Original Research Article

Efficacy and safety of high dose of lansoprazole pretreatment inpatients with breast cancer receiving neo-adjuvant chemotherapy

ABSTRACT

Background: High dosage of lansoprazole (LPZ) can be used to control acidic microenvironment surrounding the cancerous cells thus improving tumor response.
Aim: The study aimed at investigating the possible antitumor efficacy and safety of high dose of LPZ pretreatment in patients with breast cancer (BC) receiving neo-adjuvant chemotherapy (NAC).
Study design: Single blinded, randomized placebo-controlled study.
Place and Duration of Study: The study was conducted between June 2021 and November 2022 at Clinical Oncology and Nuclear Medicine Department, Menoufia University Hospital, Egypt.
Methodology: 66 females with stage II and III BC were randomly assigned into two groups; the LPZ group (n=33) which started LPZ capsules 60 mg orally bid 4 days before starting NAC and the control group (n=33) which received placebo capsules and the same NAC regimen as LPZ group. Evaluation of tumor response was done according to the Response Evaluation Criteria in Solid Tumors (RECIST, v1.1). Permeability-glycoprotein (P-gp) and Ki-67 levels were assessed in the two groups before and after treatment. Adverse events were documented and graded using National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE, v5).**Trial registration:** ClinicalTrials.gov identifier: NCT04874935.
Results: Lansoprazole group showed more favorable response especially within luminal B/HER2 negative subtype. Lansoprazole group showed non-significant decrease in P-gp (P = 0.19) and Ki-67 (P = 0.44) levels as compared to the control group. Dyspepsia was the only significant adverse effect reported with LPZ group (P = 0.011).
Conclusion: However, LPZ didn't reveal a statistically significant anti-tumor effect as compared to placebo; it produced a clinically important improvement in tumor response which was translated by higher number of patients who achieved complete response. Furthermore, the high dose of LPZ used during this study was tolerable and safe.

Keywords: Breast cancer, Lansoprazole, RECIST, Permeability-glycoprotein, Ki-67.

1. INTRODUCTION

Breast cancer (BC) is a complicated disease that depends on many factors for its development [1]. In 2020 according to the Global cancer statistics (GLOBOCAN), the estimated global new cases diagnosed with BC were 2,261,419 (11.7% of all cancers) and the estimated number of deaths secondary to BC was 684,996 (6.9% of total cancer deaths) [2]. Since the diagnosed cases with breast cancer and related deaths increase dramatically globally every year, discovering new treatment and repurposing of old drugs seem very important. Unfortunately, discovering a new drug is a very strenuous process that needs high financial support, time, and effort. Repurposing of already approved drugs could save money and seem more easier to be assessed in clinical trials [3,4]. Proton pump inhibitors may represent a promising example for drug repurposing that allows chemo-sensitization [5,6,7,8]. Within cancerous cells, increased glucose consumption causes acidic microenvironment that surrounds these cells. Aerobic glycolysis leads to formation of lactic acid which is called Warburg effect [9,10]. Vacuolar-ATPase (V-ATPase), an ATP dependent proton pump, transports the excess protons to the extracellular compartment, in order to counteract this acidity and maintain normal, suitable pH inside the cancerous cells [11,12]. Resistance to chemotherapy may arise from acidic microenvironment, beside the increased activity of V-ATPase on intracellular lysosomal vesicles that cause drug sequestration and extrusion [6,7,13,14,15]. Basic drugs such as adriamycin and 5-fluorouracil (5-FU) can be easily ionized in this acidic condition with subsequent hindrance of their uptake inside the cells [7,14,15].

Also, acidic pH may promote P-gp activity, the drug efflux pump that is closely associated with multidrug resistance (MDR) and can result in decreasing drug concentration inside cancerous cells and consequently reducing its therapeutic effect [7,14,15,16]. Moreover, this acidity may also encourage cancerous cells proliferation, aggressiveness, and metastasis [6,7,13,14]. Targeting V-ATPase may help in avoiding or decreasing resistance to chemotherapy, with consequent better cancer management and tumor response [6,7,14]. Many former studies reported that, inhibition of V-ATPase was associated with slowed growth and increased cancerous cells death [14,17,18,19]. Proton pump inhibitors are weakly basic prodrugs that require protonation to be activated. Thus, the acidic microenvironment provides optimal conditions for PPIs activation [14,20,21,22]. Several in-vitro and in-vivo studies showed that PPIs exert an inhibitory effect on V-ATPase [7,14,23,24,25,26]. Furthermore, many studies suggest that PPIs act as P-gp inhibitor and suggested that PPIs might enhance chemotherapeutic effect [23,27]. In some in-vitro and pre-clinical studies, LPZ was reported to exert higher anti-tumor effect as compared to other PPIs [7,28]. It has been demonstrated that LPZ reaches its fulleffect of acid suppression four days after administration [21,22].

Therefore, the above-mentioned information encouraged us to conduct this study which aimed at investigating the possible antitumor efficacy and safety of high dose of LPZ with NAC in patients with BC.

2. METHODOLOGY

2.1 Study design and patients' population

This single blinded, randomized placebo-controlled study was conducted between June 2021 and November 2022 at Clinical Oncology and Nuclear Medicine Department, Menoufia University Hospital, Egypt. Sixty-six female patients withbody mass index (BMI) and body surface area (BSA) matched, diagnosed instage II and stage III BC (according to the American Joint Committee on Cancer AJCC- TNM staging system, eighth edition, 2018) were enrolled voluntary in this study. The study was carried out in accordance with International Ethical Guidelines and the principle of the Declaration of Helsinki 1964. The study was approved from the Research Ethics Committee of Tanta University (Approval code: 34615/4/21) which was accepted by Menoufia University. All participants gave their written informed consent. All data of the patients was private and confidential. The study was registered on ClinicalTrials.gov with ID: NCT04874935.

The inclusion criteria included newly diagnosed females with BC that was confirmed using core biopsy, age ≥ 18 years old, patients who were candidates for NAC which consists of 4 cycles of adraimycin and cyclophosphamide (AC) every 21 days, followed by 4 cycles of paclitaxel (Taxol) which was administered on weekly basis for 12 weeks. Neoadjuvant chemotherapy was indicated for patients who were luminal B, HER2 positive, triple negative stage II or stage III breast cancer and for luminal A with T3 and lymph node involvement. Patients with HER2 positive and hormonal receptors positive received anti-HER2 therapies and hormonal therapy directly after surgery. The exclusion criteria were pregnancy, nursing mothers, active or uncontrolled infection, presence of another malignancies, inadequate baseline blood picture (CBC), serum creatinine (S.Cr) more than 1.5 mg /dl at baseline, aspartate amino transaminase (AST) and alanine amino transaminase (ALT) more than 2.5 upper limit at baseline and history of known hypersensitivity to LPZ.

The patients were randomized through random permuted blocks method into two groups: the LPZ group (n=33), which was pretreated with LPZ 60 mg oral capsules (Loral©, manufactured by Pharco, Egypt) bid 4 days before starting NAC, 4 cycles AC every 21 days (adriamycin 60 mg/m2 diluted with 250 mL normal saline and administered intravenously over 30 min and cyclophosphamide 600 mg/m2 diluted with 500 ml normal saline and administered intravenously over 60 min), followed by 4 cycles of Taxol weekly for 12 weeks (paclitaxel 80 mg/m2 diluted with 500 mL normal saline and administered by intravenous infusion over 90 min) and the placebo group (n=33) which received placebo capsules 4 days before starting NACand the same chemotherapy regimen exactly as LPZ group. The blindness was maintained only for patients, in order to be able to manage cases with severe vomiting (grade 3) and in order to provide another suitable gastrointestinal tract protection for the placebo group which was supported by famotidine 10 mg twice daily for 3-5 days after each chemotherapy cycle.

2.2Demographic and anthropometric measurements

Demographic data including age, social status, menopausal status, complete disease and medication history, and family history were recorded, and patients' sheets were completed for all participants. Measurements of weight and height with subsequent calculation of BMI and BSA were also done according to equations: BMI = *Weight (kg) /Height (m^2)* and BSA=$\sqrt{Weight(kg) \times Height(cm)/3600}$.

2.3Evaluation of tumor response

Tumor response was assessed according to RECIST v1.1. Mammography with complementary ultrasonography was used for the assessment of lesions during the current study. For all participants, sum of diameters (Sum of D) in millimeter (mm) for all target lesions was calculated at baseline and after completion of NAC cycles using the same imaging technique. Target non-nodal lesions should have longest diameter ≥10 mm and target nodal lesions should have shortest diameter ≥15mm. The maximum target lesions adopted in this study were two including nodal lesion. Response was calculated through implication of the following equation: $\left(\frac{Sum\ of\ D\ at\ baseline - Sum\ of\ D\ at\ end\ of\ NAC}{Sum\ of\ D\ at\ baseline}\right) \times 100$.

Complete response (CR) means disappearance of all target lesions (<10mm in longest diameter for non-nodal and <15mm in shortest diameter for nodal ones), partial response (PR) means 30% decrease in Sum of D of target lesions, progressive disease (PD) means 20% increase in Sum of D of target lesions, and stable disease (SD) indicates that, there is no sufficient decrease or increase in Sum of D of target lesions.

2.4Blood sampling, biochemical and Immunohistochemical (IHC) analyses

Blood samples were withdrawn at baseline, 1 hour before starting pretreatment with LPZ or placebo before first cycle of NAC and 1 week after the last cycle of NAC for the assessment of P-gp level before and after treatment. Two mL of venous blood was withdrawn by antecubital venipuncture from each patient into EDTA test tube and then centrifugated at 3000 rpm for 20 minutes. The separated plasma was kept at -80 °C until analysis. Plasma P-gp level was determined by enzyme-linked immune-sorbent assay (ELISA) kits (Sun Red, Biological Technology Co., Ltd, Shanghai, China, Catalogue No: 201-12-172. Immunohistochemical analysis of breast tissue sections preserved on paraffin wax (by core biopsy at baseline and at surgery after NAC cycles) was done according to the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) to determine Ki-67 before and after treatment. Ki-67 cut off was set at 14% at this study, according to the central pathology laboratory of the hospital.

2.5Routine laboratory investigations (Follow-up investigations)

Routine laboratory investigations were done at baseline and before each cycle (every 21 days during patient's follow-up visits to oncology clinic). Routine laboratory investigations included assessment of kidney function through follow-up S.Cr and blood urea nitrogen (BUN) levels, assessment of liver function through evaluation of ALT and AST, and determination of CBC.

2.6Assessment of participants' adherence, drug tolerability and adverse effects

Lansoprazole and placebo capsules were supplied to the study participants during follow-up visit to oncology clinic before each cycle. Adherence was determined through the medications refilling rate and through counting the remaining capsules. All participants were followed by telephone calls to ensure their adherence and for reporting anydrug-related adverse effects. The adverse effects were also collected from the participants' laboratory data and the patients' sheets. The participants were also asked about any adverse effects related to all study medications. Any reported adverse events were graded according to National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) v5.

2.7Primary and secondary outcomes

The primary outcome was to evaluate the tumor response and the change in biological biomarkers (P-gp and Ki-67). The secondary outcome was to examine the safety of high dose of LPZ.

2.8Sample size calculation

The required sample size was calculated using G*Power software version 3.1.9.7 (Institut für ExperimentellePsychologie, Heinrich Heine Universität, Dusseldorf, Germany). The estimated sample size was 30 participants in each group which provides a statistical power of 95% to detect the outcome measured. With the assumption of an attrition rate of 10%, the required sample size was 66 patients in the two groups (33 patients in each group).

2.9Statistical analysis

The statistical analysis was performed with IBM® SPSS® Statistics v28 (SPSS Inc., 2021, USA). Data were tested for normality using Shapiro–Wilk tests. Paired student *t*-test was used to compare the data before and after treatment within the same group. Unpaired student *t*-test was applied to compare the values of the two different groups (LPZ

group and placebo group). Chi-Square test was implicated for analyzing categorical data. Fisher exact test used to analyze the reported side effects. Correlations between variables were assessed with Spearman correlation for categorical data. All results are expressed as mean±SD, number and percentage. The level of significance was set at $P<0.05$.

3. RESULTS

Patients' enrollment, randomization, and follow-up during the course of the study are demonstrated in Figure (1). A total number of 317 patients with stage II and stage III BC were assessed for eligibility, 212 women were excluded (not eligible as they underwent surgery before implication of chemotherapy) and 105 patients were eligible (as they had to receive NAC before surgery). Out of those 105 women, 32 patients declined to participate in the study and therefore 73 women with stage II and III BC were randomized into the two study groups: the LPZ group (n=38) and placebo group (n=35). During the follow-up period, a total number of (n=7) women were dropout in both groups (5 patients in LPZ group and 2 patients in the placebo group) secondary to withdrawn from the study due to non-adherence to treatment (n=4), changed hospital to another one closer to home (n=1) and loss of follow-up (n=2). The final analysis included 66 patients with 33 women in each group.

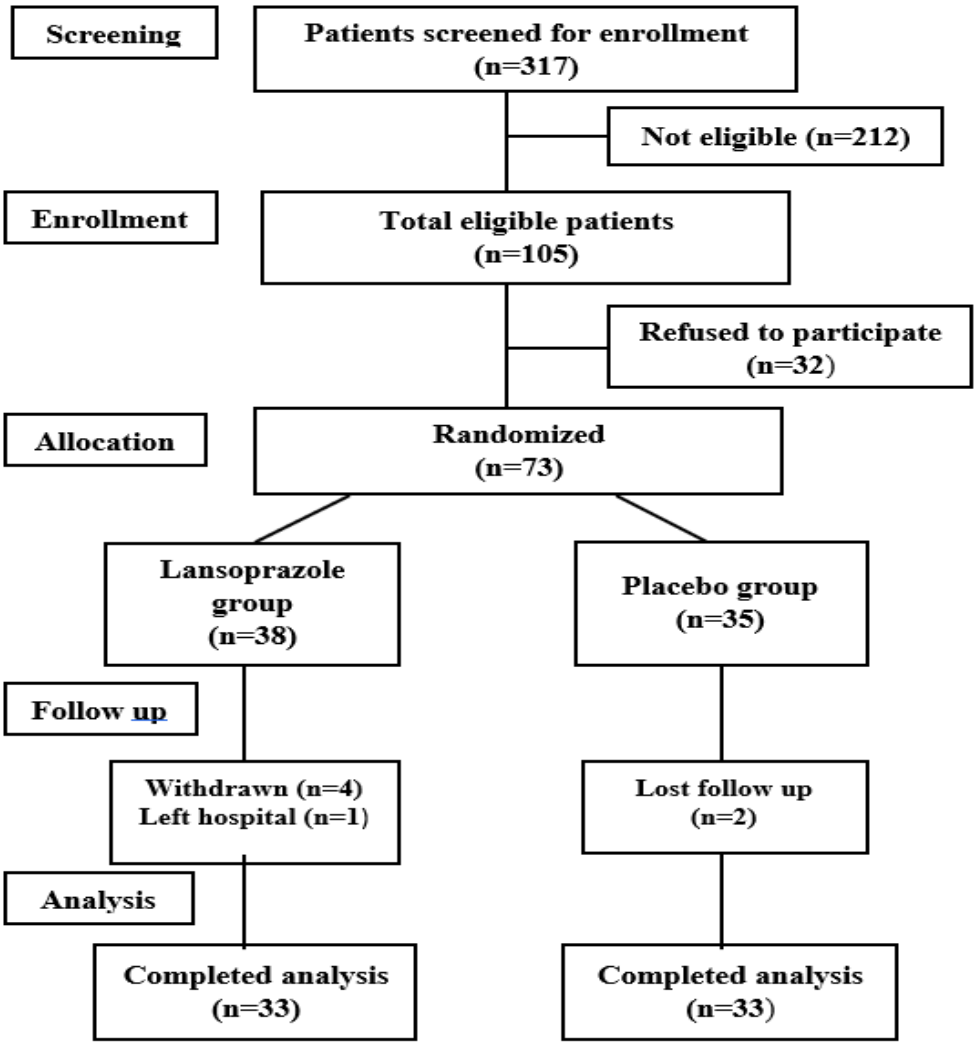

Fig. 1. Patients flow chart.

3.1 Anthropometric, demographic, and clinical data

At baseline, there was no statistically significant difference between LPZ group and placebo group (P>0.05) regarding anthropometric measurements including age, weight, height, BMI and BSA, demographic and clinical data including family history, menopausal status, chronic disease status (hypertension, diabetes mellitus and hepatitis C), type of breast carcinoma, molecular breast cancer subtypes, grade, stage of the disease, duration of treatment, cumulative doses of NAC and type of surgery after NAC as shown in Table (1).

Table 1.Baseline anthropometric, demographic, and clinical data for the two study groups.

Parameter	**Lansoprazole group (n=33)**	**Placebo group (n=33)**	***P*-value**
Age (years)	49.97±10.17	46.91±9.89	0.22
Weight (kg)	87.64±18.61	85.91±19.49	0.71
Height (m)	1.59±0.08	1.59±0.06	0.82
BMI (Kg/m^2)	34.45±7.81	33.75±7.43	0.71
BSA (m^2)	1.96±0.22	1.94±0.24	0.68
Family history	9(27.3%)	7(21.2%)	0.57
Chronic disease status			
Hypertension	6(18.2%)	5(15.2%)	0.74
DM	5(15.2%)	4(12.1%)	0.72
HCV	0(0%)	2(6.1%)	0.15
Menopausal state			
Premenopause	17(51.5%)	23(69.7%)	0.13
Postmenopause	16(48.5%)	10(30.3%)	
Type of breast carcinoma			
Invasive ductal	31(94%)	32(97%)	0.60
Invasive lobular	1(3%)	0(0%)	
Inflammatory	1(3%)	1(3%)	
Receptor status			
Estrogen receptor +ve	28(84.8%)	26(78.8%)	0.52

Progesterone receptor +ve	25(75.8%)	22(66.7%)	0.42
HER2 +ve	12(36.4%)	9(27.3%)	0.43
Molecular subtypes			
Luminal A	3(9.1%)	5(15.2%)	0.58
Luminal B/HER2-ve	15(45.5%)	16(48.5%)	
Luminal B/HER2+ve	10(30.3%)	5(15.2%)	
HER2 overexpression	2(6.1%)	4(12.1%)	
Triple negative	3(9.1%)	3(9.1%)	
Grade			
2	28(84.8%)	29(87.9%)	0.72
3	5(15.2%)	4(12.1%)	
Stage			
II	29(87.9%)	31(93.9%)	0.39
III	4(12.1%)	2(6.1%)	
Type of surgery			
MRM	30(90.9%)	29(87.9%)	0.69
BCS	3(9.1%)	4(12.1%)	
Duration of chemotherapy (months)	5.18±0.53	5.12±0.60	0.66
Cumulative dose of Adriamycin (mg)	437.58±40.24	428.48±47.71	0.41
Cumulative dose of Cyclophosphamide (mg)	4369.70±420.24	4260.61±440.81	0.31
Cumulative dose of Paclitaxel (mg)	1718.18±178.63	1683.64±201.21	0.46

Data are expressed as mean±SD for continuous values and expressed as numbers (percentages) for categorical values.
Kg: kilogram, m: meter, BMI: Body mass index, BSA: body surface area, DM: Diabetes mellitus, HCV: Hepatitis C virus infection, HER2: Human epidermal receptor 2, MRM: Modified radical mastectomy, BCS: Breast conservative surgery, mg: milligram.
*P<0.05 was considered statistically significant.

3.2 Effect of intervention on tumor response and biological markers

For tumor response evaluation, mean Sum of D (mm) was calculated at baseline and after completion of NAC cycles. At baseline before starting NAC regimen, there was non-significant difference between LPZ group and placebo group in the Mean Sum of D (mm) for target lesions (48.70±27.56 mm versus 47.30±17.16 mm; *P1* =0.81). Furthermore, after completion of NAC cycles, there was non-significant difference between LPZ group and placebo group in the Mean Sum of D (mm) for target lesions (23.09±14.71 mm versus 26.65±12.85 mm; *P2* =0.30) as illustrated in Figure (2).

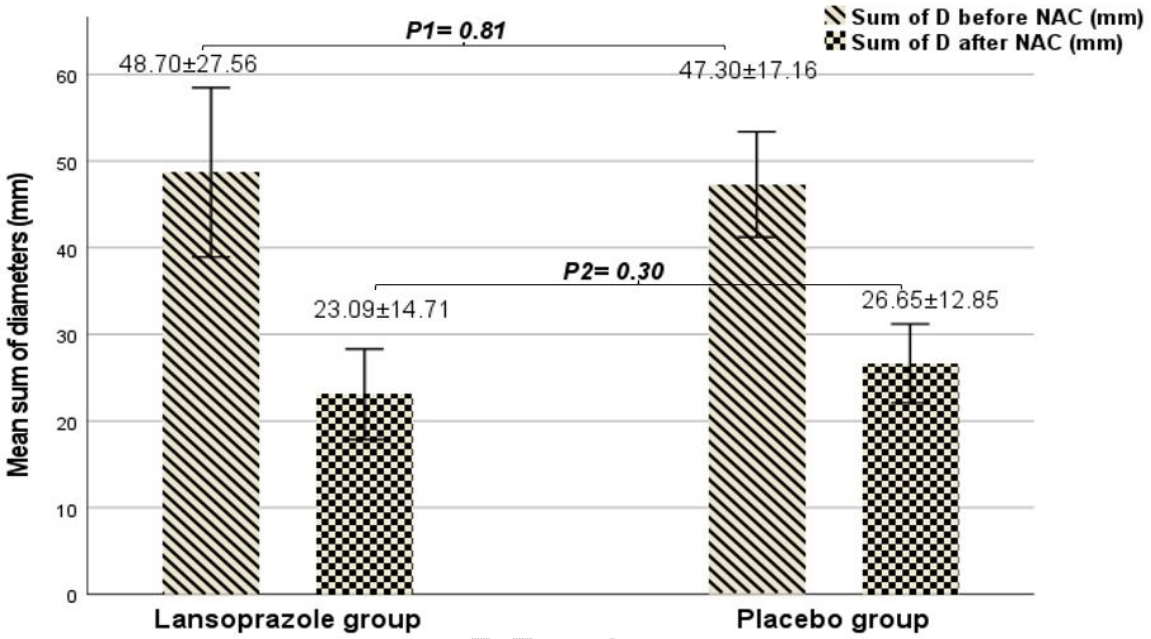

Fig. 2. Mean Sum of D in LPZ group and placebo group before and after NAC

Tumor response according to the RECIST v1.1 was evaluated, as compared to the placebo group, LPZ group showed non-significantly higher number of patients who achieved CR [5 (15.2%) versus 3 (9.1%); *P* =0.11] and PR [23 (69.7%) versus 18 (54.5%); *P* =0.11]. The number of patients who showed SD was 3 times lower in LPZ group as compared to the control group [4 (12.1%) versus 12 (36.4%); *P* =0.11]. Only 1 patient in LPZ group developed progressive disease (PD), however there was no statistically significant difference between the two groups [1 (3%) versus 0 (0%); *P* =0.11].Pathological response was recorded after surgery, and it was highly correlated to response calculated according to that of RECIST criteria (*r*=0.47; *P*<0.001). As compared to the placebo group, LPZ group showed a non-significantly higher number of patients who achieved CR [10 (30.3%) versus 6 (18.2%); *P* =0.51]. In contrast and as compared to the placebo group, LPZ group showed a non-significantly lower number of patients who achieved PR [20 (60.6%) versus 24 (72.7%); *P* =0.51]. The number of patients who showed no response was equal in both study groups [3 (9.1%) versus 3 (9.1%); *P* =0.51].At baseline, there was non-significant variation between the LPZ group and the placebo group for P-gp plasma level (8.20±7.63 ng/ml versus 8.17±5.51 ng/ml; *P* =0.98). Also, after completion of NAC cycles, the difference between the two groups regarding P-gp plasma level remained statistically non-significant (8.10±5.05 ng/ml versus 7.88±2.97 ng/ml; *P* =0.84). Further evaluation of P-gp plasma level revealed that, the number of patients with decreased P-gp plasma level as compared to baseline was non-significantly higher in LPZ group when compared to placebo group [14 (42.4%) versus 9 (27.3%); *P* =0.20]. Similarly, there was non-significant variation between the two study groups regarding Ki-67 expression (*P*>0.05). At baseline, the number of patients who had Ki-67<14% was 5 (15.2%) in LPZ group versus 6 (18.2%) in the placebo group (*P* =0.74). After completion of NAC cycles, the number of patients with Ki-67<14% was non-significantly higher in LPZ group when compared to placebo group [13 (39.4%) versus 10 (30.3%); *P* =0.44] as demonstrated in Table (2).

Table 2.RECIST response, pathological response, and biological markers in the two study groups

Parameters	Lansoprazole group (n=33)	Placebo group (n=33)	*P*-value
Response according to RECIST			
Complete response (CR)	5 (15.2%)	3(9.1%)	0.11
Partial response (PR)	23 (69.7%)	18 (54.5%)	
Stable disease (SD)	4 (12.1%)	12 (36.4%)	
Progressive disease (PD)	1 (3%)	0 (0%)	
Pathological response			
Complete response (CR)	10 (30.3%)	6 (18.2%)	0.51
Partial response (PR)	20 (60.6%)	24 (72.7%)	
No response	3 (9.1%)	3 (9.1%)	
P-gp (ng/ml)			
Plasma level before treatment	8.20±7.63	8.17±5.51	0.98
Plasma level after treatment	8.10±5.05	7.88±2.97	0.84
Paired *t* test	0.43	0.08	
Decrease in plasma level after treatment	14(42.4%)	9(27.3%)	0.20
Ki-67			
Less than 14% before treatment	5(15.2%)	6(18.2%)	0.74
Less than 14%after treatment	13(39.4%)	10(30.3%)	0.44

Data are expressed as number and percentage.
RECIST: Response Evaluation Criteria in Solid Tumors, P-gp: Permeability glycoprotein, Ki-67: proliferation marker.
*$P<0.05$ was considered statistically significant.

Subgroup analysis was done in order to evaluate the molecular subtype and the menopause state that achieved the most favorable response according to RECIST v1.1. The menopausal status showed non-significant impact on tumor response. There was non-significant difference between premenopausal and postmenopausal status regarding tumor response according to RECIST criteria for both LPZ group and placebo group (P =0.46 and P =0.43 respectively). In LPZ group, molecular subtype luminal B/HER2 negative achieved the highest response followed by luminal B/HER2 positive when compared tumor response in the group (P =0.005). In contrast, in placebo group there was no statistically significant difference between molecular subtypes in the term of tumor response (P =0.55) as shown in Figure (3).

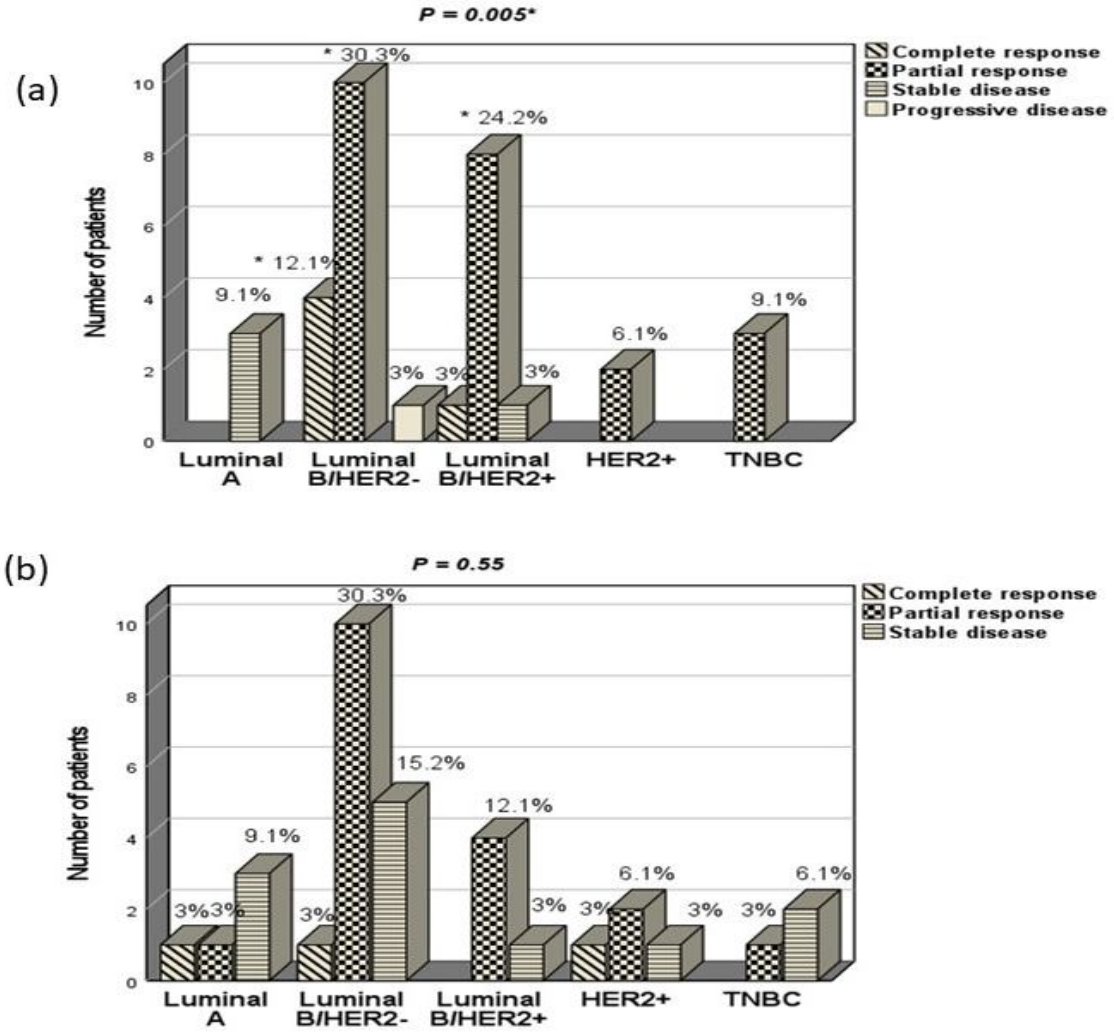

Fig. 3. Tumor response according to RECIST criteria in different molecular subtypes in both LPZ group (a) and placebo group (b).

3.3 Routine parameters

Routine parameters were investigated at baseline and after first, fourth and eighth chemotherapy cycles. The data obtained revealed that, there was non-significant variation between the two groups regarding liver function, kidney function and CBC ($P>0.05$) as shown in Table (3).

Table 3. Routine parametersat baseline and after first, fourth and eighth cycles of NAC

Parameter	First cycle		Fourth cycle		Eighth cycle	
	LPZ group	Placebo group	LPZ group	Placebo group	LPZ group	Placebo group
S.Cr (mg/dl)	0.80±0.13	0.76±0.11	0.78±0.20	0.79±0.11	0.79±0.15	0.79±0.13
P_1			0.48	0.044*	0.72	0.105
P_2	0.17		0.69		0.87	
BUN (mg/dl)	11.94±1.94	10.94±2.45	12.70±3.59	12.42±3.09	13.45±3.79	14.39±4.60
P_1			0.18	<0.001*	0.02*	<0.001*
P_2	0.07		0.74		0.37	
ATL (IU/L)	19.30±4.37	21.00±6.36	24.97±7.33	29.15±19.02	30.48±13.42	36.27±19.90
P_1			<0.001*	0.013*	<0.001*	<0.001*
P_2	0.21		0.24		0.17	
AST (IU/L)	21.06±3.02	22.45±5.06	26.48±7.92	31.06±15.59	29.18±12.02	34.70±14.63
P_1			<0.001*	0.002*	<0.001*	<0.001*
P_2	0.18		0.14		0.099	
BIL-T (mg/dl)	0.36±0.12	0.37±0.14	0.37±0.14	0.36± 0.20	0.42±0.18	0.44± 0.16
P_1			0.86	0.67	0.07	0.02*
P_2	0.95		0.73		0.73	
Hgb(gm/dl)	11.75±.80	11.94±.88	10.87±.89	10.99±.81	10.48±.91	10.58±1.00
P_1			<0.001*	<0.001*	<0.001*	<0.001*
P_2	0.36		0.58		0.65	
RBCs (10^6 /µl)	4.82±0.43	4.77±0.38	4.17±0.47	4.10± 0.45	3.79±0.49	3.48±0.48
P_1			<0.001*	<0.001*	<0.001*	<0.001*
P_2	0.65		0.58		0.98	
WBCs (10^3 /µl)	7.36±1.71	6.25±1.74	4.65±1.65	4.57±1.28	4.93±2.02	4.45±1.41
P_1			<0.001*	<0.001*	<0.001*	<0.001*
P_2	0.05		0.82		0.27	
PLT (10^3 /µl)	310.8±74.16	303.7±83.1	342.36±86.32	338.45±90.09	310.42±85.93	316.91±63.47

P_1		0.004*	0.04*	0.98	0.39
P_2	0.71	0.84		0.73	

Data are expressed as mean±SD
S.Cr: Serum creatinine, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate transaminase, BIL-T: Total bilirubin, Hgb: Hemoglobin, RBCs: Red blood cells, WBCs: White blood cells, PLT: Platelets
P_1: comparison within the same group (Paired *t*- test)
P_2: comparison between the two groups (Unpaired *t*- test)
*P< 0.05 was considered statistically significant

3.4 Lansoprazole safety and tolerability

Regarding the reported adverse effects, there was non-significant difference between the two study groups (P >0 .05) except for dyspepsia (P=0.011). These results mean that the implication of high dose of LPZ was safe, tolerable and the addition of LPZ to chemotherapy did not augment chemotherapy induced adverse effects. The reported adverse effects and their grading are shown in Table (4).

Table 4.Reported adverse effects between the two groups.

Parameter	**Lansoprazole group (n=33)**	**Placebo group(n=33)**	***P*-value**
Headache			
Grade 1	26(78.8%)	31(93.9%)	0.15
Grade 2	7(21.2%)	2(6.1%)	
Dizziness			
Grade 1	33(100%)	31(93.9)	0.49
Grade 2	0(0%)	2(6.1%)	
Diarrhea			
Grade 1	9(27.3%)	13(39.4%)	0.49
Grade 2	2(6.1%)	4(12.1%)	
Grade 3	2(6.1%)	1(3%)	
Constipation			
Grade1	3(9.3%)	1(3%)	0.59
Grade 2	2(6.1%)	2(6.1%)	
Dyspepsia			
Grade 1	22(66.7%)	31(93.9%)	0.011*
Grade 2	11(33.3%)	2(6.1%)	

Rash			
Grade 2	3(9.1%)	5(15.2%)	0.71
Increased liver enzymes			
Grade 3	0(0%)	1(3%)	1.00
Leucopenia			
Grade 1	5(15.2%)	3(9.1%)	0.85
Grade 2	8(24.2%)	7(21.2%)	
Grade 3	1(3%)	1(3%)	
Arthralgia			
Grade 1	24(72.7%)	29(87.9%)	0.25
Grade 2	8(24.3%)	3(9.1%)	
Grade 3	1(3%)	1(3%)	
Anemia			
Grade 1	2(6.1%)	7(21.2%)	.21
Grade 2	12(36.4%)	11(33.3%)	
Grade 3	0(0%)	1(3%)	

Data are expressed as numbers (percentages).
*P<0.05 considered statistically significant.
Grade 1: Asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated.
Grade 2: minimal, local or noninvasive intervention indicated.
Grade 3: Severe or medically significant but not immediately life-threatening.

3.5 Correlation analysis

The correlation analysis revealed that, both P-gp and Ki-67 were not significantly correlated with Sum of D (r=0.209; P =0.09 and r= 0.162; P =0.19 respectively). Furthermore, both P-gp and Ki-67 were non-significantly correlated (r=0.180; P =0.15).

4.DISCUSSION

Increased activity of V-ATPase in cancerous cells leads to acidic microenvironment, hinders weakly basic drug from influx into cancerous cells secondary to ion trapping, in addition to activation of drug efflux pump, P-gp [7,14,15,16]. Lansoprazole was implicated during the current study as a possible V-ATPase and P-gp inhibitor to improve chemotherapy uptake into cancerous cells. Zhang et al., 2014 postulated the antitumor effect of LPZ in breast cancer cell lines and in mice [29]. Also, LPZ was reported to increase endosomal pH in breast cancer cell lines with subsequent increased adriamycin uptake [30]. Furthermore, it has been demonstrated that LPZ increased the sensitivity to paclitaxel in human melanoma cell lines [31].

According to the author's knowledge, this is the first clinical study aimed at evaluating the effect of high dose pretreatment of LPZ on tumor size, P-gp and Ki-67 in patients with stages II and stage III BC. The dose of LPZ used

during the current study was selected based on the finding reported by Wang et al., 2015 who postulated enhanced antitumor effect of chemotherapy with high dose of esomeprazole which seems equivalent to the selected dose of LPZ [8]. Furthermore, Hegazy et al., 2021 reported improved response rate with the implication of LPZ dose similar to that used during the current study [32].

The primary outcome was to evaluate response according to RECIST v1.1. After completion of NAC cycles, LPZ group showed more decrease in Sum of D with consequently increased number of patients with CR and PR especially in luminal B subtype as compared to placebo group. However, this difference between the two study groups is statistically non-significant, it seems clinically important. Our finding seems in matching with the findings reported by Matsumura et al., 2022 who investigated the effect PPIs on 5-FU based chemotherapy on esophageal squamous cell carcinoma in-vitro and in clinical setting [33].

The data obtained with the current study revealed that, as compared to placebo group, LPZ group showed non-significant decrease in plasma P-gp level. In-vitro and pre-clinical studies revealed that inhibition or down-regulation of P-gp was associated with improved response to chemotherapy [15,34]. Proton pump inhibitors were reported to exert an inhibitory effect on P-gp in human gastric adenocarcinoma cells both in-vitro and in-vivo [23].

Ki-67 is an important marker that gives indication about cells proliferation, BC subtype classification and helps in identification of prognosis and recurrence of the disease. During the current study, LPZ group showed non-significant but clinically important decline in Ki-67 (Ki-67 <14%), as compared to placebo group. According to European society for medical oncology (ESMO) clinical practice guidelines 2019 and the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021, the panel did not define a consistent Ki67 cut off, in this study cut off 14% was adopted for Ki-67 to be high [35,36,37,38,39].

The data obtained with the current study revealed safety and tolerability of high dose of LPZ. There was non-significant toxicity associated with LPZ except for dyspepsia. This former finding comes in consonance with previous studies demonstrated safety of PPIs administration with chemotherapy [8,32,33].

We did not observe any significant correlation between the changes in P-gp, Ki-67, and the change in Sum of D. This could be attributed to the relatively small sample size. Moreover, there was inter-patients' variability regarding the plasma level of P-gp and Ki-67 which could contribute to the lack of correlations.

The overall data obtained with the current study revealed safety of LPZ without significant antitumor efficacy. Regarding the efficacy of LPZ, the data obtained with the current study seems in conflicting with some previous studies which reported improved response, overall survival, and increased chemo-sensitivity upon PPIs co-administration with chemotherapy [8,32,33]. These contradictory results could be attributed to the variation in the type of cancer and the chemotherapeutic agents. In addition, these previous studies and the current study all considered with small sample size.

In this context, with safety consideration, we recommended multicenter, large-scale, and more longitudinal clinical studies in order to re-evaluate the antitumor effect of LPZ.

The points of strength of the current study include its design, and the use of the same brand of LPZ, throughout the study. However, the current study has some limitations including a relatively small sample size. In this context, future multicenter, large scale and longitudinal studies are still recommended.

5. CONCLUSION

Despite the implication of LPZ didn't reveal a statistically significant anti-tumor effect as compared to placebo, it produced a clinically important improvement in tumor response which was translated by higher number of patients who achieved CR and had Ki-67 less than 14%. Furthermore, the higher dose of LPZ implicated during the current study was tolerable and safe. With this proven safety of high dose of LPZ, we recommend future multicenter, large-scale, and more longitudinal clinical studies in order to re-evaluate its antitumor effect.

CONSENT

All the study participants provided their informed consent.Authors declare that written informed consent was obtained from approving authority for publication of this research.

ETHICAL APPROVAL

The study was approved by the National Research Ethics Committee of Tanta University with an approval Code (34615/4/21)which was approved and accepted by theResearch Ethics Committee Institutional Review Board of Menoufia University. The study was consistent with the Helsinki Declaration's ethical principles in 1964 and its later amendments.

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ABBREVIATIONS

5-FU: 5-Fuorouracil
ALT: Alanine amino transaminase
ASCO/CAP: American Society of Clinical Oncology and the College of American Pathologists
AST: Aspartate amino transaminase
BC: Breast cancer
BMI: Body mass index
BSA: Body surface area
BUN: Blood urea nitrogen
CBC: Complete blood picture
CR: Complete response
ELISA: Enzyme-linked immunosorbent assay
ESMO: European society for medical oncology
GLOBOCAN: Global cancer statistics
IHC: Immunohistochemistry
LPZ: Lansoprazole
NAC: Neoadjuvant chemotherapy
NCI-CTCAE: National Cancer Institute-Common Terminology Criteria for Adverse Events
PD: Progressive disease
P-gp: Permeability-glycoprotein
PPIs: Proton pump inhibitors
PR: Partial response
RECIST: Response Evaluation Criteria in Solid Tumors
SD: Stable disease
Sum of D: Sum of diameters
V-ATPase: Vacuolar-ATPase