CYP19A1 Genetic Polymorphisms rs4646 and Osteoporosis in Patients Treated with Aromatase Inhibitor-Based Adjuvant Therapy

Objective: Third-generation aromatase inhibitors (AI) are potent suppressors of aromatase activity. The aim of this study was to measure the incidence of adverse effects in breast cancer patients treated with AI-based adjuvant therapy and the relationship with the CYP19A1 genotypes.

Materials and Methods: Forty-five postmenopausal breast cancer patients (46-85 yrs) in AI adjuvant treatment were genotyped for the rs4646 polymorphisms of CYP19A1 gene and three variations were identified. Toxicities were registered at each follow-up medical examination, and classified in accord with the Common Terminology Criteria for Adverse Events.

Results: Twenty-four (53.3%) patients presented the GG genotype; 19 (42.2%) the GT, and 2 (4.4%) the TT. The AI treatment was Anastrazole for 35 patients (77.8%) and Letrozole for the others (n=10; 22.2%). Osteoporosis was significantly associated with the GG genotype (p=0.001). Treatment discontinuation (TD) was observed in 6 cases (13.3%). The only parameter able to predict TD was the appearance of severe arthralgia/myalgia (Odds Ratio, OR=23.75; p=0.009), when adjusted for age and AI treatment.

Conclusion: Our results suggest that CYP19A1 polymorphic variants may influence susceptibility to develop AI-related side effects. Further prospective studies are needed to confirm the role of the aromatase gene (CYP19A1) polymorphisms in predicting adverse effects to AI-based therapy.

Keywords: Adjuvant hormonal therapy, rs4646, aromatase inhibitor, breast cancer, CYP19A1, single nucleotide polymorphisms

Öz

Gereç ve Yöntem: 45 postmenopozal meme kanserli hasta (46-85 yaş) CYP19A1 geninin RS4646 polimorfizmlerini genetikleştirdik ve üç varyasyon tanımladık. Toksisiteler her takipteki tıbbi muayenede kaydedildi ve Advers Olaylar için Ortak Terminoloji Kriterleri ile uyumlu olarak sınıflandırıldı.

Bulgular: Yirmi dört (%53,3) hasta GG, 19’u (%42,2) GT ve 2’si (%4,4) TT genotipi gösterdi. Osteoporoz özellikle GG genotipi ile ilişkiliydi. Tedaviye devam etmeme (TD) 6 olguda (%13,3) görülüyordu. Yaşa ve AI tedavi visine göre düzeltildiğinde, TD’yi öngörebilecek tek parametre artralji/miyalji oluşumu idi (Odds Oranı, OR=23,75; p=0,009).


Anahtar Kelimeler: Adjuvan hormonal terapi, rs4646, aromataz inhibitory, meme kanseri, CYP19A1, tek nükleotid polimorfizmler
Introduction

Third-generation aromatase inhibitors (letrozole, anastrozole and exemestane) are potent suppressors of aromatase activity and represent the standard care for adjuvant therapy in postmenopausal patients with hormonal receptors-positive breast cancer [1, 2].

Aromatase is the enzyme that catalyses the peripheral conversion of androstenedione and testosterone to oestrone (E1) and estradiol (E2). In women with ovarian failure, this process may represent the main source of oestrogen production.

The inhibition of this pathway causes a profound oestrogen deficiency and low levels of oestrogen may affect many organs, including the brain, the skeleton and the skin as well as the cardiovascular and genitourinary systems [3].

Aromatase inhibitors’ adverse effects are similar to menopause-related symptoms. They cause the interruption of adjuvant treatment in 20-30% of the cases. Most relevant effects are hot flashes, myalgia, arthralgia, vaginal dryness, insomnia or other sleep problems, asthenia and loss of bone density. Oestrogens have a regulatory effect on bone metabolism by stimulating bone growth and inhibiting bone resorption, so their depletion leads to bone loss that causes a progressive osteopenic and/or osteoporotic status and a higher risk of fractures [3].

The estrogenic synthesis suppression, induced by aromatase inhibitors, seems to be related to polymorphisms of CYP19A1 gene that encodes the aromatase enzyme (cytochrome P-450, family 19, subfamily A, polypeptide 1; 15q21.1) [3].

Variability in the sex hormone levels, in serum and urine, could be account by the CYP19A1 genetic polymorphisms; Dunning et al. [4] reported that the TT genotype (rs4646) is associated to a lower circulating oestrogen levels, compared to GT and GG genotype. In postmenopausal women treated with letrozole for metastatic breast cancer, time to progression (TTP) was significantly prolonged in those with the rare T allele of rs4646 compared with homozygotes for the wild-type variant (GG) [5]. The same variants (GT and TT) were also associated with a poorer benefit from letrozole (shorter progression-free survival) compared to GG, evaluated in a neoadjuvant setting [6].

On the contrary, Colomer et al. [5] reported that patients with the allele T (TT and GT) of rs4646 polymorphisms had a better TTP compared to those patients with wild type genotype (GG). Furthermore, GG genotype was related to side effects in 56% of the cases, while GT and TT genetic variants (without distinguishing homozygous from heterozygous patients) were associated with side effects in 44% of the cases.

Two recent studies [7, 8] investigated the relationship between rs4646 polymorphisms and letrozole administration in breast cancer. Neither of them found any significant connection with AI treatment outcome.

The early identification of patients who will develop side effects is important in order to improve the compliance to treatment and to reduce its interruption.

Napoli et al. [9] and Mao et al. [10] demonstrated an association among polymorphisms rs60271534, rs700518 and side effects induced by aromatase inhibitors.

To our knowledge, no empirical studies exist addressing the relationship between polymorphisms of CYP19A1 gene (rs4646) and the onset of AI therapies related-side effects; we hypothesized that a single nucleotide polymorphisms of CYP19A1 (rs4646) may influence the occurrence of side effects in patients treated with aromatase inhibitors-based adjuvant therapy. The aim of this study was to measure the incidence of the adverse effects in breast cancer patients treated with AI-based adjuvant therapy and the relationship with the CYP19A1 genotypes.

Materials and Methods

We carried out a retrospective study. 45 postmenopausal women in third generation-aromatase inhibitor adjuvant treatment in May 2012 were enrolled in the study. Main inclusion criteria were: stage I–III histologically confirmed breast cancer; ER positive breast cancer; radical surgery (either mastectomy or breast conserving surgery) and axillary or sentinel node dissection or biopsy; diagnosis and treatment on (name) between January 2006 and January 2012. Those positive for osteoporotic, before the therapy, were excluded from the study. Letrozole (Femara®) was administered at the dose of 2.5 mg/day and Anastrozole (Arimidex®) at the dose of 1 mg/day. Written informed consent was obtained from all patients to collect blood samples and to use their clinical information from clinical chart. The study protocol has been approved by the Hospital Ethic Committee.

The National Cancer Institute’s Common Terminology Criteria for Adverse Events version 3.0 [11], has been used to grade the severity of the adverse events. Toxicities were registered at each scheduled follow-up (outpatient visits or treatment administration).

For osteopenic and osteoporotic assessment, bone mineral density (BMD) has been measured in the lumbar spine and proximal femur by dual energy x-ray absorptiometry (DXA). Lumbar spine BMD was determined using the anteroposterior projection and was calculated as the average of L1-L4. The non-dominant hip was used for proximal femur scans. BMD was measured before the AI treatment, and every 12-18 months up to the fourth year of treatment. According
CYP19A1 genotyping

The X-tractor Gene system (Corbett life Science, Australia) for automated nucleic acid extraction has been used. The analysed rs4646 single nucleotide polymorphism (SNP) was located in the 3’ untranslated region of aromatase (CYP19A1) gene. Reference sequence for CYP19A1 gene was obtained from NCBI GenBank database (http://www.ncbi.nlm.nih.gov/).

Genotyping was performed by Pyrosequencing technology (Pyrosequencer PyroMark ID system-Qiagen). Both amplification and sequencing primers were obtained by the PSQ Assay Design software (Biotage AB and Biosystems, Uppsala, Sweden). Selected primer sequences are: 5’-CATTTTATAGGCATACCTC-3’ (GG) as forward primer, biotin-5’CTGCTTTTTCTCTTGTAG-3’ (GT) as reverse primer, and 5’-CCAAGCTAGGTGCTATT-3’ as sequencing primer (TT).

Results

Twenty-four patients (53.3%) had the GG primer sequence, 19 (42.2%) the GT, and 2 (4.4%) the TT. In the whole sample the median age was 59 years (range, 46-85), and the median length of follow-up was 54 months (range, 36-70). Twenty-four patients (53.3%) were overweight (BMI: 25-29.9); nine (20.0%) were smokers; three (6.6%) had a family history of hip fracture and six (13.2%) had a personal history of fragility fracture; thirteen (28.9%) were in chemotherapy. Thirty-five (77.8%) patients were treated with Anastrozole and ten (22.2%) with Letrozole. At baseline, toxicities or treatment adverse effects were absent; and no significant differences in participants clinical features, according to genotypes of the CYP19A1, emerged except for the percent of patients in chemotherapy treatment (GG=16.7%; GT=47.4%; TT=0.0%; p=0.063).

The incidence of several adverse effects associated with the AI treatment is shown in Table 1. Despite the high incidence of fatigue, pain, vaginal dryness, nausea and vomiting, sleep diseases, hypercholesterolemia and osteoporosis, they are usually of mild severity. Osteoporosis was encountered in one out of four cases (24.4%); however 80% of the osteoporosis occurred after the first year of AI treatment. Vaginal dryness was significantly associated with Anastrozole treatment (n=15; 42.9% vs n=1; 10.0%); sleep diseases and cutaneous rash were associated with Letrozole treatment (n=5; 14.3% vs n=6; 60.0%; n=2; 5.7% vs n=3; 30.0%, respectively).

When the associations of those adverse effects with the AI treatments were adjusted for CYP19A1 polymorphisms (GG=reference) and age (<59yrs=reference), the results remained substantially unchanged. In multiple logistic regression analyses, sleep diseases and cutaneous rash were both independently predicted by Letrozole treatment (Odds Ratio, OR=9.26, 95% CI 1.24-68.91, p=0.030; OR=18.06, 95% CI 1.36–239.91, p=0.028; respectively). Vaginal dryness was independently weakly predicted by the Anastrozole treatment (OR=0.13, 95% CI 0.12–1.42, p=0.094). Differently, osteoporosis was significantly and independently associated with the GG genotype for the CYP19A1 (Table 2).

Treatment discontinuation (TD) was observed in 6 cases (13.3%); 4 (11.4%) patients treated with Anastrozole, and 2 (20.0%) with Letrozole. TD was significantly predicted by severe pain (G3; p=0.009), when adjusted for age.

Discussion

In postmenopausal women with hormonal receptor positive breast cancer, AI based adjuvant therapy reduces the risk of both death and disease relapse, however the incidence of side effects, resulting by oestrogen suppression, is still too high.

The recognition and the management of side effects, and the individuation of the predictor of toxicities could allow to reduce the risk of treatment discontinuation and to improve patient’s quality of life [13].

In this study; we explored the role of the CYP19A1 gene rs4646 polymorphism in the occurrence of side effects.

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<table>
<thead>
<tr>
<th>Table 1. Incidence of adverse effects associated with Anastrozole and Letrozole (n and %)</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Fatigue</td>
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<tr>
<td>Pain</td>
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<tr>
<td>Hyper Cholesterolemia</td>
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<tr>
<td>Vaginal dryness</td>
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<tr>
<td>Nausea &amp; vomiting</td>
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<tr>
<td>Osteoporosis</td>
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<tr>
<td>Sleep disturbances</td>
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<tr>
<td>Cephalea</td>
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<td>Cutaneous rash</td>
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<tr>
<td>Diarrhoea</td>
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<tr>
<td>Anorexia</td>
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<tr>
<td>Hyper Triglyceridemia</td>
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<td>Dysthyroidism</td>
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</tbody>
</table>

*p=Chi squared, Fisher’s exact test

Hyper Cholesterolemia (>200mg/dL); Hyper Triglyceridemia (>150 mg/dL)
The rs4646 single-nucleotide polymorphism (snp) has been already studied by many authors who demonstrated the association of CYP19A1 polymorphisms with different plasma concentrations of oestrogen; other studies, therefore demonstrated that it is associated with the clinical outcome in women treated with neoadjuvant, or metastatic therapy. Many studies evaluated the protective role of T allele of the rs4646 polymorphism against migraine. The exact migraine pathophysiology is still unknown, but surely elevate oestrogen levels are associated with a higher risk in women. The results of literature suggest that rs4646 polymorphism affect the aromatase activity and the effect of oestrogen.

To our knowledge, none of the studies investigated the role of rs4646 as predictor of IAs toxicities in adjuvant setting. The AI-adverse effects found in our sample, consistent with those described in literature, cause the interruption of adjuvant treatment in 20-30% of cases [3].

We did not find any association between the basal value of BMD and genotype, but we observed a significant association between GG genotype and osteoporosis onset.

Osteoporosis was encountered in 11 patients (24.4%) in four years; in 2 cases, the diagnosis has been posed at the first follow-up, in 5 cases one year later, in 3 cases at the third follow-up, and in the last case at the fourth follow-up. It is noteworthy that, in nearly 80% of the cases, appearance of osteoporosis occurred after the first year of AI treatment.

Skeletal homeostasis is maintained by the balance between bone formation and its resorption. Many local and systemic factors are involved in this process and oestrogens have a fundamental regulatory role. During menopause, physiological reduction of oestrogen levels produces bone mass density decrease, with a subsequent increase of fracture risk. This risk is further increased in women treated with AI [13].

Furthermore, healthy postmenopausal women lose almost 0.5-2% of bone mass density every year and also this mechanism is increased during AI therapy [15]. In our study median follow up and timing of osteoporosis onset suggested that osteoporosis may be a later side effect.

Muscular-skeletal symptoms including arthralgia and myalgia frequently occur in aging women, particularly during the transition to menopause, when plasma oestrogens precipitously decline [16, 17]. We reported that 51% of the patients had arthralgia and myalgia, but no significant association with CYP19A1 genetic variants was found. However, arthralgia was the only side effect that seemed to be a significant predictor of therapy interruption, at the univariate analysis.

Furthermore, the previous results from San Antonio Breast Cancer 2013 about the association between the baseline symptoms of patients and the discontinuation of AI treatment, are very interesting and they suggest that early management of symptom burden may avoid the discontinuity of the treatment [18].

### Table 2. Incidence of adverse effects, separately by CYP19A1 genotypes (n and %)

<table>
<thead>
<tr>
<th>CYP19A1 genotypes</th>
<th>Total sample n=45</th>
<th>GG (n=24; 53.3%)</th>
<th>GT (n=19; 42.2%)</th>
<th>TT (n=2; 4.4%)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>27 (60.0)</td>
<td>13 (54.2)</td>
<td>12 (63.2)</td>
<td>2 (100)</td>
<td>0.525</td>
</tr>
<tr>
<td>Pain</td>
<td>23 (51.1)</td>
<td>12 (50.0)</td>
<td>9 (47.4)</td>
<td>2 (100)</td>
<td>0.603</td>
</tr>
<tr>
<td>Hyper Cholesterolia</td>
<td>20 (44.4)</td>
<td>11 (45.8)</td>
<td>8 (42.1)</td>
<td>1 (50.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Vaginal dryness</td>
<td>16 (35.6)</td>
<td>11 (45.8)</td>
<td>4 (21.1)</td>
<td>1 (50.0)</td>
<td>0.190</td>
</tr>
<tr>
<td>Nausea &amp; vomiting</td>
<td>13 (28.9)</td>
<td>6 (25.0)</td>
<td>5 (26.3)</td>
<td>2 (100)</td>
<td>0.118</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>11 (24.4)</td>
<td>11 (45.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Sleep disturbances</td>
<td>11 (24.4)</td>
<td>7 (29.2)</td>
<td>2 (10.5)</td>
<td>2 (100)</td>
<td>0.031</td>
</tr>
<tr>
<td>Cephalae</td>
<td>5 (11.1)</td>
<td>1 (4.2)</td>
<td>4 (21.1)</td>
<td>0 (0.0)</td>
<td>0.255</td>
</tr>
<tr>
<td>Cutaneous rash</td>
<td>5 (11.1)</td>
<td>3 (12.5)</td>
<td>2 (10.5)</td>
<td>0 (0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5 (11.1)</td>
<td>2 (8.3)</td>
<td>2 (10.5)</td>
<td>1 (50.0)</td>
<td>0.333</td>
</tr>
<tr>
<td>Anorexia</td>
<td>5 (11.1)</td>
<td>2 (8.3)</td>
<td>3 (15.8)</td>
<td>0 (0.0)</td>
<td>0.717</td>
</tr>
<tr>
<td>Hyper Triglyceridemia</td>
<td>4 (8.9)</td>
<td>2 (8.3)</td>
<td>2 (10.5)</td>
<td>0 (0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dysthroidism</td>
<td>4 (8.9)</td>
<td>4 (16.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.269</td>
</tr>
</tbody>
</table>

*Chi squared, Fisher’s exact test

Hyper Cholesterolia (>200 mg/dL); Hyper Triglyceridemia (>150 mg/dL)
Our limit was the small sample size, depending on pilot nature of our study. Our results highlight the role of the individual susceptibility to develop side effects, and give a contribution to previous studies that demonstrated the relationship between rs4646, oestrogen basal level and therapy efficacy.

Further prospective studies are needed to confirm the role of polymorphism rs4646 (CYP19A1) in predicting adverse effects of AI-based therapy, in particular osteoporosis.

In conclusion, our study represents a promising approach to personalized therapy based on metabolic profile of patients in order to prevent severe adverse events and treatment discontinuation.

**Ethics Committee Approval:** Ethics committee approval was obtained.

**Informed Consent:** Written informed consent was obtained from patient/patients who participated in this study.

**Peer-review:** Externally peer-reviewed.


**Conflict of Interest:** The authors declared no conflict of interest.

**Financial Disclosure:** The authors declared that this study has received no financial support.

**References**