respectively. A score of 0 or 1+ was considered negative. A score of 2+ was subsequently confirmed by the FISH technique [24]. Only 2+/FISH+ were considered as erbB2 positive. A score of 3+ was considered positive as well.Treatment protocolsAll treatments had cyclophosphamide as common drug. Depending on the pathological characteristics of the tumour, four different protocols of treatment were administered to the patients as follows: FEC regimen (5-fluorouracil 600 mg/m^2, epirubicin 90 mg/m^2, cyclophosphamide 600 mg/m^2, on day 1, 15-day intervals for six cycles), FEC + docetaxel regimen (5-fluorouracil 600 mg/m^2, epirubicin 90 mg/m^2, cyclophosphamide 600 mg/m^2, on day 1, 15-day intervals for four cycles followed by docetaxel 100 mg/m^2, on day 1, 21-day intervals for four cycles), FEC + paclitaxel regimen (5-fluorouracil 600 mg/m^2, epirubicin 90 mg/m^2, cyclophosphamide 600 mg/m^2, on day 1, 15-day intervals for four cycles followed by paclitaxel 100 mg/m^2, on day 1, 7-day intervals for four cycles) and CMF (cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2, 5-fluorouracil 600 mg/m^2, on day 1, 21-day intervals for six cycles). After completion of neoadjuvant therapy, patients received either breast-conserving surgery or modified radical mastectomy depending on the tumour size. The World Health Organization criterion was applied to assess the response to therapy [25]. A complete pathologic response was defined as no evidence of residual invasive disease in the breast and lymph nodes. A <50% of response was considered when the tumour reduced its size <50%. A >50% of response was considered when the tumour reduced its size >50%. And no response was considered when the tumour did not modify its size after PST. Common Toxicity Criteria (National Cancer Institute) was used to classify the grade of adverse reaction to chemotherapy.

2.4. Genotyping

Polymorphic sites in TYMS (TSER), MTHFR (C677T), p53 codon 72 (Arg/Pro) and MDR1 (C3435T) were examined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis as previously reported [26]. Except for TSER, the amplified fragments were digested with the appropriate restriction endonucleases (New England BioLabs). After incubation at the optimal temperature, 20 μl of digested product was analyzed by gel electrophoresis on a 2–3% low melting agarose gel and visualized under ultraviolet light in a ChemiDoc System (Bio-Rad) after staining with ethidium bromide (Pierce).

2.5. Statistical analyses

Determination of the deviation from the Hardy–Weinberg equilibrium was made using the chi-square test. Chi-square test was used in the bivariate analysis of categorical variables. For multivariate analysis, binary logistic regression model was used to calculate the odds ratio and the corresponding 95% confidence interval (CI) to set the association between the polymorphisms and other variables. All tests were two-sided and differences were considered significant when p values were <0.05. All analyses were performed with SPSS for Windows (version 15.0; SPSS, Chicago, IL)

3. Results

The study population consisted of 50 women with histological confirmed breast cancer treated with 5-FU and cyclophosphamide-based neoadjuvant treatment. Age ranged from 33 to 76 years (mean 53.24, median 50.50 years) at the time of diagnosis. The majority of women were postmenopausal (58%): due to stated change in life (89.7) or due to hysterectomy (10.3%). The majority of patients responded to the neoadjuvant therapy: complete response, 30%; >50% of response, 52%; <50% of response, 8%; and no response, 6%. Demographic and clinic pathologic characteristics are detailed in Tables 1 and 2, respectively. TSER, MTHFR C677T, p53 Arg72Pro and MDR1 C3435T allelic and genotypic frequencies were estimated (Table 3). Observed allelic frequencies were (%): TSER, (2) 54 and (3) 46; MTHFR C677T, (C) 59 and (T) 41; p53 Arg72Pro, (Arg) 73 and (Pro) 27; MDR1 C3435T, (C) 52 and (T) 48. The genotype distributions were in Hardy–Weinberg equilibrium. We did not observe differences in the gene polymorphism distributions between breast cancer patients and general population from our region ($p > 0.05$). The distribution of initial clinical stage, tumour size, estrogen or progesterone receptor status, erbB2