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Assessment of clinical outcomes in breast cancer patients treated with taxanes: multi-analytical approach

Sonam Tulsyan [a], Pankaj Chaturvedi [a], Abhishek Kumar Singh [a], Gaurav Agarwal [b], Punita Lal [c], Sushma Agrawal [c], Rama Devi Mittal [d], Balraj Mittal [a,*]

[a] Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226014, India
[b] Department of Endocrine & Breast Surgery, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226014, India
[c] Department of Radiotherapy, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226014, India
[d] Department of Urology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226014, India

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ABSTRACT

Polymorphisms in genes encoding CYPs (Phase I) and ABCB1 (Phase III) enzymes may attribute to variability of efficacy of taxanes. The present study aims to find the influence of CYP and ABCB1 gene polymorphisms on taxanes based clinical outcomes. 132 breast cancer patients treated with taxanes based chemotherapy were genotyped for CYP3A4*1B, CYP3A5*3, CYP1B1*3, CYP2C8*3, ABCB1 1236C>T, 2677G>T/A and 3435C>T polymorphisms using PCR-RFLP. Associations of genetic variants with clinical outcomes in terms of response in 58 patients receiving neo-adjuvant chemotherapy (NACT), and chemo-toxicity in 132 patients were studied. Multifactor dimensionality reduction (MDR) analysis was performed to evaluate higher order gene–gene interactions with clinical outcomes. Pathological response to taxane based NACT was associated with GA genotype as well as A allele of CYP3A5*3 polymorphism ($P_{corr} = 0.0465$, $P_{corr} = 0.0465$). Similarly, association was found in dominant model of CYP3A5*3 polymorphism with responders ($P_{corr} = 0.0465$). Haplotype analysis further revealed A_{CYP3A4}–A_{CYP3A5} haplotype to be significantly associated with responders ($P_{corr} = 0.048$). In assessing toxicity, significant association of variant (TT) genotype and T allele of ABCB1 2677G>T/A polymorphism, was found with 'grade 1 or no leucopenia' ($P_{corr} = 0.0465$, $P_{corr} = 0.048$). On evaluating higher order gene–gene interaction models by MDR analysis, *CYP3A5*3*; ABCB11236C>T and ABCB1 2677G>T/A; ABCB1 3435C>T and CYP1B1*3 showed significant association with treatment response, grade 2–4 anemia and dose delay/reduction due to neutropenia ($P = 0.024$, $P = 0.004$, $P = 0.026$), respectively. Multi-analytical approaches may provide a better assessment of pharmacogenetic based treatment outcomes in breast cancer patients treated with taxanes.

1. Introduction

Taxanes are commonly used cyto-toxic drugs in breast cancer treatment. Paclitaxel and docetaxel — the two common taxanes interfere with the microtubules at the mitotic spindle, resulting in cell cycle arrest during mitosis and finally cell death (Abal et al., 2003). Taxanes are part of the adjuvant treatment of breast cancer, as well as neo-adjuvant chemotherapy (NACT) for locally advanced breast cancers (LABC). However, there is a considerable inter-individual variation in the response to taxanes based chemotherapy, and many breast cancer patients have taxanes resistant tumors (Gehrmann et al., 2008). The severity of drug toxicity and adverse events, mostly in the form of myelosuppression also varies considerably. Pharmacogenetic studies can help identify individuals predisposed to a high risk of toxicity and resistance to taxanes containing chemotherapeutic regimen.

Polymorphisms in genes encoding taxane metabolizing enzymes and transporters can affect drug treatment outcomes. Both paclitaxel and docetaxel are metabolized by CYP3A4, CYP3A5 and CYP2C8 to inactive hydroxylated metabolites in the liver (Marsh, 2006). Taxanes are substrates for CYP1B1 enzymes (Bournique and Lemarie, 2002) and are responsible for their catalytic activity (Hanna et al., 2000; Li et al., 2000; Shimada et al., 1999). The drug metabolites are substrates for ABCB1 transporters or P-glycoprotein which are located at the biliary canalicular membrane, enabling taxanes elimination from hepatocytes (Sparreboom et al., 1997). Polymorphisms in CYPs and ABCB1 genes have been studied in context with taxanes dependent toxicities (Dong et al., 2012; Rizzo et al., 2010; Tsai et al., 2009). As noted for anthracyclines, some studies have found ABCB1 genotypes and haplotypes to correlate with taxanes

Abbreviations: NACT, neo-adjuvant chemotherapy; ACT, adjuvant chemotherapy; NCI, National Cancer Institute; CTCAE, Common Toxicity Criteria for Adverse Events; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; OR, odds ratios; CI, confidence intervals; FDR, False Discovery Rate Test; MDR, multifactor dimensionality reduction; CVC, cross-validation consistency; LABC, locally advanced breast cancer.

* Corresponding author at: Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India.
E-mail address: bml_pgi@yahoo.com (B. Mittal).

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dependent toxicities (Bosch et al., 2006; Sissung et al., 2006; Yamaguchi et al., 2006), while other studies have not shown any correlation (Goh et al., 2002; Kim et al., 2012; Marsh and McLeod, 2007; Marsh et al., 2007).

Considering that these candidate genes have a role in taxane activation, metabolism and transportation, we aimed to find out the influence of 7 SNPs (CYP3A4*1B, CYP3A5*3, CYP1B1*3, CYP2C8*3, ABCB1 1236C>T, ABCB1 2677G>T/A and ABCB1 3435C>T) with taxanes based breast cancer treatment outcomes in terms of response to NACT and chemo-toxicity using multi-analytical approaches.

2. Methodology

2.1. Patient selection

This prospective study was approved by the research ethics committee of SGPGIMS, Lucknow, India. A total of 132 histologically confirmed breast cancer patients treated with taxanes based chemotherapy in the Departments of Endocrine & Breast Surgery and Radiotherapy, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, were recruited in the study after taking their informed consent. Patients were staged according to AJCC-TNM classification system (Edge and Compton, 2010). Patients who did not complete their treatment as planned, or those for whom complete clinical, pathologic, operative or outcome data were unavailable were excluded.

2.2. Drug administration

Study patients either received Docetaxel or Paclitaxel as adjuvant or NACT in sequential manner after four cycles of combination chemotherapy consisting of anthracyclines (doxorubicin or epirubicin), 5-fluorouracil and cyclophosphamide. Patients treated with concomitant anthracyclines and taxanes were excluded. Paclitaxel was administered at the standard dose of 80 mg/m^2 infused i.v. over 1 h q 1 weekly for 12 cycles, and docetaxel was administered at the dose of 100 mg/m^2 infused i.v. over 2 h q 3 weekly for four cycles.

2.3. Evaluation of treatment outcomes

According to the Response Evaluation Criteria in Solid Tumors (RECIST criteria) (Therasse et al., 2000), response was assessed in 58 breast cancer patients treated with taxanes based NACT at the end of taxanes therapy. The patients with complete and partial pathological response were considered as responders while patients with static and progressive disease were considered as non-responders. Chemo-toxicity in 132 patients receiving taxanes based adjuvant or NACT was graded according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE), version 3.0 (http://ctep.cancer.gov). According to the NCI-CTCAE, grade 2–4 toxicity was recorded in terms of grade 2–4 anemia (hemoglobin < 10 g/dl), leucopenia (TLC < 3000/mcL) and thrombocytopenia (platelets count < 75,000/mcL). Patients who suffered from no or grade 1 toxicity are included in "No grade 2–4 toxicity". Chemo-toxicity in the form of dose delay or reduction due to febrile neutropenia was also recorded.

2.4. Genotyping

Venous blood (5 ml) was collected from each patient and the genomic DNA was extracted from peripheral blood using the standard salting out method (Miller et al., 1988). The quality and quantity of DNA were checked by using Nanodrop spectrophotometer (Thermo Fisher Scientific/Nanodrop Products, Wilmington, Delaware, USA).

Polymorphisms in CYP3A4, CYP3A5, CYP1B1, CYP2C8 and ABCB1 were determined by Polymerase chain reaction (PCR)-Restriction fragment length polymorphism (RFLP) (Supplementary Table S1). Ten percent of the samples from patients including samples of each genotype were re-genotyped by other laboratory personnel. No discrepancy was found after sequencing randomly selected 5% samples.

2.5. Statistical evaluation

Descriptive statistics of patients was presented as mean and standard deviations for continuous measures and frequencies and percentages for categorical measures. Correlation between various genotypes and treatment outcomes was examined using binary logistic regression. Association was expressed as odds ratios (OR) or risk estimates with 95% confidence intervals (CI). Statistical analysis was done using SPSS statistical analysis software, version 17.0 (SPSS Inc., Chicago, IL, USA). Statistical analysis of the haplotype estimation and linkage disequilibrium was conducted using the SNPstat Software (Sole et al., 2006). Benjamini–Hochberg False Discovery Rate Test (FDR) is used in multiple hypothesis testing to correct for multiple comparisons. In a list of rejected hypotheses, FDR controls the expected proportion of incorrectly rejected null hypothesis (type I errors). Multifactor dimensionality reduction (MDR) analysis was done for the detection and characterization of gene–gene interactions (Hahn et al., 2003). The best gene–gene interaction model was selected across all multi-locus models on the basis of maximum testing accuracy (rounded up to 2 decimal places), cross-validation consistency (CVC) and permutation results were considered to be statistically significant at the 0.05 level (rounded up to 3 decimal places).

3. Results

3.1. Patient characteristics

The mean age of breast cancer patients was 48.06 ± 10.97 years. Detailed characteristics of patients including assessment of hormone receptors (estrogen and progesterone) and HER 2-neu status; lymph nodes involved, metastasis, menopausal status and histology are summarized in Table 1. Out of 132 patients recruited in the study, paclitaxel was administered to 68 (51.51%) patients while docetaxel was given to 64 (48.48%). 58 patients in all received NACT.

3.2. Association of genetic variants with treatment response

Of the 58 locally advanced breast cancer (LABC) patients treated with taxanes based NACT, 22 (37.9%) were non-responders whereas 36 (62.1%) patients were responders (Table 2). For CYP3A5*3 polymorphism, border-line association of AG genotype was observed with responders ($P_{corr} = 0.0465$, OR = 0.29). Similarly, significant association of variant allele A ($P_{corr} = 0.0465$, OR = 0.31) and in dominant model ($P_{corr} = 0.0465$, OR = 0.26) was seen with responders. However, no association was found with other genetic variants of CYP and ABCB with response (Table 2).

3.3. Association of genetic variants with chemo-toxicity

Out of 132 patients treated with taxanes based adjuvant or NACT, overall grade 2–4 chemo-toxicity was observed in 78 (59.1%) patients. No association of CYP or ABCB1 polymorphisms was observed with chemotherapy induced grade 2–4 toxicity (Table 3).

Grade 2–4 anemia was noticed in 72 (54.5%) patients. For genetic variants in either CYP or ABCB1, no association was observed with grade 2–4 anemia at the genotypic level or at the allelic level (Supplementary Table S2). Furthermore, grade 2–4 leucopenia and thrombocytopenia were recorded in 32 (24.2%) and 7 (5.3%) patients, respectively. For ABCB1 2677G>T/A, significant association of GT genotype was observed with patients having grade 1 or no leucopenia ($P_{corr} = 0.0465$, OR = 0.15). Similarly, a marginal association of T allele was seen ($P_{corr} = 0.0465$, OR = 0.54) with grade 1 or no leucopenia (Supplementary Table S3). However, due to less patient