Comparing the Efficacy and Side Effects of PDLASTA® (Peg-Filgrastim) with PDGRASTIM® (Filgrastim) in Breast Cancer Patients; A Non-inferiority Randomized Clinical Trial

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Abstract

Background: The objective of this study was to compare the efficacy and side effects of to evaluate the efficacy and safety of a single dose (Peg-Filgrastim or PDL) or repeated six daily injections (Filgrastim or PDG) during chemotherapy courses in breast cancer patients in a non-inferiority clinical trial.

Methods: In this randomized clinical trial, 80 patients recruited and allocated randomly in two equal arms. In one group, a single subcutaneous dose of 6 mg of PDL was injected the day after receiving a chemotherapy regimen in each cycle. The second arm received a subcutaneous injection of 300 micrograms per day for six consecutive days in each course of treatment. Side effects of GCF treatment and its effect on blood parameters were compared in each cycle and during eight courses of chemotherapy.

Results: hematologic parameters showed no significant difference in each course of treatment between two groups of study. The comparison of the WBC (p=0.527), Hgb (p=0.075), Platelet (p=0.819), Neutrophil (p=0.575), Lymphocyte (p=705) and ANC (p=0.675) changes during eight courses of treatment identified no statistically significant difference between two study groups. Side effects including headache, injection site reaction and muscle pain had a lower frequency in patients receiving PDL drugs.

Conclusion: Regarding our results, PDL is completely non- inferior in efficacy and also less toxic compared to PDG. Prescribing in single-dose and lower expenses of PDL introduces it as a cost-effective drug in the treatment of chemotherapy-induced neutropenia.

Trial registration number, date of registration: IRCT20190504043465N1, May 2019

https://www.irct.ir/search/result?query=IRCT20190504043465N1

Background

G-CSF is the main cytokine in the control of neutrophil production, which is used clinically for the treatment of congenital and acquired neutropenia[1]. This cytokine in vitro increases the number of circulating neutrophils and improves their performance[2]. More than 90% of patients respond to G-CSF by an increase in ANC (Absolute Neutrophil Count) of more than 1×10^9/L [3, 4]. These patients benefit greatly from G-CSF [5, 4]; such as a significant improvement in the quality of life, including health, performance in society and socioeconomic status, reduction in the frequency and severity of infections, fever, use of antibiotics, hospitalization, oral ulcers and severity, and increased survival [6–9, 5, 10–12]. Also, treating children with severe congenital neutropenia severely reduces the risk of sepsis [13]. This cytokine significantly improves the quality of life of patients. Treatment with rhG-CSF improves all previous chronic infections, decreases the frequency of new episodes of infection, and discontinues the administration of prophylactic antibiotics [6]. Various studies have been conducted on the side effects of G-CSF. Increased spleen size has been reported in most patients. Also, the effect of G-CSF on bone marrow stimulation and development appears as early bone pain [8]. According to the SCNIR, side effects of these patients include bone pain, splenomegaly, thrombocytopenia, osteoporosis, and leukemia/MDS [14, 4] and also fever, myalgia, and erythema [15]. G-CSF has several effects on the granulocytic cell line. Not only does it stimulate the growth and differentiation of myeloid precursors, but it also enhances the activity of adult neutrophils [16]. According to numerous studies, side effects of this drug include splenomegaly, thrombocytopenia, osteopenia and osteoporosis, bone pain, vasculitis, skin rash, eosinophilia, monocytosis and malignant changes to AML/MDS [7, 2, 17, 18, 3, 19, 10, 4]. In other disparate studies, hyperplasia, glomerulonephritis, myalgia, erythema, dyspnea, hypotension, sweating, and hot flashes have also been observed [20, 15, 2]. The most important of these complications is the advancement of AML / MDS, which is still

unclear whether G-CSF is the cause of this transformation or because of the inherent tendency of the congenital neutropenic disease to progress to MDS / AML, the increased survival of congenital patients by G-CSF Provides a good opportunity to develop this malignancy [21, 22]. Cytogenetic abnormalities have been reported with a high risk of these malignancies: CSF3R mutation (G-CSF receptor), ELA2 gene mutation, Rascogenic activity, chromosome 7 monosomal, and chromosomal changes that in studies conducted in a number of patients, resistance to G-CSF therapy and the continuation of severe infections that are often life-threatening are reported, with HSCT, is the only treatment available [23, 3, 24].

As already stated PDGRASTIM or Filgrastim (PDG) and PDLASTA or Peg-Filgrastim (PDL) had been a comparison in a head to head trial with brand one (GCSF and Neulasta) and show non-inferiority results. So most of the patients should take some kinds of GCSF during dense dose treatment if the treatment is GCSF, the patient should come to the center every day after chemo for at least 6 days, but if they take PDL just one shoot required, so the objective of this study is to compare the drug effect and side effects between two drugs in breast cancer patients in a non-inferiority clinical trial.

Methods

This interventional study compared the efficacy and safety of the PDL product of Pooyesh Darou Biopharmaceuticals Company with PDG in breast cancer patients as a non-inferiority parallel groups Randomized Clinical Trial. The flow diagram has been demonstrated in Fig. 1.

Patients and criteria

This study included 80 patients diagnosed with breast cancer. They were treated with adjuvant and neoadjuvant therapy with a dose-dense AC*4-T*4 regimen which consists of four courses of Adriamicine plus Cyclophosphamide and four courses of Taxen based drugs. They referred to Motamed Cancer Institute for Chemotherapy.

Inclusion criteria were:

- Age > 18 years
- Investigator diagnosis of breast cancer candidate of adjuvant therapy
- Absolute neutrophil count $\geq 1.5 \times 10^9$/l
- platelet count $\geq 100 \times 10^9$/l
- Serum creatinine < 1.5 × upper limits of normal
- indication of receiving GCSF

Exclusion criteria consisted of:

- Bilirubin > upper limit of normal; or aspartate transaminase and/or alanine transaminase > 1.5 × upper limits of normal, concomitant with alkaline phosphatase > 2.5 × upper limit of normal
- Radiation therapy within 4 weeks of randomization into this study
- Prior bone marrow or stem cell transplantation
- Total lifetime exposure to doxorubicin > 240 mg/m^2 or epirubicin > 600 mg/m^2
- Ejection Fraction < 40%

- Liver cirrhosis

In case of any serious complications due to the use of PDL, the patient was excluded from the study.

In this study, breast cancer patients who were under adjuvant and neo_adjuvant chemotherapy with a dose-dense AC*4-T*4 regimen, considering inclusion and exclusion criteria, signed informed consent and recruited the study. They were randomly assigned to the drug group (PDL) or to the control group (PDG) and demographic and clinical characteristics were recorded. Those drugs were prescribed free of charge and were injected with the supervision of a doctor at the treatment center. Patients in the two groups received up to 8 courses of chemotherapy, which the first 4 courses consisted of Adriamycin and the second half of treatment courses consisted of taxen based drugs. Hematologic parameters and the possible side effects of the drug were recorded according to Cell Blood Count and patients' symptoms on start day and 7th and 15th days of each chemotherapy course.

The incidence of Febrile Neutropenia in each cycle was assumed as a primary efficacy outcome. according to Holmes's study[25], the rate of success in PDL and PDG in a lower incidence of neutropenia was 0.91 and 0.82, respectively. Considering the Non-inferiority margin of 0.1, the Allocation ratio of 1/1, α error equal to 0.05, and 80% power of the study, 40 patients were recruited to the study.

The patients were trained to notify any complications by telephone to the design interface who was presented in the consent form. If a patient had fever and neutropenia in the first week or the second week after treatment, at least three additional doses with appropriate antibiotics were prescribed and, if the physician advised, she was admitted to the hospital. In patients with Grade 3 or 4 of neutropenia, PDL would change to PDG up to the end of treatment. Those patients were excluded from the PDL group. If the patient was admitted, all drug costs were paid by the Pharmaceutical Drug Company.

Intervention:

Patients in the PDL group received a single subcutaneous injection of 6 mg Peg-Filgrastim on the second day of each chemotherapy cycle. In the PDG group, in each chemotherapy cycle, 300 micrograms Filgrastim per day was injected subcutaneously for six consecutive days.

Outcome

The outcome of interests were hematologic parameters consisting of WBC, Hgb, Platelet, Neutrophil, Lymphocyte, and ANC which their values were compared between two groups in each course and during eight courses of chemotherapy. ANC was calculated by multiplying the percentage of neutrophils by the total number of WBCs (in thousands). The short and long term side effects of the drugs were recorded in both groups during the study.

Randomization and blinding

Randomization was performed using quadruple blocks. Blocks of random allocation consisted of pockets provided by corresponding researcher. Concealment was supervised by one the clinic personnel who was not involve in enrollment of patients. The oncologist assigned participants to interventions. Due to the different protocols of administration of the two drugs and the need for supervision of an oncologist, blinding of the patient and the therapist was not possible. The statistical analyzer was not informed about the assignment of patients to the groups.

Statistical analysis

An Interim analysis was achieved after completing one third of sample size. Since no side effect was noticed in two groups, the recruitment was continued up to the end of study. The frequency of demographic and clinical characteristics of the two groups was demonstrated by descriptive statistics. The randomized allocation of two groups

of the study was assessed by chi-square and t-student tests. Kolmogorov-Smirnov test was applied to evaluate the normality of outcome variables' distribution. Most variables did not show the normal distribution and nonparametric tests were applied in the next steps of analysis.

The mean and median of hematologic variables (WBC, HgB, platelet, neutrophil, lymphocyte, and ANC) distribution and frequency of complications were compared in PDG and PDL groups.

The changes in hematologic variables (WBC, HgB, platelet, neutrophil, lymphocyte and ANC) during start point, 7th and 15th days in each course of chemotherapy were evaluated in both groups by Friedman analysis. This variation of repeated measurements of outcome between two groups of the study was compared by Generalized Estimation Equation (GEE) analysis. Hematologic parameters' changes during 8 courses of chemotherapy, were compared between two groups by Generalized Estimation Equation analysis too. Statistical analysis was performed by SPSS software version 22.

Ethical considerations

Patients entered the study by signing a written informed consent for drug intake. All information such as emphasizing the process of implementation, right of exclusion of study during the treatment, covering expenses, possible side effects and emergency phone number for consultation and reporting side effects had been included in the informed consent form. This research was approved and registered in the Ethics Committee of Breast Cancer Research Center with code number: IR.ACECR.IBCRC.REC.1395.19. Also, this study was registered in the Iranian Registry of Clinical Trials (IRCT) in https://www.irct.ir/ with registration code: IRCT20190504043465N1.

Results

Eighty patients were recruited to PDL and PDG groups equally. Table 1 shows the comparison of demographic and clinical characteristics of breast cancer patients treated by PDG or PDL. The mean age of PDG and PDL groups were 47.8 ± 9.04 and 43.7 ± 9.23, respectively. There was no significant difference between two groups in terms of age, BMI, tumor size, excised LN, involved LN, Ki-67 index, education, marital, employment, ER, PR, and HER2 status.

Table 1
Demographic and clinical characteristics of patients in two study groups

Variable	PDG	PDL	P-value
	Mean ± SD	Mean ± SD	
Age, year	47.8 ± 9.04	43.7 ± 9.23	0.05
BMI, kg/m^2	27.8 ± 4.88	26.6 ± 4.06	0.261
Tumor size, cm	3.0 ± 1.1	3.1 ± 0.9	0.744
Excised LN, n	7.9 ± 4.6	8.7 ± 6.1	0.566
Involved LN, n	2.1 ± 2.7	2.1 ± 2.6	0.958
Ki-67, %	36.0 ± 23.2	34.6 ± 26.5	0.819
	No (%)	No (%)	
Age			0.116
< 50	24 (60)	30 (75)	
≥ 50	16 (40)	10 (25)	
Education status			0.362
Illiterate / Elementary	16 (42.1)	19 (48.7)	
Diploma / Academic	22 (57.9)	20 (51.3)	
Marital status			0.387
Married	33 (82.5)	33 (87.5)	
Single / Divorce / Widow	7 (17.5)	5 (12.5)	
Employment status			0.293
Housewife	30 (75.5)	33 (82.5)	
Employed	10 (25)	7 (17.5)	
ER			0.150
Negative	11 (28.2)	6 (15.8)	
Positive	28 (71.8)	32 (84.2)	
PR			0.061
Negative	19 (48.7)	11 (28.9)	
Positive	20 (51.3)	27 (71.1)	
HER2			0.587
Negative	31 (79.5)	30 (78.9)	
Positive	8 (20.5)	8 (21.1)	

Outcome measurements were achieved at the start point, 7th and 15th days in each course of chemotherapy. The distribution of hematologic variables in eight courses of chemotherapy in both groups of study has been demonstrated in Table 2.

Table 2
Changes of hematologic variables during eight courses of chemotherapy in two groups

Time	variable	PDG	PDL	Time	variable	PDG	PDL
		Mean ± SD	Mean ± SD			Mean ± SD	Mean ± SD
Start of study	WBC	9068.42 ± 1433.59	8361.58 ± 1992.03	**5th course (7th day)**	WBC	2593.42 ± 538.79	2455.52 ± 688.89
	Hgb	12.75 ± 1.04	12.98 ± 1.2		Hgb	11.78 ± .93	11.93 ± .79
	Platelet	298972.22 ± 130889.81	281910.53 ± 104249.08		Platelet	104027.78 ± 12112.53	100473.68 ± 29235.82
	Neutrophil	74.5 ± 6.62	75.37 ± 6.09		Neutrophil	50.53 ± 6.55	52.63 ± 5.54
	Lymphocyte	24.97 ± 6.38	22.92 ± 6.92		Lymphocyte	47.79 ± 9.516	46.55 ± 6.41
	ANC	6659 ± 1263	6388 ± 1682		ANC	1312 ± 360	1299 ± 406
1st course (7th day)	WBC	3623.68 ± 1272.46	5997 ± 12404	**5th course (15th day)**	WBC	5339.47 ± 1450.54	5121.08 ± 1699.03
	Hgb	12.56 ± 1.01	12.8 ± 1.01		Hgb	11.68 ± .96	11.89 ± .76
	Platelet	141722.22 ± 77288.18	136584 ± 32865		Platelet	153888.89 ± 21372.58	158657.89 ± 42731.9
	Neutrophil	46.5 ± 6.19	47.6 ± 6.3		Neutrophil	88.24 ± 6.32	88.58 ± 4.18
	Lymphocyte	52.97 ± 6.57	51.3 ± 6.6		Lymphocyte	11.63 ± 6.41	10.87 ± 3.4
	ANC	1662 ± 737	2896 ± 6062		ANC	4681 ± 1328	4554 ± 1597
1st course (15th day)	WBC	5618.42 ± 1216.74	5827.63 ± 1127.22	**6th course (7th day)**	WBC	3515.79 ± 701.65	3531.58 ± 889.23
	Hgb	12.52 ± .95	12.76 ± 1.107		Hgb	11.6 ± 1.03	11.85 ± .71
	Platelet	182694.44 ± 35740.19	185578.95 ± 32961.59		Platelet	118111.11 ± 22193.66	116078.95 ± 17653.4
	Neutrophil	85.18 ± 5.4	84.66 ± 5.54		Neutrophil	51.66 ± 6.84	52.6 ± 6.82
	Lymphocyte	14.45 ± 4.75	15 ± 5.57		Lymphocyte	47.55 ± 7.03	46.95 ± 7.17
	ANC	4655 ± 1292	4891 ± 1054		ANC	1809 ± 460	1879 ± 755

Time	variable	PDG Mean ± SD	PDL Mean ± SD	Time	variable	PDG Mean ± SD	PDL Mean ± SD
2nd course (7th day)	WBC	2786.84 ± 850.17	3553.16 ± 3202.25	6th course (15th day)	WBC	7200 ± 1567.23	7056.58 ± 1948.95
	Hgb	12.36 ± 1.03	12.44 ± 1.06		Hgb	11.58 ± .9	11.76 ± .72
	Platelet	109055.55 ± 19608.95	117118.42 ± 20749.29		Platelet	173972.22 ± 19983.54	171236.84 ± 37220.62
	Neutrophil	47.71 ± 6.21	50.52 ± 8.98		Neutrophil	88.73 ± 5.23	88.37 ± 3.97
	Lymphocyte	51.23 ± 6.09	48.21 ± 10.82		Lymphocyte	11.13 ± 5.2	11.63 ± 3.97
	ANC	1324 ± 476	1819 ± 1891		ANC	6371 ± 1558	6244 ± 1792
2nd course (15th day)	WBC	5405.52 ± 5803.10	4693.42 ± 831.65	7th course (7th day)	WBC	5722.37 ± 7130.64	4190.79 ± 757.38
	Hgb	12.25 ± .96	12.4 ± 1.18		Hgb	11.52 ± .88	11.69 ± .73
	Platelet	152444.44 ± 12622.98	160131.58 ± 20145.64		Platelet	124611.11 ± 15331.16	122815.79 ± 16204.53
	Neutrophil	86.73 ± 5.75	86.34 ± 4.39		Neutrophil	52.87 ± 5.5	68.34 ± 98.78
	Lymphocyte	14.02 ± 8.14	12.89 ± 3.67		Lymphocyte	46.34 ± 5.82	46.18 ± 8.47
	ANC	4550 ± 4351	4009 ± 808		ANC	3086 ± 4284	2923 ± 4491
3rd course (7th day)	WBC	2610.52 ± 1345.12	2660.52 ± 1277.09	7th course (15th day)	WBC	11797.37 ± 14999.43	8007.9 ± 2431.03
	Hgb	12.14 ± .93	15.23 ± 17.99		Hgb	11.48 ± .95	11.63 ± .76
	Platelet	97802.78 ± 14290.07	103476.31 ± 132901.69		Platelet	183500 ± 21285.14	181289.47 ± 31302.28
	Neutrophil	47.4 ± 6.9	51.18 ± 4.57		Neutrophil	89.44 ± 4.85	90.63 ± 3.83
	Lymphocyte	51.97 ± 6.52	47.55 ± 5.25		Lymphocyte	9.05 ± 3.38	10.64 ± 10.17
	ANC	1215 ± 582	1359 ± 636		ANC	10515 ± 13354	7273 ± 2273

Time	variable	PDG Mean ± SD	PDL Mean ± SD	Time	variable	PDG Mean ± SD	PDL Mean ± SD
3rd course (15th day)	WBC Hgb Platelet Neutrophil Lymphocyte ANC	3961.31 ± 668.86 12.06 ± .92 142861.11 ± 14204.93 87.1 ± 5.05 12.81 ± 5.11 1864 ± 431	3993.68 ± 717.65 15.14 ± 17.99 145589.47 ± 27305.87 87.87 ± 4.47 12 ± 4.6 2010 ± 450	**8th course (7th day)**	WBC Hgb Platelet Neutrophil Lymphocyte ANC	5396.05 ± 1612.13 11.15 ± 1.91 128583.33 ± 20230.63 53.55 ± 6.03 45.39 ± 8.08 2924 ± 1057	5010.53 ± 945.41 11.51 ± .81 127594.74 ± 17224.53 52.21 ± 7.29 46.63 ± 7.65 2624 ± 648
4th course (7th day)	WBC Hgb Platelet Neutrophil Lymphocyte ANC	2284.47 ± 1369.58 11.92 ± .92 90383.33 ± 28554.37 49.1 ± 10.91 50.29 ± 10.68 1148 ± 881	1976.58 ± 380.76 12.07 ± .84 92842.1 ± 11083.29 50.26 ± 4.92 47.68 ± 6.63 3711 ± 968	**8th course (15th day)**	WBC Hgb Platelet Neutrophil Lymphocyte ANC	13300 ± 6704.21 11.52 ± 1.24 193444.44 ± 26144.04 88.94 ± 7.64 10.68 ± 7.39 11861 ± 6426	13448.68 ± 17942.84 11.41 ± .87 194526.31 ± 41315.36 90.05 ± 4.77 9.71 ± 4.79 12133 ± 16165
4th course (15th day)	WBC Hgb Platelet Neutrophil Lymphocyte ANC	4213.15 ± 4968.81 11.61 ± 1.95 131355.55 ± 27377.83 86.52 ± 7.29 13.34 ± 7.21 3563 ± 3892	3415.79 ± 789.98 11.98 ± .78 134000 ± 27496.44 86.79 ± 4.89 13.08 ± 4.74 996 ± 222				

Figure 2 shows the changes in ANC values during eight courses of chemotherapy in PDG and PDL groups. Similar trends of ANC values are notifiable between two groups.

Within and between groups' variation of blood counts was analyzed during start point, 7th and 15th days in each course of chemotherapy. (Table 3) The results showed that in PDL and PGL groups, all of the hematologic components (WBC, Hgb, Plt, Neut, Lymph and ANC) had significant changes during each course of chemotherapy. Decreasing the hematologic component and increasing after GCF injection in each course is the prominent pattern of data variations. But in the fifth course of PDL injection, Hgb showed no significant change (P = 0.095).

Table 3
Comparing within and between groups blood counts measured at the start point, 7th and 15th days in each course of chemotherapy

Courses		PDL Group (Median)			p-value*	PGL Group (Median)			p-value*	p-value**
		D0	**D7**	**D15**		**D0**	**D7**	**D15**		
1	**WBC**	8900	4100	5850	< 0.001	9550	3850	5750	< 0.001	0.295
	Hb	13.20	13.00	13.00	< 0.001	12.80	12.35	12.25	< 0.001	0.159
	Plt	280000	124000	173000	< 0.001	248500	124000	168000	< 0.001	0.416
	Neut	75	50	85	< 0.001	70	50	85	< 0.001	0.312
	Lymph	25	50	15	< 0.001	27	50	15	< 0.001	0.130
	ANC	6545	2050	5025	< 0.001	6830	1660	4600	< 0.001	0.327
2	**WBC**	5850	3100	4400	< 0.001	5750	3000	4500	< 0.001	0.866
	Hb	13.00	13.00	12.90	< 0.001	12.25	12.20	12.20	< 0.001	0.307
	Plt	173000	111000	156000	< 0.001	168000	107000	156000	< 0.001	0.468
	Neut	85	50	85	< 0.001	85	50	86	< 0.001	0.504
	Lymph	15	50	13	< 0.001	15	50	14	< 0.001	0.243
	ANC	5025	1575	3838	< 0.001	4600	1400	3895	< 0.001	0.950
3	**WBC**	4400	2450	3950	< 0.001	4500	2500	3950	< 0.001	0.820
	Hb	12.90	12.90	12.30	< 0.001	12.20	12.00	12.00	< 0.001	0.107
	Plt	156000	101000	139000	< 0.001	156000	100000	146000	< 0.001	0.302
	Neut	85	50	90	< 0.001	86	50	90	< 0.001	**0.023**
	Lymph	15	50	10	< 0.001	14	50	10	< 0.001	**0.006**
	ANC	3838	1305	1975	< 0.001	3895	1200	1900	< 0.001	0.229

p-value: Repeated measurements within groups (Friedman test)*

*p-value**: Repeated measurements Between groups (GEE analysis)*

4	**WBC**	3950	1900	3100	< 0.001	3950	2100	3400	< 0.001	0.443
	Hb	12.30	12.05	12.05	< 0.001	12.00	12.00	12.00	< 0.001	0.473
	Plt	139000	92500	128500	< 0.001	146000	98000	132000	< 0.001	0.969
	Neut	90	50	90	< 0.001	90	50	90	< 0.001	0.317
	Lymph	10	50	11	< 0.001	10	50	10	< 0.001	0.143
	ANC	1975	955	2738	< 0.001	1900	1020	2872	< 0.001	0.548
5	**WBC**	3100	2500	4500	< 0.001	3400	2500	4900	< 0.001	0.341
	Hb	12.05	12.00	12.00	**0.095**	12.00	12.00	11.50	< 0.001	0.237
	Plt	128500	100000	151000	< 0.001	132000	102000	159000	< 0.001	0.974
	Neut	90	50	90	< 0.001	90	50	90	< 0.001	0.413
	Lymph	11	50	10	< 0.001	10	50	10	< 0.001	0.590
	ANC	2738	1350	3895	< 0.001	2872	1250	4410	< 0.001	0.289
6	**WBC**	4500	3200	6750	< 0.001	4900	3450	6800	< 0.001	0.581
	Hb	12.00	12.00	12.00	0.001	11.50	11.40	11.30	< 0.001	0.206
	Plt	151000	111500	159500	< 0.001	159000	110000	174000	< 0.001	0.919
	Neut	90	50	90	< 0.001	90	50	90	< 0.001	0.663
	Lymph	10	50	10	< 0.001	10	50	10	< 0.001	0.743
	ANC	3895	1725	5948	< 0.001	4410	1900	6120	< 0.001	0.543
7	**WBC**	6750	4200	8400	< 0.001	6800	4600	9100	< 0.001	0.050
	Hb	12.00	11.90	11.50	< 0.001	11.30	11.30	11.30	0.002	0.342

p-value: Repeated measurements within groups (Friedman test)*

*p-value**: Repeated measurements Between groups (GEE analysis)*

	Plt	159500	121000	170500	< 0.001	174000	124000	186000	< 0.001	0.559
	Neut	90	50	90	< 0.001	90	50	90	< 0.001	0.306
	Lymph	10	50	10	< 0.001	10	50	10	< 0.001	0.898
	ANC	5948	2100	7560	< 0.001	6120	2300	8330	< 0.001	0.146
8	**WBC**	8400	4950	10000	< 0.001	9100	5100	10550	< 0.001	0.395
	Hb	11.50	11.35	11.35	< 0.001	11.30	11.25	11.25	< 0.001	0.528
	Plt	170500	124000	186500	< 0.001	186000	124000	194500	< 0.001	0.637
	Neut	90	50	90	< 0.001	90	50	90	< 0.001	0.781
	Lymph	10	50	10	< 0.001	10	50	10	< 0.001	0.838
	ANC	7560	2525	9000	< 0.001	8330	2575	9601	< 0.001	0.434
p-value: Repeated measurements within groups (Friedman test)*										
*p-value**: Repeated measurements Between groups (GEE analysis)*										

Applying GEE analysis showed no significant difference between the trend of hematologic values during the most courses of chemotherapy. There were two exceptions in the third course of chemotherapy regarding Neutrophil count (p = 0.023) and Lymphocyte count (p = 0.006) with lower fluctuations in the PDL group.

Hematologic parameters' changes during 8 courses of chemotherapy, were compared between two groups by Generalized Estimation Equation analysis. The comparison of the WBC (p = 0.527), Hgb (p = 0.075), Platelet (p = 0.819), Neutrophil (p = 0.575), Lymphocyte (p = 705) and ANC (p = 0.675) changes during eight courses of treatment identified no statistically significant difference between two study groups.

Since the probability of neutropenia frequency fluctuates during treatment, the changes in blood parameters were compared between the first four courses of chemotherapy with the second four courses. In the PDG group, the mean value of WBC, Plt, and ANC in the first half of treatment were 6281, 153171, and 3358 and in the second half of 6280, 151064 and 5220, respectively. P-Value of WBC, Plt and ANC the mean difference between two halves was < 0.001, o.543 and < 0.001. In the PDL group, the mean value of WBC, Plt, and ANC in the first half of the treatment were 4446, 154095 and 3249 and in the second half of 6820, 152298 and 5575, respectively. P-Value of the WBC, Plt and ANC mean difference between two halves was < 0.001, o.651 and < 0.001.

Results showed that WBC (p = 0.439), Hgb (p = 0.052), Platelet (p = 0.7), Neutrophil (p = 0.324), Lymphocyte (p = 0.463) and ANC (p = 0.571) changes during two halves of treatment were not statistically significant between the two study groups.

The comparison of side effects between the two groups is shown in Table 4. There was no side effect in 50% of patients of the PDL group compared to 12.5% in the PDG group. The most common side effects in the PDG group were

musculoskeletal pain with a relative frequency of 47.5% compared to 15% in PDL groups. Headache (30%), injection site reaction (25%), leukocytosis (20%), and bone pain (17.5%) were other common side effects in the PDG group.

Table 4
Comparison of very common side effects between two groups

Side effect	PDG N (%)	PDL N (%)	Total N (%)
No	5 (12.5)	20 (50%)	25 (31.25)
Headache	24 (30)	10 (25)	34 (42.5)
Bone pain	7 (17.5)	3 (7.5)	10 (12.5)
Nausea	3 (7.5)	4 (10)	7 (8.75)
Musculoskeletal pain	19 (47.5)	6 (15)	25 (31.25)
Fever	-	1 (2.5)	1 (1.25)
Injection site reaction	10 (25)	1 (2.5)	11 (13.75)
Leukocytosis	8 (20)	4 (10)	12 (15)
Non cardiac chest pain	1 (2.5)	-	1 (1.25)
Anaphylaxis	1 (2.5)	-	1 (1.25)

Three patients were excluded from the study. In the PDL group, two patients were excluded from the study because of fever and neutropenia. The first patient received PDL in two courses of chemotherapy. In the third course, ANC on the 7th day of injection decreased to 490. In the second case of this group, an ANC of 750 was recorded at the end of the third course of chemotherapy. Both patients received antibiotics out patiently, and PDL was not continued. In PDG group one patient was hospitalized because of fever and neutropenia after one course of injection. Her ANC on first course of chemotherapy decreased from 5440 to 70 during 15 days of injection. She had oral mucositis and high grade of fever. After three days of antibiotic therapy in hospital, she was recovered.

Discussion

All of the chemotherapy regimens which were used in this study are those chemotherapy drugs that cause more than 10% neutropenia without GCF. The chemotherapeutic agents which were used in the study had eight courses. The first four courses are completely similar and composed of Doxorubicin, Cyclophosphamide which cause more neutropenia and fever. But the next four courses consisted of just Docetaxol which cause neutropenia much less than the previous courses. The results showed that WBC ($p = 0.439$), Hgb ($p = 0.052$), Platelet ($p = 0.7$), Neutrophil ($p = 0.324$), Lymphocyte ($p = 0.463$) and ANC ($p = 0.571$) changes during two halves of treatment were not statistically significant between the two study groups. Hematologic parameters' changes during the eight courses of chemotherapy identified no statistically significant difference between the two study groups.

In several studies, the efficacy of the PDG drug has been proven. Due to the number of injections administered daily, the drug was slowly released. The Pegylated form of this drug has been effective and safe in clinical trials. Pegylation of drugs improves the clinical level of drugs, such as increased solubility [26] protects the enzyme degradation drug [27], decreases renal clearance [28], physical and thermal stability [29], the longer half-life of antigenicity and toxicity [30]. PDL is a G-CSF quadrilateral conjugate formulation whose efficacy and efficacy are comparable to PDG [31–33]. The half-life of PDL is 12 times longer than the half-life of non-conjunctive drugs. Polyethylene glycol binding to G-CSF

reduces renal secretion and prevents its proteolysis, resulting in an increase in drug levels up to 14 days after single-dose administration. Following the usual chemotherapy, the number of leukocytes and the appearance of CD34 in the peripheral blood after PDL occur faster and sooner than G-CSF [34]. PDG has been used in chemotherapy-induced neutropenic patients and has recently been used in children's neutropenia [32]. In a study by Holmes et al. in 2002, 154 female breast cancer patients were enrolled in the study, 129 of whom received PDL, and 25 received G-CSF. Five patients had unbearable side effects that resulted in discontinuation of the drug, one of these patients suffering from renal insufficiency at a dose of 100 micrograms per kilogram and four others with a dose of at least 30 micrograms had the following side effects: fever, diarrhea, nausea and dehydration. Other side effects seen in all patients were mild to moderate bone pain that was similar to PDL and G-CSF (35%) and 7% of patients needed to use narcotic to relieve their pain [24]. There was no side effect in 50% patients of PDL group compared with 12.5% in PDG group. Also, the most common side effects in PDG group was musculoskeletal pain with 47.5% compared to 15% in PDL groups, wherase, the injection site reaction was the most common side effect with 25% in PDG group. Headache (30% vs. 25%), injection site reaction (25% vs. 2.5%), leukocytosis (20% vs. 10%), and bone pain (17.5% vs. 7.5%) were other common side effects in PDG group.

In a study of 310 adjuvant chemotherapy patients taking Docetaxel 75 mg daily and Doxorubicin 60 mg per square meter of weight surface area for the first day of each cycle for a maximum of 4 cycles, patients on the second day of the cycle received PDL 100 micrograms per kilogram weight were compared with patients who received PDG 5 micrograms per kilogram weight that the results were comparable in two groups and ANC values in both groups were not significantly different and neutropenia with fever was less common in patients who took PDL. The PDL was tolerated and the side effects profile of the two groups was similar [25]. In another study, which compared the multiple doses of PDL with filgrastim randomly in breast cancer patients, a dose of PDL 100 micrograms per kilogram weight had efficacy and a profile of favorable side effects [24]. In a double-blind, phase III trial with a fixed dose of 6 mg PDL, febrile neutropenia was less than G-CSF (13% versus 20%) [3]. Every chemotherapeutic regimen can cause neutropenia, but when the absolute neutrophil counts down below 1000, it causes the risk of fever neutropenia and sepsis which is very high. So most of the authors believe that GCF should be used to prevent the decrease of absolute neutrophil count below 1000.

In a study that examined women who received chemotherapy during pregnancy and received G-CSF and PDL, there was no significant change in the age of birth, embryonic anomalies or child weight and in these patients; myelopoiesis is stopped at the first stage of growth (the promyelocyte/myelocyte stage) [17]. In a study by Calderwood et al. in 2001, splenomegaly was reported in all patients and mild hyperplastic hypertrophy was observed in a few others, and no short-term drug toxicity was observed [2]. As in the previous study stated, these generic drugs which were used in this study have been compared to brand type in previous studies. The authors stated the ANC and platelet count charts have the most valuable things in our curves. But after these, the lymphocyte count is valuable too. The authors chose headache, bone pain and the injection site reaction as the most common side effects of these drugs. we also mentioned other side effects but these three are the most important types which are needed to scrutinize.

Conclusions

Regarding our results, PDL is completely non-inferior in efficacy and also less toxic compared to PDG. Prescribing in single-dose and lower expenses of PDL introduces it as a cost-effective drug in the treatment of chemotherapy-induced neutropenia.

Declarations

Funding

A grant from Pooyesh Darou Company funded the leading research.

Conflicts of interest/Competing interests

Authors have no conflict of interest.

Ethics approval

This research was approved and registered in the Ethics Committee of Breast Cancer Research Center with code number: IR.ACECR.IBCRC.REC.1395.19. Also,

Consent to participate

All participants signed a written Informed Consent.

Consent for publication

Not applicable

Availability of data and materials

All data and materials are available for the journal.

Code availability

This study was registered in the Iranian Registry of Clinical Trials (IRCT) in https://www.irct.ir/ with registration code: IRCT20190504043465N1

Authors' contributions

SN and SH contributed to designing and supervising the project, data analysis, interpretation of data. MA and VK contributed to data collection, managing the project. All authors have participated in providing the first draft of the manuscript and they have approved the final version.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

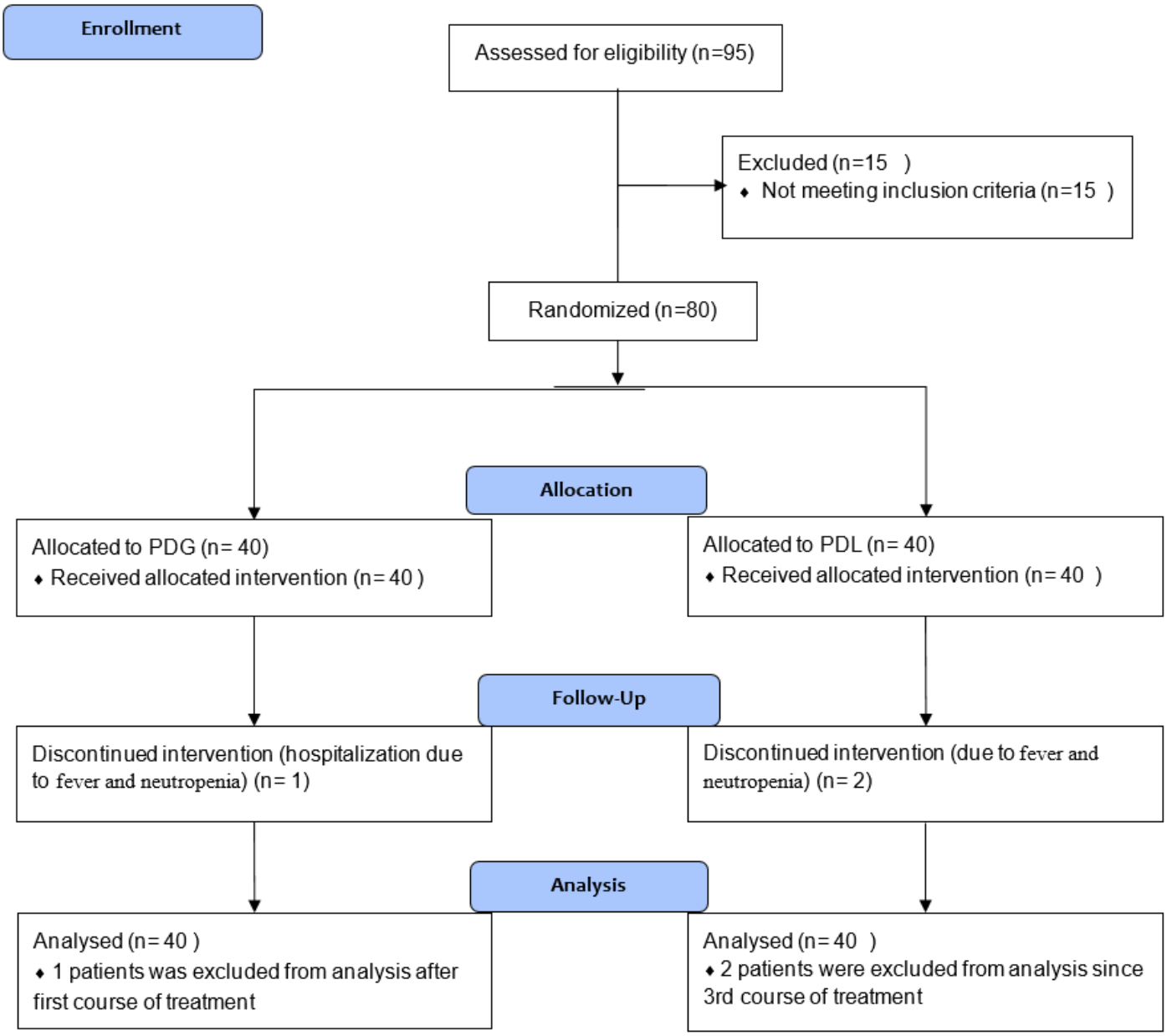

Figure 1

CONSORT flow diagram

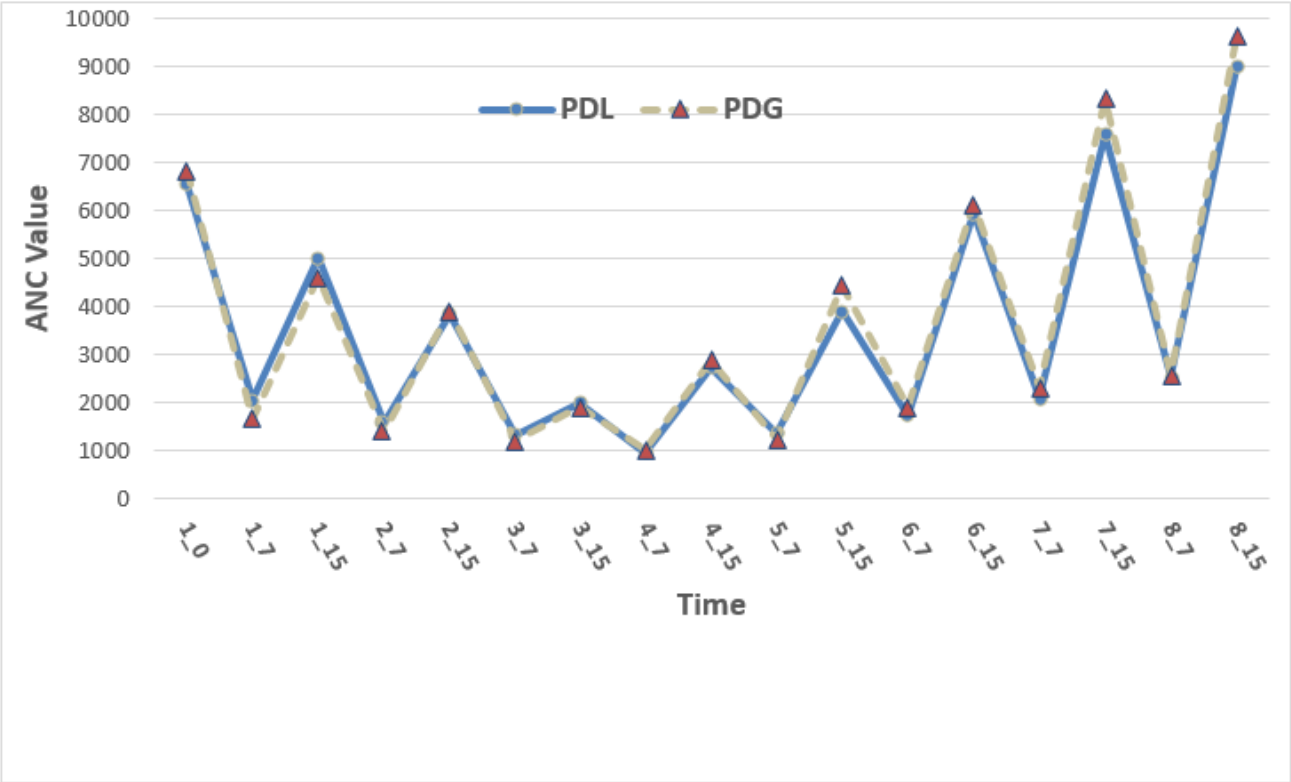

Figure 2

Changes of ANC values during eight courses of chemotherapy between two groups

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