Original Article / Özgün Araştırma

Molecular Subtype Distribution and Cyclooxygenase-2 Expression Situation in Inflammatory Breast Cancer

Enflamatuvar Meme Kanserinde Moleküler Alt Tip Dağılımı ve Siklooksijenaz-2 Ekspresyon Durumu

Püsem Patır[1], Burçak Karaca[2], Alper Şener[3], Osman Zekioğlu[3], Güray Saydam[1], Canfeza Sezgin[2]

[1]Ege University Faculty of Medicine, Department of Internal Medicine, Division of Hematology, İzmir, Turkey
[2]Ege University Faculty of Medicine, Department of Internal Medicine, Division of Medical Oncology, İzmir, Turkey
[3]Ege University Faculty of Medicine, Department of Pathology, İzmir, Turkey

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Address for Correspondence/Yazışma Adresi:
Püsem Patır MD,
Ege University Faculty of Medicine,
Department of Internal Medicine, Division of Hematology, İzmir, Turkey
Phone : +90 232 390 35 40
E-mail : pusemp@yahoo.com

Abstract

Objective: Inflammatory breast cancer (IBC) is a rare and aggressive form of locally advanced breast cancer. Currently, almost all women with IBC have lymph node involvement and approximately one-third have distant metastases. Therefore, there is still need for understanding of the molecular biology and new therapy alternatives in IBC. The purpose of this study was to determine the potential role of the cyclooxygenase-2 (COX-2) expression pattern in the aggressive and fatal course of IBC and to investigate the possibility of using COX-2 inhibitors as therapy options in the treatment of IBC.

Materials and Methods: IBC samples obtained from breast cancer patients treated Between 2000-2009 in Ege University Faculty of Medicine Department of Medical Oncology were retrospectively evaluated. Immunohistochemical analysis was performed by a special breast cancer pathologist and manually assessed using an immunohistochemical scoring for both staining intensity and percentage.

Results: In this study, the molecular subtypes identified in IBC patients were: basal (31%), luminal B/human epidermal growth factor receptor 2 (HER2)- (22%), luminal B/HER2+ (22%), HER2 (17%) and luminal A (8%). COX-2 expression was found to be positive in 92.6% of patients.

Conclusion: In this context, it was concluded that the relatively high expression rate of COX-2 could be a reason for poor prognosis and also might lead to the use of COX-2 inhibitors not only as a single agent but also in combination with current chemotherapeutic agents in patients with IBC.

Öz

Amaç: Enflamatuvar meme kanseri (EMK), lokal ilerlemiş meme kanserinin agresif bir alt tipidir. Günümüzde EMK'lı kadınların hemen hepsi lenf nodu tutulumuna ve yaklaşık üçte biri de uzak metastaza sahiptir. Bu yüzden EMK'nın moleküler biyolojisinin anlaşılmasına ve yeni tedavi seçeneklerine ihtiyaç vardır. Bu çalışmanın amacı EMK'nın agresif ve fatal seyrinde moleküler doğasının rol oynayabileceğini ve siklooksijenaz-2 inhibitörlerinin EMK tedavisinde hedefe yönelik tedavi seçeneği olabileceğini göstermektir.

Gereç ve Yöntemler: Ege Üniversitesi Tıp Fakültesi Medikal Onkoloji Anabilim Dalı'nda 2000-2009 yıllarında meme kanseri tedavisi gören hastalardan sağlanan EMK örnekleri retrospektif olarak değerlendirildi. İmmünohistokimyasal analiz özel

meme kanseri patoloğu tarafından yapıldı ve immünohistokimyasal puanlama boyanma yoğunluğu ve yüzdesi kullanılarak belirlendi.
Bulgular: EMK hastalarının %31'i bazal tip, %22'si luminal B/insan epidermal büyüme faktörü reseptörü 2 (HER2)- tip, %22'si luminal B/HER2+ tip, %17'si HER2 tip, %8'i luminal A tip olarak tanımlanmıştır. Hastaların %92,6'sında siklooksijenaz-2 ekspresyonu pozitif bulunmuştur.
Sonuç: Bu bağlamda, EMK'nın kötü prognoza ve sağkalıma sahip olmasının nedenlerinden birinin EMK'nın moleküler doğası olabileceği ve ayrıca EMK'da siklooksijenaz-2 yolağının önemli bir hedefe yönelik tedavi seçeneği olabileceği görülmüştür.

Introduction

Inflammatory breast cancer (IBC) is an aggressive subtype of locally advanced breast cancer. The term IBC was first used in 1924 by Lee and Tannenbaum (1). It is very rare and accounts for 0.5 to 2% of invasive breast cancers diagnosed in the United States, but may be higher elsewhere (2,3). IBC survival rates are still low at 35 to 40% compared to other breast cancers despite the increase in survival with multimodality treatment. Breast cancer is a complex disease that includes a lot of clinical, morphological and molecular differences. Profiles of breast cancer are progressing significantly with the development of techniques such as array-based gene expression. Breast cancer can be divided into six molecular subtypes based on gene expression profiling. These are classified as: luminal A (estrogen receptor (ER)+ and/or progesterone receptor (PR) +, human epidermal growth factor receptor 2 (HER2)-), luminal B (ER+ and/or PR+, HER2+), HER2 (ER- , PR-, HER2+), basal-like (ER-, PR-, HER2-), normal breast-like and claudin-low subtypes. These molecular subtypes demonstrate both prognostic and predictive factors for breast cancer. This different classification of subtypes of breast cancer has been important not only for genetic array testing but also for immunohistochemical (IHC) features in terms of prognostic approaches. IHC studies have opened new windows for breast cancer treatment. For this reason, the positivity of cyclooxygenase-2 (COX-2) enzyme illustrated by IHC may be very important for decisions regarding therapy and determination of prognosis. The aim of this study was to provide a rationale in terms of the relationship of COX-2 expression and possible role of the molecular nature of IBC with aggressive and fatal course and whether that may be the target for the future therapeutic strategies for IBC.

Materials and Methods

Clinical Samples and Definition of Molecular Subtype

We retrospectively evaluated the medical report files of all breast cancer patients (n=3194) who were treated between 2000 and 2009 in to the Medical Oncology Division of Ege University Faculty of Medicine. We detected the patients who were diagnosed with IBC. Sex, age, date of diagnosis, menopausal status at diagnosis, body mass index, histological type, ER expression, PR expression, HER2 expression, HER2 fluorescence in situ hybridization (FISH), and Ki-67 expression were registered from the medical report files. Informed consents were obtained from all patients. One percent and higher expressions of ER and PR were assessed as positive as indicated in the literature (4). Ki-67 expression value was assessed as positive in patients with expression rates of 13.25% and higher (4). We have enrolled patients having HER2 (3+) by IHC or FISH positivity. Molecular subtypes were classified as Luminal type A (ER+ and/or PR+, HER2-, Ki-67 <13.25), luminal type B/HER2- (ER+ and/or PR+, HER2-, Ki-67 ≥13.25), luminal type B/HER2+ (ER+ and/or PR+, HER2+), HER2 type (ER-, PR-, HER2+), basal-like type (ER-, PR-, and HER2-).

Immunohistochemical Method

Biopsy specimens of breast tissue containing tumor were included and the expression of COX-2 was studied by IHC in samples of the primary tumor. Formalin fixed paraffin embedded tissue blocks as 5µm thick sections were mounted on coated slides and dehydrated for 20 minutes at 95 ºC. Sections were incubated for 15 min each with the diluted anti-COX-2 (Clone CX294, Dako Kits). IHC evaluation was made by a special breast cancer pathologist

and visually identified using an IHC scoring for both staining intensity and percentage. Staining intensity and percentage was graded as: negative expression (0 and 1), or positive expression (2 and 3) as indicated in previous publications (4).

Statistical Analysis

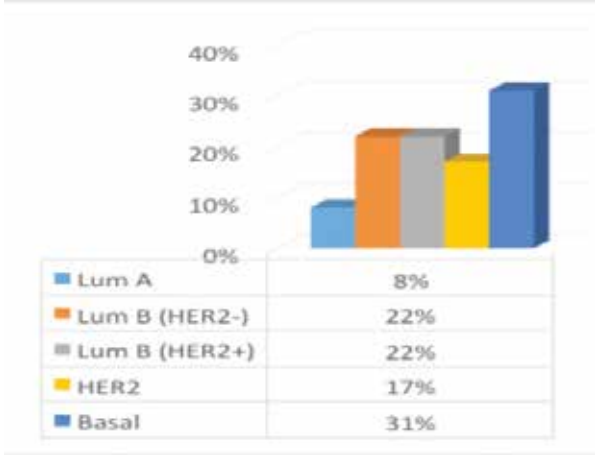
Frequency and distribution of the clinical and IHC observation was assessed using Statistical Package for the Social Sciences version 20.0.

Results

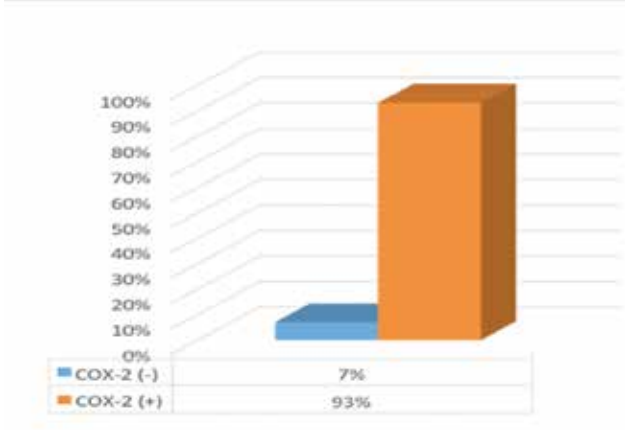
A hundred and seventeen (3.6%) patients were diagnosed with IBC based on the pathology reports. Samples from 95 patients were re-evaluated and confirmed as IBC by the Pathology Department of Ege University Faculty of Medicine. A total of 83 patients were enrolled in the study because the results of HER2 FISH were not found in seven patients, HER2 expressions and Ki-67 expression were not examined in the pathological investigation in three patients, and tumor tissues could not be obtained in two patients. All the patients were female. The mean age of the patients was 49±12 years. Forty-three (51.8%) patients were post-menopausal. The mean body mass index of the patients was 28.0±5.8 kg/m^2. The molecular subtypes of IBC were defined as: basal in 26 (31%) patients, luminal B/HER2- in 18 (22%) patients, luminal B/HER2+ in 18 (22%) patients, HER2 in 14 (17%) patients, and luminal A in 7 (8%) patients. The molecular subtypes of IBC in the patients are shown in Graphic 1. We found that COX-2 expression was positive in 77 (92.6%) patients (Figure 1, 2). COX-2 expressions in the patients are illustrated in Graphic 2.

Discussion

IBC is a rare, different and aggressive form of invasive breast cancer. IBC still has a poor prognosis and low survival rate despite multimodal treatment approaches including anthracycline-based neoadjuvant therapy. Molecular subtype distribution and the status of the COX-2 expression were examined by IHC method in 83 patients who have been diagnosed with IBC in this study. The most important weakness of the study is that its retrospective design and the small number of patients enrolled. However, referring to the literature, a small number of patients is seen



Graphic 1. Molecular subtype distribution in inflammatory breast cancer
HER2: Human epidermal growth factor receptor 2, Lum: Luminal



Graphic 2. Cyclooxygenase-2 expression pattern in inflammatory breast cancer
COX-2: Cyclooxygenase-2

in the studies because IBC is a rare condition. Gene expression profiling studies of breast cancer provide a tremendous background for the biology of breast cancer heterogeneity and in combination with clinical parameters of these studies may contribute to find a way to make a better classification of these tumors. Gene expressions may have a clinical significance in predicting prognosis and treatment response or may facilitate the identification of novel molecular targets for drug development. New molecular classification of breast cancer includes at least three subtypes: luminal, HER2 and basal-like types. Each subtype has specific molecular, clinical and/or pathological features. Molecular classification in our patients revealed that 31% of patients were with basal-like

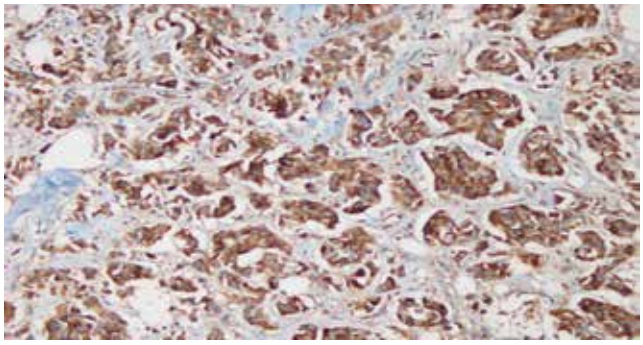


Figure 1. Figure points intense staining of cyclooxygenase-2 at x20 high power magnification in inflammatory breast cancer patient

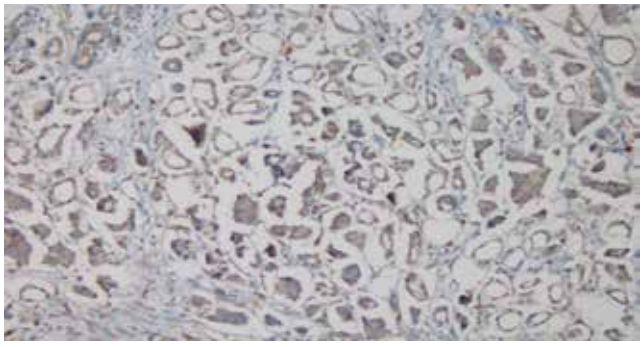


Figure 2. Figure mostly points negative staining of cyclooxygenase-2 at x20 high power magnification in inflammatory breast cancer patient

type, 22% - with luminal B/HER2- type, 22% - with luminal B/HER2+ type, 17% - with HER2 type, and 8% of patients were with luminal A type breast cancer. This study showed that IBC frequency was higher in HER2 positive (luminal B/HER2+ and HER2+ type) and basal-like subtypes. The distribution of IBC molecular subtypes was similar to that in the literature (5,6). Basal-like type was found to be the most common subtype of IBC, which is more critical compared to other subtypes due to its poor prognosis and a higher relapse rate and the fact that targeted therapy cannot be used. For this reason, for prolonged overall survival, we need novel treatment options for IBC that target molecules different from the ER/PR, HER2. COX-2, which is a pro-inflammatory enzyme, is one of the important enzymes involved in the arachidonic acid metabolism. The relationship between COX-2 expression and clinicopathological parameters in breast cancer tissues is controversial, but evidence suggests that COX-2 expression has a close positive relationship with the parameters defined as poor prognosis (7,8). In the light of this information, COX-2 expression status was evaluated in IBC patients. COX-2 expression was positive in 92.6% of patients in this study. In a meta-analysis made by Glover et al. (9), COX-2 expression by IHC methods was found in 53% of ductal carcinoma in situ studies and 42% in invasive breast cancer patients. COX-2 expression was found at a quite high rate in IBC in this study. This result suggests that induction of COX-2 and overproduction of prostaglandins have played an important role in the pathogenesis of IBC. The incidence of breast cancer remarkably reduces with the long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs). This result was demonstrated within a group of 4876 patients treated with indomethacin in 1980 (10). In a meta-analysis of epidemiological studies about selective COX-2 inhibitors and breast cancer risk, Ashok et al. (11) have shown that selective COX-2 inhibitors have been found to be more protective than the non-specific NSAIDs in breast cancer. Outside of their prophylactic effects, COX-2 inhibitors could also be used in the adjuvant chemotherapy of breast cancer. Concomitant use of COX-2 inhibitors and taxanes seems to be an encouraging combination. Because microtubule-interfering agents stimulate the transcription of COX-2, which leads to a reduction of monotherapy activity (12). Other than this, Falandry et al. (13) offered that the combination of COX-2 plus aromatase inhibitors therapy could be an utility treatment option in hormone-dependent breast cancers. In this instance, combination of COX-2 inhibitors and trastuzumab should also be taken into consideration in HER2 positive breast cancers. On the other hand, Arun and Goss (14) compared the groups that were treated with the combination of FEC (5-FU 500 mg/m^2, epirubicin 75 mg/m^2, cyclophosphamide 500 mg/m^2) chemotherapy plus celecoxib (400 mg) and only FEC chemotherapy in neoadjuvant treatment of locally advanced breast cancers. There was no significant difference between the clinical and pathological outcomes of both groups. In this context, more and comparative studies are need to define the exact role of COX-2 inhibitors in patients with inflammatory and non-inflammatory breast cancers. As a result, the presence of high positive COX-2 expression suggests that the COX-2 pathway might be an important targeted therapy option in IBC. These findings should be evaluated and verified with

further research. Thus, novel drugs can be developed for IBC treatment.

Conclusion

In this study, COX-2 expression was positive in 92.6% of patients with IBC. This finding suggests that the development of new reliable side effect profiles of COX-2 inhibitors may increase the chances of success in the treatment of IBC.

Ethics

Ethics Committee Approval: The study were approved by the Ege University of Local Ethics Committee (approved on 15.12.2011 and B.30.2.EGE.0.20.05.00/OY/1592/635 number is given).

Informed Consent: Consent form was filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Püsem Patır, Burçak Karaca, Canfeza Sezgin, Concept: Püsem Patır, Burçak Karaca, Canfeza Sezgin, Design: Püsem Patır, Burçak Karaca, Canfeza Sezgin, Data Collection or Processing: Püsem Patır, Alper Şener, Osman Zekioğlu, Analysis or Interpretation: Püsem Patır, Burçak Karaca, Alper Şener, Osman Zekioğlu, Güray Saydam, Canfeza Sezgin, Literature Search: Püsem Patır, Burçak Karaca, Writing: Püsem Patır, Güray Saydam, Canfeza Sezgin.

Conflict of Interest: No conflict of interest was declared by the authors.

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