

Research Article



Expression of HMG-Coareductase in Breast Carcinoma and its Association with Estrogen Receptor and Progesterone Receptor Status

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Abstract | Statins are the drugs that target 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR). Several studies have reported the anti-tumoral properties of statins by exhibiting anti-proliferative properties in HMG-CoAR positive breast carcinomas. Increased efficacy has also been observed when these were used as combination therapy along with tamoxifen in estrogen receptor (ER) and HMG-CoAR positive breast carcinomas. However, in our local population, expression of HMG-CoAR is not investigated. The objective of our study was to examine the expression of HMG-CoAR in primary breast carcinomas and determine its association with ER/PR expression.

Material & Methods: Cross-sectional analytical study was conducted to examine the immunohistochemical expression of HMG-CoAR in 75 cases of breast carcinoma. Study was carried out in postgraduate medical institute, Lahore. The frequency and percentage were calculated for histological grade of the tumor and expression of HMG-CoAR, ER and PR. Fischer exact test was applied to determine the association between HMG-CoAR and ER/PR expression.

Results: HMG CoAR, ER and PR were found positive in 69 (92%), 50 (66.67%) and 38 (50.67%) cases, respectively. HMG CoAR expression showed significant association with ER ($p=0.003$) as well as with PR ($p=0.014$).

Conclusion: Through results of our study, it is concluded that there is positive association between HMG CoAR and ER/PR statuses. Clinical trials are needed to establish the predictive role of HMG-CoAR expression as a target for statin therapy in breast carcinoma.

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Keywords | Association, Breast, Carcinoma, Estrogen, HMG-CoAR, Progesterone Receptor.

Introduction

Breast cancer is the most frequently diagnosed cancer in women accounting for 25% of all the cancers.⁽¹⁾ Among Asian countries; the highest incidence rate of breast cancer is seen in Pakistan. Each year, approximately 90,000 women are diagnosed with breast carcinoma with 50% disease specific mor-

tality.⁽²⁾ The heterogeneous nature of the breast cancer has always focused researchers' attention to explore new more specific agents for better prognosis and treatment of breast carcinoma.⁽³⁾

For decades, 3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A Reductase (HMG-CoAR) is used as a major target for cholesterol-lowering drugs. Now, it has be-

come subject of interest regarding its role and utilities in breast carcinoma. HMG-CoAR is the rate-limiting enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids.⁽⁴⁾ Various studies on mevalonate pathway have shown that it is an important factor for regulation of cellular proliferation and transformation. Study results suggest that deficient feedback control of HMG-CoAR results in increased HMG-CoAR activity in the tumor cells as compared to normal cells.^(5,6) These abnormalities promote cell growth by increasing the cellular reserves of mevalonic acid (MVA) and its end-products.⁽⁷⁾ Statins, which inhibit HMG-CoAR are also seen to play role in carcinogenesis. Recent epidemiological studies have shown that patients taking certain statins for hypercholesterolemia management showed a decreased risk of developing various cancers.⁽⁸⁾ Lower incidence of estrogen receptor (ER) negative breast carcinoma is found among lipophilic statin users.⁽⁹⁾

However, in our local population, to date no epidemiological study has been conducted to evaluate the role of HMG-CoAR in primary breast carcinoma. Present study was designed to investigate immunohistochemical expression of HMG-CoAR in primary breast carcinoma in the local population. We also aimed to determine the association between HMG-CoAR and ER and progesterone receptor (PR) expression.

Material and Methods

Cross-sectional analytical study was carried out on 75 cases of breast carcinoma in Pathology Department of Postgraduate Medical Institute (PGMI), 06- Abdul Rahman Chughtai Road, Lahore, Lahore General Hospital, Services Hospital Lahore and Mayo Hospital Lahore. Non-probability, purposive sampling was done to collect the samples of breast carcinoma over the period of one year from patients who reported for treatment. The study was started after taking approval from hospital ethical committee and making sure that there is no ethical issue involved in this study. Patients were included after taking informed consent. Specimens of breast carcinoma were taken from only female patients of age ranging from more than 20 years to 80 years. These patients underwent various surgical procedures including mastectomy, trucut biopsies and lumpectomy. Histopathologically diagnosed cases of primary invasive breast carcinoma were included in

the study. Patients already taking chemotherapy, radiotherapy or hormone therapies were excluded from the study. Also patients taking statin therapy or antibiotic cover for more than three weeks, patients suffering from any inflammatory condition or autoimmune disease, patients using non-steroidal anti-inflammatory drugs (NSAIDs) and diabetic patients were not included.

After surgical procedures, specimen were fixed in 10% neutral buffer solution and brought into the histopathology laboratory. These were allocated a laboratory number. Grossing was done according to standard guidelines of handling of surgical specimens.⁽¹⁰⁾ The representative sections were processed in an automated processor. The sections were mounted on glass slides and dried completely at 60 °C for 30 minutes (min).⁽¹¹⁾

Hematoxylin and Eosin staining of the sections was done as per standard protocol.⁽¹²⁾ Heat induced epitope retrieval (HIER) technique was carried out for Anti-Human Estrogen Receptor (ER): ID5 and Anti-Human Progesterone Receptor (PR): ID5 (DAKO, USA) using a pressure cooker. The results of ER/PR expression were given according to Allred scoring guidelines.⁽¹³⁾ HIER technique was carried out for Monoclonal rabbit Anti-human antibody to HMG-CoAR (Anti-HMGCR [EPR1685(N)] antibody ab174830) using PT link (Dako, USA).

Cytoplasmic staining Intensity (SI) for HMG-CoAR was subjectively evaluated and scored as: Absent = no immunoreactivity, Weak = faint immunoreactivity, Moderate = medium immunoreactivity and Strong = distinct immunoreactivity. Fraction of stained cells (FSC) showing cytoplasmic staining was evaluated as: a) <2%, b) 2-25%, c) > 25-75% and d) >75%. A 'Staining Score' was calculated for each sample by adding both SI and FSC. Staining score '0' was considered as "negative" while staining scores '1', '2' and '3' were considered as "positive".⁽¹⁴⁾

Data was entered and analyzed through IBM SPSS 20. Mean and standard deviation (SD) was calculated for quantitative variables like age, weight and size of sample. Frequency and percentage was calculated for qualitative variables like morphological findings, HMG CoAR and ER/PR expression. Fisher exact test was applied to determine the association of HMG-CoAR with ER/PR expression in primary

breast carcinoma. P-value of ≤ 0.05 was considered as significant.

Results

HMG-CoAR expression was evaluated in different intensities and fractions in the cytoplasm of tumor cells (Figure 1a,b,c,d).

HMG-CoAR expression was found to be positive in 69 (92%) out of 75 cases. Six cases (8%) were HMG-CoAR negative. Out of 75 cases, 56 (74.7%) cases were positive for ER while 19 (25.3%) cases were negative for ER. Out of 75 cases, 50 (66.67%) cases were positive for PR while 25 (33.33%) cases were negative for PR (Figure 2).

Table 1: Association between HMG-CoAR expression and ER status

Receptor status		HMG-CoAR		Total
		Positive	Negative	
ER	Positive	55	1	56
	Negative	14	5	19
Total		69	6	75

Fisher Exact test, p -value = 0.003

HMG-CoAR expression was found to be associated with ER expression (Table 1). Significant association was calculated between HMG-CoAR expression and PR expression (Table 2).

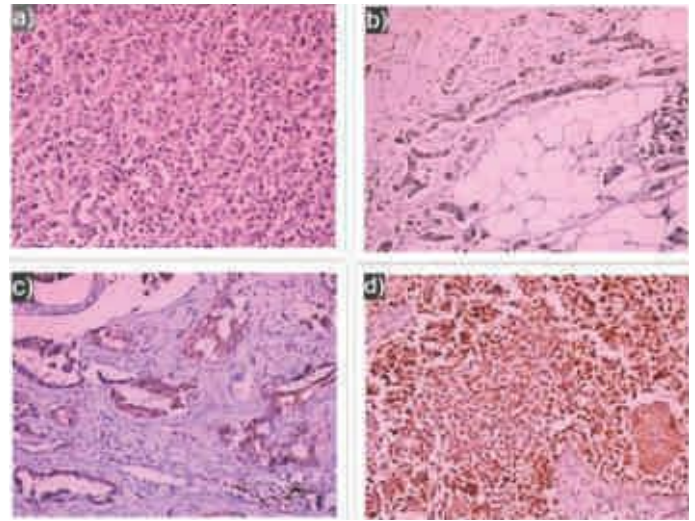


Figure 1: HMG-CoAR immunohistochemical expression in different grades of invasive ductal carcinoma scored as: a) Histological grade II, Staining score 0; b) Histological grade III, Staining score 1; c) Histological grade II, Staining score 2; d) Histological grade III, Staining score 3

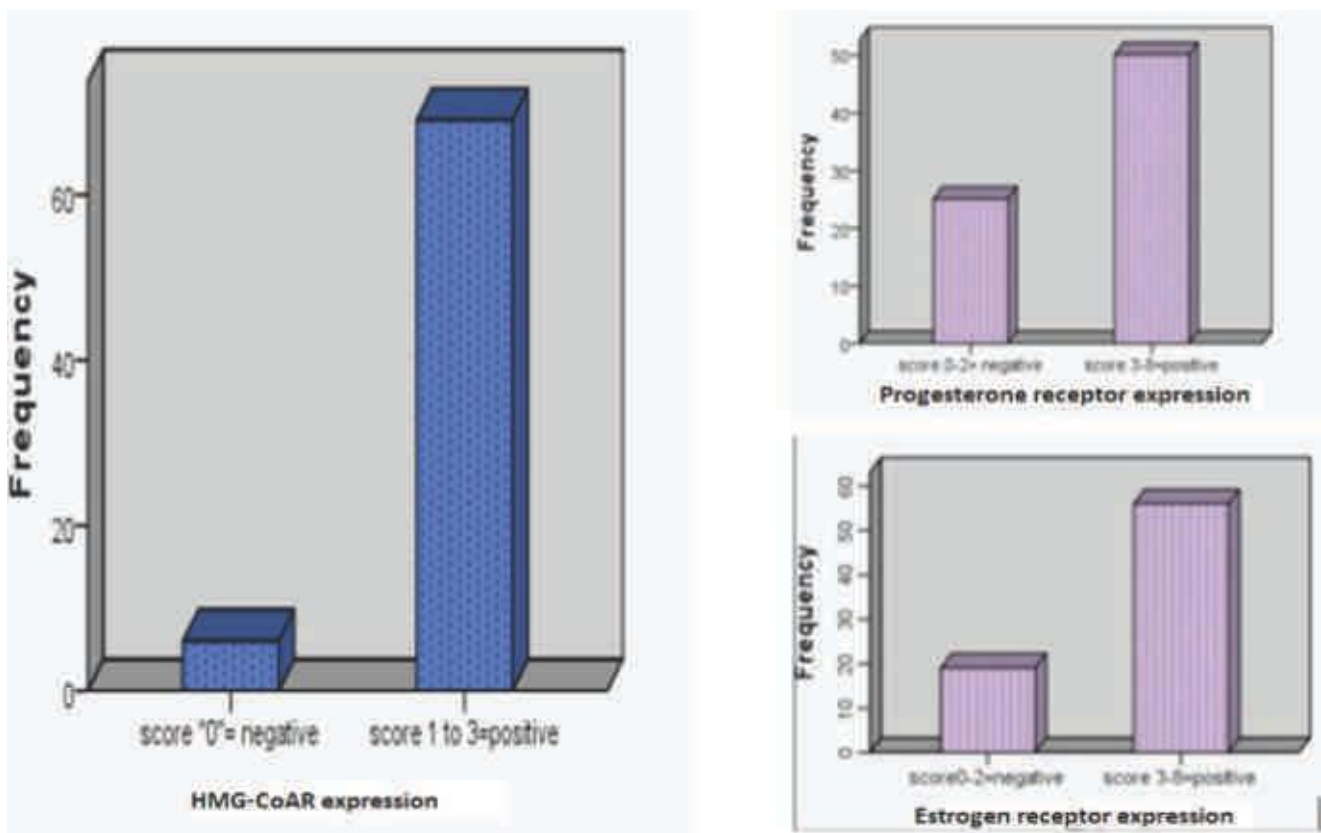


Figure 2: Bar graphs representing frequency of HMG-CoAR, ER and PR immunohistochemical expression in 75 cases of breast carcinoma.

Table 2: Association between HMG-CoAR expression and PR status

Receptor status	HMG-CoAR		Total
	Positive	Negative	
PR	Positive	49	50
	Negative	20	25
Total	69	6	75

Fisher Exact test, p -value = 0.014

Discussion

Our results revealed that HMG-CoAR is expressed in various intensities and fractions in the cytoplasm of tumor cells in breast carcinoma. HMG-CoAR expression was positive in 69 out of 75 cases (92%). Our results were similar to many studies, which were done in the past. Butt et al. (2014) described very close results to our study. He found HMG-CoAR positivity in 93% of the cases.⁽¹⁵⁾ In 2008, Borgquist et al. have found HMG-CoAR positive cases in lower frequency (82% of the cases).⁽¹⁶⁾ The variations in the frequency of immunostaining can be explained by regional and genetic differences.

Previous studies have shown ER expression in 70% of the patients with breast carcinomas.⁽¹⁷⁾ These figures support the results of present study which showed 56(74.6%) cases with ER positivity and 50(66.7%) cases with PR expression. In contrast, in another study low frequency of ER+ and PR+ cases was reported (63.6 and 58.9 % respectively).⁽¹⁸⁾

The association between HMG-CoAR and ER/PR status has been previously reported by researchers.⁽¹⁹⁾ Data from a study showed significant association HMG-CoAR and ER expression ($p = 0.01$) while no association was found between HMG-CoAR and PR expression ($p = 0.79$).⁽¹⁹⁾ In present study HMG-CoAR significant association was found between HMG-CoAR and both ER and PR (i.e. $p = 0.003$ and $p = 0.014$). A study supporting our results revealed significant association of HMG-CoAR with both ER ($p = 0.03$) and PR ($p = 0.01$) expression post treatment with statin therapy.⁽²⁰⁾

Conclusions

It was concluded from the present study that in local population, HMG-CoAR is expressed in primary breast carcinoma patients. Significant association was

observed between HMG-CoAR expression and ER/PR status in local population.

Author's Contribution

Zartasha Anjum: Selection of the study, acquisition of data, analysis and interpretation of data, collection and drafting of data.

Rakhsindah Bajwa: Substantial contributor to conception and design of study, interpretation of data, and revised the article critically.

Munazza Hassan: Analysis and interpretation of data and revised the article critically

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