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The expression of hypoxia inducible factor-1 alpha in diffuse large B-cell lymphoma (DLBCL) patients: a cross-sectional study in Indonesia

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Introduction: Hypoxia fuels cancer growth by supporting blood vessel formation, suppressing immune response, and helping cancer cells adapt to harsh surroundings. This happens when cancer cells react to low oxygen levels by activating hypoxia inducible factor-1 alpha (HIF-1α). High levels of HIF-1α can indicate an aggressive form of cancer and resistance to treatment in diffuse large B-cell lymphoma (DLBCL) patients. This study aimed to identify which factors are linked to HIF-1α distribution using immunohistochemistry in DLBCL patients.

Method: This study conducted at a hospital in Indonesia between 2020 and 2022 aimed to investigate factors associated with HIF-1α expression in DLBCL patients. Newly diagnosed DLBCL patients were categorized into two groups based on HIF-1α distribution (< 40% and ≥ 40%). Various factors were analyzed between the two groups using statistical tests such as $\chi 2$, Mann–Whitney U, and Spearman correlation tests.

Results: In this study, 40 participants diagnosed with DLBCL were divided into two groups based on their HIF-1α distribution. The group with HIF-1α distribution greater than or equal to 40% had a higher incidence of extranodal involvement, including primary extranodal disease, compared to the group with less than 40% distribution. This difference was statistically significant. The authors also found that haemoglobin level statistically significant ($P = 0.041$) in this research. The Spearman test analysis showed negative correlation between haemoglobin ($P = <0.05$, $r = -0.44$) and positive correlation of soluble interleukin-2 receptor (sIL-2R) ($P = <0.05$, $r = 0.5$) with vascular endothelial growth factor (VEGF), as well as between tumour volume ($P = <0.05$, $r = 0.37$) with sIL-2R. Additionally, there was a positive correlation between VEGF and sIL-2R ($P = <0.05$, $r = 0.5$).

Conclusion: Patients with higher HIF-1α expression (≥ 40%) had more extranodal involvement and primary extranodal disease in this study of 40 DLBCL patients. Haemoglobin level, sIL-2R, and VEGF were also identified as potential biomarkers.

Keywords: diffuse large B-cell lymphoma, Hypoxia inducible factor-1 alpha, cancer, correlation

Introduction

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of lymphoproliferative malignancies with different patterns of behaviour and responses to treatment. The most common form of NHL is diffuse large B-cell lymphoma (DLBCL), which accounts for 30–35% of cases of NHL[1]. DLBCL is an aggressive malignancy and is often diagnosed at an advanced stage[2]. A multicenter epidemiological study of B-cell NHL in Indonesia between 2008 and 2010 from 13 haematology centres showed that there were 170 cases of B-cell non-Hodgkin lymphoma, with DLBCL histology results in 116 cases (68.2%). Another study conducted by the Department of Anatomic Pathology in Yogyakarta from 2010 to 2014 showed that there were 834 cases of lymphoma, with an incidence of 370 cases of DLBCL[3].

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In the 1950s, Tomlinson and Gray first discovered a condition of hypoxia in human tumours. Hypoxia is generally defined as a status in which the partial pressure of oxygen (pO2) in tissues is below the physiological level of about 5–10 mm Hg[4]. The physiological pO2 level in the liver and brain is 20 mmHg, while in the kidney it is 70 mmHg. The result of tissue oxygenation below the physiological level can disrupt the maintenance of normal cell function. Generally, pO2 levels below 10 mmHg will activate hypoxia inducible factor-1 (HIF-1) and other adaptive molecular pathways to maintain cellular function[4,5]. Hypoxia refers to a very low oxygen level, with oxygen levels between 5 and 10 mmHg[4,6].

Various tissues are exposed to different oxygen tensions, and when the oxygen demand exceeds the supply, it can lead to hypoxia. Hypoxia triggers a comprehensive transcriptional program that involves the activation of HIF transcription factors. HIF plays a role in inducing various genes that have roles in angiogenesis, cell proliferation, cell death, and autophagy[7]. Aggressive cancer growth and stromal cells lead to the development of irregular neovascularization (angiogenesis), which results in poor structure, leakiness, and meandering of the blood vessels[7,8]. Temporary occlusion of these abnormal new blood vessels can cause disruptions in blood flow within the tumour tissue, resulting in acute hypoxia or perfusion-related hypoxia. This hypoxic state then stimulates the cytokine vascular endothelial growth factor (VEGF) as the main regulator of angiogenesis, where VEGF is a transcriptional target of HIF-1α and HIF-2α[2,8].

Dysregulation of HIF-1α has been implicated in the development and progression of various diseases, including cancer. In certain investigations, it was discovered that greater tumour sizes in a variety of malignancies, including breast, pancreatic, and renal cell carcinoma, correlated with higher levels of HIF-1 expression[9,10].

Although many studies have shown a poor prognosis for HIF-1α overexpression with survival in diseases such as oesophageal cancer, oral squamous cell carcinoma, lung cancer, and glioma, according to research by Evens *et al.*[11], overexpression of HIF-1α actually showed better prognosis in DLBCL patients who received R-CHOP therapy compared to those with normoexpression of HIF-1α. This can be explained by the fact that HIF-1α expression can also be triggered by reactive oxygen species (ROS) through the regulation of HIF-1α mRNA and protein levels. According to the research by Gupta and colleagues, ROS can increase CD20 expression in Burkitt lymphoma cells. Therefore, it can be concluded that in patients with HIF-1α overexpression, ROS may play a role in increasing CD20 expression, which is followed by a better response to regimens using Rituximab[12].

To our knowledge, there is no study that has investigated the correlation between[11,13] VEGF serum levels and other variables in DLBCL patients with varying distributions of tumour hypoxia. The purpose of this study is to investigate which factors are associated with the distribution of HIF-1α expression in DLBCL patients experiencing tumour hypoxia.

HIGHLIGHTS

- First study in Indonesia.
- This study aimed to identify factors linked to hypoxia inducible factor-1 alpha (HIF-1α) distribution in diffuse large B-cell lymphoma patients using immunohistochemistry.
- The study found a significant correlation between high HIF-1α distribution ($\geq 40\%$) and increased incidence of extranodal involvement in diffuse large B-cell lymphoma patients.
- This suggests that HIF-1α may play a role in promoting the spread of cancer cells beyond the lymph nodes, highlighting its potential as a prognostic indicator and therapeutic target.

Method

This was a cross-sectional study conducted at a hospital in Indonesia. The research subjects were newly diagnosed DLBCL patients with non-Hodgkin malignant lymphoma at the hospital between 2020 and 2022. Anatomical pathology examination and immunohistochemical expression of HIF-1α were analyzed by two independent hematopathologists. The diagnosis of DLBCL was established by standard staining and immunohistochemistry CD20, CD79a/CD45, and Ki67. This research has been reported in line with the STROCSS criteria[14].

Samples were collected using a non-probability purposive sampling technique, and all newly diagnosed DLBCL non-Hodgkin malignant lymphoma patients who met the inclusion and exclusion criteria were included in the study. Eligible research participant candidates will be invited to a room to receive detailed information and complete study procedures, which will be explained by the principal researcher. Participants will be provided with written approval from the researcher confirming that there are no contraindications to participating in the study, undergoing further examination, and completing the study.

Inclusion and exclusion criteria

The inclusion criteria for this study were confirmed newly diagnosed DLCBL patients by morphology-histopathology and immunohistochemistry, aged 18–65 years and agreed to participate in this research.

The exclusion criteria were anaemia (haemoglobin < 11 g/dl), obstructive lung disease (based on X-ray and/or spirometry), heart disorders (based on electrocardiogram examination and/or echocardiography left ventricular ejection fraction), cerebrovascular disease, severe liver dysfunction (total bilirubin ≥ 2 mg/dl), severe kidney dysfunction (glomerular filtration rate ≤ 30 ml/min), diabetes mellitus (fasting glucose ≥ 126 mg/dl or random glucose test ≥ 200 mg/dl), on medication of metformin or metronidazole therapy, suffered from infection or inflammation, Eastern Cooperative Oncology Group (ECOG) performance status greater than or equal to 2, pregnancy before or during chemotherapy, history of previous chemotherapy for non-Hodgkin lymphoma cases, and allergic reactions to chemotherapy treatment.

Immunohistochemistry staining

Tissue sections with 4-μm-thickness were made from paraffin-embedded tissue blocks and dried overnight at 60°C before being deparaffinized twice in xylene solution. The tissue sections were then rehydrated using alcohol and heated in EDTA buffer (pH

8.9) in a microwave (800 Watt for 7 min or 300 Watt for 15 min) to release the antigen. After the heating process, the tissue was cooled at room temperature for 20 min and washed with water before being placed in tris-buffered saline for 5 min.

The primary antibody was incubated at room temperature. The EnVision kit's peroxidase blocking solution was used to inhibit endogenous peroxidase for 25 min, and the preparation was then washed with tris-buffered saline. Immuno detection was performed using the Tech-Mate Instrument (Dako) and EnVision method, adjusting the instructions from the manufacturer's catalogue.

The interpretation of the HIF-1α immunohistochemistry was carried out by D.P and H.I., who served as the pathological anatomists in this study. The HIF-1α distribution results will be divided into 2 categories based on the distributions: HIF-1α distribution less than 40% and HIF-1α distribution greater than or equal to 40%. Immunohistochemistry technique was used to perform additional examinations such as BCL-2 and MYC expression.

Serum sample collection

Serum samples were collected from DLBCL patients at the time of diagnosis. Blood was drawn into serum separator tubes and allowed to clot for 30 minutes at room temperature before centrifugation at 1000 × *g* for 10 min. The resulting serum samples were stored at – 80°C until analysis. Serum levels of VEGF were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions. All other analyses such as haemoglobin, white blood cell, platelet, lactate dehydrogenase and soluble interleukin-2 receptor (sIL-2R) were performed according to the manual instructions provided with each assay kit.

Data collection

The variables of age, sex, early tumour size, body surface area, BMI, B symptoms, international prognostic index score, extranodal involvement, primary extranodal involvement, subtype of the DLCBL, bulky mass, ECOG, staging of the DLCBL were collected for this study. B symptoms, also known as systemic symptoms, refer to a set of general symptoms of fever above 38° C, drenching night sweats, and weight loss 10% of body mass in the previous 6 months. These symptoms commonly associated with lymphomas, including DLBCL. The presence of B symptoms is an important factor in determining the stage and prognosis of DLBCL[8].

Outcome

The primary outcome of this study was to identify the variables that were associated with the distribution of expression of HIF-1α in patients with DLBCL. The secondary outcome was to find the correlation between baseline variables, serum hypoxia, and angiogenesis in DLBCL patients with hypoxic tumours.

Statistical analysis

The data were collected and analyzed descriptively to examine the baseline characteristics by looking at the median values (minimum–maximum). The Statistical Program for Social Science (SPSS) 25 ver. was used to analyze the research data. A normality test using Shapiro–Wilk was conducted to assess the distribution of the research data and it was found that the data distribution was not normal.

The $\chi 2$ test was used to analyze the bivariate categorical data, and Mann–whitney U test was used to compare numerical data between the less than 40% and greater than or equal to 40% distributions of HIF-1α groups. Spearman correlation test was used to examine the correlation between numerical variables. To compare the levels of VEGF serum and the distribution of HIF-1α expression, we used receiver operating characteristic (ROC) curve analysis. The ROC curve was used to assess the diagnostic performance of VEGF levels in discriminating between HIF-1α distribution less than 40% and HIF-1α distribution greater than or equal to 40%. The area under the ROC curve was calculated to evaluate the overall diagnostic accuracy of the test. The optimal cutoff value was determined based on the Youden index. The confidence interval was set at 95%, and a *P* value less than 0.05 was considered statistically significant.

Ethical approval

This study has been approved by the ethical review board at the hospital (Ethical clearance number: No.736/EC/KEPK/-RSDK/2021).

Results

A total of 40 participants with a diagnosis of DLBCL were included in this study. Out of a total of 40 samples, 20 patients had HIF-1α distribution less than 40% and 20 patients had HIF-1α distribution greater than or equal to 40% (Fig. 1 and Fig. 2). The Table 1 presents the distribution of different variables among patients with DLBCL based on the distribution of HIF-1α. The median age was 56.5 years (range: 39–66) for patients with HIF-1α distribution less than 40% and 61.5 years (range: 18–65) for those with HIF-1α distribution greater than or equal to 40%; $P = 0.99$.

In the group with HIF-1α distribution greater than or equal to 40%, 12 patients (60%) had non-germinal-centre B-cell-like subtype of DLBCL, while in patients with HIF-1α distribution less than 40%, 17 patients (85%) had non-germinal-centre B-cell-like subtype of DLBCL. There was no significant difference between the two groups ($P = 0.77$). Additionally, there was a slightly higher occurrence of bulky mass in patients with HIF-1α distribution < 40%, with 12 patients (60%), compared to 11 patients (55%) in the HIF-1α distribution greater than or equal to 40% group. However, this difference was not statistically significant ($P = 0.749$).

The study found that patients with HIF-1α distribution greater than or equal to 40% had a significantly higher incidence of extranodal involvement, with 14 patients (67%) having extranodal involvement compared to 33% in patients with HIF-1α distribution less than 40%. The difference was statistically significant ($P = 0.027$). Furthermore, patients with HIF-1α distribution greater than or equal to 40% were more likely to have primary extranodal disease compared to those with HIF-1α distribution less than 40%, with nine patients (45%) having primary extranodal disease compared to three patients (15%) in the other group. This difference was statistically significant ($P = 0.038$). However, there were no significant differences observed between the two groups in terms of other factors such as B symptoms, international prognostic index score, gender, ECOG

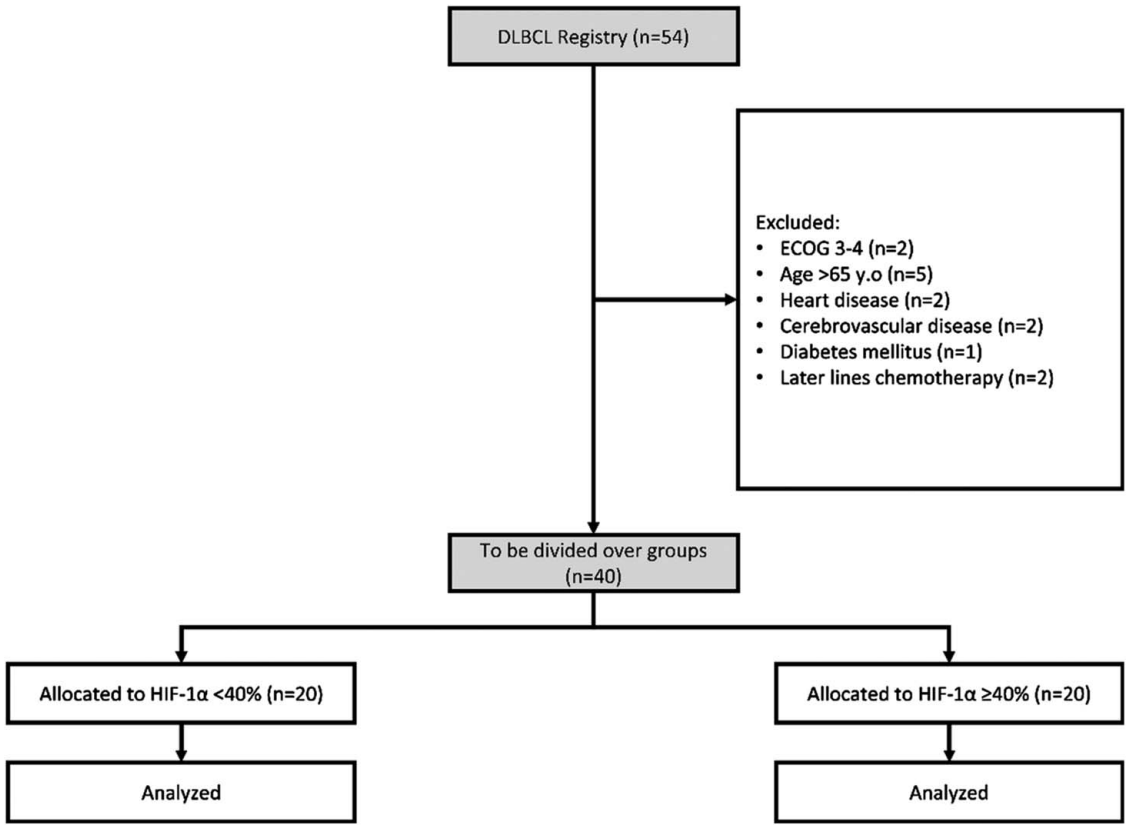

Figure 1. Flowchart of the study.

performance status, high-risk status, BCL-2 expression, MYC gene expression, and staging.

In high-risk DLBCL in this study was considered as double expressor lymphoma (DEL). DEL is defined by the co-expression of two proteins: MYC and BCL-2 or BCL-6. MYC is a proto-oncogene involved in cell proliferation, while BCL-2 and BCL-6 are anti-apoptotic proteins that promote cell survival. The simultaneous overexpression of MYC and either BCL-2 or BCL-6 indicates dysregulation in these key cellular processes. The presence of double expressor status has important clinical implications. DEL cases tend to have more aggressive disease behaviour and a poorer prognosis compared to other DLBCL subtypes. They are associated with a higher risk of treatment failure, disease relapse, and shorter overall survival[15,16].

The study found that patients with HIF-1α distribution less than 40% had a significantly lower median haemoglobin level of 11.4 g/dl (ranging from 10 to 15.6), compared to those with HIF-1α distribution greater than or equal to 40% who had a median haemoglobin level of 13.5 g/dl (ranging from 10.3 to 14.9). The difference was statistically significant with a *P* value of 0.04.

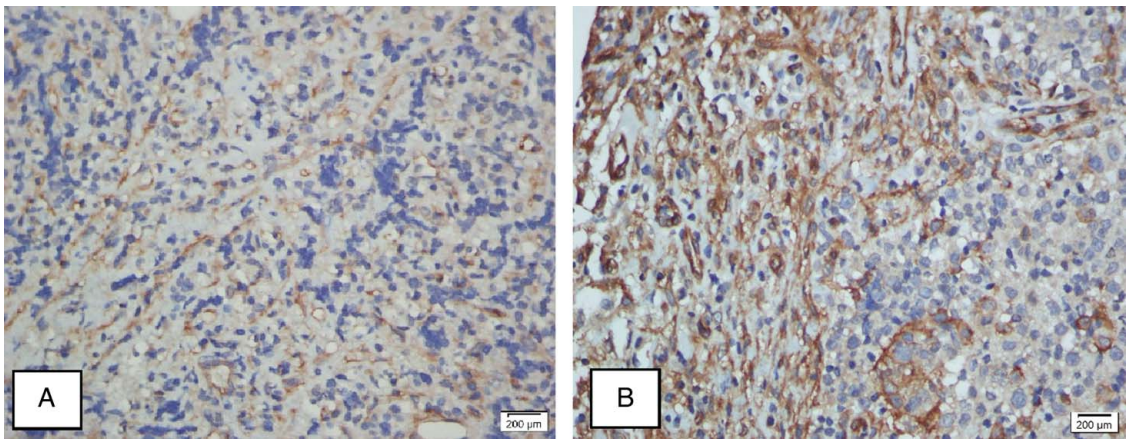

Figure 2. Representative images of HIF-1α immunohistochemistry. (A) Distribution <40%; (B) Distribution ≥ 40%.

Table 1
Baseline characteristics

Variable	HIF-1α distribution		P
	HIF-1α distribution < 40% expression, [*n*, (%)] (*n* = 20)	HIF-1α distribution HIF ≥ 40% expression [*n*, (%)] (*n* = 20)	
Age	56.5 (39–66)	61.5 (18–65)	0.99[a]
Sex			0.75[a]
Male	12 (60)	11 (55)	
Female	8 (40)	9 (45)	
Subtype of DLBCL			0.77[a]
GCB	3 (15)	8 (40)	
Non-GCB	17 (85)	12 (60)	
Bulky mass			0.75[a]
Yes	12 (60)	11 (55)	
No	8 (40)	9 (45)	
Extranodal involvement			0.03[a]
Yes	7 (35)	14 (70)	
No	13 (65)	6 (30)	
Primary extranodal			0.04[a]
Yes	3 (15)	9 (45)	
No	17 (85)	11 (55)	
B symptoms			0.38[a]
Yes	18 (90)	16 (80)	
No	2 (10)	4 (20)	
IPI score			0.51[a]
1–2	8 (40)	6 (30)	
3–4	12 (60)	14 (70)	
ECOG			0.86[a]
0–1	16 (80)	17 (85)	
2	4 (20)	3 (15)	
High-risk status			0.49[a]
DEL	5 (25)	7 (35)	
Non-DEL	15 (75)	13 (65)	
BCL-2			1.00[a]
≥ 40%	17 (85)	17 (85)	
< 40%	3 (15)	3 (15)	
MYC gene			0.75[a]
≥ 40%	8 (40)	9 (45)	
< 40%	12 (60)	11 (55)	
Staging Ann Arbor			0.69[a]
1–2	15 (75)	11 (55)	
3–4	5 (25)	9 (45)	
Early tumour volume	123.4 (0.3–1417)	31 (2.7–764.8)	0.59[b]
BSA	1.5 (1.3–1.9)	1.4 (1.3–1.9)	0.13[b]
BMI	22.1 (15.79–32.89)	21.39 (17.71–39.54)	0.81[b]
Haemoglobin	11.4 (10–15.6)	13.5 (10.3–14.9)	0.04[b]
Leucocyte	9.1 (3–21.7)	9.4 (3.1–35.9)	0.77[b]
Trombocyte	333.8 (41–859)	351.5 (266–441)	0.80[b]
LDH	796.5 (416–1928)	846.5 (408–1470)	0.55[b]
VEGF serum	98.6 (14.9–565.4)	51.5 (3.3–277.3)	0.02[b]
sIL-2r	182.5 (13–1897)	134 (24–707)	0.72[b]

BCL-2, B-cell lymphoma 2; BSA, body surface area; DEL, double expressor lymphoma; DLBCL, diffuse large B-cell Lymphoma; ECOG, eastern cooperative oncology group performance Status; GCB, germinal centre B-like-lymphoma; HIF-1α, hypoxia inducible factor-1 alpha; IPI, international prognostic Index; LDH, lactate dehydrogenase; sIL-2r: soluble interleukin-2 receptor; VEGF, vascular endothelial growth factor.
[a]$\chi 2$ analysis.
[b]Mann–Whitney Analysis.

Patients with HIF-1α distribution less than 40% also had a significantly higher serum level of VEGF (vascular endothelial growth factor) at 98.6 ng/ml (ranging from 14.9to 565.4) compared to those with HIF-1α distribution greater than or equal to 40%, which was 51.5 ng/ml (ranging from 3.3 to 277.3), with a *P* value of 0.024. However, there were no statistically significant differences observed between the two groups in terms of other numerical variables, as shown in Table 1.

After conducting a thorough analysis of all the numerical variables, the Spearman test revealed several correlations that were statistically significant. The results of this analysis showed a significant negative correlation between haemoglobin and VEGF, with a *P* value less than 0.05 and a correlation coefficient of – 0.44. Moreover, the study found a positive correlation between sIL-2R and VEGF, as well as tumour volume, with *P* value less than 0.05 and correlation coefficients of 0.5 and 0.37, respectively. Additionally, the study found a positive correlation between VEGF and sIL-2R, with a *P* value less than 0.05 and a correlation coefficient of 0.5. These findings are further illustrated in Fig. 3, which presents a clear depiction of the observed correlations between the variables.

In order to compare the levels of VEGF serum and the distribution of HIF-1α expression, we utilized the ROC curve. By doing so, we were able to establish a cutoff value of 61.8 ng/ml. Our investigation yielded significant findings, demonstrating a considerable difference in VEGF values between the two groups, with a *P* value of 0.037. (Fig. 4 and Table 2).

Discussion

Due to the high rate of cellular growth that is characteristic of DLBCL, there is an excessive demand for nutrients and oxygen. Low oxygen levels, or hypoxia, are a frequent feature of solid and haematological malignancies, including DLBCL. The HIF family of transcription factors, which control the expression of genes involved in angiogenesis, cell survival, and metabolism, are involved in the signalling cascade that cells activate in response to hypoxia[6,7,17]. The HIF-1 transcription factor complex contains the HIF-1α subunit, which is essential for the body's response to hypoxia. Hypoxia causes HIF-1α to translocate to the nucleus, where it binds to hypoxia response elements in the promoters of target genes. Under normoxic conditions, HIF-1α is quickly destroyed by the ubiquitin-proteasome system. Elevated HIF-1α expression has been seen in tumour tissue samples from DLBCL, and it is expected to support angiogenesis, metabolic reprogramming, and immune surveillance by encouraging tumour growth and survival. Targeting HIF-1α signalling may be a promising therapeutic approach for DLBCL, as HIF-1α has also been associated to chemotherapy resistance in this cancer[15,17].

Various theories have been proposed to explain the relationship between bulky mass and HIF-1α expression in tissue hypoxia cancer, but the precise connection remains uncertain. One possibility is that a bulky mass may experience tumour hypoxia, which occurs as the oxygen supply to the tumour tissue decreases. This phenomenon arises due to the increased distance between tumour cells and blood arteries[18]. Second, bulky mass may also stimulate the production of growth factors and cytokines that can activate signalling pathways that support the development of HIF-1α. For instance, it has been demonstrated that the cytokines interleukin-6 and TNF-alpha promote the production of HIF-1α in cancer cells[18]. The metabolism of cancer cells may be impacted by bulky mass, which would result in elevated amounts of ROS. Through a number of processes, including the stability of the HIF-1α protein and the activation of downstream signalling pathways, ROS can promote the production of HIF-1α[9,18].

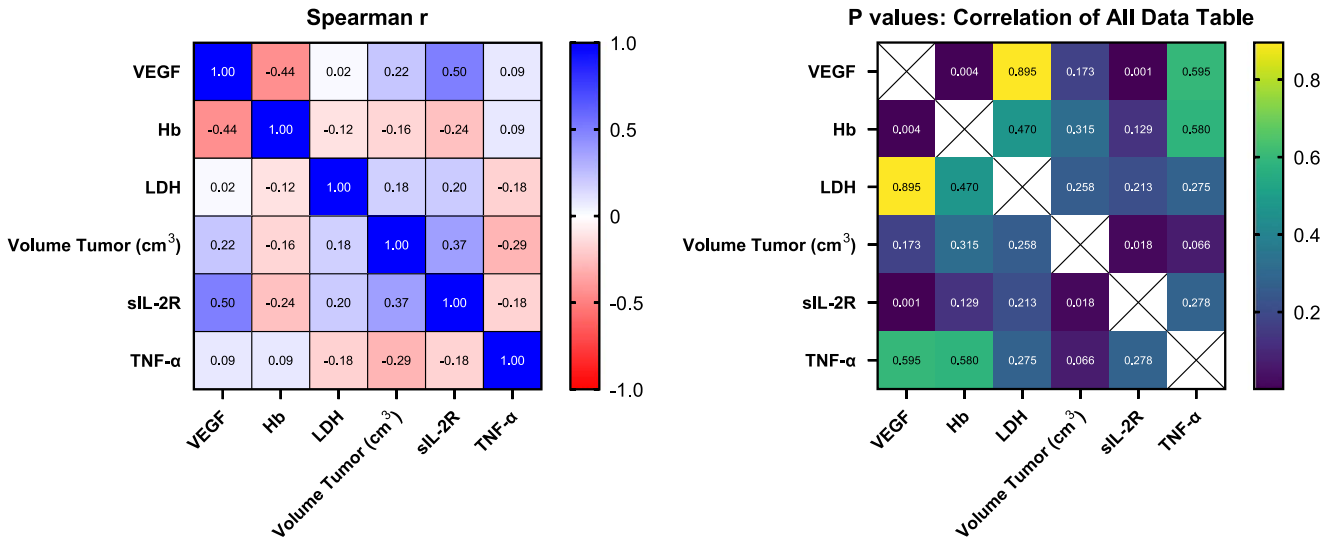

Figure 3. Spearman test analysis of the numerical variables.

However, in this study there was no significant differences between bulky tumour status and the level of HIF-1α distribution.

This study found significant differences between involvement in extranodal and nodal regions and the distribution of HIF-1α expression. Patients with a higher distribution of HIF-1α expression tend to have more extranodal involvement. DLBCL arises from extranodal organs in ~30% of cases. Its prognosis and risk of recurrence in the central nervous system vary depending on the primary site of origin. This extranodal involvement shares a high variety of biological features, such as the MYD88/CD79B-mutated (MCD) genotype and concurrent MYC and BCL-2 or BCL-6 rearrangements, which can be characterized as double-hit or triple-hit lymphoma[16].

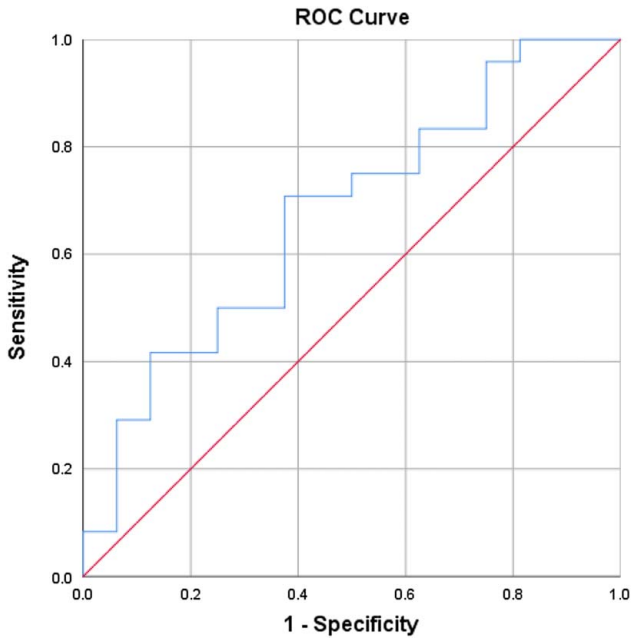

Figure 4. ROC curve of VEGF serum.

Double-hit lymphomas frequently involve extranodal locations in over 60% of cases, with secondary spread to sites such as the blood, bone marrow, central nervous system, or stomach/intestine. However, there is limited information on the frequency of double-hit lymphomas among primary extranodal lymphomas[19]. The significant differences in extranodal involvement in this study may be associated with gene arrangements, which were not evaluated in this study. Therefore, further investigation is necessary to better understand this phenomenon.

The negative correlation between haemoglobin, which reflects the oxygen-carrying capacity of red blood cells, and VEGF suggests that low oxygen tension may stimulate VEGF expression a potent pro-angiogenic factor that promotes endothelial cell proliferation, migration, and survival via HIF-1α and contribute to the development of a hypoxic microenvironment in DLBCL[18]. This phenomenon may play a crucial role in the angiogenic switch that drives tumour progression and metastasis[15,20]. Secondly, the positive correlation between sIL-2R and VEGF, as well as between sIL-2R and tumour volume, suggests that the immune system may play a role in modulating tumour angiogenesis and growth in DLBCL. sIL-2R is a marker of T-cell activation and proliferation, and may reflect the distribution of immune infiltration and activation within the tumour microenvironment[5,18].

The positive correlation between sIL-2R and VEGF suggests that T-cell activation may stimulate the production of VEGF and promote angiogenesis in DLBCL. The positive correlation between sIL-2R and tumour volume suggests that T-cell activation may also contribute to tumour growth and progression, possibly by enhancing the survival and proliferation of DLBCL cells[15,17,20]. To the best of our knowledge, no studies have yet investigated the correlation between HIF-1α expression and serum VEGF levels in patients with DLBCL. In our previous preliminary research study, it was found that the expression of HIF-1α was positively correlated with the expression of VEGF in tissues[5]. The lower serum VEGF levels in the HIF-1α distribution greater than or equal to 40% group, compared to the HIF-1α distribution less than 40% group, may be due to the larger size of the lymphoma tumour being influenced by inflammation rather

Table 2

Correlation between HIF distribution and VEGF serum

HIF-40% distribution	VEGF (< 61.8 ng/ml), *n* (%)	VEGF (≥ 61.8 ng/ml), *n* (%)	*P*
HIF-1α distribution <40% (*n* = 24)	7 (29)	17 (71)	0.037
HIF-1α distribution ≥ 40% (*n* = 16)	10 (62)	6 (38)	

HIF-1α, hypoxia inducible factor-1 alpha; VEGF, vascular endothelial growth factor.

than angiogenesis [21]. The level of VEGF in serum may not fully reflect the expression of VEGF in tissues[1,11,22,23]. The previous study concluded that only a small quantity of VEGF present in the serum is likely to originate from lymphoma tissue[15].

One limitation of this study is that we did not have access to the distribution of HIF-1α expression in normal tissue, which would have provided a better understanding of the disease. Moreover, the study was conducted only at one hospital facility, and the measurement of HIF-1α expression is challenging to perform in all cancer centres. Therefore, further investigation is needed to assess the feasibility of using HIF-1α serum as a non-invasive marker for diagnosing, prognosing, and monitoring treatment response in patients with DLBCL.

CONCLUSION

This study analyzed 40 patients with DLBCL and found that those with higher levels of HIF-1α (≥ 40%) had a significantly greater incidence of extranodal involvement and primary extranodal disease. Haemoglobin level was also statistically significant. Positive correlations were observed between sIL-2R and VEGF, as well as between tumour volume and sIL-2R. These results suggest that HIF-1α distribution, haemoglobin level, and biomarkers such as sIL-2R and VEGF may be important factors to consider in the diagnosis and management of DLBCL.

Ethical approval

The informed consent for this study has been approved by the committee ethics of the hospital where this study was conducted and according to The Declaration of Helsinki (Ethical clearance number: No.736/EC/KEPK/-RSDK/2021) in 7 February 2021.

Consent

Written informed consent was obtained from the patients for publication and any accompanying images. Copies of written consent are available on request.

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Author contribution

E.A.P., D.R., K.T., R.M.N., S.P.K., H.I., D.P., A.G.S., B.S., D.S., S.M.H., and C.S. all contributed to the research and writing of this article. All authors have reviewed and approved the publication of this research article.

Conflicts of interest disclosure

None.

Research registration unique identifying number (UIN)

1. Name of the registry: Research Registry.
2. Unique Identifying number or registration ID: researchregistry9033 (updated).
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): https://www.researchregistry.com/browse-the-registry#home/registrationdetails/6464a5a56cab730028af8fc1/.

Guarantor

Eko Adhi Pangarsa.

Data availability statement

The corresponding author can provide the datasets utilized in the current study upon reasonable request.

Provenance and peer review

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