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Comparison of CYP1A1 and CYP1B1 expressions in tissue biopsies obtained from with diagnosis invasive breast lobular carcinoma

İnvaziv lobuler meme karsinomadan sağlanan doku biyopsisinde CYP1A1 ve CYP1B1 ekspresiyonlarının karşılaştırılması

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SUMMARY

Objective: Expression of CYPs may provoke tumor development targeting activation of procarcinogens or increase in development and proliferation of breast tumor cells. Moreover, it affects the etiology of breast cancer and they may also change response to treatment. So, we aimed to compare the protein expression of CYP1A1 and CYP1B1, which are the most commonly expressed in breast cancer, which is so important.
Method: Breast biopsy specimens from at least 14 invasive breast lobular carcinoma were used and they obtained from Adiyaman University Pathology Department. The protein expression of CYP1A1, CYP1B1 were determined by immunohistochemically method. Staining intensity was graded as 0 if no staining was observed, +1 if weak staining was present, +2 if moderate staining was observed, and +3 if strong staining was present.
Results: Results were assessment using the total number of participants. When pathologic and histological glands are considered, for right breast, as +3 staining CYP1A1 and CYP1B1 protein expressions were found to be 83.33 % and 16.67 % respectively. In +2 staining CYP1A1 protein expressions was found to be 16.67 %, but CYPB1 not expressed. For left breast, CYP1A1 protein was strongly stained by 50 %. In account of moderate staining. In terms of moderate staining, both CYP1A1 and CYP1B1 proteins were expressed in 33.33 % percent. Besides, weak staining results to be found. CYP1A1 and CYP1B1 were found to be 16.67 % and 33.33 %, respectively. No statistically significant difference was found between CYP1A1 and CYP1B1 ($P>0.05$).
Conclusions: CYP1A1 was expressed in all cases, whereas CYP1B1 was expressed less or not at all. These results indicate that CYP1A1 can an important marker in breast cancer cases.
Keywords: CYPA1, CYPA2, breast cancer, invasive breast lobular carcinoma, immunohistochemistry

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ÖZET

Amaç: CYP'lerin ekspresyonu prokarsinojenlerin aktivasyonunu hedefleyen tümör gelişimine neden olabilir veya meme tümör hücrelerinin gelişiminde ve proliferasyonunda artış sağlayabilir. Ayrıca, meme kanseri etiyolojisini etkiler ve tedaviye yanıtı değiştirebilir. Bu nedenle, meme kanserinde en yaygın şekilde ifade edilen CYP1A1 ve CYP1B1'in protein ekspresyonunu, karşılaştırmayı amaçladık.

Yöntem: En az 14 invaziv meme lobüler karsinomundan meme biyopsi örnekleri kullanılmış ve bunlar Adiyaman Üniversitesi Patoloji Anabilim Dalı'ndan alınmıştır. CYP1A1, CYP1B1'in protein ifadesi immünohistokimyasal yöntemle belirlenmiştir. Boyanma gözlenmediyse, boyanma yoğunluğu 0 olarak, +1 ise zayıf boyanma, +2 ise orta derecede boyanma ve güçlü boyanma mevcutsa +3 olarak derecelendirildi.

Bulgular: Sonuçlar toplam katılımcı sayısını kullanarak değerlendirildi. Patolojik ve histolojik bezler düşünüldüğünde sağ meme için +3 boyanma olarak CYP1A1 ve CYP1B1 protein ekspresyonlarının sırasıyla % 83.33 ve % 16.67 olduğu bulundu. +2 boyanmada CYP1A1 protein ifadeleri % 16.67 olarak bulundu, ancak CYPB1 ekspreslenmedi. Sol meme için, CYP1A1 proteini % 50 oranında kuvvetle boyandı. Orta boyanma açısından, hem CYP1A1 hem de CYP1B1 proteinleri % 33.33 oranında ifade edildi. Ayrıca, zayıf boyama sonuçları bulundu. CYP1A1 ve CYP1B1 sırasıyla % 16.67 ve % 33.33 olarak bulundu. CYP1A1 ve CYP1B1 arasında istatistiksel olarak anlamlı bir fark bulunmadı (P> 0.05).

Sonuç: CYP1A1 tüm durumlarda, CYP1B1 ise daha az veya hiç ifadelenmemiştir. Bu sonuçlar, CYP1A1'in meme kanseri vakalarında önemli bir belirteç olabileceğini göstermektedir.

Anahtar sözcükler: CYPA1, CYPA2, m eme kanseri, invaziv meme lobüler karsinoma, immünohistokimya.

INTRODUCTION

In breast cancer, cytochrome P450 (CYP) metabolizes both endogenous substrates (i.e. estradiol) and exogenous substrates (i.e. anticancer drugs), which is associated not only with tumor development and progression but also with efficacy of cancer treatment [1]. In the literature, data on this subject limited to case reports. So, we performed to compared of CYP1A1 and CYP1B1 protein expression.

Cytochrome P450 CYP1A1 is one of the three members of the CYP1 family and CYP1A1 catalyzed by different reactions. This mechanism associate with hydroxylation at a vacant position of an aromatic ring. Substrates of the CYP1A1 enzyme, the cytosolic receptor AhR, is mediated by its translocation to the nucleus and subsequent formation of a dimer [2,3]. CYPB1 is an enzyme associated with catalysis of formation genotoxic 4-hydroxyestradiol and it has an important role in metabolic control of estrogen homeostasis, and highly induced in breast tumors [4-8].

MATERIAL AND METHODS

Primer and seconder antibodies Normal Swine Serum, Normal Goat Serum, Avidin Biotin Complex Horse Radish Peroxidase were purchased from Santa Cruz. Ethanol, Methanol were purchased from Merc. Sodium Citrate and Citric acid were purchased from Sigma-Aldrich.

Sample collect

Breast biopsy specimens from at least 14 invasive breast lobular carcinoma women whose diagnosis and treatment protocols were determined between 09.07.2007 - 01.12.2018 were used at Adiyaman University Medical Pathology Department. This study was performed by Ethics Committee with resolution of 2019 / 1-28.

Immunohistochemistry

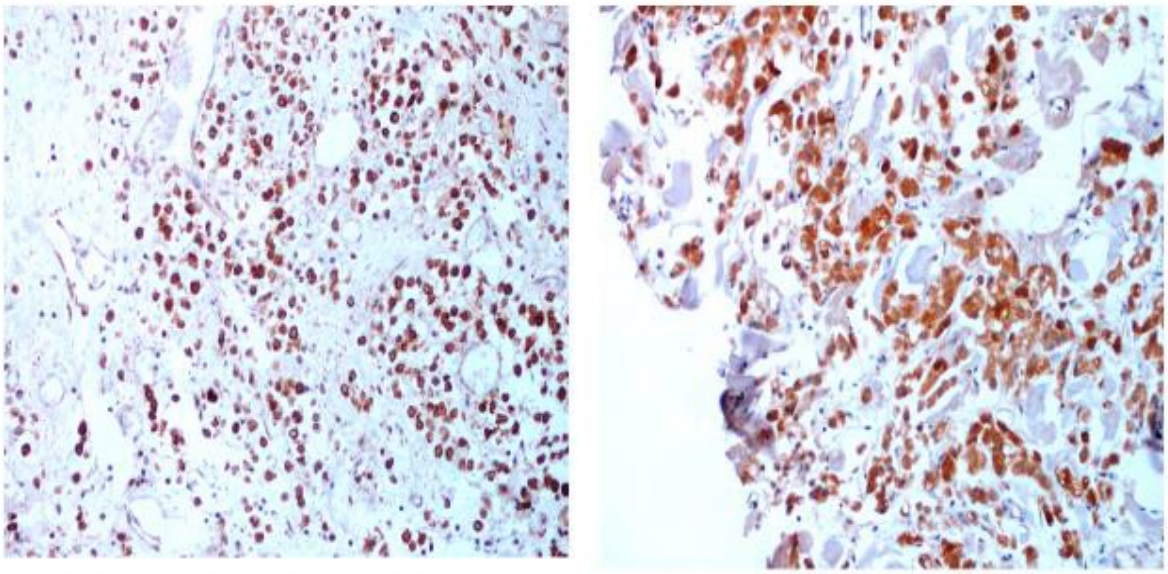
The tissues were fixed in 10 % buffered formalin and embedded in paraffin blocks. Then, 5-mm thick sections were cut. Tissues was kept in the oven at 70 ºC for 1 hour. Then kept to 10 minutes at room temperature to cool down. Samples kept in alcohol and washed the distilled water Peroxidase activity was blocked by incubating the sections in 1% hydrogen peroxide (v/v) in methanol for 20 min at room temperature (RT). The sections were subsequently washed with distilled water Antigen retrieval was performed in a domestic pressure cooker for 3 min using 0.01 M After washing with distilled water, Then, primer antibody of 1 mL was added Washed 3 times with PBS, Then, sections covered seconder antibody and washed with PBS. DAPP was kept in paint for 5 minutes. Washed distilled water and stained with hematoxylin and then the sections were dehydrated and treatment with alcohol then examined under a light microscope. Staining intensity was graded as 0 if no staining was observed, +1 if weak staining was present, +2 if moderate staining was observed, and +3 if strong staining was present.

RESULTS and DISCUSSION

In the total number of cases, CYP1A1 protein expression, strong staining was observed in 10 (71.42 %) of 14 case, moderate staining in 3 (21.42 %) of 14 case and weak staining in 1 (7.14%) of 14 patients. When the total number of cases for CYP1B1 protein expression was examined, strong moderate staining and weak staining were observed in 1 (7.14 %) of 10 patients, in 2 (14.28 %) and weak grade staining in 9 (64.28 %), respectively. No statistically significant difference was found between CYPA1 and CYPB1 ($P > 0.05$).

When compared with premenopausal (age < 50) and post-menopausal (age ≥ 50) status, both CYP1A1 and CYP1B1 protein expressions increased in post-menopausal period according to all staining degrees. When the staining values for premenopausal age were examined, strong and mediate staining for CYP1A1 protein expression was 80% and 20%, whereas strong and mediate strain was not observed in CYP1B1 protein expression. When the staining values were examined according to postmenopausal age, strong, mediate and weak staining were 66.66 %,22.22 % and 11.11 % for CYP1A1, while strong, mediate and weak straining for CYP1B1 were 11.11 %, 11.11 % and 66.66 %, respectively ($P=0.1$).

When compared according to premenopausal and postmenopausal status, estrogen positive receptor was expressed as on average 80 % in premenopausal period and 66.66 % in postmenopausal period. In this study, Estrogen receptor 62 ± 0.36 % for invasive lobular carcinoma left glands, but for right glands was 68 ± 0.45 %. For right breast, as strong staining CYP1A1 and CYP1B1 protein expressions were found to be 83.33 % and 16.67 % respectively. For left breast, CYP1A1 protein was strongly stained by 50 %, but CYP1B1 not expressed. These results showed that Estrogen Receptor (ER) associated with activation of AhR-ligand are related to each other and induced signaling pathways in breast carcinoma, but CYP1B1 expression could reduce due to was accompanied by increased AhR expression and constitutive activity of the receptor.



Invasive lobular carcinoma of the breast with CYPA1 status immunohistochemistry 3+ stainingx40

+Invasive lobular carcinoma of the breast with CYPB1 status immunohistochemistry 3+ stainingx40

Figure 1: Comparison of CYP1A1 and CYP1B1 immunohistochemically staining.

CONCLUSION

CYP1A1 protein was expressed in all cases, whereas CYP1B1 was expressed less frequently or not at all. These results indicate that CYP1A 1 can be an important marker in breast cancer cases. Further studies should consider as detailed association between CYP1A1 and AhR mediated Estrogen Receptor activation targeting gene expression using both PCR and Western blot methods and the number of cases should be increased and

expanded to the extent possible within the region and country.

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