# **Original Research Article**

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# Biopsy of sonologically detected peripheral lymph nodes: diagnostic value in HIV positive patients

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# **ABSTRACT**

**Background:** One of the most common manifestations in human immunodeficiency virus (HIV) is generalized lymphadenopathy. Biopsy of these nodes can help in diagnosing associated conditions. Biopsy of clinically non-palpable lymph nodes can help physician to obtain an early diagnosis of associated diseases in people living with HIV (PLHIV). Present research was undertaken to study diagnostic yield of sonologically detected peripheral lymph node biopsy in symptomatic PLHIV.

**Methods:** One hundred ten PLHIV above age of 18 years referred to surgery department for excision biopsy of sonologically detected peripheral lymph nodes were included. Specimen was sent in normal saline to laboratory for testing and part of the specimen was fixed in formalin for further evaluation. Gram stain, Zeil Nelson stain, histopathological examination and genotype MTBDR plus test were conducted. Comparison of quantitative variables and qualitative variables was done by using Kruskal wallis test and Chi-square test / Fisher's exact test respectively.

**Results:** Most common diagnosis obtained was tuberculous lymphadenopathy followed by reactive hyperplasia. Significantly higher percentage of patients having weight loss was diagnosed with tuberculous lymphadenopathy. Percentage of tuberculous lymphadenopathy patients was higher in patients who were not on anti-retroviral therapy (ART) as compared to those who were on ART. Median duration of HIV in tuberculosis lymphadenopathy patients was less as compared to patients with malignancy. Sensitivity, and specificity was 91.7%, and 61.5% respectively for diagnostic yield of USG in non-palpable lymph nodes.

**Conclusions:** USG is a sensitive tool for early detection of clinically non-palpable pathological lymph nodes in symptomatic PLHIV.

**Keywords:** Biopsy, Human immunodeficiency virus, People living with HIV, Tuberculous lymphadenopathy, Ultrasonography

# INTRODUCTION

One of the most common manifestations in human immunodeficiency virus (HIV) is generalized lymphadenopathy. Biopsy of these nodes can help in diagnosing associated conditions, occurring either due to immune suppression or lymphotrophic nature of HIV. A trend has started to biopsy clinically non-palpable lymph

nodes that many a times can help the physician to obtain an early diagnosis of associated diseases in people living with HIV (PLHIV).<sup>2-4</sup> Studies show correlation between CD4 count and appearance of lymphadenopathy and lymphoid pathology.<sup>5</sup>

A multitude of conditions can cause lymphadenopathy, including HIV itself, opportunistic or other infections, or

malignancies. Diseases like lymphoma and tuberculosis (TB) are common in HIV positive patients and can be diagnosed by lymph node biopsy with high accuracy.

Worldwide, tuberculosis is the most common opportunistic infection affecting HIV-seropositive individuals, and it remains the most common cause of death in patients with acquired immunodeficiency syndrome (AIDS).<sup>6</sup> HIV infection has contributed to a significant increase in worldwide incidence of TB.<sup>7</sup> Although HIV-related TB is both treatable and preventable, incidence continues to increase in developing nations like India, wherein HIV infection and TB are endemic and resources are limited.

Even if clinical lymphadenopathy has not developed, i.e. lymph nodes are not palpable, early features of disease may be seen on ultrasonography (USG).<sup>8</sup> Thus, subjecting HIV positive patient not having clinical lymphadenopathy and presenting with constitutional symptoms like fever, anorexia, weight loss, cough etc. to lymph node biopsy of sonologically detected lymph nodes may help early detection of certain conditions.<sup>8,9</sup>

Present research was undertaken to study diagnostic yield of sonologically detected peripheral lymph node biopsy in symptomatic PLHIV.

#### **METHODS**

All HIV positive in-patient and outpatient department patients more than 18 years of age accessing health care in Poona Hospital and Research Centre, Pune (India) between February 2015 and May 2016 and referred to the surgery department for excision biopsy of sonologically detected peripheral lymph nodes and ready to participate were included. Permission was obtained from ethics committee and scientific advisory committee of the institution.

# Inclusion criteria

Inclusion criteria were HIV positive patients confirmed by serological tests; patients presenting with varied symptoms like fever, weight loss, anorexia, cough with lymph nodes detected on USG neck/axilla.

#### Exclusion criteria

Exclusion criteria were HIV positive patients having clinically palpable lymphadenopathy; contraindication for surgical procedures; absence or withdrawal of informed written consent. Based on previously published study, setting an alpha error at 0.05, and power at 80%, sample size of 110 was calculated for present observational study by a formula. Demographic and clinical data was collected, e.g. age, gender, current CD4 count, antiretroviral therapy (ART) status (naïve/experienced), duration of HIV diagnosis, past medical history etc. Patients were clinically examined for lymphadenopathy

and patients with palpable lymph nodes were excluded. Written informed consent was obtained from all the patients after explaining them the study in detail. Biopsy of the selected node / nodes was performed according to the site indicated on USG, and sent for histopathological examination (HPE). Only one patients underwent axillary lymph node biopsy, rest 109 patients underwent cervical lymph node biopsies.

Once biopsy site was chosen, lymph node/nodes were selected. All lymph node biopsies were performed under local anaesthesia and single/multiple nodes were excised from a single incision. The largest, most suspicious lymph node (not necessarily most accessible) was selected. A planned incision of 3-4 cm was outlined with a marker (preferably in a skin crease) and infiltrated with 1% lidocaine with 1:100,000 epinephrine + 0.25% bupivacaine (about 15-20ml as per weight). The sternocleidomastoid muscle was split (not divided unless required for exposure) and retracted with Langenbach's retractors. Potts forceps with teeth were used to grasp the cervical fascia. Fascia was divided with electro-cautery with care to avoid iatrogenic injury to superficial vessels. Extreme care was taken so that the lymph node was not grasped directly to avoid any crush or pull artifact. Atraumatic forceps were used. The use of cautery was minimized until excision of lymph node to avoid trauma to the node. Artery forceps were used for blunt dissection. Specimen was sent in normal saline to the laboratory for testing and part of the specimen was fixed in formalin for further evaluation. Gram stain, Zeil Nelson (ZN) stain, HPE and genotype MTBDR plus test (done if AFB detected in either ZN stain or HPE) were conducted. Reports were recorded and analyzed.

Data analysis was done by using Statistical Package for Social Sciences (SPSS) for Windows version 20, IBM Corporation, Chicago, USA. Qualitative data is expressed as frequency and percentage (%). Quantitative data is expressed as median. Chi-square test / Fisher's exact test was used to study the significance between final diagnosis with various factors for qualitative data variables. To compare duration of HIV infection and CD4 count with final diagnosis we have used Kruskal wallis test. P<0.05 was considered as significant.

#### **RESULTS**

Of 110 patients, 9 (8.2%) were below the age of 31 years, 38 (24.5%) were between age of 31 and 40 years, 48 (43.6%) were between age of 41 and 50 years, 13 (11.8%) were between age of age 51 and 60 years, whereas 2 (1.8%) were above the age of 60 years. Mean age of the study population was 41.8 years. Of 110 patients, 83 (75.5%) were males and 27 (24.5%) were females. Eighty three (75.5%), 57(51.8%), 57(51.8%) and 41 (37.3%) patients had fever, weight loss, anorexia, and cough respectively. Fifty six (50.9%), 30 (27.3%) and 24 (21.8%) patients had HIV duration  $\leq 1$  year, 1-5 years, and > 5 years respectively. CD4 count was  $\leq 200$ , 201-500

and >500 in 61.8%, 30% and 8.2% patients respectively. Seventy one (64.5%) patients were on ART whereas 39 (35.5%) patients were not on ART. Majority of the patients 109/110 had cervical excision biopsy. Sixty seven (60.9%), 26 (23.6%), 13 (11.8%), 3(2.7%) and 1 (0.9%) patients were diagnosed as tubercular lymphadenitis, reactive hyperplasia, malignancy, others and fungal infection respectively. Of 13 patients of

malignancy, 7 had Hodgkin's lymphoma, 5 had Non-Hodgkin's lymphoma and 1 had metastatic adenocarcinoma with an unknown primary for which further evaluation was advised. Three patients had other poorly defined histological patterns such as hyalinised sclerosis with focal calcification, paracortical hyperplasia with immunoblastic proliferation, and necrotizing lymphadenitis.

Table 1: Symptoms of HIV and final diagnosis.

	Final diagnosis						
Symptoms	Tuberculous lymphadenopathy N (%)	Reactive hyperplasia N (%)	Malignancy N (%)	Fungal infection N (%)	Others N (%)	Total n (%)	P value
Fever							
Yes	54 (65.1)	17 (20.5)	9 (10.8)	1 (1.2)	2 (2.4)	83 (100.0)	0.429
No	13 (48.1)	9 (33.3)	4 (14.8)	0 (0.0)	1 (3.8)	27 (100.0)	0.429
Weight loss							
Yes	42 (73.7)	8 (14.0)	6 (10.5)	1 (1.8)	0(0.0)	57 (100.0)	0.008
No	25 (47.2)	18 (34.0)	7 (13.2)	0 (0.0)	3 (5.6)	53 (100.0)	0.008
Cough							
Yes	19 (46.3)	14 (34.2)	6 (14.6)	0 (0.0)	2 (4.9)	41 (100.0)	0.073
No	48 (69.6)	12 (17.4)	7 (10.2)	1 (1.4)	1 (1.4)	69 (100.0)	0.073
Anorexia							
Yes	32 (56.1)	14 (24.6)	7 (12.3)	1 (1.8)	3 (5.2)	57 (100.0)	0.458
No	35 (66.0)	12(22.7)	6 (11.3)	0 (0.0)	0(0.0)	53 (100.0)	0.430

Fisher's exact test was used.

Table 2: CD4 count and final diagnosis.

	HPE final diagnosis					_
	Tuberculous lymphadenopathy	Reactive hyperplasia	Malignancy	Fungal infection	Others	P value
Median CD4 count	175.0	193.0	97.0	24.0	312.0	0.012

 $Kruskal\hbox{-}Wall is \ test \ was \ used; \ HPE\hbox{-}\ Histopathological examination}.$ 

Table 3: ART status and final diagnosis.

	Final diagnosis						
ART	Tuberculous lymphadenopathy N (%)	Reactive hyperplasia N (%)	Malignancy N (%)	Fungal infection N (%)	Others N (%)	Total n (%)	P value
Yes	36 (50.7)	22 (31.0)	9 (12.7)	1 (1.4)	3 (4.2)	71(100.0)	0.022
No	31 (79.5)	4 (10.3)	4 (10.3)	0 (0.0)	0 (0.0)	39 (100.0)	0.022
Total	67 (60.9)	26 (23.6)	13 (11.8)	1(0.9)	3 (2.7)	110 (100.0)	

Fisher's exact test was used; ART- Anti-retroviral therapy.

Table 4: USG findings and final diagnosis.

	Final diagnosis			р
USG findings	HIV associated diseases n (%)	Reactive hyperplasia n (%)	Total n (%)	value
Sonologically characteristic findings	77 (88.5)	10 (11.5)	87 (100)	0.001
Sonologically unremarkable	7 (30.4)	16 (69.6)	23 (100)	0.001
Total	84 (76.4)	26 (23.6)	110 (100)	

Chi-Square test was used.

Table 5: Comparison of diagnosis yield of various studies.

Author	Country of study	Sample size	Diagnosis (%)
Kamana et al <sup>12</sup>	India	300	<ul><li>(57.7) Tuberculous lymphadenopathy</li><li>(29.7) Reactive hyperplasia</li><li>(2.3) Lymphoma</li><li>(2.3) Fungal infection</li><li>(1.7) Suppurative lymphadenitis</li></ul>
Bogoch et al <sup>14</sup>	USA	107	<ul><li>(42.9) Malignancy</li><li>(49.5) Reactive hyperplasia</li><li>(2.8) Tuberculous lymphadenopathy</li><li>(4.7) Other</li></ul>
Bottles et al <sup>15</sup>	USA	121	<ul><li>(50.0) Benign hyperplasia</li><li>(20.0) Non-Hodgkin lymphomas</li><li>(17.0) Tuberculous lymphadenopathy</li><li>(10.0) Kaposi sarcoma</li><li>(3.0) Hodgkin lymphoma</li></ul>
Vanisri et al <sup>16</sup>	India	36	(58.3) Tuberculous lymphadenopathy (36.1) Reactive hyperplasia
Jayaram et al <sup>17</sup>	Malaysia	39	(53.8) Tuberculous lymphadenopathy (25.6) Reactive hyperplasia
Tirumalasetti et al <sup>18</sup>	India	129	<ul> <li>(41.9) Tuberculous lymphadenopathy</li> <li>(35.6) Reactive hyperplasia</li> <li>(12.4) Suppurative lymphadenitis</li> <li>(4.7) Malignancy</li> <li>(0.78) Fungal infection</li> <li>(0.78) Other</li> </ul>
Ramos et al <sup>19</sup>	Brazil	210	<ul> <li>(50.2) Tuberculous lymphadenopathy</li> <li>(23.3) Reactive follicular hyperplasia</li> <li>(9.0) Lymphoma</li> <li>(5.7) Fungal infection</li> <li>(2.9) Metastatic cancer</li> <li>(4.8) Inconclusive</li> </ul>
Present study	India	110	(60.9) Tuberculous lymphadenopathy (23.6) Reactive Hyperplasia

There was no statistically significant difference between age group and gender with final diagnosis. As depicted in Table 1, there was a statistically significant difference between weight loss and final diagnosis. Significantly higher percentage of patients having weight loss were diagnosed with tuberculous lymphadneopathy.

The median HIV infection duration for final diagnosis of tuberculous lymphadneopathy, reactive hyperplasia, malignancy, other diseases, and fungal infection was 2, 24, 60, 24 and 84 months respectively which was statistically significant (p=0.003). As depicted in table 2, median CD4 count was 175, 193, 97, 24 and 312 in patients diagnosed with tuberculous lymphadenopathy, reactive hyperplasia, malignancy, fungal infection and others respectively which was statistically significant. As depicted in Table 3, there was statistically significant difference between HPE findings and history of ART. Percentage of tuberculosis lymphadenopathy patients was significantly higher in those who were not taking ART as compared to those who were on ART.

To analyse the statistical significance of USG findings, we clubbed together HIV associated diseases (tuberculous lymphadenopathy, malignancy, fungal, others) and compared them with reactive hyperplasia. Loss of fatty hilum, loss of nodal architecture, matted nodes, hypoechoic nodes, intranodal necrosis, intranodal calcification, perinodal tissue edema (periadenitis) were considered sonologically characteristic findings whereas enlarged nodes without any other characteristic finding were included in 'sonologically unremarkable' group.

As shown in Table 4, percentage sonologically characteristic findings was higher in HIV associated diseases (tuberculous lymphadenopathy, malignancy, fungal, others) in clinically non-palpable nodes predicted by USG as compared to reactive hyperplasia. Sensitivity, specificity, positive predictive value, and negative predictive value was 91.7%, 61.5%, 88.5%, and 69.6% respectively for diagnostic yield of USG in non-palpable lymph nodes.

#### **DISCUSSION**

The present research was conducted on 110 HIV positive patients to study diagnostic yield of non-palpable peripheral lymph node biopsies in sonologically proven enlarged/suspicious nodes in PLHIV.

More than three-fourths (78.1%) of the study population was between 31 and 50 years of age. The age range is similar to studies conducted for evaluation of peripheral lymphadenopathy in HIV positive patients.<sup>12,13</sup>

In the present study the final diagnoses obtained after histopathological analysis, in order of frequency were tuberculouas lymphadenopathy, reactive hyperplasia, malignancy and fungal infection. There are many past studies evaluating diagnoses obtained after fine needle aspiration cytology (FNAC) of enlarged lymph nodes but there are no studies in which only sonologically detected non-palpable lymph node biopsy was done. Table 5 compares the diagnosis yield of FNAC with the present study.

Lymphadenopathy is likely to be reactive or malignant in developed countries which are non-tuberculosis endemic regions. Also, since the studies are based on FNAC reports, many patients might have been misdiagnosed as reactive hyperplasia. Most of the differences in the clinical presentation among patients from various geographic areas were driven by the epidemiology of TB and HIV in the countries of origin. H

# Tuberculous lymphadenopathy

It can be seen from table 5 that in developed country like USA, the prevalence of tuberculosis was less as compared to developing countries.<sup>14,15</sup> In developing countries like India, Malaysia, and Brazil, the prevalence of tuberculosis was high as reported by many studies.<sup>12,16-19</sup> In the present research prevalence of tuberculosis was comparable with the studies conducted in developing countries.

# Reactive hyperplasia

Reactive lymphadenopathy/ hyperplasia/lymphadenitis indicates persistent generalized lymphadenopathy in HIV positive patients. In the present research 26/110 (23.6%) patients had reactive lymphadenopathy. The prevalence of reactive lymphadenopathy in our study was consistent with FNAC studies conducted in tuberculosis endemic countries like India. <sup>12,16,17</sup>

# **Malignancy**

In the present study, 13/110 (11.8%) patients were diagnosed with malignancy. The prevalence of malignancy in our study was higher compared to previous FNAC studies in India and in Malaysia. <sup>12,16-18</sup> This can be explained by confirmatory diagnosis obtained by excision biopsy after HPE due to adequate specimen and with

preserved morphology. Higher prevalence of malignancy was reported in studies conducted in USA. <sup>14,15</sup>

# Fungal infection

In the present research only one patient was diagnosed with histoplasma infection. Histoplasmosis is an underrecognized disease in India and should be considered in the differential diagnosis of patients of immune-compromised status with prolonged fever, oral ulcers and granulomatous disease on histopathology without response to anti-tubercular treatment. Similar findings were reported in India <sup>18</sup> whereas higher prevalence of fungal infection was reported in other studies. <sup>12,17,19</sup>

# Effect of HIV duration and final diagnosis

In the present study, median duration of HIV in patients diagnosed with malignancy was 5 years. Increased duration of HIV correlates with higher likelihood of the diagnosis being malignancy (Hodgkin's lymphoma, Non-Hodgkin's lymphoma). Median duration of diagnosis of Hodgkin's lymphoma in PLHIV was approximately 7 years and 6 months in one study.<sup>20</sup> In a retrospective study of 45 patients with HIV associated Hodgkin's lymphoma, the mean interval between the diagnosis of HIV and HIV associated Hodgkin's lymphoma was 5.2 years.<sup>21</sup> Our results are similar to this study.

# CD4 count and final diagnosis

In the present study, the median CD4 count was 175, 193, 97 and 24 in patients diagnosed with tuberculous lymphadenopathy, reactive hyperplasia, malignancy, and fungal infection respectively. This suggests that lower CD4 count increases the incidence of malignancy and fungal infection in HIV positive patients. It was reported that risk factors for the development of Non-Hodgkin's lymphoma in HIV include a low CD4 T-cell count, high HIV viral load and higher age. 22,23 The incidence of Lymphomas in HIV/AIDS has changed since the era of highly active ART, with a lower incidence of lymphomas correlating with improved CD4 counts. 24

# USG and final diagnosis

In the present study, there was statistical significance between USG findings and final diagnosis after biopsy of lymph nodes. The findings on USG like hypoechoic nodes, loss of fatty hilum, loss of nodal architecture, intranodal calcifications, intranodal necrosis, matted nodes, periadenitis were considered characteristic findings. Thus, USG can effectively guide the surgeon to undertake biopsy of the most pathological lymph node with a sensitivity of 91.7%.

The strength of the present research is no similar study was conducted to find diagnostic yield of non-palpable lymph node biopsy on the basis of USG alone. Limitations of the present study is that control group was not used to compare diagnostic yield of clinically

palpable lymph node biopsy and effect of previous anti tuberculosis treatment status (complete/incomplete). History of previous opportunistic infection was not taken into consideration. Multi-centric study with larger sample size is required to evaluate usefulness of sonologically detected peripheral lymph node biopsy in PLHIV.

# **CONCLUSION**

USG is a sensitive tool for the early detection of clinically non-palpable pathological lymph nodes in symptomatic PLHIV. The most common diagnosis obtained after sonologically detected peripheral lymph node biopsy in symptomatic PLHIV was tuberculous lymphadenopathy, followed by reactive hyperplasia. Significantly higher percentage of patients having weight loss were diagnosed with tuberculosis lymphadenopathy. The prevalence of tuberculous lymphadenopathy was higher in patients who were not on ART. Diagnosis of HIV associated diseases significantly varied with the duration of HIV. Median duration of HIV in tuberculosis lymphadenopathy patients was less whereas malignancy had a longer duration of HIV. Sensitivity, and specificity was 91.7%, and 61.5% respectively for diagnostic yield of USG in non-palpable lymph nodes.

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