Window-of-opportunity Study Testing the Antiproliferative Effect of Atorvastatin in Egyptian Patients with Operable Breast Cancer

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Abstract Background and purpose: Cholesterol-lowering drugs (statins) appear to have pleiotropic effects independent of cholesterol level. The aim of this study was to assess the effect of pre-operative therapy with a statin (atorvastatin) on tumor proliferation in patients with newly diagnosed breast cancer. Methods: Thirty cases with histologically proven breast cancer were subjected to treatment by atorvastatin 80 mg /day for at least 14 days before the final surgical procedure (MRM or BCS). Immunohistochemical expression of Ki-67 staining of breast tumor cells was evaluated to assess tumor proliferation in biopsy tissues before treatment with atorvastatin then in final surgical tissues specimens. Results: The median age of the patients was 49.5 ranging from 29 to 61 years. The vast majority of the patients had invasive duct carcinoma (IDC) and was positive for estrogen and progesterone receptors. The mean pretreatment Ki67 index was high in the majority of patients and was significantly associated with both tumor grade and estrogen receptor status ($P = 0.001$ and $P = 0.003$, respectively). The Ki67 index had decreased in the post-treatment samples after final surgery in 19 cases, increased in 8 cases and unchanged in 3 cases compared to the pre-treatment specimens. Tumor grade is a significant predictor of treatment response ($p=0.05$). Conclusion: Atorvastatin decreased tumor proliferation in breast cancer especially in high grade tumors and its role should be considered in the future studies.

Keywords Atorvastatin, Breast Cancer, Statins, Ki67, Tumor Proliferation, Window-of-opportunity Study

1. Introduction

Statins, the cholesterol lowering drugs, appear to have

pleiotropic effects independent of cholesterol level in both cardiovascular diseases and cancer for many decades [1]. Many preclinical studies suggest that statins suppress breast cancer progression, but clinical studies have little evidences to support their protective effect on breast cancer prevalence with consistent evidences supporting a protective effect on disease recurrence [2]. Statins interrupt the synthetic mevalonate pathway which affects several other downstream pathways essential for tumor growth and proliferation, so, these biological mechanisms may have potential anti-cancer effects [3]. The association of statins and triple negative breast cancer was evaluated in a large cohort searching for the effect of statins exposure on this subtype of breast cancer, in which there is a relative frequency of estrogen receptor negative breast cancers reduced in cases taking statins for more than one year [4]. Regarding the lipid solubility, Lipophilic statins interrupt the synthesis of mevalonate in the liver and in peripheral tissues, demonstrating cholesterol and pleiotropic effects but lipophobic statins mediate their effect in hepatocytes only, so, when studies examined the effect of lipophilic statins, they showed a protective role of lipophilic statins in prevention of breast cancer [5-8], also other studies demonstrated in their animal models that lipophilic statins cause growth inhibition in breast cancer cell lines [9-11]. Data from phase II studies on statins implicated in the pre-operative setting showed reduced tumor cellular proliferation and increase in apoptosis in cases with high grade breast cancer, also, statins' anti-proliferative effects were confirmed in other studies, in which stain anti-proliferative effects were demonstrated in terms of changes of tumor levels of Ki67 index [12]. Ki67 is a biomarker widely used clinically for assessment of the proliferation of a breast cancer cells and is expressed in active phases during cell cycle, so, its changes are a good indicator for the effect of treatment [13]. Based on the encouraging results of the previous studies, we conducted

this study to investigate the effect of statin (atorvastatin) on tumor proliferation in breast cancer patients in our country with expectation of the same results previously reported with continuation of researches in this era of cancer treatment.

2. Patients and Methods

This study includes thirty patients chosen according to inclusion criteria below and was conducted at general surgery, pathology and clinical oncology departments, Zagazig University hospitals, Zagazig, Egypt, during the period between March 2016 and April 2017, This research was approved by the local ethical committee of our department and signed informed consent from every patient was taken before the procedure.

Patient Eligibility

Females with pathologically confirmed operable invasive breast cancer, other inclusion criteria were: age $\geq$ 18 years, no prior chemotherapy or radiotherapy, Eastern Cooperative Oncology Group (ECOG) Performance Status 0-2 and normal renal and hepatic functions. The patient was excluded if on anti-diabetic and anti-hyperlipidemic drugs, with known potential hypersensitivity with atorvastatin, or on drugs with a known interaction with atorvastatin (e. g., clarithromycin, HIV protease inhibitors, and itraconazole).

Study Design

This is a window-of-opportunity study in which atorvastatin was administered during the pre-operative period between the biopsy and final surgical operation either modified radical mastectomy (MRM) or breast conservative surgery (BCS).

Research Plan

All patients were diagnosed as breast cancer by true cut biopsy and tumor proliferation was measured before the start of statin treatment, after that, all patients received atorvastatin 80mg /day for at least 14 days, then subjected to the suitable surgical procedure (modified radical mastectomy or breast conservation). The effect of atorvastatin on tumor proliferation was tested by immunohistochemical analysis of Ki67 expression in formalin fixed, paraffin-embedded tissue of core biopsies and final surgical tissues before and after statin therapy respectively. Immunostain was performed by using Dako Autostainer (Dako Corporation, Carpinteria, CA, USA) through using Envision system for detection. Ki-67 antibody (clone MIB-1, 1: 50 dilution; Dako). A Ki67 index of 14% or more Ki67-positive tumor nuclei was taken as the cut point, where, Ki67 index of $<14\%$ was

considered low and a Ki67 index $\geq 14\%$ was considered high index.

Endpoint

The primary endpoint of this study was the statin-induced decrease in tumor proliferation measured by the change in Ki67 expression.

Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA). Shapiro Walk test and Mann Whitney U test were used to check the normally distributed data and to compare between two groups of non-normally distributed data respectively. Kruskal Wallis H test was used for more than two groups and Wilcoxon signed-rank test was used to compare two dependent groups of normally distributed data. The nonparametric tests were used because although the distributions of Ki-67 index tended to be skewed and log-transformation of pre, post treatment change values led to approximate normality, the difference in log-transformed values failed the Shapiro–Wilk test for normality. Percent of categorical variables were compared using Pearson's Chi-square test or Fisher's exact test when was appropriate. The trend of change in the distribution of relative frequencies between ordinal data was compared using Chi-square test for trend. Paired categorical variables were compared using McNemar's test. All tests were two sided. p -value < 0.05 was considered statistically significant.

3. Results

Patients Characteristics

The clinicodemographic parameters of patients included in this study are shown in (Table 1). The median age of the patients was 49.5 ranging from 29 to 61 years. Of 30 patients in this study, nine patients (30%) had pathological tumor size (pT) less than 2 cm and twenty-one (70%) had (pT) more than 2 cm, axillary lymph nodes were positive for malignancy in nineteen cases (63.3%) and negative in eleven patients (36.7%). Regarding the tumor grade, there were four cases (13.3%) with grade I, sixteen (53.3%) with grade II and ten (33.3%) with grade III. The vast majorities of the patients in this study had invasive duct carcinoma (IDC) and were positive for estrogen and progesterone receptors. In this study, human epidermal growth factor receptor 2 (HER2) was negative in seventeen (56.7%) patients and positive in thirteen (43.3%). The mean pretreatment (baseline) Ki67 was high in the vast majority of patients in this study and was significantly associated with both tumor grade and estrogen receptor status ($P = 0.001$ and $P = 0.003$, respectively) (Table 3).

Table 1. Basic characteristics of the studied patients

Characteristics	All patients (N=30)	
	No.	%
Age (years)		
Mean $\pm$ SD	48.86 $\pm$ 6.74	
Median (Range)	49.50 (29 – 61)	
≤ 35 years	1	3.3%
> 35 years	29	96.7%
Tumor size		
≤ 2 cm	9	30%
>2 cm	21	70%
Lymph node		
Negative	11	36.7%
Positive	19	63.3%
Grade		
Grade I	4	13.3%
Grade II	16	53.3%
Grade III	10	33.3%
Histology		
IDC	19	63.3%
ILC	7	23.3%
Other	4	13.3%
ER		
Negative	10	33.3%
Positive	20	66.7%
PR		
Negative	12	40%
Positive	18	60%
HER2/neu		
Negative	17	56.7%
Positive	13	43.3%
Ki67 pretreatment		
Mean $\pm$ SD	16.10 $\pm$ 2.23	
Median (Range)	16 (12 – 20)	
Low	5	16.7%
High	25	83.3%

Figure 1. Waterfall plot of change from baseline (%) in Ki-67 after atorvastatin administration in the studied patients

Table 2. Pretreatment and posttreatment Ki67

Ki-67	Pretreatment B(N=30)		Post-treatment(N=30)		p-value
Mean $\pm$ SD	16.10	$\pm$ 2.23	13.90	$\pm$ 2.05	0.001•
Low	5	(16.7%)	15	(50%)	0.013‡
High	25	(83.3%)	15	(50%)	

•Wilcoxon signed ranks test; ‡ McNemar's test; $p < 0.05$ is significant

Treatment Outcome

The change in the Ki67 index following at least two weeks of atorvastatin treatment was evaluated in 30 paired tumor tissues. The Ki67 index had decreased in the post-treatment samples after final surgery in 19 cases, increased in 8 cases and unchanged in 3 cases compared to the pre-treatment samples (Fig. 1). In the pre-treatment sample, the mean Ki67 index was 16.10 range [12-20], in comparison, in post treatment samples, the mean Ki67 index was 13.90 range [10-19] with average absolute reduction 2.2 percentage points ($P = 0.001$). (Table 2)

The relation between clinicopathological variables and Ki67 changes were analyzed to predict the factors that may affect the response to treatment (Table 3). Pathological tumor size (pT), lymph node (LN) status and tumor

histology showed non-comparable changes in Ki67 index, it is noticed that tumors with pT more than 2 cm showed decline in Ki67 in 57.1% of cases compared to 77.8% in those with pT less 2 cm ($p = 0.448$), in cases with LN positive there were decrease in Ki67 in 57.9% compared to 72.7% in other LN negative cases ($p=0.696$) also, tumor histology had not any impact in treatment response ($p=0.148$). The molecular prognostic factors (ER, PR, HER2) were evaluated, there was statistically insignificant effect in ER negative, PR negative and HER2 positive tumors ($p=0.300$, $p=0.306$ and $p=0.215$ respectively). The only variable has a significant impact on treatment response in this study was the tumor grade, in cases with high grade tumors there is significant decline in Ki67 index in 90% of cases compared to low grade tumors ($p=0.005$).

Table 3. Relation between clinicopathological variables and Ki67 changes

Variables		Ki67 change						p-value
	N	Unchanged (n=3)		Decrease (N=19)		Increase(N=8)		
		No.	(%)	No.	(%)	No.	(%)	
Age								
≤ 35 years	1	0	(0%)	0	(0%)	1	(100%)	0.241‡
> 35 years	29	3	(10.3%)	19	(65.5%)	7	(24.1%)	
Tumor size								
≤ 2 cm	9	1	(11.1%)	7	(77.8%)	1	(11.1%)	0.448‡
>2 cm	21	2	(9.5%)	12	(57.1%)	7	(33.3%)	
Lymph node								
Negative	11	1	(9.1%)	8	(72.7%)	2	(18.2%)	0.696‡
Positive	19	2	(10.5%)	11	(57.9%)	6	(31.6%)	
Grade								
Grade I	4	2	(50%)	1	(25%)	1	(25%)	0.005§
Grade II	16	1	(6.3%)	9	(56.3%)	6	(37.5%)	
Grade III	10	0	(0%)	9	(90%)	1	(10%)	
Histology								
IDC	19	3	(15.8%)	12	(63.2%)	4	(21.1%)	0.148‡
ILC	7	0	(0%)	3	(42.9%)	4	(57.1%)	
Other	4	0	(0%)	4	(100%)	0	(0%)	
ER								
Negative	10	0	(0%)	8	(80%)	2	(20%)	0.300‡
Positive	20	3	(15%)	11	(55%)	6	(30%)	
PR								
Negative	12	0	(0%)	8	(66.7%)	4	(33.3%)	0.306‡
Positive	18	3	(16.7%)	11	(61.1%)	4	(22.2%)	
HER2/neu								
Negative	17	3	(17.6%)	9	(52.9%)	5	(29.4%)	0.215‡
Positive	13	0	(0%)	10	(76.9%)	3	(23.1%)	

‡ Chi-square test; § Chi-square test for trend; p<0.05 is significant.

4. Discussion

The goal of this window-of-opportunity study was to determine the antiproliferative effect of a lipophilic statin (atorvastatin) on invasive breast cancer in Egyptian patients. To our knowledge, this is the first clinical study conducted on Egyptian patients to assess the antiproliferative effect of pre-operative statins on invasive breast cancer. In this study, we evaluated the changes in Ki67 index as an indicator of tumor proliferation before and after short-term administration of atorvastatin. The anti-cancer effect of statins and its use as anti-cancer agents in patients with breast cancer were evaluated in previous preclinical and clinical studies [3, 10, 14]. Based on results of these studies combined with many recent reviews, the need for further studies that evaluate the anti-proliferative and hence the anti-cancer effect of statins as inhibitors of HMG-CoA reductase (HMGCR) is emerging [15]. As previously shown, statins were inhibitors of HMGCR

which is differentially expressed in breast cancer, and so, this led to hypothesis that statins may considered as potential therapeutic agents in breast cancer management [3, 16, 17]. All patients in our study received 80 mg daily atorvastatin which is considered high dose. This high dose was well tolerated during treatment period without significant side effects. Regarding to baseline Ki67, in this study, the mean pretreatment (baseline) Ki67 was high in vast majority of patients and we observed that it was significantly associated with both high grade and negative estrogen receptor tumor, these results are nearly the same that obtained by Bjarnadottir et al, who reported in their study that the baseline Ki67 was high in tumors with high grade and estrogen and progesterone receptors negative [1]. Our finding regarding changes of Ki67 after atorvastatin was that there was decreased Ki67 index in 19 cases, increased in 8 cases and unchanged in 3 cases, these results also nearly similar to those obtained by Garwood et al, who reported in their study in which they investigated the

antiproliferative effect of statin (fluvastatin) in high grade breast cancer patients [3]. The effect of tumor basic characteristics on treatment response was analyzed in which various variables were used and our results showed that there is no relation between pT, LN status and tumor histology in the degree of Ki67 changes, although these results appeared contrary to what we expected, this may be due to influence of other factors that contribute to tumor behavior and aggressiveness. The molecular factors (ER, PR, HER2) also were evaluated to determine its impact on Ki67 changes, our finding showed that tumors not expressing estrogen receptors (ER-ve) and progesterone receptors (PR-ve) had more reduction in Ki67 index compared with those positive for these receptors but with statistically insignificant difference, these findings were similar to those obtained by Garwood et al study and differ from those obtained in Bjarnadottir et al who reported that following treatment, the Ki67 changes were not associated with the baseline tumor characteristics. HER2, the human epidermal growth factor receptor 2, one of the most prognostic factors in breast cancer was evaluated for its impact on treatment response. We found that tumors positive for HER2 had more decline in Ki67 compared with those negative for it but with statistically insignificant difference, these finding also observed in Yulian et al study who investigated the role of statin (simvastatin) in inhibiting proliferation of breast cancer cells, but they found significant difference in both HER2 positive and HER2 negative tumors [18], our expectation was that these molecular factors would significantly affect the treatment response in term of comparable decrease in Ki67 index, but the results were contrary to our expectation. In our study, we found that the most factor that has impact on post-treatment ki67 changes is tumor grade, regarding the tumor grade, there were four cases (13.3%) with grade I, sixteen (53.3%) with grade II and ten (33.3%) with grade III, after treatment with atorvastatin there was remarkable Ki67 changes regarding tumor grade, in grade I cases, 50% of them had unchanged Ki67, 25% increased and 25% decreased, in grade II cases, 6.3% of them had unchanged Ki67, 56.3% decreased and 37% increased compared with those with grade III, in whom Ki67 decreased in 90% of them ($p=0.05$) with statistically significant difference (Table 3), these results were similar in various studies that were conducted to evaluate the role of statins in breast cancer [1, 3, 18, 19]. Finally, the antiproliferative effect of statins, which was established in this study, was supported by previous in-vitro and in-vivo studies not only in breast cancer, but also in other malignancies [20-22] and encouraging further researches to incorporate statins in the neo-adjuvant and adjuvant breast cancer treatment.

5. Conclusions and Recommendation

In conclusion, this study suggests that short-term therapy

with the statin (atorvastatin) has biologic effects on breast cancer. Atorvastatin decreased tumor proliferation manifested by the decline in Ki67 expression, especially in high grade tumors. These findings in concordance with health promoting advantages and safety of statins should support further studies of atorvastatin and other lipophilic statins as potential therapeutic agents for breast cancer treatment.

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Conflict of Interest

All authors declare that they have no conflict of interest.

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