Evaluation of Ultrasonography and Colour Doppler in Cervical Lymphadenopathy Taking Histopathology as Comparison

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ABSTRACT

BACKGROUND
Grey scale ultrasonography can be used as a first line imaging modality along with histopathology for evaluation of cervical lymphadenopathy. Morphologic features like size, shape, hilum and necrosis within the lymph node and vascularity are analysed. We wanted to identify the role of Grey scale ultrasonography and colour Doppler in characterisation of enlarged cervical lymph nodes, evaluate findings in benign and malignant cervical lymphadenopathy and assess the sensitivity, specificity, predictive value, likelihood ratios and accuracy in malignant and benign cervical lymph node enlargement.

METHODS
This was a cross sectional study with diagnostic test evaluation. All suspected cases of cervical lymphadenopathy referred for sonological evaluation to the Department of Radiodiagnosis of Govt. T.D. Medical College, Alappuzha were included in the study. About 75 patients included in the study, underwent Grey scale and colour Doppler sonography of cervical lymphadenopathy.

RESULTS
When three criteria were positive for malignancy, the sensitivity and specificity of grey scale ultrasonography and colour Doppler were 100 % and 73.80 %, and when four criteria were positive, the sensitivity and specificity were 72.73 %, and 90.48 % respectively. When we analysed individual variables, status of hilum (absence / presence) has reasonably good sensitivity and specificity, positive predictive value, negative predictive value and accuracy (90.09 %, 92.90 %, 90.09 %, 92.90 % and 92.0 % respectively). In our study the important criteria positive for malignant lymph nodes were size > / 11 mm in short axis diameter, round shape, absent hilum and peripheral and central + peripheral type of vascularity of cervical lymph nodes. For benign lymph nodes, oval shape, presence of hilum and central vascularity were the important criteria noted.

CONCLUSIONS
Grey scale ultrasonography can be complemented by colour Doppler ultrasonography (USG) in patients presenting with cervical lymphadenopathy to differentiate between benign and malignant lesions by reducing unnecessary biopsies by more accurate discrimination.

KEY WORDS
Grey Scale Ultrasonography, Malignant, Benign Lymph Nodes, Histopathology
Lymphadenopathy is an abnormality in the size or character of lymph nodes caused by the invasion or propagation of inflammatory or neoplastic cells to the lymph nodes. It usually presents as enlargement of lymph nodes and are very common in developing countries like India. The lymph nodes affected are abnormal in size, consistency or number. It may be generalised or localised involving specific groups of lymph nodes. Causes for cervical lymphadenopathy may vary. They include primary or secondary malignant neoplasms of lymph node, bacterial, viral, fungal and protozoal infections, autoimmune conditions or drugs. Majority of the patients may have benign lymphoid hyperplasia or reactive lymphadenitis. Lymphadenopathy may be the only clinical finding or one of the several nonspecific findings in some patients. Early detection of malignancy is of great clinical significance. It improves the patient survival by proper surgical and radiation treatment. Pre-treatment staging and therapeutic planning in patients with primary malignant tumours is possible with sonographical evaluation.

Biopsy and other pathological tests are invasive and time consuming. But, Ultrasonography is easily available, cost effective, non-invasive, radiation free and safe investigation. For assessing cervical lymph node, sonography is a useful imaging tool. It is a non-invasive, non-ionising imaging technique. This Technique with the help of ultrasound waves provides valuable diagnostic information with a high diagnostic accuracy. The conventional ultrasound uses a grey scale to detect the various anatomical structures of body and study their morphological characteristics. Even small blood vessels in lymph nodes can be identified with ultrasound. Grey scale sonography is widely used in the evaluation of number, size, site, shape, borders, matting, adjacent soft tissue oedema and internal architecture of cervical lymph node. The differentiation of benign from malignant lymphadenopathy is of critical importance in both treatment planning and prognosis. The use of higher frequency sonic wave’s results in an improvement in spatial and contrast resolution and also shows the internal architecture of lymph nodes. This readily identifies normal, inflammatory and malignant lymph node.

Colour Doppler can show flow in all the lymph nodes including benign and malignant lesions of lymph node. The analysis of patterns of nodal vascularity helps to differentiate benign from malignant lesions. Transducer and the equipment used determines the ability to detect a colour flow pattern within a small lymph node. High frequency transducer wave has the capacity to detect superficial low velocity signals. Vessels in inflamed lymph nodes are dilated. But vessels in lymph nodes affected with metastasis may be compressed by tumour cells. This can be easily detected with the help of colour Doppler. Presence of extra hilar blood vessels is another sign of malignancy in the sonographic diagnosis of lymph node. Presence of calcification and necrosis is also more common in malignant lymph nodes. So, in our study, size, shape, border, echogenicity, hilum, necrosis, calcification and matted / discrete nature of lymph nodes are studied for distinguishing benign and malignant lymph nodes by sonological methods.

This study has been conducted to evaluate the efficacy of ultrasonography and colour Doppler to differentiate benign from malignant cervical lymphadenopathy.

Our study is a cross sectional study with diagnostic test evaluation. It was conducted at the Department of Radio diagnosis, Govt. T.D.M.C, Alappuzha with a study period of 18 months from January 2016 to June 2017.

**Sampling Technique**

Patients with suspected cervical lymphadenopathy from other departments (Surgery, General Medicine, and E.N.T) referred to the Department of Radiodiagnosis, for sonographic evaluation of neck were included for the study. Cervical lymph nodes measuring 5 mm or more than 5 mm in long axis diameter by ultrasonography were included for the study in patients from 5 to 80 years. Patients not giving consent and in whom follow up lost were excluded from the study.

By using the formula, \( N = (Z \alpha)^2 \times pq / d^2 \) one would get the total number of positive cases \( n \) which is required for the study. Where \( Z \alpha \) is 1.96 at 5 % \( \alpha \).

\( P \) is expected sensitivity, \( q \) is \((100 - p)\), \( d \) is precision, which can be any value between 5 to 20 % of \( p \) (taking as 10 % of \( p \) in this study). Hence the total number of positive cases, \( N = (1.96)^2 \times 87.5 \times 12.5 / 12^2 = 29.37 \) approximately 30 cases. From the cases referred to the Department of Radio diagnosis, On examining records, an average of 40 % prevalence was noted.

Hence the total sample size \( N \) would be equal to total number of positive cases \( N \) required for the study divided by prevalence \( P \).

Sample size \( N = n / P = 30 \times 100 / 40 = 75 \).

Cervical lymph nodes are characterised on grey scale by the shape, size, border, hilum and calcification of the lymph nodes and their vascular pattern on Colour Doppler. All lymph nodes were evaluated by histopathology to differentiate malignant or benign.

Data was collected from a total of 75 cases referred for the ultrasonography of neck. All sonographic examinations were done in GE Logic F Series ultra-sonographic and Colour Doppler equipment with a linear array high frequency \( (7 \times 13 \text{ MHz}) \) transducer. Data from all histopathological evaluations were collected. Data was entered in Microsoft excel.

**Statistical Analysis**

Analysis of the data was done in SPSS version 16.0 and DAG_Stat (Diagnostic & Agreement Statistics analysis package). Frequencies of variables, sensitivity, specificity, positive predictive value, negative predictive values were calculated.
TP - True positive is both gold standard and test parameter positive
TN - True negative is both gold standard and test parameter negative
False positive (FP) is test parameter positive and gold standard negative
False negative - (FN) is test parameter negative and gold standard positive
Sensitivity - (TP) / (TP + FN)
Specificity: (TN) / (TN + FP)
Positive predictive value (PPV) - (TP) / (TP + FP)
Negative predictive value (NPV) - (TN) / (TN + FN)
Accuracy - (TN + TP) / (TN + TP + FN + FP)
Receiver Operating Characteristics (ROC) Analysis
ROC curve (Receiver-Operating Characteristics Curve) was plotted between sensitivity and 1 - specificity.
For a given diagnostic test, the true positive rate (TPR) against false positive rate (FPR) can be measured, where TPR= TP / (TP + FN) FPR = FP / (FP + TN)
TPR is equivalent to sensitivity and FPR is equivalent to (1 - specificity). A point in ROC space is represented by a TPR and corresponding FPR, which shows the trade-off between sensitivity and 1 - specificity at different cut offs. This means that, the increase in sensitivity is accompanied by a decrease in specificity or increase false positive rates. 

### RESULTS

| Size of the Lymph Node | Malignant No. (%) | Benign No. (%) | Total No. (%) |
|------------------------|-------------------|---------------|--------------|
| < 10 mm                | 6 (18.80 %)       | 39 (92.85 %)  | 45 (60 %)    |
| ≥ / > 10 mm            | 27 (81.20 %)      | 3 (7.15 %)    | 30 (40 %)    |

**Table 1. Size of the Cervical Lymph Node (Short Axis Diameter) with Histopathology (Malignant vs. Benign)**

**Figure 1. Percentage Distribution of the Sample According to Age**

**Figure 2. Distribution of Sample as per Shape, Matted / Discrete Nature, Vascularity of Lymph Node**

**Figure 3. Percentage Distribution of Sample as per Border, Status of Hilum, Necrosis, Echogenicity and Calcification of Lymph Node**

| Border | Status of hilum | Necrosis | Echogenicity | Calcification |
|--------|----------------|----------|--------------|--------------|
| 42.4   | 95.2           | 92.9     | 90.9         | 90.9         |
| 4.8    | 9.1            | 7.1      | 9.5          | 9.1          |
| 42.4   | 95.2           | 92.9     | 90.9         | 90.9         |
| 42.4   | 95.2           | 92.9     | 90.9         | 90.9         |
| 42.4   | 95.2           | 92.9     | 90.9         | 90.9         |
After the completion of the study, the following observations were made from the analysis. Out of the 75 patients who presented with cervical lymphadenopathy, ultra-sonographic examination and histopathological correlation was done in all the cases.

The analysis was done under 3 main subdivisions. This included Descriptive statistics, Analysis by Gray-scale ultrasonographic findings, analysis by colour Doppler sonographic findings.

**Descriptive Statistics**
In the study total participants were 75 (100 %), out of which 52 (69.3 %) were males & 23 (30.7 %) were females. Out of this 50.7 % were below 39 years and 33.3 % were 40 to 59 years and 16 % were above 59 years old.

**Grey Scale Ultra Sonographic Findings**

*Analysis of Cervical Lymph Node by Size*
Test positive criteria: size of the cervical lymph node (short axis diameter) > / = 10 mm. Out of 75 lymph nodes, 30 nodes (40 %) were > / = 10 mm in short axis diameter and 45 (60 %) < 10 mm in short axis diameter. Out of the 33 (100 %) malignant lymph nodes, 27 (81.20 %) were > / = 10 mm in short axis diameter and 6 (18.80 %) were < 10 mm in short axis diameter. Out of 42 (100 %) benign lymph nodes, 39 (92.85 %) lymph nodes were < 10 mm in short axis diameter and 3 lymph nodes (7.15 %) were > / = 10 mm.

Statistical indices showing comparison of size of lymph nodes with histopathology (Malignant Vs Benign) showed the following results. By comparing the size of the lymph node, the sensitivity to differentiate benign and malignant and benign lymph nodes were 81.8. Specificity was 92.9. False Negative was 18.2 and false positive was 7.1. Positive predictive value was 90. Negative Predictive Value was 86.7. Positive Likelihood ratio was 11.5. Negative Likelihood ratio was 0.2. Accuracy of the test was 88.

*Analysis of Cervical Lymph Node by Shape*
Shape of the cervical lymph node correlated with histopathology (Malignant vs. Benign) using test positive criteria as round shape. Out of the 33 malignant lymph nodes, 28 (84.84 %) were round in shape and 5 (15.12 %) were oval in shape. Out of the 42 benign lymph nodes, 37 (90.1 %) were oval in shape and 5 (11.90 %) were round in shape. Statistical indices showing comparison of shape of lymph node with histopathology (Malignant vs. Benign) were done. Sensitivity of the test was 84.8. Specificity was 88.1. False negative was 15.2. False positive was 11.9. Positive Predictive value was 84.8. Negative Predictive value was 88.1. Positive Likelihood ratio was 7.1. Negative Likelihood ratio was 0.2. Accuracy of the test was 86.7.

*Analysis of Cervical Lymph Node by Border*
Test positive criteria for analysis of cervical lymph node was irregular border of the lymph node. Out of 33 malignant nodes, 14 cases (42.42 %) were with irregular borders and 19 cases (57.58 %) were with regular borders. Out of 42 benign nodes 40 (95.40 %) were with regular borders and 2 cases (4.60 %) were with irregular borders. Positive Predictive value was 84.8. Negative Predictive value was 88.1. Accuracy was 86.7.

*Analysis of Cervical Lymph Node by Hilum*
Out of 33 malignant lymph nodes, 30 cases (90.90 %) showed absent hilum and 3 cases (9.1 %) showed preserved hilum. Out of 42 benign nodes 39 Cases (92.90 %) showed presence of hilum and 3 cases (7.1 %) showed absence of hilum. Test positive criteria is absent hilum of lymph node. Sensitivity was 90.9. Specificity was 92.9. False Negative was 9.1. False positive was 7.1. Positive predictive value was 90.9. Negative predictive value was 92.9. Positive Likelihood ratio was 12.7. Negative Likelihood ratio was 0.1 Accuracy was 92.

*Analysis of Cervical Lymph Node by Necrosis*
Test positive criteria was presence of necrosis in lymph node. Out of 33 cases of malignant cervical lymph nodes 14 cases (42.42 %) were with necrosis and 19 (57.58 %) were without necrosis. Out of 42 lymph nodes, only 4 cases (9.58 %) showed presence of necrosis. 38 cases (90.42 %) were without necrosis. Sensitivity was 42.4. Specificity was 90.5. False Negative was 57.6. False positive was 9.5. Positive predictive value was 77.9 negative predictive value was 66.7. Positive Likelihood ratio 4.5. Negative Likelihood ratio was 0.6. Accuracy was 69.3.

*Analysis of Cervical Lymph Node by Hypochoegenecity*
Test positive criteria was hypochoegenecity of lymph node. Out of 33 malignant cases, 30 (90.9 %) cases were hypoechoic and 3 (9.1 %) cases were hetero-hypoechoic. Out of 42 benign cases, 41 (97.38 %) cases were hypoechoic and 1 (2.62 %) cases were hetero-hypoechoic. Sensitivity of the test was 90.9. Specificity was 2.4. False negative was 9.1. False positive was 97.6. Positive predictive value was 42.3. Negative predictive value was 25. Positive likelihood ratio was 0.9. Negative likelihood ratio was 3.8. Accuracy was 41.3.

*Analysis of Cervical Lymph Node by Calcification*
Test positive criteria was presence of calcification in lymph node. Out of 33 lymph nodes, 2 (6.06 %) showed calcification. 31 cases (93.94 %) showed no calcification. All 42 benign
lymph nodes showed no calcification. Sensitivity of the test was 93.9. Specificity was 0. False negative was 6.1. False positive was 100. Positive predictive value was 42.5. Negative predictive value was 0. Positive likelihood ratio was 0.9. Accuracy was 41.3.

**Analysis of Cervical Lymph Node by Matted / Discrete Nature**

Test positive criteria was discrete nature of lymph node. Out of 33 malignant lymph nodes, 31 (93.93 %) lymph nodes were discrete and 2 (6.06 %) were matted. Out of 42 benign lymph nodes, 35 (83.33 %) were discrete and 7 (16.66 %) were matted. Sensitivity was 93.9. Specificity was 16.7. False negative was 6.1. False positive was 83.3. Positive predictive value was 47. Negative predictive value was 77.8. Positive likelihood ratio was 1.1. Negative likelihood ratio was 0.4. Accuracy was 50.7

**Analysis of Cervical Lymph Nodes by Colour Doppler Sonographic Findings**

Among 33 malignant lymph nodes 14 cases (42.42 %) were with peripheral vascularity and 19 cases (57.58 %) with peripheral and central vascularity. Only 3 cases (9.09 %) showed central vascularity. Among 42 benign nodes 34 cases (80.95 %) showed central vascularity and 8 (19.04 %) cases showed peripheral and central vascularity. Test positive criteria was peripheral / Central + peripheral vascularity of lymph node. Sensitivity was 90.9. Specificity was 91. False negative was 9.1. False positive was 90. Positive predictive value was 78.9. Negative predictive value was 91.9. Positive likely ratio was 4.8. Negative likely ratio was 0.1. Accuracy was 85.3.

**Histopathological Evaluation of the Sample**

Out of 33 malignant lymph nodes, 25 cases (75.75 %) were metastasis from various primaries, 8 cases (24.24 %) were diagnosed as lymphoma on histopathology. Out of 42 cases diagnosed as benign, 29 cases (69.04 %) were reactive, 11 cases (26.19 %) were tuberculosis and 2 cases (4.76 %) sarcoidosis.

**Analysis Taking Multiple Variables (Malignant v/s. Benign)**

When analysing the multiple criteria as test positive, the following observations were made from our study. When one or two criteria were positive for malignancy the sensitivity, specificity and accuracy were 100 %, 0 % and 44 % respectively. When three criteria were positive for malignancy, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 100 %, 73.80 %, 75.0 %, 100 % and 85.33 % respectively. When four criteria were positive for malignancy, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 72.73 %, 90.48 %, 85.70 %, 80.85 % and 82.67 % respectively. When five criteria were positive for malignancy, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 72.73 %, 90.48 %, 85.70 %, 80.85 % and 82.67 % respectively. When six criteria were positive for malignancy, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 9 %, 100 %, 100 %, 58.33 % and 60.0 % respectively.

When seven or all criteria were positive for malignancy, the sensitivity, specificity, and accuracy were 0 %, 100 % and 56 % respectively. From analysing all these data, we got a reasonably good sensitivity when three criteria were positive for malignancy and good specificity when four criteria were positive for malignancy as previously described.

Receiver-Operating Characteristics Curve (ROC Curve) for size of lymph node (short axis diameter) with cut off as > / = 10 mm was put. Area Under the curve was 0.907. From the above ROC curve, a reasonably good cut off value with acceptable specificity was found at 11.0 mm short axis diameter of lymph node. As per the new cut-off of the short axis diameter of lymph node as 11 mm, out of the 33 malignant lymph nodes, 27 were > / = 11 mm and 6 were < 11 mm. Out of 42 benign lymph nodes, 3 were > / = 11 mm and 39 were < 11 mm. We obtained a sensitivity of 81.82 %, specificity of 92.86 % and accuracy of 88 %. Positive predictive value was 90.00 % Negative predictive value was 86.67 %. Positive Likelihood Ratio was 11.45. Negative Likelihood Ratio was 0.20.

**DISCUSSION**

Out of the 75 patients studied, 44 % (33 cases) were malignant and 56 % (42 cases) were benign. 49 % (37 cases) were in the age group of more than 40 years, which was the common age group of majority of patients presenting with malignant lesions. Most common clinical presentation of patients was swelling in the neck.

Majority of the malignant lymph nodes (81.8 %) 27 cases have a short axis diameter > / = 10 mm and majority of benign lymph nodes (92.9 %) 37 cases have a short axis diameter <10 mm. Thus size of the lymph node has got a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 81.8 %, 92.9 %, 90 %, 86.7 % and 88 % respectively.

Majority of the malignant lymph nodes, 28 cases (84.84 %) were round in shape and majority of benign lymph nodes 37 cases (90.1 %) were oval in shape. So shape of lymph node is a very important grey scale sonological parameter in deciding the character of lesion. In our study it has got a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 84.8 %, 88.1 %, 84.8 %, 88.1 % and 86.7 % respectively.

When analysing the hilum of the lymph node, absent hilum was noted in 90.9 % of malignant lymph nodes and hilum was preserved in 92.9 % cases of benign lymph nodes. So the presence or absence of hilum helps in characterizing the lesion. In our study it has got a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 90.9 %, 92.9 %, 90.9 %, 92.9 % and 92.0 % respectively.

Intranodal necrosis is another important parameter in characterising the cervical lymphadenopathy whether it is benign or malignant. In this study, necrosis was noted in 14 cases (42.42 %) of malignant nodes and 4 cases (9.58 %) of benign lymph nodes. In our study it has got a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 42.4 %, 90.5 %, 77.8 %, 66.8 % and 89.3 % respectively.
Calcifications within the malignant lymph nodes was usually noted in papillary or medullary carcinoma of thyroid.\textsuperscript{32,33} 2 cases (6.06\%) in our study which showed calcifications were proved malignant. In our study it has got a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 93.9\%, 0\%, 42.5\%, 0\% and 41.3\% respectively. This variable has got good sensitivity but not specific.

Another important parameter in characterising the cervical lymphadenopathy is vascularity within the lymph node.\textsuperscript{34,35} Benign nodes usually have a central hilar vascularity. Majority of the benign nodes especially reactive nodes will have this pattern.\textsuperscript{36,37} But malignant lymph nodes have peripheral or central+ peripheral type of vascularity. In our study 34 cases (80.95\%) of benign nodes have central vascularity and 30 cases (90.9\%) of malignant nodes showing peripheral, central + peripheral type of vascularity.\textsuperscript{38,39} In our study it has got a sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 90.9\%, 81.0\%, 78.9\%, 91.9\% and 85.3\% respectively.

When analysing the multiple criteria as test positive, the following observations were made from our study.
- When one or two criteria were positive for malignancy the sensitivity, specificity and accuracy were 100\%, 0\% and 44\% respectively.
- When three criteria were positive for malignancy, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 100\%, 73.80\%, 75.0\%, 100\% and 85.33\% respectively.
- When four criteria were positive for malignancy, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 72.73\%, 90.48\%, 85.70\%, 80.85\% and 82.67\% respectively.
- When five criteria were positive for malignancy, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 36.36\%, 100\%, 100\%, 16.67\% and 72.0\% respectively.
- When six criteria were positive for malignancy, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 9\%, 100\%, 100\%, 58.33\% and 60.0\% respectively.
- When seven or all criteria were positive for malignancy, the sensitivity, specificity, and accuracy were 0\%, 100\% and 56\% respectively.

From analysing all these data, we got a reasonably good sensitivity when three criteria were positive for malignancy and good specificity when four criteria were positive for malignancy as previously described. When analysing individual variables, it was found that absence of hilum of a cervical lymph node could detect malignancy with a reasonably good sensitivity and specificity of 90.9\% and 92.9\% respectively. Hence, it could be considered as a strong variable by itself to characterize cervical lymph node as malignant or benign.

**Relevance of the Study**

Although histopathology is the gold standard (100\%) in evaluation of cervical lymphadenopathy, Grey scale ultrasonography and colour Doppler can be used a first line investigative modality, provided this study and further studies in the area prove that it has high sensitivity, specificity, positive predictive value, negative predictive value and accuracy in the evaluation of cervical lymphadenopathy. CT scan, MRI, FDG-PET can be used, but not used widely due to its inaccessibility and the radiation exposure. Grey scale ultrasonography and colour Doppler ultrasonography is non-invasive, painless, readily available and relatively easy to apply, and it entails no radiation exposure. It is especially useful in patients in whom radiation exposure has to be avoided like pregnant patients, paediatric patients, patient with known history of malignancy who need multiple CT scans for follow up.

**CONCLUSIONS**

Grey scale ultrasonography and colour Doppler have a sensitivity and specificity of 100\% and 73.80\% respectively, in detecting malignancy in cervical lymphadenopathy when three criteria are positive. As the number of criteria increases the specificity increases, but sensitivity decreases. When analysing individual variables, absence of hilum has got a good sensitivity and specificity, positive predictive value, negative predictive value and accuracy. In our study, the most important criteria positive for malignant lymph nodes were size of lymph node > / 11 mm in short axis diameter, round shape, absent hilar and peripheral and central+ peripheral type of vascularity of cervical lymph nodes.

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