RESEARCH ARTICLE

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The Impact of Tumor Hypoxia Modulation on sIL-2R Levels in Newly Diagnosed Diffuse Large B Cell Lymphoma (DLBCL) Patients Undergoing Chemotherapy: A Randomized Clinical Trial

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Abstract

Objective: Tumor hypoxia induces the production of Hypoxia-Inducible Factor (HIF)-1 alpha, which interacts with NF-kB, leading to cancer proliferation and metastasis. This study investigated the effect of tumor hypoxia modulation using carbogen (95% O2 and 5% CO2) and nicotinamide on reducing soluble interleukin-2 receptor (sIL-2R) levels in newly diagnosed DLBCL patients with tissue overexpression of HIF-1α ≥10%. **Material and Methods:** A prospective randomized controlled clinical trial was conducted at Dr. Kariadi Hospital in Semarang, Indonesia, from 2021 to 2022. Newly diagnosed DLBCL patients with tissue HIF-1α ≥10% were randomized into an intervention group (nicotinamide 2,000 mg + carbogen 10 liters/min during R-CHOP) and a control group (R-CHOP alone) for one cycle. sIL-2R levels were measured in the blood before and after intervention. **Results:** The intervention group showed a significant reduction in sIL-2R levels after chemotherapy (p=0.026), with 85% of samples exhibiting a decrease. In contrast, only 45% of samples in the control group demonstrated a decrease in sIL-2R levels (p=0.184). The median sIL-2R level decreased from 139.50 pg/mL to 70.50 pg/mL in the intervention group, while the control group exhibited an increase from 182.50 pg/mL to 250.00 pg/mL following one cycle of chemotherapy. **Conclusion:** Tumor hypoxia modulation led to a significant decrease in serum sIL-2R levels, potentially through improvements in the crosstalk between hypoxia and inflammation pathways.

Keywords: lymphoma- chemotherapy- treatment- soluble interleukin-2 receptor

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Introduction

Non-Hodgkin Lymphoma (NHL) is the most prevalent hematologic malignancy, with an estimated 77,240 new cases reported in the United States in 2021. Among the various subtypes of NHL, Diffuse Large B Cell Lymphoma (DLBCL) accounts for approximately 30% to 40% of these cases, representing the most common subtype [1]. DLBCL encompasses a diverse array of biological entities that lead to aberrant clonal proliferation of B cells from germinal or post-germinal centers.

Hypoxia, characterized by lower-than-normal oxygen levels in tissues ranging from 4% to 9%, is a distinctive feature observed in solid tumors. It serves as an adverse prognostic indicator in various cancers, including prostate, cervix, breast, head, and neck cancers [2]. This condition triggers the activation of Hypoxia-inducible factor-1α (HIF-1α) transcription factor and other adaptive molecules in pathways to maintain cellular function [3]. HIF-1α serves as a pivotal regulator of gene transcription, controlling the expression of over 200 genes in response to cellular hypoxia. The genes regulated by HIF-1α influence cancer growth, metastasis, and contribute to therapy resistance, establishing hypoxia as a well-known barrier to cancer treatment. Inflammatory processes, a hallmark of cancer, are closely linked to hypoxia in various pathological conditions [4]. Nuclear Factor-κB (NF-κB), a transcription factor associated with inflammation, engages in extensive and intensive interactions with HIF-1α. Additionally, NF-κB can be directly activated by hypoxic

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conditions [5].

The soluble form of the interleukin-2 receptor (sIL-2R) is expressed on the membrane of various cancer cells, including lymphoma. Several studies have associated higher levels of sIL-2R in DLBCL with larger tumor sizes, greater burdens, and worse clinical outcomes [6, 7]. The transcription factor NF-κB is directly and indirectly linked to sIL-2R levels, as IL-2R is a direct target gene of this transcription factor. Additionally, NF-κB plays a role in the activation of tumor-associated macrophages (TAM), which release cytokines such as matrix metalloproteinase (MMP) 1, MMP3, and MMP9, leading to the cleavage of IL-2R into its soluble form (sIL-2R) [8]. In this study, we aimed to assess the effectiveness of modulating tumor hypoxia through the inhalation of carbogen (95% O2 and 5% CO2) and nicotinamide in reducing sIL-2R levels in newly diagnosed DLBCL subjects with tumor hypoxia characterized by tissue overexpression of HIF-1α ≥10%.

Materials and Methods

This prospective randomized open-label true experimental pre-post-test control group study was conducted at Dr. Kariadi Hospital in Semarang, Indonesia, between 2021 and 2022. The research subjects were newly diagnosed DLBCL patients confirmed morphologically and immunohistochemically. Pathological anatomy examinations and HIF-1α immunohistochemical expression were analyzed by two independent hematopathologists.

The trial protocol and any modifications received approval from the institutional ethics body. Prior to enrollment, all patients provided written informed consent. The trial was conducted in adherence to the approved protocol, its amendments, and Good Clinical Practice standards. The clinical trial was registered with the International Standard Randomized Controlled Trial Number (ISRCTN) database under registration number ISRCTN77237304, demonstrating our commitment to transparency and accountability in clinical research by providing a publicly accessible record of the trial's design and protocol.

Inclusion and exclusion criteria

Patients who met the eligibility criteria were assessed based on the study's inclusion and exclusion criteria. Inclusion criteria comprised newly diagnosed DLBCL patients with immunohistochemical tissue examination showing increased expression of HIF-1α (> 10%); aged between 18 and 65 years; absence of anemia (Hemoglobin ≥ 11 g/dl); absence of obstructive lung disease (normal chest x-ray and/or spirometry); absence of heart disorders (normal electrocardiogram (ECG) and/or echocardiography left ventricular ejection fraction (LVEF)); absence of cerebrovascular disorders; no severe liver dysfunction; no severe kidney dysfunction; absence of diabetes mellitus (fasting blood glucose < 126 mg/dL or random blood glucose < 200 mg/dl); not receiving metformin or metronidazole therapy; absence of infection or inflammation; and willingness to participate in the study.

Exclusion criteria included Eastern Cooperative Oncology Group (ECOG) score ≥ 2; current pregnancy; previous chemotherapy for LNH cases; allergic reactions to chemotherapy treatment.

Sample population

Eligible participants for the research were invited to a designated location where the principal investigator provided detailed information and explanations about the research procedures. Participants received written approval from the researcher confirming their eligibility. Prior to engaging in the study, participants underwent preliminary examinations, including a review of medical history, a comprehensive physical examination, ECG, echocardiography if necessary, chest X-rays, and spirometry to assess lung function. Additional tests included LDH levels, kidney function assessments (urea, creatinine), and liver function evaluations (total bilirubin, direct/indirect bilirubin, SGOT, SGPT). Screening for hepatitis B and anti-HCV was also conducted to ensure participant safety and eligibility. If any contraindications or health issues were identified, appropriate measures were taken, and participants were informed accordingly. Subsequently, eligible participants underwent further examinations and participated in the research, involving one cycle of chemotherapy.

Randomization

Randomization was conducted using a random number table generated using Microsoft Excel, and eligible patients were assigned to their respective groups based on the randomization schedule.

Intervention

The intervention group received an oral supplement of 2000 mg nicotinamide orally 2-3 hours before chemotherapy. Additionally, they underwent carbogen gas inhalation (95% O2 and 5% CO2) starting 10 minutes before chemotherapy and continuing until the completion of the chemotherapy procedure. Inhalation was administered through a modified non-rebreathing mask at a flow rate of 10 liters/minute to minimize room air inhalation during carbogen administration. Conversely, the control group only underwent chemotherapy without any supplementary interventions.

sIL-2R Measurement

Serum samples were collected and stored following the guidelines of Quantikine® ELISA Human sIL-2R R&D System, Inc. in Minneapolis, USA. The process involved retrieving serum stored at room temperature, followed by centrifugation and sedimentation. A specially prepared microplate with monoclonal antibodies for sIL-2R was used. Each well on the microplate was filled with diluent RD1F and the serum to be examined. After sealing and a 2-hour incubation, well contents were discarded, and the microplate was washed. Subsequent steps included refilling wells with conjugate, incubation, washing, adding substrate solution, and further incubation. Stop solution was then added. Measurement results were obtained by reading the optical density (OD) of each well using a

micro ELISA Reader. sIL-2R levels were calculated using a standard curve as a regression curve from OD values. This standardized and measurable method provided an assessment of sIL-2R levels in serum samples.

Measurements

The initial assessment involved the examination of venous blood samples and magnetic resonance imaging (MRI) of the largest tumor region. The measurement of serum sIL-2R levels was conducted immediately before the intervention and chemotherapy using commercially available kits and once again one week afterward. The study focused on observing the effects of the intervention during one cycle of R-CHOP chemotherapy. Adverse events were monitored during the intervention and one week thereafter, with grading based on the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0 [9].

Several other variables were assessed, including age, Ann Arbor staging, tumor volume measured using the McGill University method [10], National Comprehensive Cancer Network International Prognostic Index (NCCN-IPI) score, subtypes using Hans Algorithm [11], immunohistochemistry expression of B-cell lymphoma 2 (BCL-2) and Myc in tumor tissue, bulky disease (≥7.5 cm), extranodal status, primary extranodal involvement, ECOG performance status, and B symptoms. B symptoms refer to a set of common symptoms such as fever above 38°C, excessive night sweats, and a weight loss of 10% or more of body mass within the previous 6 months.

Outcomes

The primary objective of the study was to assess the impact of a one-cycle intervention on serum sIL-2R levels in patients newly diagnosed with DLBCL and tissue overexpression of HIF-1α. Secondary outcomes included evaluating clinical and laboratory correlations with serum sIL-2R levels. Safety assessments were conducted across both groups, encompassing all patients involved in the study.

Statistical Analysis

Numeric data with a normal distribution were reported as mean ± standard deviation, while non-normally distributed data were reported as median (minimum – maximum range). Categorical data were presented as proportions (percentages). The paired T-test was used to examine the relationship between the intervention and changes in the dependent variable sIL-2R levels within each group, specifically when the data followed a normal distribution. Conversely, the Wilcoxon test (non-parametric) was used when the data distribution was non-normal. Differences between two sets of data in the intervention and control groups were examined using the independent T-test or Mann-Whitney test.

Results

The research was conducted between April 2021 and May 2022. During the participant sample collection period, a total of 83 newly diagnosed DLBCL samples were evaluated for eligibility. Out of these 83 participant samples, 28 did not meet the inclusion criteria, leaving only 55 participant subjects who met the inclusion criteria before randomization.

Participant subjects who met the inclusion criteria were then requested to provide informed consent to participate in the study. Participants underwent screening for HIF-1α immunohistochemical expression. The screening results indicated that 6 subjects had an expression of HIF-1α antibodies < 10%, so they were not included in the randomization. One subject experienced loss of follow-up, and 2 subjects died due to deterioration, resulting in only 46 participant subjects meeting the inclusion criteria at the end of screening, with a total of 40 participants completing the study. Out of these 40 participants, they were grouped into the intervention group (n=20) and the control group (n=20) (Figure 1).

The overall median age of all participants was 57 years (18-65), with a median of 55 years (24-65) in the intervention group and 60 years (18-65) in the control group, showing no statistically significant difference between the two groups. The male gender was slightly more prevalent in both groups without significant differences. There were no significant differences between variables such as ECOG, B-symptoms, cell of origin subtypes, stage, bulky disease status, extranodal status, primary extranodal, NCCN International Prognostic Index, BCL-2 and Myc expression, as well as tumor volume between the two groups (Table 1).

In the initial assessment of serum sIL-2R levels, a non-normally distributed data spread was observed, necessitating the use of non-parametric statistical tests. The intervention group exhibited sIL-2R levels of 139.50 pg/mL (13.00-1,897.00), which were lower compared to the control group with levels of 182.50 pg/mL (24.00-1,625.00). However, no significant difference was observed in the Mann-Whitney test analysis (p=0.82). Following one cycle of chemotherapy, a decrease in the median sIL-2R level was observed in the intervention group to 70.50 pg/mL (13.00-612.00), while the control group showed an increase to 250.00 pg/mL (24.00-1,557.00). Nonetheless, no significant difference in median post-chemotherapy sIL-2R levels was found between the two groups (p=0.08) (Table 2).

In the intervention group, 17 (85%) samples experienced a decrease in sIL-2R levels pre- and post-chemotherapy, with a significant decrease found using the Wilcoxon test (p=0.026). Conversely, in the control group, only 9 (45%) samples showed a decrease in sIL-2R levels (p=0.184). The median Δ decrease in serum sIL-2R levels pre-post chemotherapy was 11.50 pg/mL (-301.00-1721.00) in the intervention group, while the control group exhibited an increase of 41.50 pg/mL (-1,246.00-892.00). A significant difference between the two groups was observed (p=0.01) (Figure 2 and Figure 3).

When considering the decrease of serum sIL-2R as a dependent variable, a total of 26 (65%) research subjects experienced a decrease in serum sIL-2R levels after one cycle of chemotherapy. In the treatment group, 17 (65.4%) subjects showed a decrease in sIL-2R levels compared to only 9 (21.4%) in the control group (p=0.01).

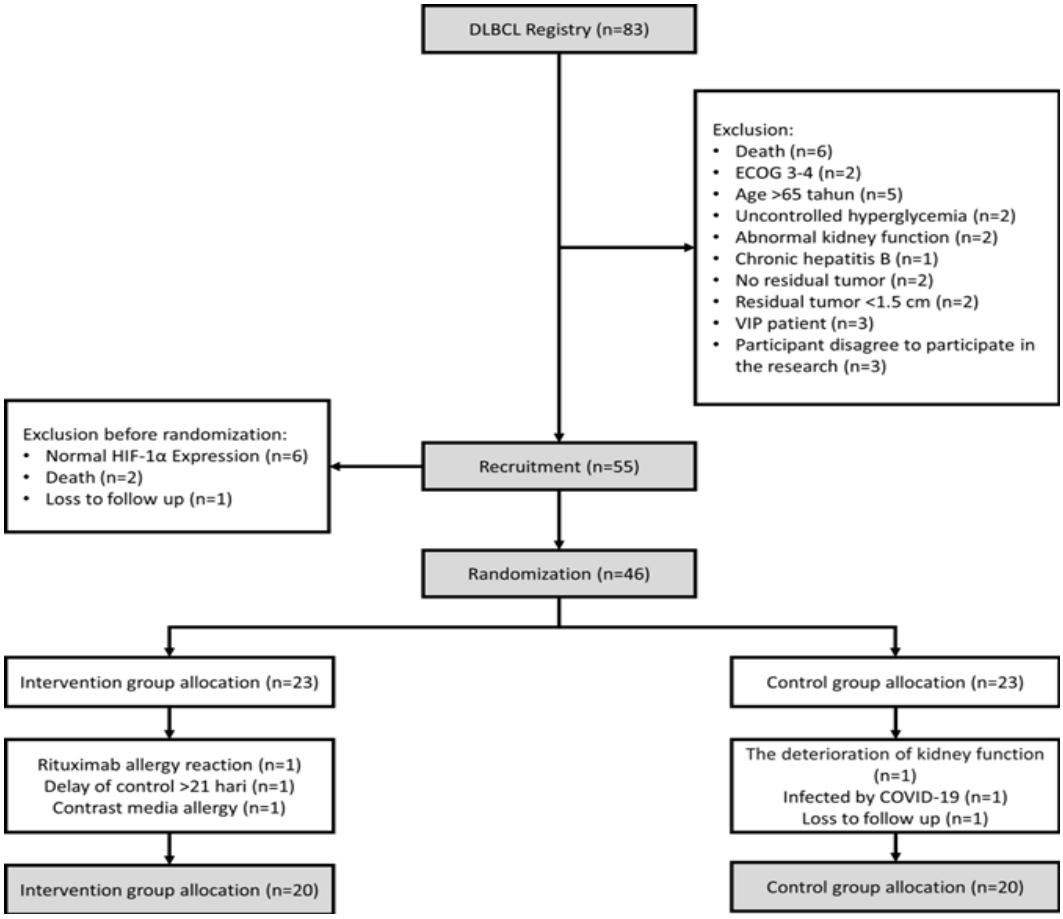

Figure 1. Consort of the Study

Additionally, in the non-GCB group, subjects tended to experience a more challenging decrease in sIL-2R levels compared to the GCB group, with 13 (92.9%) subjects showing an increase in sIL-2R levels from the non-GCB group (p=0.03). Meanwhile, subjects with bulky tumors more frequently experienced a decrease in sIL-2R levels compared to patients without bulky tumors (p=0.04). Multivariate analysis of treatment group, subtype, bulky disease, and Myc expression variables indicated that the intervention group and subtype variables had the most significant impact on the decrease in serum sIL-2R levels (Table 3).

In the correlation analysis of various baseline numeric variables using the Spearman test, a significant positive correlation was found between sIL-2R levels and tumor volume (r=0.36; p=0.02), and a negative correlation was

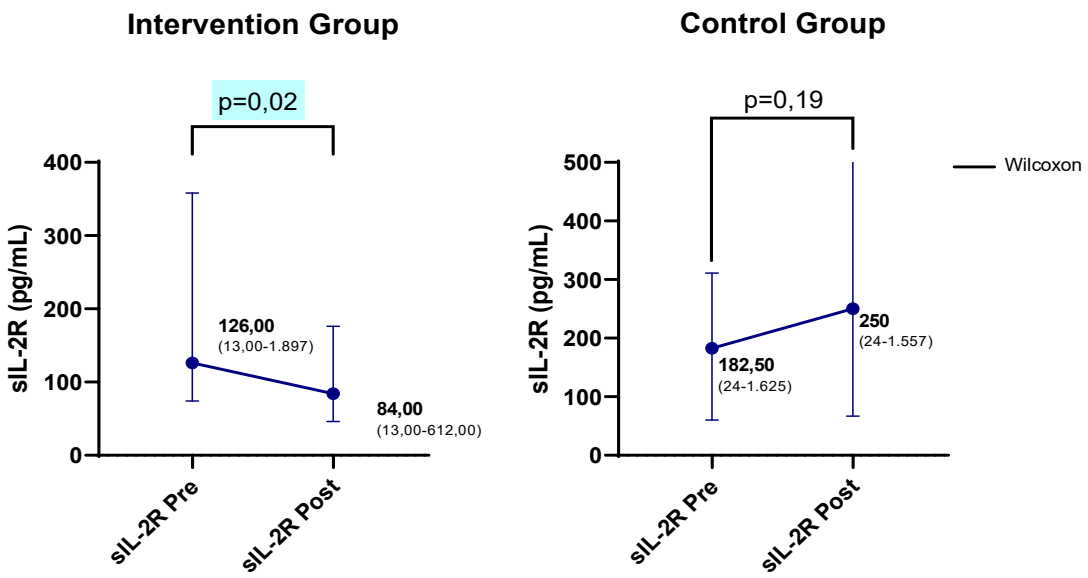

Figure 2. The Response of Carbogen-Nicotinamide Inhalation to Serum sIL-2R

Table 1. Baseline Characteristics

Variable	Group (Median/n, %)		p
	Intervention (n=20)	Control (n=20)	
Age (years)	55,0 (24-65)	60 (18-65)	0.211*
Gender			0.500‖
Male (n, %)	11 (55%)	12 (60%)	
Female (n, %)	9 (45%)	8 (40%)	
ECOG			
0-1	17 (85%)	16 (80%)	0.667*
2	3 (15%)	4 (20%)	
B Symptoms			1‖
Yes (n, %)	17 (85%)	17 (85%)	
No (n, %)	3 (15%)	3 (15%)	
Subtype			0.480‖
GCB (n, %)	4 (20%)	7 (35%)	
Non-GCB (n, %)	16 (80%)	13 (65%)	
Stage			
1 (n, %)	1 (5%)	2 (10%)	0.183*
2 (n, %)	13 (65%)	8 (40%)	
3 (n, %)	4 (20%)	2 (10%)	
4 (n, %)	2 (10%)	8 (40%)	
Bulky Disease			
Yes (n, %)	12 (60%)	11 (55%)	0.500‖
No (n, %)	8 (40%)	9 (45%)	
Extranodal			0.527‖
Yes (n, %)	9 (45%)	12 (60%)	
No (n, %)	11 (55%)	8 (40%)	
Primary Extranodal			1.000‖
Yes (n, %)	6 (30%)	6 (30%)	
No (n, %)	14 (70%)	14 (70%)	
NCCN IPI Score			
Low – Low Intermediate (n, %)	9 (45%)	5 (25%)	0.320‖
High intermediate – High (n, %)	11 (55%)	15 (75%)	
BCL2 Expression			0.182‖
>40% (n, %)	15 (75%)	19 (95%)	
<40% (n, %)	5 (25%)	1 (5%)	
Myc Expression			0.200‖
>40% (n, %)	6 (30%)	11 (55%)	
<40% (n, %)	14 (70%)	9 (45%)	
Haemoglobin (g/dL)	11,95 (10.0-15.60)	11,85 (10,10-14,90)	0.678*
LDH	842 (368-1.722)	809 (416-1.928)	0.799*
Tumor volume (cm3)	63.82 (0.36-1,417)	97.03 (3.65-1,258)	0.583*

*BCL2, B-cell lymphoma 2; ECOG, Eastern Cooperative Oncology Group; LDH, Lactate dehydrogenase; NCCN IPI, National Comprehensive Cancer Network International Prognostic Index; *, Mann Whitney Test; ‖, Wilcoxon test

observed between sIL-2R levels and hemoglobin levels (r=-0.32; p=0.05) (Table 4).

Three participants experienced adverse events in this study. One participant had grade 1 flushing, and two participants had grade 1 rash. All of these events resolved without the need for symptomatic medication within one hour of observation after chemotherapy. No participants reported adverse events that led to the discontinuation of the intervention.

Discussion

Hypoxia, characterized by a decrease in blood oxygen levels, acts as a pivotal physiological and pathological

Table 2. The Response of Carbogen-Nicotinamide Inhalation to Serum sIL-2R in Each Study Group.

sIL-2R Serum Level	Intervention (n=20)	Group (n=20)	P
Pre-test, median (pg/mL) (min-max)	139.5 (13.00-1,897.00)	182.5 (24.00-1.625)	0.82*
Post-test, median (pg/mL) (min-max)	70,50 (13.00-612.00)	250 (24.00-1.557)	0.08*
Post – Pre level			
Negative Ranks	17	9	
Positive Ranks	2	10	
Ties	1	1	
p (Wilcoxon post-pre)	0.01‖	0.19‖	
ΔsIL-2R			
Median (pg/mL) (min-max)	11,50 (-301.00-1,721)	-41.5(-1.246-892)	0.01*

*, Mann Whitney Test; ‖, Wilcoxon test

Table 3. Univariate and Multivariate Analysis of Decrease in Serum sIL-2R Levels

Variable	Decrease in serum sIL-2R levels				
	Univariate analysis			Multivariate analysis	
	Yes (n=26)	No (n=14)	p	HR (95% CI)	p
Group			0.01 *		
Intervention	17 (65.4%)	3 (21.4%)		18.01 (2.52-128.28)	0.01
Control	9 (34.6%)	11 (78.6%)			
Gender			0.63‖		
Male (n, %)	13 (50%)	10 (71.4%)			
Female (n, %)	13 (50%)	4 (28.6%)			
ECOG					
0-1	22 (84.6%)	11 (78.6%)	0.67*		
2	4 (15.4%)	3 (21.4%)			
B Symptoms			1‖		
Yes (n, %)	22 (84.6%)	12 (85.7%)			
No (n, %)	4 (15.4%)	2 (14.3%)			
Subtype			0.03‖		
GCB (n, %)	10 (38.5%)	1 (7.1%)		20.30 (1.56-262.69)	0.02
Non-GCB (n, %)	16 (61.5%)	13 (92.9%)			
Bulky Disease					
Yes (n, %)	18 (69.2%)	5 (35.7%)	0.04‖	4.68 (0.75-29.21)	0.01
No (n, %)	8 (30.8%)	9 (64.3%)			
Extranodal					
Yes (n, %)	14 (53.8%)	7 (50%)	1.00‖		
No (n, %)	12 (46.2%)	7 (50%)			
Primary Extranodal			1.00‖		
Yes (n, %)	8 (30.8%)	4 (28.6%)			
No (n, %)	18 (69.2%)	10 (71.4%)			
NCCN IPI Score					
Low – Low Intermediate (n, %)	8 (30.8%)	6 (42.9%)	0.50‖		
High intermediate – High (n, %)	18 (69.2%)	8 (57.1%)			
BCL2 Expression			0.40‖		
>40% (n, %)	23 (88.5%)	11 (78.6%)			
<40% (n, %)	3 (11.5%)	3 (21.4%)			
Myc Expression			0.17‖		
>40% (n, %)	9 (34.6%)	8 (57.1%)		0.10 (0.051-1.988)	0.13
<40% (n, %)	17 (65.4%)	6 (42.9%)			

*BCL2, B-cell lymphoma 2; ECOG, Eastern Cooperative Oncology Group; NCCN IPI, National Comprehensive Cancer Network International Prognostic Index

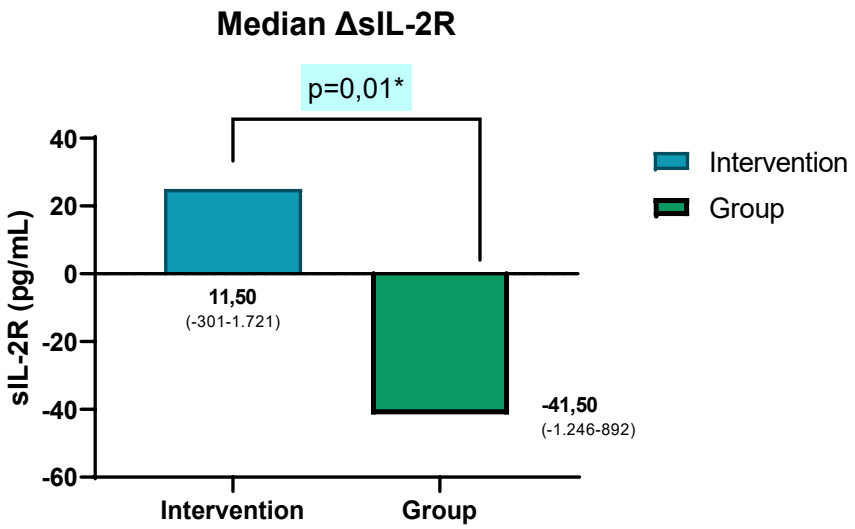

Figure 3. The Difference in Median ΔsIL-2R Serum Levels in Each Group.

Table 4. Spearman Correlation Test between Variables

	sIL-2R	Baseline Tumor Volume	Age	LDH	Hemoglobin
sIL-2R	1	0.36 (p= 0.02)	0.13 (p= 0.44)	0.21 (p= 0.20)	-0.32 (p= 0.05)
Baseline Tumor Volume	0.36 (p= 0.02)	1	-0.01 (p= 0.97)	0.18 (p= 0.26)	-0.16 (p= 0.31)
Age	0.13 (p= 0.44)	-0.01 (p= 0.97)	1	-0.13 (p= 0.41)	-0.11 (p= 0.51)
LDH	0.21 (p= 0.20)	0.16 (p= 0.26)	-0.13 (p= 0.41)	1	-0.12 (p= 0.47)
Hemoglobin	-0.32 (p= 0.05)	-0.16 (p= 0.31)	-0.11 (p= 0.51)	-0.12 (p= 0.47)	1

*LDH, Lactate dehydrogenase; sIL-2R, Soluble interleukin 2 receptor

stimulus in cellular responses. Different tissues experience varying levels of oxygen tension, and when the demand for oxygen surpasses its supply, hypoxia ensues. The regulation of oxygen sensitivity is governed by oxygenase enzymes, namely prolyl-hydroxylases (PHD) and factor inhibiting HIF (FIH). PHD modulates HIF stability by interacting with a ubiquitin ligase complex containing the tumor suppressor von Hippel Lindau (VHL), while FIH regulates HIF's transcriptional activity through interactions with coactivators p300 and CREB binding protein (CBP), thereby influencing HIF's transcriptional activity. However, cellular responses to hypoxia extend beyond HIF, with other transcription factors like NF-κB being induced under hypoxic conditions [12].

In quiescent cells, NF-κB is sequestered in the cytoplasm by binding to the inhibitor κB (IκB) protein family [13], which includes IκB-α, IκB-β, and IκB-ε. Typically, NF-κB activation results from its liberation from IκB molecules by IκB kinase or the IKK complex. Besides being activated by HIF-1α, hypoxia can directly activate NF-κB through PHD and FIH. A seminal study by Cormac Taylor's group demonstrated that PHD can activate NF-κB through its direct involvement in the Inhibitor of κB kinase complex (IKK) activity [14]. Hypoxia and inflammation have been associated with various pathological conditions [15]. Hypoxia serves as an activator for both HIF and NF-κB in an IKK-transforming growth factorβ-activated kinase 1 (TAK1)-dependent pathway. There is a new theory regarding a negative feedback mechanism where HIF can regulate IKK-TAK1 and cell division protein kinase 6 (CDK6) dependent pathways that can activate the NF-κB transcription factor [5].

Furthermore, NF-κB can directly modulate HIF expression in inflammation and hypoxia. The interplay between the HIF and NF-κB pathways is extensive and intensive. This interaction, both functional and physical, responds to various stimuli with overlapping regulators between HIF and NF-κB. Therefore, it is not surprising to find functional involvement of HIF where NF-κB is active, such as in infection and inflammation processes [5]. This intricate interplay presents new avenues for therapeutic interventions in diseases such as cancer, stroke, and inflammatory conditions. While the signaling mechanisms linking cellular hypoxia to NF-κB transcriptional activation are still under investigation, hypoxia acts as a robust activator of the HIF-dependent pathway and a more moderate modulatory stimulus for the NF-κB pathway [16]. The modulation of hypoxia in this study aims to elucidate the cross-talk between hypoxia and

inflammation through the HIF-1α and NF-κB pathways, ultimately impacting the serum marker sIL-2R. As previously discussed, the IL-2R gene is a transcriptional target of NF-κB, which also plays a crucial role in regulating tumor-associated macrophage (TAM) function, leading to the release of various matrix metalloproteinases (MMP1, MMP3, and MMP9) that cleave CD25 into its soluble form, sIL-2R [17]. Elevated levels of serum sIL-2R are prevalent in various hematolymphoid malignancies, including acute lymphoid leukemia, chronic lymphoid leukemia, and multiple myeloma [6]. In hematolymphoid malignancies, the majority of high sIL-2R levels originate from neoplastic cells and their tumor microenvironment, as indicated by Yoshida et al.'s [18] study. This underscores sIL-2R's potential as a marker for tumor burden and disease activity [18]. High sIL-2R levels are associated with poorer progression-free survival (PFS) and overall survival (OS), as demonstrated by Okamoto et al. In their study, the 3-year PFS rates for low and high sIL-2R groups were 60.4% (95% CI, 46.2-71.9) and 37.5% (95% CI, 15.7-59.4%, $p<0.001$), respectively. The 3-year OS rates were 82.2% (95% CI, 69.7-89.9) and 37.4% (95% CI, 13.8-61.4; $p<0.001$) [19]. Similarly, Yoshida et al. [18] reported that higher sIL-2R levels in DLBCL patients were associated with a lower 5-year OS rate compared to those with lower sIL-2R levels, specifically 62% and 76% ($p<0.005$), respectively [18].

The administration of a combination of carbogen (95% O2 and 5% CO2) has dual effects. Firstly, the carbon dioxide component helps maintain tumor blood flow by counteracting oxygen-induced vasoconstriction [20]. Secondly, it enhances oxygen delivery by shifting the hemoglobin-oxygen dissociation curve to the right. While some studies suggest that carbogen breathing improves tumor oxygenation and blood flow, others do not. Additionally, nicotinamide can alleviate tumor hypoxia by improving the intermittent vessel occlusion process commonly observed in hypoxic tumor blood vessels. Several studies indicate that the combination of carbogen inhalation and nicotinamide can ameliorate tumor hypoxia [21, 22].

In this study, the baseline sIL-2R levels in the intervention group were slightly lower than those in the control group, although the difference was not statistically significant. However, the addition of carbogen and nicotinamide to conventional chemotherapy resulted in a significant reduction in sIL-2R levels in the intervention group, a change that was not observed in the control group. The study unveiled a noteworthy positive correlation between serum sIL-2R levels and the initial tumor volume. This finding is consistent with previous research by Kusano et al. [23] which demonstrated a significant positive correlation between sIL-2R levels, larger tumor size, and poorer progression-free survival (PFS) [23]. This correlation supports the notion that activated lymphoma and T cells can produce MMP-9 in their microenvironments, leading to increased sIL-2R levels in the serum. Given that the tumor microenvironment (TME) in DLBCL is abundant in immune response systems mediated by T cells and macrophages, it can be inferred that larger tumors have more extensive TMEs [24].

The precise relationship between sIL-2R and hypoxia remains uncertain. However, according to Yoshida et al., in a case of non-small cell lung cancer (NSCLC) with hypoxemia due to checkpoint inhibitor pneumonitis (CIP), a notable increase in sIL-2R levels was observed [18]. This suggests a potential direct activation of sIL-2R by hypoxia, although conclusive evidence supporting this hypothesis is currently lacking. The role of nicotinamide in reducing sIL-2R levels may be elucidated by an in vitro study conducted by Hu et al. [25] where mice with breast cancer treated with nicotinamide displayed an improved T-cell immune profile [25]. Additionally, a study by Huo et al. [26] indicated a significant decrease in sIL-2R levels in DLBCL patients, which was directly linked to a more favorable prognosis in terms of both progression-free survival (PFS) and overall survival (OS) [26].

In this study, it was observed that the non-germinal center B-cell (non-GCB) subtype faced significant challenges in reducing sIL-2R levels compared to the germinal center B-cell (GCB) group. The non-GCB group can be further classified into activated B cell-like (ABC) and unclassified subtypes. Patients with diffuse large B-cell lymphoma (DLBCL) of the ABC subtype typically exhibit heightened expression of NF-κB target genes in comparison to the GCB subtype. Over 80% of DLBCL cases classified as the ABC subtype harbor genetic abnormalities that instigate abnormal NF-κB activation [27]. Genetic mutations affecting B-cell receptor regulators (CARD11, CD79A, CD79B) and Toll-like receptor signaling pathways (MYD88) can lead to downstream NF-κB activation, as well as the dysregulation of negative NF-κB regulators (TNFAIP3, encoding A20) [28]. Consistent with these findings, a study by Compagno et al. [29] demonstrated that more than 50% of DLBCL patients with the ABC subtype, as well as some with the GCB subtype, carry somatic mutations in various negative (TNFAIP3/A20) and positive (CARD11, TRAF2, TRAF5, MAP3K7/TAK1, and TNFRSF11A/RANK) NF-κB regulatory genes. This complexity may contribute to the greater difficulty encountered by the non-GCB group in achieving a reduction in serum sIL-2R levels [29].

While this study did not directly evaluate the clinical outcomes of each group beyond the first cycle, there is a strong likelihood that reducing sIL-2R levels could positively impact overall outcomes. The study's limitations include the restriction to a single-cycle intervention due to financial constraints. This investigation marks the initial effort to investigate the modulation of sIL-2R levels through the hypoxia pathway and its relationship with the inflammatory pathway. Despite promising initial findings, such as the decrease in sIL-2R levels observed with the addition of carbogen and nicotinamide to R-CHOP chemotherapy, further research involving longer intervention durations and follow-up is imperative to validate these results.

In conclusion, the intervention group exhibited a notable reduction in serum sIL-2R levels, potentially stemming from enhancements in the interplay between the hypoxia and inflammation pathways. This decrease could also be linked to a reduction in tumor mass, suggesting a correlation with sIL-2R levels.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

Scientific Statement

This research is part of the student thesis.

Ethical Clearance

The clinical trial had been registered with the ISRCTN database under the registration number ISRCTN77237304.

Availability Data

Article's data is available upon request to the corresponding author

Conflict of Interest

None declared.

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