Liposomal Doxorubicin based Chemotherapy in the treatment of Relapsed Diffuse Large B-cell Lymphoma

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Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) is the most frequent type of non-Hodgkin's lymphoma (NHL) in adults. About 30-40% of NHL will suffer from relapse. Although anthracyclines are associated with a high response rate in aggressive NHL, these extended treatment regimens may result in cardiotoxicity and higher incidence of other toxic side effects. Pegylated liposomal doxorubicin (PLD) has been shown to allow for extended treatment with anthracycline in other tumor types, with a much lower cardiac toxicity risk.

The present study aimed to assess the response rate, survival and cardiac toxicity risk of patients with relapsed DLBCL treated with PLD.

Patients and Methods: Thirty patients with relapsed DLBCL were treated with liposomal encapsulated doxorubicin (30 mg/m²) in combination with cyclophosphamide, vincristine and predinisolone (PLD-COP) for 6 cycles in the period between January 2017 to December 2018,with median follow up 17 months (5-36 months). Survival analysis was done using Kaplan-Meier method to determine OS and PFS.

Results: Nine patients had complete response (30%), 15 patients (50%) had partial remission, 5 patients (16.67%) had progressive disease and 1 patient (3.33%) had stationary disease. The progression-free survival was 75.9% at 12 months and 57.3% at 24 months, while overall survival was 83.3% and 65.8% at 12 and 24 months. The median ejection fraction pre and post treatment remained the same denoting a trivial cardiac effect.

Conclusion: PLD offers another choice to patients seeking palliation from their lymphoma recurrence with a response rate of 80% that was well tolerated and had a minimal cardiac toxicity.

Key words: Relapsed Diffuse large B cell lymphoma, Pegylated liposomal doxorubicin, Cardiac toxicity

Introduction:

Non-Hodgkin lymphoma (NHL) is the most prevalent hematologic malignancy worldwide. NHL ranks among the top ten most common malignancies accounting for 2.8% of all new cancer diagnoses globally [1]. Diffuse large B-cell lymphomas (DLBCL) represent the most common lymphoid neoplasms in adults, accounting for approximately 30% of NHL diagnosed annually [2].

Anthracycline-based chemotherapy is the cornerstone in the treatment of patients with NHL. Salvage therapy with an anthracycline-based regimen cannot be used as the cumulative doses of doxorubicin should not exceed more than 450 mg/m² [3]. Cardiotoxicity is still a key medical concern because the cumulative toxicity of anthracyclines is dose limiting and irreversible [4].

Liposomal formulations have been developed with the aim of improving the therapeutic index of doxorubicin by reducing the drug's cardio-toxicity while maintaining its anti-tumor efficacy [5]. Liposomal doxorubicin is a doxorubicin formulation in which the drug is encapsulated in liposomes. Pegylated liposomal doxorubicin (PLD) is a special formula in which a polyethylene glycol layer surrounds the doxorubicin-containing liposome as the result of a process termed pegylation that can delay uptake by the mononuclear phagocyte system and allow for a more prolonged circulation time (t1/2 of approximately 55h) [6]**.** Pegylation can "mask" the agent from the host's immune system (reduced immunogenicity and antigenicity). It increases the hydrodynamic size which prolongs its circulatory time by reducing renal clearance and enhances selective accumulation in the tumor vascular bed [7]**.** This results in 100 fold prolongation in action compared to standard doxorubicin. PLD has been

shown to have improved cardiac safety profiles compared to conventional doxorubicin [6].

This study evaluated the efficacy and safety of replacing doxorubicin with the liposomal formula in relapsed DLBCL patients pre-treated with anthracycline-based compounds which represents a contraindication to the use of standard doxorubicin-based regimens.

The primary endpoint were response rate and toxicity, where response rate determined by physical examination and radiologic assessments using Response Evaluation Criteria in Solid Tumors (RECIST) [8]**.** Secondary endpoints were progression-free survival and overall survival.

Patients and Methods:

This is a prospective, single arm, single center trial carried out in Medical Oncology department, South Egypt Cancer Institute, Assuit University in the period between January 2017 to December 2018,with median follow up 17 months (5-36 months). This study was approved by Institutional Review Board (IRB) and the ethical committee of South Egypt Cancer Institute. Informed consent was signed from patients at the time of enrollment in the study.

Inclusion Criteria were: Patients of both genders age more than 18 years, histological diagnosis of DLBCL according to WHO classification, patients with documented relapsed disease following previous chemotherapy according to criteria proposed by Cheson et al where relapsed disease reflects the appearance of new lesions either after attainment of complete remission after 1 year of CHOP (cyclophosphamide , doxorubicin, vincristine and predinisolone) or after 2^{nd} or 3^{rd} line chemotherapy regardless the duration of treatment [9,10]**.** All patients should have an Eastern Cooperative Oncology (ECOG) performance status of ≤ 2 and adequate bone marrow function with absolute neutrophil count $\geq 1.5x10^9$ /L and platelet count $\geq 75x10^9$ /L, except for those with documented bone marrow infiltration.

Exclusion Criteria included patients resistant to anthracycline (tumor Progression during or within 6 months of treatment completion), CNS involvement, pregnancy or breast-feeding, serious concomitant disorders that would compromise the patient's ability to complete the study, a second primary malignancy, cardiac comorbidity and <45% of left ventricular fractional shortening.

This study was conducted prospectively on 30 patients diagnosed with relapsed DLBCL. Most of patients (19) received PLD-COP after one line of chemotherapy (CHOP), 4 patients after 2 lines and 7 patients after ≥ 3 lines.17 patients received Rituximab with CHOP.

Pretreatment evaluation was full history and clinical examination. In addition complete laboratory investigations were done before starting treatment, with every cycle and then every 3 months during follow-up. Computed tomography of the neck, chest, abdomen and pelvis was arranged pretreatment, after every three cycles of PLD and at the end of treatment.BM aspirate and biopsy were done to all patients before treatment. PET (positron emission tomography) with computed tomography was performed for 10 patients pre and posttreatment. Echocardiography was done pre & posttreatment to estimate the ejection fraction. Ejection fraction should be >45% and was used as a baseline to monitor doxorubicin-induced cardiotoxicity.

Post treatment follow-up after complete response include both clinical and radiological methods according to The National Comprehensive Cancer Network (NCCN) guidelines [11]. Clinical follow-up included history, physical examination and laboratory investigations every 3-6 month for 2 years. Radiological methods included neck, chest, abdomen and pelvis CT scan with contrast every 6 month for 2 years after completion of treatment.

Treatment schedule: The regimen used was COP-PLD. PLD, Cyclophosphamide, vincristine and prednisone were given every 3 weeks for 6 cycles.

PLD evaluated was Caelyx. PLD was used at a dose 30 mg/m² diluted on 500 ml glucose and given as I.V infusion for 1 hour. Cyclophosphamide dose was 750 mg/m² added to 500 ml NaCl and infused intravenously over 1 hour. 1.4 mg/m² of vincristine added to 100 ml NaCl and injected I.V in 10 minutes. Prednisone was given orally in a dose of 60 mg/m² for 5 days.

Toxicities were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 [12].

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 22 [13]. Quantitative data which are normally distributed were statistically described in terms of mean ± standard deviation, while those which are not normally distributed were statistically described in terms of median (Range). Frequencies (number of cases) and percentages were used for qualitative data. Wilcoxon Signed Ranks Test was performed to compare paired data. Kaplan-Meier test [14] was performed to calculate overall and progression free survival.

Results:

Thirty patients were recruited into this study between january 2017 and december 2018. The patients' clinical characteristics are shown in ***Table I***. In summary, the patients were typical for a group with relapsed DLBCL; the median age was 49 years (range 23-57) and 57% of the patients had advanced stage disease at presentation (stage III or IV). All patients had prior treatment with CHOP. At the start of the

study nineteen patients (63.33%) had performance status 2.

Nine patients (30%) achieved complete remission, 15 patients (50%) achieved partial remission, 5 patients (16.67%) had progressive disease and 1patient (3.33%) had Stationary disease ***(table 2)***.

Overall survival 93.3% at 6 months, 83.3% at 12 months, 71.8% at 18 months, 65.8%, at 2 years and 65.8% at 3 years Progression-free survival 89.9% at 6 months, 75.9% at 12 months, 71.7% at 18 months, 57.3% at 2 years and 57.3% at 3 years (***Figure 1 & 2***).

The median dose of Caelyx delivered was 180 mg/m² (30-300 mg/m²). The median dose of non-encapsulated doxorubicin prior to PLD was 350 mg/m². So the median total dose of anthracycline (non-encapsulated doxorubicin and PLD) was 530 mg/m² (380-650 mg/m²).

The main toxic effects observed were mucositis, myelosuppression (neutropenia) and skin toxicity (skin rash) in 3 patients (10%), 2 patients (6.7%) and 1 patient (3.3%) respectively. 1 patient (3.33%) had cardiac toxicity in the form of decrease in ejection fraction >10 %, this was not associated with clinical signs or symptoms of cardiotoxicity. All cases were grade I, except neutropenia was grade II in one patient and received granulocyte colony stimulating factors and also one patient with grade II mucositis.

The median baseline LVEF was 65% ranged 57-74%. Posttreatment median of LVEF didn't show significant changes (p-value 0. 429). Only one patient had a posttreatment ejection fraction of less than 50%.

Table 1: The demographic characters of the 30 relapsed DLBCL patients in the study

Variable	No.	Percent
Median age, years (range)	49 (23-57)	
Sex		
- Male	13	43.33
- Female	17	56.67
Stage at presentation		
- Stage 1	10	33.33
- Stage 2	3	10.00
- Stage 3	14	46.67
- Stage 4	3	10.00
Performance status	11	36.67
- ≤1	19	63.33
- 2		
IPI		
- Low-intermediate	13	43.33
- High-intermediate	6	20.00
- High	11	36.67

Table 2: Response after COP-caelyx:

Response	No.	Percent
Complete remission	9	30.00
Partial remission	15	50.00
Stationary disease	1	3.33
Progressive disease	5	16.67

Table 3: Patterns of treatment related Toxicity

Toxicity	No.	Percent
Not documented	23	76.67
Neutropenia	2	6.67
Oral mucositis	3	10.00
Skin rash	1	3.33
Cardiac	1	3.33
Hand and foot syndrome	0	0.00

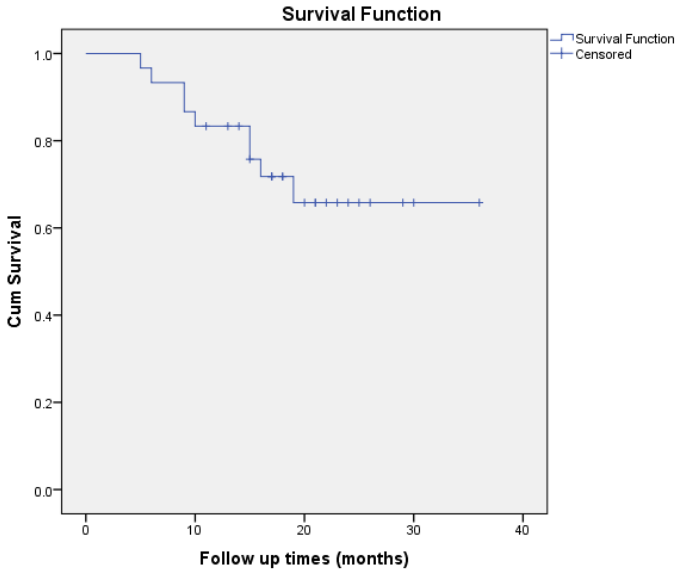

Figure (1): Overall survival analyses (Kaplan-Meier Estimation).

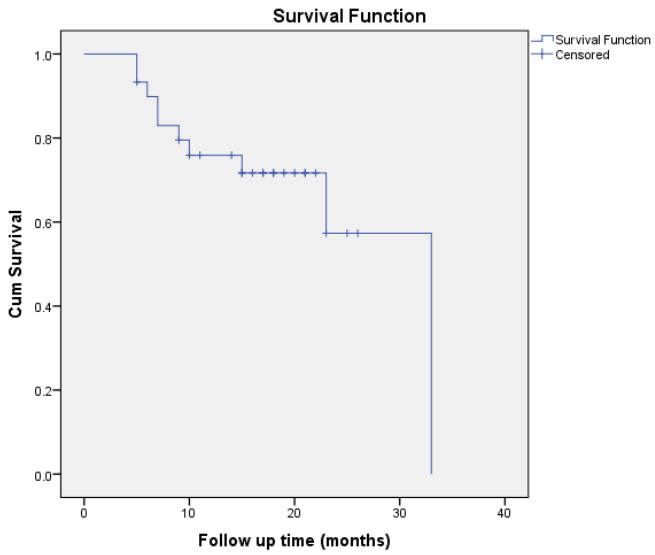

Figure (2): Progression free survival analyses (Kaplan-Meier estimation)

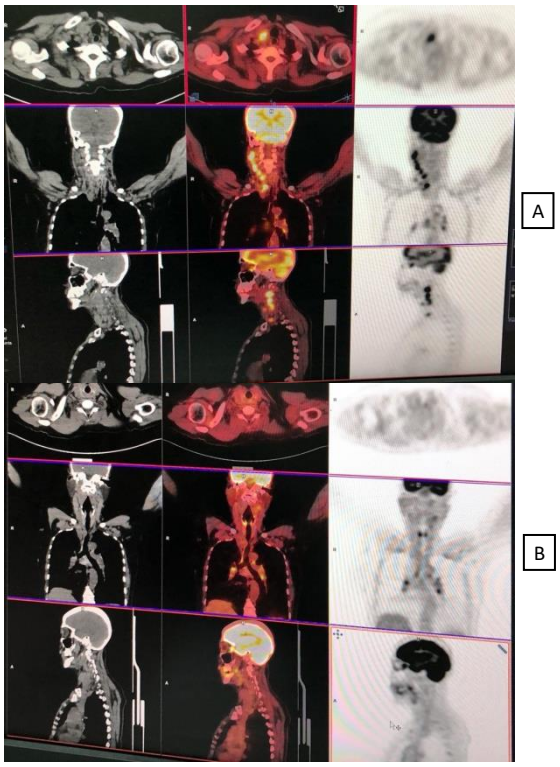

Figure (3): PET assesment of DLBCL case: A-Pre COP-PLD regimen
B- Post COP-PLD regimen

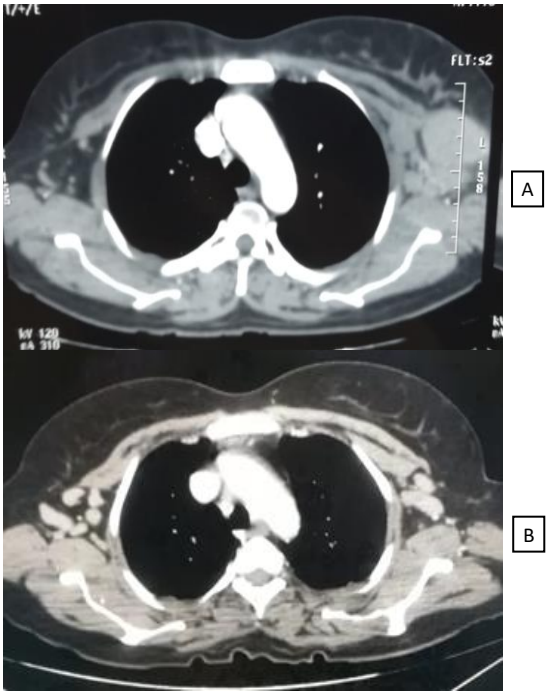

Figure (4): MSCT. Assessment of DLBCL case:
A- Pre COP-PLD regimen : Contrast enhanced MSCT chest shows multiple enlarged left axillary LNs
B- B- Post COP-PLD regimen : Contrast enhanced MSCT chest shows no significantly enlarged axillary LNs

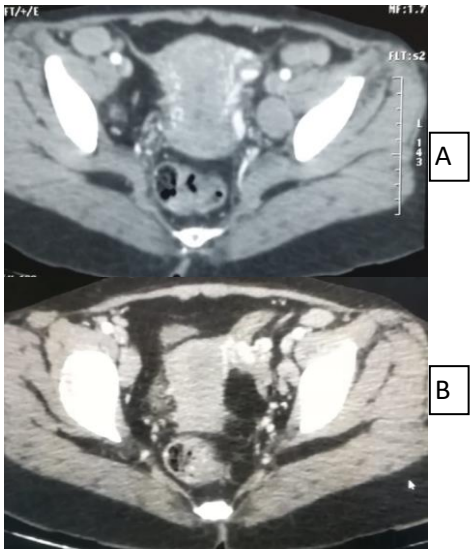

Figure (5): MSCT assessment of DLBCL case:
A- Pre COP-PLD regimen: Contrast enhanced MSCT of the pelvic region shows multiple enlarged bilateral iliac LNs.
B- B- Post COP-PLD regimen: Contrast enhanced MSCT of the pelvic region shows marked reduction in the size of enlarged bilateral iliac LNs.

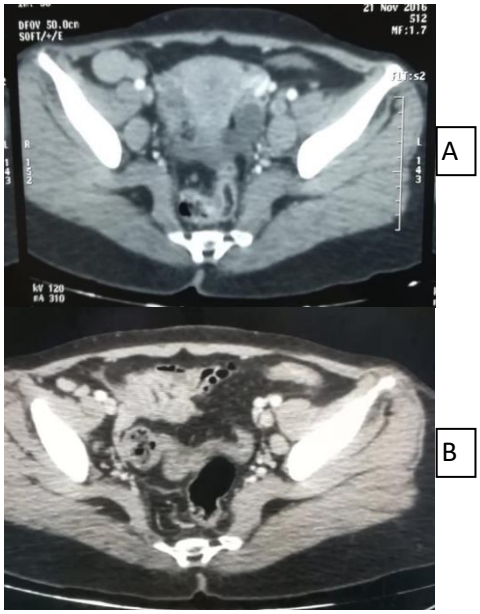

Figure (6): MSCT assessment of DLBCL case:
A- Pre COP-PLD regimen: Contrast enhanced MSCT of the pelvic region shows multiple enlarged bilateral iliac LNs.
B- B- Post COP-PLD regimen: Contrast enhanced MSCT of the pelvic region shows marked reduction in the size of enlarged bilateral iliac LNs.

Discussion:

Liposomal formulations have been developed with the aim of improving doxorubicin therapeutic index by reducing its cardio-toxicity and maintaining its anti-tumor efficacy [5].

The aim of this study was to delineate the efficacy and safety of the replacement of doxorubicin with the liposomal formulation in patients with DLBCL with clinical conditions that would have contraindicated the use of standard doxorubicin-based regimens such as relapsed patients pre-treated with anthracycline-based compounds

In this study, 30 patients diagnosed as relapsed DLBCL were treated with COP-PLD. PLD was used at a dose 30 mg/m². Two studies previously reported the use of PLD as a second line treatment in relapsed NHL where ***Macpherson et al.*** [15] used PLD in a dose of 40-50mg/m² as single agent in 18 patients, 44% were DLBCL and ***Visani et al*** [16]**,** used PDL in a higher dose 40 mg/m² in combinations with COP plus rituximab (5 untreated and 8 pretreated relapsed NHL patients).

Majority of our study patients (80%) showed excellent response where 9 patients (30%) had CR and 15 patients (50%) had PR. This was a better performance than the response rate in ***Macpherson et al.*** [15] study, which reported 23% PR (4 patients) and none had a complete response. This may be attributed to the inclusion of more aggressive subtypes of NHL where Mantle cell represented 39% in which PLD could not give a good response as a single agent. On the other hand**,** ***Visani et al.*** [16] who used PLD in a combination regimen reported similar results 53% CR and 31% PR. However, this study combined the response of untreated (one third of cases) and previously treated cases.

Regarding, non-PLD based regimens used as salvage treatment in relapsed NHL, less response rates were reported. The CORAL study is an international randomized intergroup study conducted in 2012 which evaluated the response using two second-line chemo and immunotherapy combination regimens (R-ICE and R-DHAP), in approximately 500 relapsed or refractory DLBCL patients. The overall response rates (ORRs) were 63% and 64% after R-ICE and R-DHAP respectively, but only 37% of the patients attained CR in both groups. 4-year event free survival rate according to disease status before transplantation was 26% with R-ICE compared with 34% with R-DHAP (P=0.2) and the 4-year OS rate was 43% and 51%, respectively (P=0.3) [17].

COP-PLD regimen used in our study appears to be well tolerated as no major toxicity (WHO grade III/IV) was observed. ***Visani et al***. had no major toxicity too but they gave G-CSF to all patients a as prophylaxis during treatment. ***Macpherson et al***. who used PLD as a single agent but at a higher dose, reported 5/18 grade III neutropenia and 7/18 hand-foot syndrome which necessitated dose reduction in 7 patients.

We did not observe significant cardiotoxicity with the PLD treatment. The median cumulative dose of PLD was 180 mg/m².The mean ejection fraction didn't change with PLD treatment, but one patient did have a decline in ejection fraction to below 50% after 3 cycles of PLD. This patient received 8 cycles of conventional doxorubicin in a cumulative dose of 400 mg/m² and only 3 cycles of PLD (total dose received 90 mg/m²) and progressed. Cardiotoxicity is generally a long-term effect of conventional doxorubicin treatment and the overall risk is related to cumulative doxorubicin dose. The maximum cumulative dose allowed for doxorubicin is 450 mg/m² to avoid cardiac toxicity [18,19]. However, Previous retrospective analysis suggested that doxorubicin associated cardiotoxicity may occur more frequently and at lower doses (≤300 mg/m²) [20]**.** PLD has been shown to have improved cardiac safety profiles compared to conventional doxorubicin.

Overall survival was 93% at 6 months, 83% at 12 months. This was better than Macpherson et al. [15], where overall survival was 50% at 6 months and 39% at 12 months. Similar results were found in Progression free survival analysis. PFS was 89% at 6 months, 76% at 12 months compared to 33% at 6 months and was 28% at 12 months in Macpherson study.

Safra et al. [21] examined the safety of PLD in 42 patients with advanced malignancy. Doses exceeded 500 mg/m² (range 500–1500 mg/m²) and none of the patients had clinical congestive heart failure, while only five patients had a 10% drop in ejection fraction.

PLD was incorporated in first-line chemotherapy in patients with aggressive NHL**.** ***Tulpule et al.,*** examined incorporation of PLD into a CHOP-like regimen with escalating doses starting at high doses of 40 mg/m² in newly diagnosed aggressive NHL. Most of the patients enrolled were treated at a dose of 50 mg/m² per cycle. They reported a high incidence of hematologic toxicity where 23/25 patients had grade 3-4 neutropenia [22].

On the other hand, CHOP and rituximab treatment with PLD substituted for doxorubicin was examined in elderly patients with aggressive NHL. Six cycles of treatment were given with PLD at dose similar to our study 30mg/m² [23]**.** This was well tolerated and no cardiac toxicity was observed.

Another study reported the use of PLD in a dose of 20 mg/m² in combination with gemcitabine, vinorelbine as salvage regimen for patients with aggressive non-Hodgkin's lymphoma. 25 patients with relapsed/refractory NHL (13 had DLBCL) were included together with cases with Hodgkin's lymphoma (10 in number). NHL patients showed a lower response rate than Hodgekin's (36 vs. 80%, P=0.023), with a median PFS 3 month for NHL. Grade III/IV neutropenia was noted in 34% of patients from both types, while no cardiac toxicity were observed except for 2 patients, who developed elevated ST segment without symptoms [24]**.** This regimen appears to be less effective with more toxic side effects than the COP-PLD regimen used in our study.

Conclusion:

Thus, replacement of doxorubicin with the liposomal formulation at small doses 30mg/m² in combination with COP is effective and safe salvage treatment for patients with relapsed DLBCL patients.

List of abbreviations:

NHL=Non-Hodgkin lymphoma
DLBCL=Diffuse large B-cell lymphomas
LD= Liposomal doxorubicin
PLD= Pegylated liposomal doxorubicin
RECIST=Response Evaluation Criteria in Solid Tumors
ANC=Absolute neutrophil count
CTCAE=Common Terminology Criteria for Adverse Events
LVEF=Left ventricular ejection fraction
EF= Ejection fraction
CR=Complete remission
PR= partial remission

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