Correlation between Reference Tissue Normalized 18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Standardized Uptake Value Max of Nodal and Extranodal Sites in Lymphomas: An Empirical Study

Abstract

Context: Extranodal (EN) lymphomas involve sites other than lymph nodes (LNs), spleen, thymus, and the pharyngeal lymphatic ring. The highest standardized uptake value (SUV) max of the LN can aid in the diagnosis of EN site lymphomatous infiltrations over inflammation or infection especially when there are no contrast-enhanced computed tomography (CT) changes. Aims: The purpose of this study was to find the significance of correlation between absolute SUVmax and mediastinal blood pool (mbSUVmax) and liver (lvSUVmax) normalized SUVmax of EN sites and the most fluorodeoxyglucose (FDG) avid LN in patients with primary and secondary EN involvement in Non-Hodgkin’s and Hodgkin’s Lymphoma. Settings and Design: This was a retrospective study of 70 patients with histopathologically proven lymphoma in whom 18F-FDG positron emission tomography CT was performed for pretherapy staging. Materials and Methods: Images were used to detect EN sites of disease and SUVmax of mediastinal blood pool, liver, highest SUVmax LN, and highest SUVmax EN site were calculated. Statistical Analysis Used: Karl Pearson’s coefficient of correlation (r) was used to correlate the highest SUV max of LN and EN site and corresponding highest blood pool corrected and liver corrected SUV max. In view of small sample size, t-test for paired samples at 5% and 10% significance was conducted to validate the findings. Two-tailed t-test for independent samples was also used to compare means of SUVmax values between data grouped according to gender and lymphoma subtype (Non-Hodgkin lymphoma and Hodgkin lymphoma).

Results: r = 0.54 for the highest LN SUVmax-highest EN SUVmax values and on further validation by one- and two-tailed paired t-test at significance levels of 5% and 10%, P = 0.00052 and 0.00103 respectively which denoted significant positive and moderate correlation. r = 0.59 for highest LN lvSUVmax-highest EN vSUVmax and P = 0.00032 and 0.00065 showing positive and moderate correlation. r = 0.82 for highest LN mbSUVmax-highest EN mbSUVmax values and P = 0.00034 and 0.00068 revealing positive and strong correlation. Conclusion: Significant positive and strong correlation exists between nodal and EN mbSUVmax. This is stronger than the correlation between nodal and EN absolute SUVmax and lvSUVmax. Since normalization of lesion SUVmax to reference tissues reduces the variability of SUV, this can be a useful adjunct to determine whether high SUVmax of the EN site is due to lymphomatous infiltration.

Keywords: 18-fluorodeoxyglucose positron emission tomography/computed tomography, blood, extranodal, liver, lymphoma

Introduction

Extranodal lymphomas (ENLs) are considered to involve sites other than lymph nodes (LNs), spleen, thymus, and the pharyngeal lymphatic ring. Non-Hodgkin lymphoma (NHL) is up to 8 times more common than Hodgkin disease with the incidence varying widely with environmental and genetic factors. Almost 25% all NHL and very rarely Hodgkin lymphoma (HL) can arise from tissue other than LNs or tissue devoid of any lymphoid tissue. Origin of tumor from non lymph nodal tissue is termed as primary ENL, whereas hematogenous spread of disease from LNs to extranodal (EN) tissue is secondary ENL. The male to female ratio of EN NHL ranges between 2.31:1 in India. HL shows a peak incidence in the 20–30-year age group, with a second peak in the elderly population. The incidence of NHL increases exponentially with age after 20 years. The incidence of NHL is more than that of HL.

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of EN NHL is rising faster than that of nodal NHL. EN involvement in NHL is multifactorial and depends on the age of the patient, the presence of preexisting immunodeficiency, and the pathological subtype of lymphoma. EN involvement upgrades the disease regardless of the site of primary adenopathy. Fluorodeoxyglucose-positron emission tomography (FDG-PET) is more sensitive than computed tomography (CT) because of its ability to identify splenic and bone marrow infiltration, PET or PET/CT can upstage as many as 40% of cases, though the CT component remains essential, for example, in low-grade lymphoma and in the lungs, where small nodules may be below the resolution of PET technology.

Materials and Methods

This retrospective study was conducted between January 2021 and April 2021 at our institute utilizing the PET CT database from March 2017 to April 2021. Approval of the Institutional Ethics Committee was obtained.

Inclusion criteria

Patients with histopathologically proven lymphoma who underwent LN biopsy and subsequent 18F-FDG PET CT for pretreatment staging and were found to have at least one EN site of involvement (Lugano Stage I–III with or without S).

Exclusion criteria

Patients with histopathologically proven Hodgkin and NHL with no18F-FDG PET CT evidence of EN disease (Lugano Stage I–III with or without S).

Scanning technique and imaging parameters

18F-FDG PET CT was performed on a 16 slice Biograph Horizon clinical PET CT system with TrueV-4 Ring (Siemens Healthcare Erlangen Germany) and Siemens (VJ21B) PET syngo acquisition workplace user interface. All cases were injected with 5–10 mCi (1 mCi/10 kg) of 18F-FDG intravenously approximately 40 min prior to scan. Patients blood glucose level was below 150mg/dL at the time of injection.

The examination started with a low dose contrast-enhanced routine spiral CT scan from the vertex to the mid-thigh for attenuation correction using 60–80 ml of nonionic iiodinated contrast material (Omnipaque 350mgI/mL, GE Healthcare) at 1.5–2 mL/s. The venous phase images were acquired 65 s postinjection. The parameters of the CT scan were 130 kV, 80–150 mAs (CAREDOse4D auto mAs), slice thickness of 5 mm, 512 × 512 reconstruction matrix, display matrix of 1024 × 1024, scan length-1024mm, transverse Field of View (FOV)-700mm, pitch of 0.95, 0.6 mm slice collimation, gantry rotation time of 0.6 s and kernel B31s for reconstruction. Then, PET imaging was performed at 1 min/bed position for 7 beds, 4 mm slice thickness, 256 matrix and covering the same field of view using a Gaussian filter with full-width at half-maximum of 5 mm and reconstructed with iterative plus Time of Flight (TOF) method (attenuation-weighted, three iterations, and 10 subsets, matrix size of 256, zoom of 1, isotropic CT resolution of 24lp/mm with 0.21 mm uniform resolution throughout the FOV) and temporal resolution up to 105 ms.

Image analysis and data interpretation

Whole-body PET and CT images in DICOM 3.0 format were loaded on three-dimensional workstations for visual evaluation and data analysis (Siemens syngo. via VB10, Siemens AG, healthcare sector, Erlangen, Germany). Multiplane visual assessment of on-attenuation corrected images, attenuation-corrected, as well as maximum intensity projections of CT, PET, and fused PET-CT images (overlay with 50% transparency) was done.

On contrast-enhanced CT (CECT), the EN sites were identified as positive in the presence of mass lesion, abnormal postcontrast enhancement, or standardized uptake value (SUV) max greater than blood pool SUVmax. Based on PET, the organ was positive if there was increased FDG uptake with SUVmax higher than physiologic hepatic background activity (SUVmax).

Lesions were measured and a three-dimensional volume of interest (VOI) of approximately 1cm3 was drawn in each PET CT positive lesion using response evaluation criteria in solid tumor/WHO and isocontour and ruler (version 1.1). Image Analysis Software (Siemens syngo. via VB10, Siemens AG, healthcare sector, Erlangen, Germany) was used for calculation of SUVmax using the following formula:

\[
SUV (SUV = \text{standardized uptake value}) = \frac{\text{tissue radioactivity concentration}}{(\text{mCi} / \text{ml}) / (\text{injected dose}[\text{mCi}] \times \text{body weight}[\text{kg}])}.
\]

The reference tissue SUVs were calculated as follows: mediastinal blood pool SUVmax was calculated by drawing a VOI of 1cm3 in the arch of aorta avoiding the wall of aorta and any associated plaques and calcifications. The liver SUVmax was calculated by tracing a VOI of 1cm3 in the sixth segment of the right lobe devoid of any obvious diffuse or focal lesions. mbSUVmax = tumor SUVmax divided by blood pool SUVmax and lvSUVmax = tumor SUVmax divided by liver SUVmax.

SUVmax, mbSUVmax, and lvSUVmax of the hottest LNs and hottest EN sites were quantified.

Statistical analysis

Collected data were coded and tabulated, then statistical analysis was done using MedCalc Statistical Software version 19.2.6 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2020) and IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.
Descriptive analysis was done for numerical variables and was presented as mean ± standard deviation (SD). Categorical variables (qualitative data) were presented as number and percentage. Pearson’s correlation analysis (r) was used to evaluate coefficient of correlation between the SUVmax of the hottest LN and the SUV max of EN sites of lymphoma followed by the mbSUVmax and lvSUVmax values of the same, r = 0 – 0.2 (poor correlation), 0.2 – 0.5 (moderate), 0.5 – 0.7 (strong), and 0.7 – 0.9 (very strong).

In view of small sample size, one- and two-tailed t-test for paired samples at 5% and 10% significance level was then conducted to validate the significance of the Pearson’s correlation coefficients.

Two-tailed t-test for independent samples at 5% and 10% significance level was also used to compare means of absolute and normalized SUVmax values between data grouped according to gender and histopathological subtypes (NHL and HL).

**Results**

**Demographic profile of sampled respondents**

Out of the 70 patients who underwent PET CT, 45 (64.29%) were male and 25 (35.71%) were female. The age ranged from 4 years to 82 years, mean 48 ± 20 SD. The mean age for males was 46.44 years ± 21.02 SD and that for females was 53.08 years ± 20.42 SD. The number of males with NHL was 30 and with HL was 15. The number of females with NHL was 18 and with HL was 7 [Figure 1].

Majority of the patients were males in both the non-Hodgkins (66.66%) and Hodgkins (68.18%) lymphoma groups. Ten male and 8 female patients were aged 60–69 years. The highest SUVmax before and after normalization in both LN and EN sites was seen in female patients, with the only exception being the highest LN SUVmax which was seen in a male patient.

The mean LN SUVmax in the NHL group was 13.6 ± 9.2 for males (mbSUVmax-9.54 ± 7.08, lvSUVmax-7.85 ± 5.85) and 13.73 ± 8.63 for females (mbSUVmax-10.91 ± 8.37, lvSUVmax-8.23 ± 6.66). The mean EN SUV max was 11.24 ± 7.82 (mbSUVmax-7.49 ± 4.99, lvSUVmax-5.6 ± 3.58) and 12.31 ± 5.98 (mbSUVmax-9.5 ± 6.97, lvSUVmax-7.44 ± 5.66) respectively. The Hodgkin’s Lymphoma group showed a mean LN SUVmax of 11.68 ± 5.24 for males (mbSUVmax-7.79 ± 3.87, lvSUVmax-5.51 ± 2.53) and 14.03 ± 4.45 for females (mbSUVmax-18.79 ± 25.18, lvSUVmax-7.85 ± 5.85). The corresponding EN SUVmax was 6.85 ± 3.68 (mbSUVmax-4.75 ± 2.98, lvSUVmax-6.18 ± 2.76) and 9.46 ± 5.66 (mbSUVmax-12.62 ± 17.46, lvSUVmax-4.02 ± 2.47), respectively.

The sample of n = 70 patients was subject to Karl Pearson’s coefficient of correlation and t-tests.

The P values for the highest SUVmax (LN and EN, both absolute and normalized) when divided according to histopathology into NHL and HL, were not significant even at 10% in this study. The SUVmax and normalized SUVmax values when correlated to gender showed only the P value mbSUVmax of EN lymphomas (both NHL and HL) being significant at 5% level of significance [Table 1]. The effect size of the t-test as calculated by Hedge’s g = 0.519 (medium effect). This may be due to the fact that the highest normalized SUVmax values were seen in females in this study, especially in the EN blood pool corrected values (males-6.58 ± 4.58, females-10.37 ± 10.61). This difference was not seen when the values were divided according to histopathology even though the mean SUVmax rose in both groups, probably because of the smaller sample sizes.

Pearson’s coefficient of correlation (r) was 0.54 for the highest LN SUVmax-highest EN SUVmax values and on further validation by paired t-test at significance level of 5% and 10%, the one- and two-tailed P values are significant at 0.00052 and 0.00103 which denotes a significant positive and moderate correlation. The r = 0.59 for the highest LN lvSUVmax-highest EN lvSUVmax values and on further validation P = 0.00032 and, 0.0065 denoting a significant positive and moderate correlation. The r = 0.82 for the highest LN mbSUVmax-highest EN mbSUVmax values and P values are significant at 0.00034 and 0.00068 which reveals a significant positive and strong correlation [Table 2].

This is in concordance with a study by Ömür et al.[5] in which there was a high positive correlation between the maximum SUVs of the highest 18F-FDG-accumulating LNs and EN sites (r = 0.67) in 137 patients with nodal and EN involvement. In another study by Othman et al.[6] there was a significant positive moderate correlation between SUVmax of the EN lesions and hottest LN (r = 0.45).

In our study, the heat map on the scatter graph shows a concentration of the correlates in the blood pool and liver normalized SUVmax groups [Figure 2a-c]. This can be explained by the mean SUVmax of blood pool (n = 70) being 1.53 ± 0.55, mean SUVmax of the liver being 2.06 ± 0.67 and mean SUVmax of lymph nodal
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Division of the LN and EN SUVmax by the blood pool and liver SUVmax would have yielded a mean mbSUVmax LN at 10.45 ± 10.27 and mean mbSUVmax EN at 7.94 ± 7.47. In the liver normalized SUVmax graph, mean lvSUVmax LN was at 7.28 ± 5.33, and mean lvSUVmax EN was at 5.4 ± 4.1 which is seen as a clustering of data points in the lower left-hand corner.

Discussion

Ideally, SUVmax is a semiquantitative measure of tissue glycolysis which serves to negate individual patient confounding factors such as region of interest volume, body weight, and injected dose. But it is subject to variation due to the time between injection and imaging acquisition, partial volume effects, extravasation of administered isotope at the site of injection, residual activity in the syringe, decay of the injected dose, technological characteristics and parameters. It has been repeatedly shown that at least some of these problems can be reduced or eliminated if SUV is normalized to the SUV of a suitable reference region. One of the approaches which has been suggested by Kinahan and Fletcher to assist reduction in variability of SUV measurements incorporates the use of “reference tissue” SUVmax values and normalization of lesion/target SUV measures to those of selected reference tissues.

Table 1: T-test results of absolute maximum standardized uptake value, mediastinal blood maximum standardized uptake value and liver maximum standardized uptake value of nodal and extranodal sites (Hodgkin’s lymphoma and Non-Hodgkins lymphoma) in male and female patients

|                  | Male         | Female        | Two-tailed t-test results at 5% and 10% level of significance (t, P) |
|------------------|--------------|---------------|-------------------------------------------------------------------|
| Mean LN SUVmax ±SD | 12.96±8.08   | 13.82±7.6     | −0.4319, 0.667 (NS)                                                |
| Mean EN SUVmax ±SD | 9.78±7       | 11.51±5.92    | −1.047, 0.298 (NS)                                                |
| Mean LN mbSUVmax ±SD | 8.96±6.21   | 13.11±14.87   | −1.642, 0.105 (NS)                                               |
| Mean EN mbSUVmax ±SD | 6.58±4.58   | 10.37±10.61   | −2.081, 0.411 (S)                                                |
| Mean LN lvSUVmax ±SD | 6.58±5.08   | 7.65±5.85     | −0.436, 0.664 (NS)                                               |
| Mean EN lvSUVmax ±SD | 4.8±3.28    | 6.49±5.17     | −1.669, 0.099 (NS)                                               |

SD: Standard deviation, NS: Not significance, S: Significance, SUVmax: Maximum standardized uptake value, mbSUVmax: Mediastinal blood SUVmax, LN: Lymph nodes, EN: Extranodal, lvSUVmax: Liver SUVmax

Table 2: Pearsons coefficient of correlation and t-test results for absolute maximum standardized uptake value, maximum standardized uptake value and liver maximum standardized uptake value of nodal and extranodal sites (Hodgkin’s lymphoma and Non-Hodgkins lymphoma) with scatter plots

| Sample size (n=70) | Highest LN-highest EN SUVmax | Highest LN-highest EN mbSUVmax | Highest LN-highest EN lvSUVmax |
|--------------------|-----------------------------|-------------------------------|-----------------------------|
| Pearson’s correlation coefficient (r) | 0.54 | 0.82 | 0.59 |
| Significance level (P) (P<0.001-significant) | <0.0001 | <0.0001 | <0.0001 |
| 95% CI | 0.3494-0.6877 | 0.7246-0.8845 | 0.4117-0.7277 |
| T-test for paired samples (at 5% and 10% level of significance) | | | |
| Mean difference | −2.86 | −2.51 | −1.87 |
| SD of differences | 7.0014 | 5.90 | 4.39 |
| Test statistic t | −3.427 | −3.559 | −3.574 |
| P (P<0.001-significant) | One-tailed | 0.00052 | 0.00034 | 0.00032 |
| Two-tailed | 0.00103 | 0.00068 | 0.00065 |
| Scatter diagram (y axis-extranodal, x axis-nodal) | | | |

SD: Standard deviation, CI: Confidence interval, SUVmax: Maximum standardized uptake value, mbSUVmax: Mediastinal blood SUVmax, LN: Lymph nodes, EN: Extranodal, lvSUVmax: Liver SUVmax

A number of tissues have been advocated as reference tissue including, blood pool, liver, lung, and cerebellum. The liver and blood pool are the most widely used references because they maintain a nearly constant SUV level over time following the injection of 18F-FDG. Recent studies have shown that the tumor to normal liver SUV

sites = 13.27 ± 7.87 and mean EN SUVmax = 10.4 ± 6.64. Division of the LN and EN SUVmax by the blood pool and liver SUVmax would have yielded a mean mbSUVmax LN at 10.45 ± 10.27 and mean mbSUVmax EN at 7.94 ± 7.47. In the liver normalized SUVmax graph, mean lvSUVmax LN was at 7.28 ± 5.33, and mean lvSUVmax EN was at 5.4 ± 4.1 which is seen as a clustering of data points in the lower left-hand corner.
In a study by Chiaravalloti et al., in patients with HL, liver 18F-FDG SUV is variable and related to several factors such as the beginning of chemotherapy, body weight, and the severity of the illness at staging. These findings suggest that these aspects should be considered when using liver SUV as a parameter for comparing SUVmax of other abdominal organs in the evaluation of involvement by lymphoma or for early therapy response in HL. Steatotic changes or diffuse liver disease such as cirrhosis or mineral/amyloid/drug metabolite deposition below imaging threshold can also alter SUVmax values normalized to the liver.

As stated by Ömür et al. and Othman et al., the highest SUVmax of the nodal sites can help in the differential diagnosis of organ infiltrations over other 18F-FDG-avid benign conditions, such as inflammation or infection, especially in patients with high 18F-FDG accumulation in EN sites without mass lesions. However, this may not always be the case in the Indian population which hosts a third of the world’s tuberculosis as well as HIV burden. Granulomatous infections can have protein manifestations including extrapulmonary and disseminated disease. Lymphomas and tuberculosis also share common predisposing factors, clinical and radiological features.[15] Active tuberculous lesions often exhibit a high degree of FDG uptake. No characteristic pattern has been identified as yet that will definitely and consistently differentiate them from cancerous lesions.[16] Histopathology and bacteriological investigations to differentiate the two should therefore be performed in all such cases irrespective of the PET-CT findings.

To the best of our knowledge, this is the first study which correlates mediastinal blood pool and liver corrected SUV max of most avid LN to EN site SUVmax. Ratios of tumor/background values standardized to each patients reference background tissue, especially mbSUVmax, are a useful adjunct for comparing EN and nodal SUVmax due to a strong positive correlation.

The main limitation of this study is that it a retrospective study of a small sample. A pitfall of F18-FDG is accumulation at physiological sites and in benign conditions such as infection and inflammation but all areas of FDG avidity were not biopsied for practical reasons and instead CECT findings were correlated to the best possibility. This limitation is important since granulomatous infections such as tuberculosis need to be excluded in the Indian scenario. Lymphomas showing low 18F-FDG avidity may have displayed subthreshold FDG activity. Further larger patient cohort investigations involving newer, more specific tracers, biopsy correlation of EN disease sites with multi-institutional cooperation is needed to improve the statistical results, cover the limitations and validate the findings of this study.
**Conclusion**

Significant positive and strong correlation exists between nodal and EN mbsUVmax. This is stronger than the correlation between nodal and EN absolute SUVmax and lvSUVmax. Since normalization of lesion SUVmax to reference tissues reduces the variability of SUV, this can be a useful adjunct to determine whether high SUVmax of the EN site is due to lymphomatous infiltration.

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**Conflicts of interest**

There are no conflicts of interest.

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