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THE EFFECT OF CHOP OR RCHOP CHEMOTHERAPY REGIMENT ON D-DIMER LEVELS OF NON-HODGKIN LYMPHOMA PATIENTS

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Abstracts

Background: *One of the therapeutic modality in patients with Non-Hodgkin's lymphoma (LNH) is chemotherapy. Chemotherapy drugs given to Non-Hodgkin's lymphoma patients could lead to hypercoagulability state due to damage of tumour and endothelial cells. The hypercoagulable state can increase the thromboembolic event rates in Non-Hodgkin's lymphoma patients receiving chemotherapy.*

The impact of chemotherapy drugs was measured by D-dimer examination as a marker of coagulation cascade activation and fibrinolysis.

Objective: *To determine the effects of CHOP or RCHOP chemotherapy on D-dimer levels in Non-Hodgkin's lymphoma patients.*

Material and Methods: *This was an observational analytic study with pre- and post-test studies. Followed by 30 Non-Hodgkin's lymphoma patients who have never received chemotherapy. D-dimer level was measured by ELFA examination before and after 3 cycles of CHOP or RCHOP chemotherapy.*

Results: Most of the patients were men with mean age 49 years, stage III, and histopathology classification of International Working Formulation diffuse of large cell intermediate grade also undergoing RCHOP chemotherapy. The mean D Dimer level of pre chemotherapy was 890.92+ 436.44 ng/ml and the mean of post chemotherapy D-Dimer level was 1714+ 789.34 ng/ml. Wilcoxon sign rank test on D Dimer level pre and post chemotherapy showed significant difference. Analysis of pre and post chemotherapy D Dimer in different regimen namely CHOP and RCHOP also showed significant differences but tended to be higher in RCHOP regimen (p= 0.015).

Conclusion: *There was a significant increase in post-chemotherapy D Dimer level in patients receiving CHOP or RCHOP regimen*

Keywords: *chemotherapy, D-dimer, Non-Hodgkin's lymphoma, hypercoagulation, thrombosis.*

Introduction

Patients with non-Hodgkin's lymphoma (LNH) often come with an advanced disease state suggesting chemotherapy is the only option available. Some chemotherapy drugs contribute to increase coagulation activity in patients with malignancy. Increased coagulation activity elevates the likelihood of thromboembolism, also increases the morbidity and mortality of cancer patients [*Caine GJ et al., 2002, Lechner D et al., 2007*]. Although it has been widely mentioned in other studies but recently in Indonesia, there has been no study specifically to evaluate the relationship between chemotherapy and thrombotic risk in non-Hodgkin's lymphoma patients.

Some studies mention the high incidence of thrombosis in cancer patients given chemotherapy. Research conducted by Zhou X et al. (2010) found that 72 of the 422 lymphoma-treated chemotherapy-based patients doxorubicine or methotrexate experienced episodes of thromboembolism in the form of deep vein thrombosis (DVT), pulmonary embolism, or both [*Zhou X et al., 2010*]. In the end it prolongs hospitalization, causes delayed cancer therapy, and adds unnecessary maintenance costs. Some of these patients require re-hospitalization

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due to bleeding or recurrent thromboembolic attacks. The burden of expenditures due to the incidence of thromboembolism spends more than 20,000 US dollars [*Lyman GH, Khorana AA, 2009*]. Episodes of thromboembolism due to chemotherapy might even cause deaths from arterial occlusion such as myocardial infarction and stroke as reported by Dieckmann KP and co-authors (2010). Therefore, without enough anticipation to this problem, the morbidity, mortality and the cost of treatment will steady increase.

The pathogenesis of thrombosis in cancer-related patients given chemotherapy drugs was still unclear but is suspected to be associated with tumor cell lysis and toxicity of chemotherapy drugs to endothelial cells. Both of these lead to activation of intrinsic and extrinsic clotting factors, platelet activation and decreased fibrinolytic activity resulting in thrombosis. Increased incidence of thromboembolism in cancer patients given chemotherapy drugs might occur even in the first exposure [*Mallat Z et al., 2000; Berckmans RJ et al., 2001; Chirinos JAet al., 2005; AdamsRL, Bird RJ, 2009; Torrisi JM et al., 2011*]. However, it is still need an evidence about coagulation activity in LNH patients due to 72% of thromboembolic events in these patients occur during chemotherapy [*Mohren M et al., 2005*].

Coagulation activation can be measured by various tests including fibrinopeptide A (FPA), prothrombin fragments (F1 + 2), thrombin-antithrombin complex (TAT), fibrinogen degradation product (FDP) and D-dimer. D-dimers were markers that generally indicate the activation of hemostasis and fibrinolysis. This marker was the fibrin degradation product produced when crossed linked fibrin was broken down by plasmin-induced fibrinolytic activity [*Ay C et al., 2012*]. Compared to other markers, D-dimers have several advantages, among others, daily, fast, with good sensitivity and specificity. With these considerations, the researchers chose to use D-dimer as a marker of coagulation and fibrinolysis activation. The research was still needed to evaluate the effects of chemotherapy (RCHOP or CHOP) on the level of D-dimer that marks the state of hyper coagulation in patients with non-Hodgkin's lymphoma. The regimen CHOP consists Cyclophosphamide, Doxorubicin, Oncovin and Prednison, while RCHOP regimen consist same drugs plus adding Rituximab for CD20+ LNH patients. If it was proven that chemotherapy was associated with hypercoagulation that increases the risk of thrombosis, certain actions could be taken to prevent the occurrence of such complications.

MATERIAL AND METHODS

Patients

There were 32 non-Hodgkin's lymphoma patients who had never undergone chemotherapy/radiotherapy before and will undergo chemotherapy CHOP or RCHOP in RSUD dr. Soetomo Surabaya. Out of the 32 samples, 2 patients dropped out because 1 patient died and 1 patient refused to continue their participation in the study so that 30 LNH patients were still subjected to the study.

Protocol

New non-Hodgkin's lymphoma patients diagnosed by clinical examination, physical examination and histopathology, fulfill both inclusion and exclusion criteria examined for D-dimer prior to chemotherapy. After undergoing chemotherapy for 3 cycles, the patient underwent a D-dimer re-examination. The data obtained were analyzed statistically.

D-dimer checking

D-dimers, as a marker of hypercoagulation in people with non-Hodgkin's lymphoma, were fibrin degradation product markers produced when cross-linked fibrin was broken down by plasmin-induced fibrinolytic activity [*Ay C et al., 2012*]. This check was performed before chemotherapy CHOP or RCHOP first cycle and after chemotherapy CHOP or RCHOP third cycle. D-dimers ware examined from peripheral blood by ELFA (enzyme linked fluorescent assay) method and use VIDAS D-dimer Exclusion kit produced by Biomerieux SA, unit ng/mL. The examination was done at the laboratory of the PRODIA clinic. This research has been approved by ethical clearence committe of Dr. Soetomo General Hospital

Statistic Analysis

Data analysis were using SPSS Statistic 20. In normal data distribution the difference of D-dimer pre and post chemotherapy analyzed using paired t-test. Wilcoxon signed-ranked test were used to analyze whether there is difference in each group (CHOP vs RCHOP) pre and post chemotherapy D Dimer [*Dahlan M, 2012*].

Results

Characteristics of Research Subject

There were 30 LNH patients, aged 25- 73 years old with mean 49 years and the standard deviation of 13 years. Majority of patients were male (63.3%). Of the 4 stages of LNH patients, the study sample consisted of stage II and III, most patients were in stage III (73.3%) while stage I and IV were not present. Of the grade of malignancy, the most common was intermediate grade (56.7%) but no low-grade LNH patients were found. Immunohistochemical results (IHC) of open biopsy materials were mostly CD20+ (60%) and finally underwent RCHOP chemotherapy (56.7%). In the group receiving CHOP chemotherapy, there was 1 CD20+ patient who was supposed to get the RCHOP regiment, but because the financial problem finally did not get Rituximab (R). Data on the subjects' characteristics are described in table 1.

Table 1.

Patients' characteristics

Characteristic	Total	Percentage
Sex :		
Male	19	63.3%
Female	11	36.7%
Age: Range (25 – 73) Year Old		
Age groups:		
21 – 30 Years Old	2	6.6%
31 – 40 Years Old	6	20%
41 – 50 Years Old	8	26.6%
51 – 60 Years Old	7	23.3%
61 – 70 Years Old	5	16.6%
71 – 80 Years Old	2	6.6%
Mean ± SD	49.167 ± 12.820	
Stage		
I	-	-
II	8	26.7%
III	22	73.3%
IV	-	-
Classification IWF		
Low grade	0	0
Intermediate grade:	17	56.7%
High grade :	13	43.35%
Immunohistochemical results (IHC):		
CD 20-	12	40%
CD 20+	18	60%
Chemotheraphy :		
CHOP	13	43.3%
RCHOP	17	56.7%

Based on the type of chemotherapy performed by the patient, the result of the characteristic description of the research sample was divided into CHOP and RCHOP group. At CHOP chemotherapy, the highest number were the male patients, younger age (50 years and under), stage III, intermediate and high malignancy, IHC CD20-. In RCHOP chemotherapy, the highest number of male patients, older age (51 years and over) stage III, degree of intermediate malignancy, IHC CD20+. The results of the test using mann whitney test and independent t-test resulted in conclusion that there were differences in the characteristics of patients undergoing CHOP chemotherapy by RCHOP by age and IHC, while other characteristics were concluded there was no significant difference in CHOP and RCHOP chemotherapy as in table 2

Results Measurement of D-dimer Levels of Non-Hodgkin's Lymphoma Patients Before and After Chemotherapy CHOP Or RCHOP

In general, D-Dimer values of patients with non-Hodgkin's lymphoma who underwent chemotherapy either CHOP or RCHOP were increased, mean pre chemotherapy were 890.92 *ng/mL* and post chemotherapy value up to reach 1714.99 *ng/mL* or al-

Table 2.

The descriptive research data based on type of chemotherapy

Characteristics	Chemotherapy		p value
	CHOP	RCHOP	
Gender			
Male	9 (69.2%)	10 (58.8%)	
Female	4 (30.8%)	7 (41.2%)	0.421
Age (years)			
Mean	42.8 ± 10.06	54.0 ± 12.84	0.015
Age groups			
21-30	1 (7.70%)	1 (5.90%)	
31-40	4 (30.8%)	2 (11.8%)	
41-50	5 (38.5%)	3 (17.6%)	0.020
51-60	3 (23.1%)	4 (23.5%)	
61-70	0 (0.00%)	5 (29.4%)	
71-80	0 (0.00%)	2 (11.8%)	
Stage			
II	5 (38.5%)	3 (17.6%)	
III	8 (61.5%)	14 (82.4%)	0.341
Grade			
Low	0 (0%)	0	
Intermediate	7 (53.8%)	10(58.8%)	
High	6 (46.2%)	7 (46.2%)	0.837
Immunohistochemical results (IHC)			
CD20+	12 (92.3%)	0 (0.0%)	
CD20-	1 (7.7%)	(100.0%)	0.00

most 2-fold. The results of D-dimer concentrations of pre- and post-chemotherapy research subjects were described in table 3. Pre- and post-chemotherapy D-dimer levels based on the characteristics of subjects were described intable 4. The table showed that overall D-dimer levels were increasing. D-dimer levels tend to be higher in women, age range 51-60 years, stage II, intermediate grade malignancy grade, and RCHOP chemotherapy.

TABLE 3.

D-Dimer pre and post chemotherapy

D-Dimer	Mean±SD	Median (Range)
Pre-Chemotheraphy	890.92±436.44)	733.5 (289.74-1799.62)
Post Chemotheraphy	1714.99±789.34	1701.5 (421.77-3270.45)

TABLE 4.

The Results of D-Dimer Measurement Based on Patient Characteristics

Characteristics	D Dimer Pre Chemotherapy	D Dimer Post Chemotherapy
Gender		
Male	838.31 ± 402.73	1613.04 ± 659.04
Female	981.80 ± 496.09	1891.08 ± 985.50
Age groups		
21-30	389.15 ± 140.59	673.27 ± 355.67
31-40	973.47 ± 398.71	1639.77 ±794.23
41-50	990.47 ± 492.78	1870.77 ± 746.75
51-60	935.16 ± 524.61	1719.31 ± 977
61-70	848.36 ± 380.93	1875.85 ± 770.2
71-80	698.43 ± 238.97	1941.94 ±391.89
Stage		
I	897.12 ± 508.00	1800.59 ±1010.85
II	888.67± 420.72	1683.86 ±718.33
Grade		
Intermediate	907.99 ± 438.49	1753.05 ± 690.37
High	868.61 ± 450.57	1665.22 ± 930.33

Comparison of D-dimer Levels of LNH Patients Received CHOP and RCHOP

Analysis of D Dimer level in each chemotherapy regimen were described in table 5. Wilcoxon test showed p-value value 0.001 thereby concluding that the D-Dimer values of patients with pre and post conditions of chemotherapy CHOP showed significant differences The median D-dimer pre chemotherapy 679.87 *ng/mL* was lower than after chemotherapy 1147.59 *ng/mL* indicating that there was a significant increase in D-dimer in patients undergoing CHOP chemotherapy. The same conclusion also shown in patients receiving RCHOP regimen with D-Dimer pre chemotherapy was 837.48 *ng/mL* lower than after chemotherapy of 1782.55 *ng/mL*, indicating that there was a significant increase in D-dimer in patients undergoing chemotherapy RCHOP.

DISCUSION

This avarage age of the subjects were 49 years. The mean age was lower compared to other studies in Asia involving 51-year-old LNH [*Park LC et al., 2012*], 63 years [*Sase T et al., 2005*], 60 years [*Kamikura Y et al., 2006*] and 69 years [*Yokoyama K et al., 2012*]. Most patients were aged between 41 to 50 years old. Epidemiological studies in the United States through the SEER program (surveillance, epidemiology, and end result) mentioned the trend of decreasing the mean age of LNH sufferers to the age group of 45-54 years, especially the black group in the 1993-1998 survey year. According to the study, this downward trend was related to immunodeficiency status that could be caused by HIV infection or adult onset of diabetes. Exposure to tobacco smoke acquired at a young age also contributes to a decline in the mean age of patients diagnosed with LNH [*Clarke CA, Glaser SL, 2002*].

TABLE 5.

Analysis of D Dimer level pre and post chemotherapy on CHOP or R-CHOP

Regimen	CHOP		R-CHOP		p value
	Mean±SD	Median (Range)	Mean±SD	Median (Range)	
D dimer pre ChemoTx	960.80 ±512.42	679.87 (488.56 - 1799.62)	837.48 ± 375.91	837.48 (289.74 - 1492.62)	0.001
D dimer post ChemoTx	1626.63 ± 901.98	1147.59 (767.1 - 3270.45)	1782.55 ± 712.68	1811.9 (767.1 - 3270.45)	0.001
ΔD Dimer pre-post	598.75±398.49	506.46 (101.9 - 1425.74)	979.83 ± 555.5	1099 (132.03 - 2053.94)	0.0015

In addition, as in previous studies, in this study the largest participants of male sex with male and female ratio of 1.7: 1. Previous studies by Sase T. (2005), Kamikura Y. (2006), Yokoyama K. (2012), Park L. (2012) and co-authors showed male dominance as the subject of the study with a ratio of 1.6:1, 1.5:1, 1.4:1, 1.3:1, respectively. Male has a high ratio ranging from 1.5:1 and 3:1 in almost all histopathologic subtypes of LNH. The cause of this high ratio, among others, due to exposure to chemicals while working. Farmers associated with LNH due to exposure to pesticides. Printing workers, teachers, carpenters, dry cleaning workers, industrial workers, barbers and hairdressers are at risk for LNH due to exposure to organic solvent containing trichlorethylene (RR 1.23, 95% CI, 1.07 to 1.42). Other types of work that increase the risk of LNH are butcher or meat manufacturing workers and auto mechanics (exposure to benzene) (HR 1.53, 95% CI, 1.05-2.48 and 1.74, 95% CI, 1.19-2.54) [*Skrabek P et al., 2013*].

In our study, most patients were in stage III, intermediate grade, especially diffuse large cell. Our results were similar to that of Park L. (2012), Sase T. (2005) and co-authors for example dominance type diffuse large B cell type lymphoma of 39.6% and 39.5% [*Sase T et al., 2005, Park LC et al., 2012*]. The type of Diffuse large B-cell lymphoma being the most common type of LNH encountered with an incidence that increased 3% -4% per year, regardless of gender, unrelated to a particular race and covering all adult age groups [*Flowers CR et al., 2010*]. Men have an incident 1.5 times higher than for women to suffer from diffuse large B-cell lymphoma [*Flowers CR et al., 2010*] and it also accounts for the majority of diffuse large B-cell lymphoma histopathology types in our study subjects. Another factor that causes high LNH diffuse large B-cell lymphoma type was the low intake of flavonoids from vegetables and fruits. Flavonoid intake may reduce the risk of LNH especially diffuse large B-cell lymphoma with odds ratio (OR) 0.39, 95% CI, 0.22-0.71. Individual clinical conditions that may affect the risk of occurrence of diffuse large B-cell lymphoma are viral infections and diseases and therapies that cause immunosuppression of the immune system. Infectious agents associated with the risk of LNH events are Epstein-Barr virus, Kaposi sarcoma-associated human herpes virus 8, Helicobacter pylori, Chlamydia pistachio, and viral hepatitis C. These infectious agents are responsible for specific mutations in the host which causes lymphomagenesis, antigenic stimulation that stimulates cell proliferation, increased potential for errors during cell replication, as well as decreased immune system (immunosuppression) that stimulates tumor cell growth [*Flowers CR et al., 2010*].

Most of the subjects have aggressive type LNH so chemotherapy became the treatment option. First-line chemotherapy options are CHOP and RCHOP (addition of Rituximab monoclonal antibodies to the CHOP regiment). The addition of rituximab to CHOP increases response rates by more than 90% in indolent and aggressive lymphoma patients [*Flowers CR et al., 2010*]. Studies conducted by the Groupe d'Etude des Lymphomes de l'Adulte in 2002 compared CHOP and RCHOP in patients over 60 years old. The results of this study obtained a complete response rate (CR) was higher in the RCHOP group than CHOP (76% vs 63%, p = 0.0005) and the 2-year survival increased from 57% to 70% (p = 0.007) so RCHOP choice of therapy for LNH derived from B cells [*Feugier P et al., 2005*]. In our study the most chemotherapy regiment was RCHOP.

The highest proportion of LNH patients involved in this study was stage III, in contrast to the research conducted by Sase T. (2005), Kamikura Y. (2006), Yokoyama K. (2012) and co-authors the majority being stage IV. This might be due to the divergence of diagnostic modalities used more fully including CT scan, lymphangiography, skeletal X-ray and bone marrow biopsy, while in RSUD Dr. Soetomo in addition to physical examination, facilities that support to determine the stadium is based on X-ray chest and abdominal ultrasound so that affect the sharpness of staging LNH stage [*Sase T et al., 2005, Kamikura Y et al., 2006, Yokoyama K et al., 2012*].

In this research, the average D-dimer value before chemotherapy was higher than the research done by Sase T et al. (2005) that is 890,92 ng/L [*Sase T et al., 2005*]. This might be due to the high grade histology. High levels of D-dimer prior to chemotherapy suggest a hypercoagulatory state in LNH patients contributing to tumor progression and angiogenesis so that it was indirectly related to patient prognosis [*Sase T et al., 2005*]. High levels of D-dimer in malignant patients were not always associated with thrombus but might reflect tumour biology (highest levels of breast, prostate and in-

testinal cancers) [*Knowlson L et al., 2010*].

D-dimer levels of LNH patients before undergoing chemotherapy turned out to be higher in women. Lee A. (1995), Giansante C. (1994) and co-authors have reported that D-dimer results in women were significantly higher than men and thought to be due to hormonal influences. The estrogen hormone can increase the level of markers of thrombin and fibrin formation and decrease the activity of fibrinolytic inhibitors (plasminogen activator inhibitor 1 PAI-1) [*Teede HJ et al., 2000*]. Hormonal effects on increased coagulation and fibrinolysis activity have been demonstrated in a study by Teede HJ and co-authors (2000) in 42 post-menopausal healthy women who received hormone replacement therapy for 6 weeks [*Teede HJ et al., 2000*]. The results of this study showed an increase of coagulation markers (prothrombin fragments 1 + 2, 0.20 ± 0.06 vs 0.06 ± 0.04 *nmol/L*, p = 0.0005; soluble fibrin, 2.3 ± 0.4 vs 0.25 ± 0.3 *μg/mL*, p = 0.0004), decreased activity of fibrinolysis inhibitors (PAI-1, -0.67 ± 0.16 vs 0.24 ± 0.21 *U/mL*, p = 0.002) and fibrinolysis increase (D-dimer, 24 ± 12 vs -6 ± 8 *ng/mL*, p = 0.02) than placebo, along with a positive correlation between soluble increase in fibrin and D-dimer (r = 0.59, p = 0.02).

D-dimer levels increased with age as in this study was in the age range 41-50 years. These findings differ from those of Rumley A and co-authors (2006) in the British Regional Heart Study involving 3861 men aged 60-79 years. The results of research by Rumley et al found an increase of hemostasis marker and inflammation with age of fibrin D-dimer (90%), CRP (57%), vWF antigen (23%), tissue plasminogen activator antigen (11%), factor VIII 10%), and fibrinogen (8%). Increased D-dimer at increased age reflects increased turn-over fibrin due to endothelial impairment and worsening age-related atherosclerosis [*Lee AJ et al., 1995*]. The differences in the findings of this study may be due to differences in sample populations taken. The authors used samples from normal population, did not suffer from malignancy.

In addition to the above two factors, the increase in D-dimer levels is also higher in the histology of small non-cleaved cells, which is a type of high-grade LNH pathology characterized by rapid turnover of cells and diffuse spread. Sase T and co-authors (2005) in his study concluded that the D-dimer levels increased with increasing degree of malignancy [*Sase T et al., 2005*]. They also suggested that spontaneous TF-induced expression of TF (induced by other causes, eg chemotherapy drugs) arises from inflammatory mediators that lead to high D-dimer levels in lymphoma patients.

The angiogenesis process in LNH increases with the degree of malignancy and high levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)reflects active tumor neovascularization. The components of the hemostasis system affect the angiogenesis responsible for the growth and spread of cancer. The coagulation system affects angiogenesis through two mechanisms, namely dependent and independent. Coagulation-dependent mechanisms are caused by tissue factor-induced generation of thrombin and cross-linked fibrin deposition in the kestraseluler matrix. VEGF and bFGF bind to insolvent fibrinogen in and on the surface of high-affinity fibrinogen or immobilized fibrin. Cross-linked fibrin tissue becomes the foundation of angiogenic peptides to firmly place angiogenesis and stimulate adhesion, proliferation and migration of endothelial cells during angiogenesis. Independent coagulation mechanisms are the production of proangiogenic proteins such as VEGF or bFGF released from activated cells during the coagulation process [*Wrobel T et al., 2006*].

Angiogenesis of the tumor will produce an irregular blood vessel endothelium so that coagulation activation was easy to occur. The more VEGF, the greater the neovascularization, coagulation activation and plasma fibrinogen levels. In addition to VEGF produced by oncogenes, tumor cells that stimulate coagulation cascade will activate platelets and endothelial cells resulting in increased release of VEGF and bFGF. The coagulation process was always followed by the fibrinolysis process so that the results of fibrinolysis activation tests such as D-dimer are also improved [*Wrobel T et al., 2006*].

This type of chemotherapy regiment affects post-chemotherapy D-dimer levels. In our study we found a higher D-dimer result after RCHOP than CHOP. Elice F and co-authors (2008) suggests that bevacizumab (a type of monoclonal antibody to VEGF) in addition to increasing survival rates in patients with advanced disease but increasing the potential for thrombotic events although it is difficult to separate the effects of other potentially thrombotic chemo-

therapy drugs on a chemotherapy regiment. This suggests that the more components of chemotherapy drugs used in one regiment the higher the potential for thrombosis.

In our study we found that the initial D-dimer values were higher in stage II and intermediate grade than in higher stages and histologic degrees. The results of this examination differ from the research conducted by Sase T and co-authors (2005) that higher D-dimer levels in stage IV and high grade. This difference may be due to the presence of other potentially powerful influencing factors but unknown to the researcher.

The patient's D-Dimer values at pre and post chemotherapy conditions (no differentiating regiment type) showed significant differences (p = 0.000). This finding differs from that of Kamikura and co-authors (2006) who studied levels of D-dimer patients with chemotherapy lymphoma. Autors found no significant differences in hemostatic marker abnormalities including D-dimers in patients with pre- and post-chemotherapy lymphoma. This difference may be due to only checking the levels of D-dimer before and serial after first chemotherapy until day 7. The time of post-chemotherapy in Kamikura and co-authors (2006) was not in line with previous research by Ottinger H et al. (1995) on the highest incidence of thromboembolism in the 3rd cycle (64%).

When the chemotherapy type was separated according to the CHOP and RCHOP regiments, the D-dimer levels also increased significantly (p = 0.001 and p = 0.000) but the increase in D-dimer levels was significantly greater in the RCHOP regiment (p = 0.015) than the CHOP regiment. The authors have not found a study comparing the effects of chemotherapy on CHOP and RCHOP on coagulation activity. The difference in D-dimer increase between CHOP and RCHOP is thought to be due to 2 things, namely age and the presence of rituximab drugs. In the RCHOP group it was found that 7 of the 17 patients (41.18%) were over 60 years of age. The advanced age contributes to the improvement of D-dimers as a marker of coagulation and fibrinolysis activation in circulation as suggested by Rumley A et al. (2006).

The mechanism of aging (aging) causes hypercoagulation state consists of several factors: prokoagulan disorders, anticoagulant disorders, fibrinolysis disorders and platelet function disorders. Procoagulants such as FV, FVII, FVIII, FIX, FXIII levels increase with age. Anticoagulants such as protein C and protein S increase without ATIII changes. Although anticoagulants also increase with age but not as high as procoagulants resulting in an imbalance. Plasminogen activator inhibitor 1 (PAI-1) and thrombin activatable fibrinolysis inhibitor (TAFI) are increased so that the fibrinolysis process decreases. High procoagulant, low anticoagulant and inhibition of fibrinolysis accompanied by low platelet threshold for aggregation leads to thrombosis [*Faillace C, De Carvalho JF, 2012*].

Rituximab can lead to the release of proinflammatory cytokines during the process of B cell apoptosis and the formation of human anti-chimeric antibody (HACA) against rituximab which gives rise to the rituximab-HACA complex as a form of rituximab elimination in the host [*Suzuki K et al., 2009; Hansel TT et al., 2010*].

The case of the role of rituximab causing the occurrence of thromboembolism has been reported by Faillace C, De Carvalho JF (2012) in SLE patients receiving rituximab. These patients had no classic risk factors for stroke and no antiphospholipid antibodies (aPL) were found before rituximab was administered. The incidence of thromboembolism appeared 2 weeks after the third rituximab was accompanied by positive aPL test results so that rituximab thought could lead to antiphospholipids syndrome (APS).

Antiphospholipids syndrome is a protrombotic disorder consisting of vascular occlusion and the presence of aPL which is an auto antibody that causes platelet and endothelial activation. Coagulation cascade in APS is triggered by the presence of tissue factor whose expression is increased due to lipopolysaccharide stimulation, complement activation and proinflammatory cytokines. The immune complexes of rituximab and human antichimeric antibody (HACA) will activate complementary processes that lead to hypercoagulation in antiphospholipid syndrome (APS) [*Suzuki K et al., 2009*]. Another explanation that can be explained is that Fc rituximab mediates antibody-dependent cellular cytotoxicity and complement- dependent cytotoxicity that causes platelet aggregation to trigger hypercoagulation state [*Dada R et al., 2016*].

Due to time limitations of the study, we could not found a study sample of early-stage LNH patients. This study used a chemotherapy regiment

consisting of a combination of several drugs thus it was difficult to determine the chemotherapy drugs that most contribute to increased levels of post-chemotherapy D-dimer. This study could do not control all confounding variables (age, sex, hyperglycemia during chemotherapy).

CONCLUSION

This study subjects average age were 49 years old with the distribution of age most in the group 41 -50 years. Male gender were majority. The malignancy grade were intermediate grade, based on histopathology most was the diffuse large cell whereas for the stadium based on Ann Arbor criteria the most was stage III. Chemotherapy obtained by most research participants was RCHOP. The mean rate of pre-chemotherapy D-dimer (regardless of chemotherapy regiment type) was 890.92 ± 436.44 ng/mL whereas post-chemotherapy was 1714.99 ± 789.34 ng/mL. Pre- and post-chemotherapy D-dimer levels increased significantly with p = 0.000. The increase in post-chemotherapy D-dimer levels in LNH patients receiving RCHOP regiments was significantly higher than the CHOP regiment (p = 0.015). The hypercoagulant effects caused by the RCHOP chemotherapy regiment were stronger than the CHOP regiment.

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