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Influence of ABCB1 genetic variants in breast cancer treatment outcomes

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ABSTRACT

Background: Transportation of anticancer drugs such as anthracyclines across the membrane is regulated by P-glycoprotein encoded by the human multidrug resistance gene 1 (ABCB1). Polymorphisms in the ABCB1 gene (1236C > T, 2677G > T/A, 3435C > T) have been found to be associated with intrinsic and acquired cross resistance to these anticancer drugs. Therefore, the aim of this study is to evaluate the influence of ABCB1 gene polymorphisms in breast cancer treatment outcomes in terms of response and toxicity.

Method: Response to neo-adjuvant chemotherapy was evaluated in 100 patients while grade 2–4 toxicity was followed in 207 patients, who had undergone FEC/FAC chemotherapy. Genotyping for ABCB1 polymorphisms was done by PCR-RFLP. Chi square and logistic regression analyses were used to calculate Odd's ratio using SPSS ver 17.0. A meta analysis was also performed using Comprehensive Meta Analysis Ver 2.

Results: In response evaluation, 1236C > T polymorphism was significantly associated with treatment response for CT genotype [OR = 5.17(1.3–20.2), $P = 0.018$] and in dominant model (CC vs CT + TT) [OR = 4.63(1.25–17.0), $P = 0.021$]. In the toxicity group, the T allele of 1236C>T was associated with grade 2–4 tocxicity [OR 1.48(1.00–2.20), $P = 0.049$] and the association was also significant in the recessive model [OR 1.88(1.05–3.39), $P = 0.033$]. For other two SNPs 2677G>T/A and 3435C>T no association was seen with either treatment response or grade 2–4 toxicity. In meta analysis, no overall association was found.

Conclusion: In our study, significant association was seen for ABCB1 1236C>T polymorphism with treatment response. The meta analysis did not show overall association with treatment outcomes.

1. Introduction

Breast cancer patients are treated with chemotherapy depending upon the stage, e.g. locally advanced breast cancers are treated with neo-adjuvant chemotherapy (NACT) while early breast cancers are treated with primary surgery followed by adjuvant chemotherapy (ACT). Treatment strategies include chemotherapy – anthracyclines (epirubicin/doxorubicin), cytotoxics (cyclophosphamide, paclitaxel, docetaxel), hormone therapy anti-estrogens (tamoxifen) and aromatase inhibitors (exemestane, anastrozole, letrozole). A significant heterogeneity is observed in the response and toxicity to chemotherapeutic agents [1,2]. Genetic differences in drug transporters, enzymes of primary and secondary metabolism pathways may contribute to the inter-individual variations in treatment outcomes [3].

The multidrug resistance gene 1 (ABCB1) is responsible for energy dependent efflux of drugs, resulting in low intracellular levels and is encoded by P-glycoprotein (P-gp). Resistance to many anti-cancer drugs including anthracyclines and taxanes are found to be associated with genetic variations affecting function and expression of ABCB1 [4]. Recent clinical studies on breast cancer have shown that the expression of P-glycoprotein is associated with response to chemotherapy [5,6].

Drug transporters including ABCB1 are members of superfamily of ABC (ATP Binding Cassette) transporters and comprise of eight subfamilies. ABCB1 is located on chromosome 7, spans more than 100 kb and expressed as 4.5 kb mRNA [7,8]. More than 20 variations in the ABCB1 gene have been reported until now [9], out of which most commonly studied are 1236C>T (exon 12, rs1128503), 2677G>T/A (exon 21, rs2032582) and 3435C>T (exon 26, rs1045642). The SNPs 1236C>T (Gly412Gly) and 3435C>T (Ile1144Ile) are synonymous while 2677G>T/A results in an amino-acid change from Ala at codon 893 to Ser/Thr. The

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2677G>T/A and 3435C>T polymorphisms are in linkage disequilibrium [9,10]. Some studies report that 3435C>T plays a role in response to chemotherapy in patients with locally advanced [2] as well as advanced breast cancer [11]. However, other studies do not find any role of the polymorphism in treatment response [12,13]. Similarly, for toxicity, Tsai et al. [14] have shown that patients with ABCB1 2677G/G genotype suffered more from febrile neutropenia than other genotypes. The patients having 3435C/C genotype were more prone to leucopenia. On the contrary, Cizmarikova et al. [12] found no association of 3435C>T with hematologic toxicities in breast cancer patients. Due to such contradicting studies, the present investigation is aimed at finding the role of these polymorphisms in predicting clinical outcomes in terms of response to chemotherapy and grade 2–4 toxicity. Moreover, a meta analysis was also performed to draw overall conclusions.

2. Materials and methods

2.1. Patients and treatment regimen

Two hundred and seven breast cancer patients treated at the Departments of Endocrine & Breast Surgery; and Radiotherapy, Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGIMS), Lucknow, India were recruited in this study. Patients who were treated with FAC/FEC (Fluorouracil, Epirubicin/doxorubicin and Cyclophosphamide) chemotherapy regimens were included in this study. Written informed consent, after the approval of the ethical committee of the institute was taken. The patients were graded according to the TNM staging and treated as per standard institutional protocols, which involved surgery, radiation therapy, chemotherapy and hormonal therapy. Demographic and clinico-pathological parameters of the patients were recorded and are illustrated in Table 1. Of the 207 patients, 100 received NACT and 107 received ACT following surgery.

Tumor response was evaluated in patients receiving NACT according to RECIST criteria (Response Evaluation Criteria in Solid Tumors) [15], 3 weeks after three cycles as well as last cycle of chemotherapy. The patients with complete and partial pathological response were categorized as responders while static and progressive disease were categorized as non-responders. Surgery was performed after 3 weeks of last cycle of chemotherapy. According to NCI-CTCAE [16], grade 2–4 toxicity was recorded in 207 patients, in terms of grade 2–4 anemia (hemoglobin < 10 g/dl), leucopenia (TLC $< 3000/\mu$cL) and thrombocytopenia (platelets count $< 75{,}000/\mu$cL) [16]. Records of patients who had dose delay or reduction due to febrile neutropenia were also maintained.

2.2. Genotyping

Blood samples were collected in EDTA (ethylene-diamine-tetra-acetic acid) vials and genomic DNA was extracted from peripheral blood leukocyte pellet using a modified salting-out method [17]. The quality and quantity of DNA was checked spectrophotometrically using the Nano Drop Analyzer-1000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). The ratio of absorbance at 260 and 280 nm of DNA was between 1.7 and 1.9 and the isolated DNA was stored at $-70\,^{\circ}$C.

Polymerase chain reaction (PCR)-Restriction fragment length polymorphism (RFLP) was used to determine the genotypic frequencies of 1236C>T [10], 2677G>T/A [10] and 3435C>T [18] (representative gel pictures are shown in supplementary Figures S1a–c). 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.

See Figure S1 as supplementary file. Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.canep.2013.04.012.

2.3. Literature search strategy and data extraction for meta analysis

Literature search was carried out in PubMed, OVID and Springer, covering all the papers published until December 2012, using the keywords multidrug resistance gene, ABC transporters, breast cancer, pharmacogenetics and toxicity. Reference lists of key studies and reviews were also screened for additional related studies. The criteria used for literature selection were: (a) original papers, (b) exploring the association between the three selected SNPs and chemotherapy response and toxicity, (c) papers with crude Odd's ratio and 95% confidence interval or sufficient data to calculate overall OR at 95% CI), (d) chemotherapy response evaluation by RECIST criteria. A total of 66 related studies were found by using these research criteria. After screening according to the inclusion criteria, finally 8 publications were included for meta analysis. Information on the following data was collected for each study: first author's name, publication date, country, ethnicity, number of patients included in the study, clinical stage, treatment protocols, genotyping methods, evaluation criteria and sample origin.

2.4. Statistical analysis

Descriptive statistics of patients were presented as mean and standard deviations for continuous measures whereas frequencies and percentages were used for categorical measures. Effective sample sizes were calculated by the Quanto software version 1.2 [19]. Statistical significance of differences in genotype frequencies between patients with different treatment outcomes was estimated by the χ^2 test. Binary logistic regression was used for all analysis variables to estimate risk as odds ratio (OR) with 95% confidence intervals (95%CIs). All statistical analyses were performed using the SPSS software version 17.0 (SPSS, Chicago, IL, USA) and tests of statistical significance were two-sided.

In meta analysis, pooled odd's ratios and confidence intervals were calculated for overall toxicity and response. Analyses were weighted by trial size. Statistical heterogeneity was measured using the Q statistic ($p < 0.10$ was considered as significant heterogeneity) [20]. The effect of heterogeneity was also quantified by I^2 statistic with the following suggested cut off points: $I^2 = 0$–25%, no heterogeneity; $I^2 = 25$–50%, moderate heterogeneity and $I^2 = 75$–100% extreme heterogeneity [21]. Fixed effects model was used when no heterogeneity was found, otherwise random effects model was used. Publication bias was investigated with funnel plot, in which the standard error of log OR of each study was plotted against its OR. Funnel plot asymmetry was further assessed by the method of Egger's linear regression test [22].

3. Results

3.1. Genotypes and treatment response

According to the RECIST criteria, response assessment was made in one hundred patients who were given NACT, and it was observed that 61 (61%) patients were responders and 39 (39%) patients were non-responders (Table 2). For 1236C>T polymorphism, the CT genotype was significantly associated with adverse response to chemotherapy [OR = 5.17(1.3–20.02), $P = 0.018$]. We also observed significant results when dominant model was applied to the above polymorphism [OR = 4.63(1.25–17.0), $P = 0.021$] (Table 2). However no association was seen at the allelic level.