ABSTRACT

Introduction: The lymph node is commonly involved by a spectrum of inflammatory pathologies, some of which are associated with graven morbidity and mortality if not promptly treated.

Objectives: To analyze pathological spectrum of involvement of peripheral lymph nodes by various inflammatory lesions at the University of Uyo Teaching Hospital and a private specialist laboratory in Uyo, Akwa Ibom State, Nigeria between January 2007 and December, 2015.

Methodology: A retrospective review of all Haematoxylin and Eosin stained slides of lymph node specimens were undertaken. Relevant data were extracted from their laboratory request forms, registers and reports.

Results: A total of 66 histologically confirmed diagnoses of inflammatory lesions of superficial lymph node were made; males outnumbered females in the ratio of 1.2:1. The age of these patients ranged from 0.75 to 78 years with a mean age of 26.5 years. The modal age group was 11-20 years, accounting for 24 cases (36.4 %). Fifty - two (78.8 %) cases of inflammatory lesion occurred in patients aged below 30 years. Cervical lymph node was the most common biopsied site for inflammatory lesions, constituting 38 (57.6 %) cases; followed by axillary and inguinal lymph nodes in order of frequencies of 13 (19.7 %) and 7 (11.7 %) cases respectively. Tuberculous lymphadenitis was the commonest inflammatory lesion, accounting for 30 out of 66 cases (45.5%); this is followed by reactive hyperplasia representing 18 out of 66 cases (27.3 %). Necrotizing chronic lymphadenitis, HIV - associated lymphadenopathy, benign reticuloses, and Castleman disease were other inflammatory lesions seen in 12.1 %, 7.6 %, 4.5 %, and 3.0 % of cases respectively.

Conclusion: Tuberculous lymphadenitis and reactive hyperplasia were the most preponderant inflammatory lesions of superficial lymph node in our environment with children and cervical lymph node being more favourably affected.

INTRODUCTION

Lymph node, a vital component of reticuloendothelial system plays a crucial role in adaptive immune response in humans and reacts to various known and unknown stimuli with resultant reactive changes leading to vast array of non-neoplastic lymphoid proliferations or hyperplasias (1,2). The lymph node appears spheroidal, ovoid or reniform in shape and has a delicate fibrous capsule; it is composed of lymphadenoid parenchyma, the lymphadenoid follicles and the lymphatic sinusoids mainly (3,4). Despite its protective function, lymph node is not free from a spectrum of diseases including acute lymphadenitis, chronic lymphadenitis, reticuloses and lipodystrophies, primary lymphadenopathies, secondary lymphadenopathies, drug reactions (including certain vaccines), and non-neoplastic lymphoproliferative disorders such as Castleman and Rosai Dorfman diseases (2-13).

Most of these pathologies in the lymph node are manifested as enlargement of the lymph node otherwise known as lymphadenopathy (1-3,6-10,14-16). Thus, lymphadenopathy (LAP) is a term used to describe the conditions in which lymph nodes become abnormal in size, consistency, and number (2,15,16).
Lymphadenopathy can be categorized clinically either as localized or generalized types (1-3,6-10, 14-16). In another way, it can be classified clinically as either peripheral or visceral lymphadenopathies (5). Peripheral lymphadenopathies are easily detected by routine physical examination owing to their superficial anatomic location which make them more easily accessible to biopsy or lymphadenectomy, which is a minor surgical procedure (5). On the hand, visceral lymphadenopathy is deep seated and does require laparotomy or sophisticated imaging techniques for detection (5).

Of note, superficial nodes in the upper part of the body (cervical, supraclavicular, axillary) are preferentially biopsied than lower limb nodes (popliteal, inguinal or femoral) as the former are more likely to yield definitive diagnosis whereas the latter are often characterized by non-specific reactive or chronic inflammatory and fibrotic changes (5,17).

The causes of peripheral or superficial lymphadenopathy are diverse and may either be obvious or obscure (3,6). The causes may be broadly grouped into neoplastic and non-neoplastic. The non-neoplastic causes are largely inflammatory changes ranging from infective agents related, drug reactions, lipid storage disorders, and non-specific inflammatory conditions (1,14,17).

Aetiology of non-neoplastic lesions, largely inflammatory lymphadenitis varies with age group, anatomical and geographical locations (1,13,15).

Cervical lymph nodes are commonly involved, more often than are other lymph nodes in inflammatory lesions (15).

Although, cytological examination of lymph nodes by fine needle aspiration smears can distinguish lymphadenopathy into inflammatory, benign and malignant neoplastic lesions, excision biopsy of the lymph node biopsy still remains the "gold standard" for diagnosis of lymphadenopathy (2,9,12,15).

This study is aimed at analyzing pathological spectrum of involvement of superficial lymph nodes by various inflammatory lesions in our environment.

**MATERIALS AND METHODS**

This was a retrospective hospital-based study of inflammatory lesions seen in superficial lymph node specimens, which were received in the Histopathology laboratory of University of Uyo Teaching Hospital (UUTH) and an independent, Histo-Vision Private Specialist laboratory, Uyo, Akwa-Ibom State, Nigeria, between January 2007 and December 2015.

The relevant clinical information from the case notes, histopathology registers and reports including biodata of the patients including HIV status, anatomic location, and histological characteristics were retrieved from archive of Department of Histopathology of the University of Uyo Teaching Hospital and the specialist laboratory in Uyo, Akwa Ibom State.

Slides and paraffin-embedded blocks of the pathological specimens of the lymph node were retrieved from the archive of the department; where necessary, new sections were cut from the blocks at three to five microns in thickness sections and processed with haematoxylin and eosin stain. Special stains including Ziehl Neelsen, Periodic Acid Schiff (PAS) and Gomori's methenamine silver were used where indicated.

The slides were microscopically reviewed to confirm the diagnosis. Inclusion criterion for the study was all inflammatory lesions of the superficial lymph node biopsies while the exclusion criteria were all inflammatory lesions of the visceral lymph node biopsies, and neoplastic lesions of lymph nodes biopsies.

Descriptive statistics were used for analysing the data using SPSS version 19 and results were presented in percentage, simple frequency, tables, figures and photomicrographs. Relationship between HIV status and lymphadenopathy would be established.

**RESULTS**

A total of 66 histologically confirmed diagnoses of inflammatory lesion of
superficial lymph node were made over a period of 9 years giving an annual frequency of occurrence of 7.3 cases in the two laboratory facilities. This constituted 1.0% of a total number of samples received during the years under review with a slight male preponderance (n=36) and a male to female ratio of 1.2:1. The age of these patients ranged from 0.75 to 78 years with a mean age of 26.5 years and modal age of 20 years. The modal age group was 11-20 years, accounting for 24 cases (36.4%) equally distributed among both genders; it is slightly higher than 22.7% and 19.7% recorded in 0-10 years and 21-30 years age groups respectively. Fifty-two (78.8%) cases occurred in patients aged below 30 years while the remaining 14 (21.2%) cases occurred in the patients aged above 30 years (Table I).

Cervical lymph node was the most preponderant biopsied site for inflammatory lesions of superficial lymph node, constituting 37 (57.6%) cases. Axillary lymph node was the second commonly affected by inflammatory lesions of superficial lymph node, accounting for 13 (19.7%) cases. Inguinal lymph node was the third leading lymphoid site for inflammatory lesions of superficial lymph node in 7 (11.7%) cases. The least anatomic sites for inflammatory lesions of superficial lymph node were supraclavicular and auricular lymph nodes accounting for 4 (6.1%) cases each (Table II).

Tuberculosis was the commonest inflammatory lesions of superficial lymph node accounting for 30 out of 66 cases (45.5%) with a male preponderance giving a male to female ratio of 1.5:1. Tuberculous lymphadenitis occurred most commonly in the 11-20 years age group accounting for 12 out of 30 cases (40.0%) overtaking 7 cases seen in 0-10 and 21-30 years age groups (23.3% each) (Figures I & II). Only one case of tuberculous lymphadenitis demonstrated positivity to acid-fast bacilli using Ziehl-Neelsen stain (3.3%).

Reactive hyperplasia was the second leading inflammatory lesion, representing 18 out of 66 cases (27.3%) with a male to female ratio of 2.6:1. Of the reactive hyperplasia, follicular-type constituted the majority, accounting for

<table>
<thead>
<tr>
<th>TABLE I: DISTRIBUTION OF INFLAMMATORY LESIONS OF SUPERFICIAL LYMPH NODES ACCORDING TO THE AGE AND SEX.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (years)</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>0-10</td>
</tr>
<tr>
<td>11-20</td>
</tr>
<tr>
<td>21-30</td>
</tr>
<tr>
<td>31-40</td>
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<tr>
<td>41-50</td>
</tr>
<tr>
<td>51-60</td>
</tr>
<tr>
<td>61-70</td>
</tr>
<tr>
<td>71-80</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>
13 out of 18 and occurring frequently in the 11-20 years age group in 6 out of 13 cases with a male to female ratio of 5.5:1) (Figure III). Sinus histiocytosis accounted for the remaining 5 cases of reactive hyperplasia with bimodal peak age of presentation within 11-20 years and 41-50 years age groups and a slight female preponderance (female to male = 1.5:1) (Figure IV).

Necrotizing chronic lymphadenitis was the third leading inflammatory lesion, representing 8 out of 66 cases (12.1%) and occurring frequently in the 21-40 years age group in 4 out of 8 cases with a male to female ratio of 1.0:1.

HIV-associated lymphadenopathy accounted for 5 cases of inflammatory lesion of superficial lymph node (7.6%) with females within the 11-20 years age group being commonly affected (female to male ratio of 4.0:1).

Benign reticuloses was diagnosed in 3 out of 66 cases (4.5%), all occurring in the cervical lymph nodes with males within the 0-10 years age group being affected predominantly.

Castleman disease was diagnosed in 2 out of 66 cases (3.0%), occurring in the cervical lymph nodes and inguinal lymph node with males within 0-10 years and 21-30 age groups being involved respectively.

Six out of the thirty cases of tuberculous lymphadenitis were accompanied with positive HIV status (20.0%). Five cases of generalized lymphadenopathy were recorded with 2/5 (40.0%) of them were HIV positive.

DISCUSSION

Superficial lymph nodes are easily accessible for excision biopsy or lymphadenectomy which is important in making prompt and precise diagnosis of lymph node lesions (1,2,4-6,9,12,15-17).

In the index study, a total of 66 histologically confirmed diagnoses of inflammatory lymphadenitis were made in patients aged 0.75 to 78 years with a mean age of 26.5 years. The highest frequency of inflammatory lymphadenitis was seen in 11-20 years age group in 24 cases (36.4%) equally distributed among both genders. These findings are relatively consistent with predominance of inflammatory lesion in patients aged below

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Cervical</th>
<th>Axillary</th>
<th>Supraventricular</th>
<th>Inguinal</th>
<th>Auricular</th>
<th>Subtotal</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>6.0</td>
<td>4.0</td>
<td>1.0</td>
<td>1.0</td>
<td>-</td>
<td>8.0</td>
<td>15.0 (22.7)</td>
</tr>
<tr>
<td>11-20</td>
<td>8.0</td>
<td>4.0</td>
<td>2.0</td>
<td>4.0</td>
<td>-</td>
<td>14.0</td>
<td>24.0 (36.4)</td>
</tr>
<tr>
<td>21-30</td>
<td>4.0</td>
<td>4.0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>6.0</td>
<td>13.0 (19.7)</td>
</tr>
<tr>
<td>31-40</td>
<td>2.0</td>
<td>2.0</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>4.0</td>
<td>5.0 (7.6)</td>
</tr>
<tr>
<td>41-50</td>
<td>2.0</td>
<td>-</td>
<td>1.0</td>
<td>1.0</td>
<td>-</td>
<td>3.0</td>
<td>5.0 (7.6)</td>
</tr>
<tr>
<td>51-60</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>2.0 (3.0)</td>
</tr>
<tr>
<td>61-70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>1.0 (1.5)</td>
</tr>
<tr>
<td>71-80</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0 (1.5)</td>
</tr>
</tbody>
</table>

Subtotal 24.0 14.0 4.0 9.0 1.0 3.0 6.0 1.0 2.0 2.0 1.0 - 66.0

38.0 (57.6%) 13.0 (19.7%) 4.0 (6.1%) 7.0 (11.7%) 4.0 (6.1%) 1(1.5%) (100.0%)
TABLE III: DISTRIBUTION OF INFLAMMATORY LESIONS OF SUPERFICIAL LYMPH NODE ACCORDING TO THE HISTOLOGIC TYPES.

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Males</th>
<th>Females</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>18.0</td>
<td>12.0</td>
<td>30.0 (45.5)</td>
</tr>
<tr>
<td>Reactive hyperplasia</td>
<td>11.0</td>
<td>7.0</td>
<td>18.0 (27.3)</td>
</tr>
<tr>
<td>HIV- associated lymphadenopathy</td>
<td>1.0</td>
<td>4.0</td>
<td>5.0 (7.6)</td>
</tr>
<tr>
<td>Necrotinizing chronic lymphadenitis</td>
<td>3.0</td>
<td>5.0</td>
<td>8.0 (12.1)</td>
</tr>
<tr>
<td>Benign reticuloses</td>
<td>2.0</td>
<td>1.0</td>
<td>3.0 (4.5)</td>
</tr>
<tr>
<td>Castleman disease</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0 (3.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36.0</strong></td>
<td><strong>30.0</strong></td>
<td><strong>66.0</strong></td>
</tr>
<tr>
<td><strong>Percentage</strong></td>
<td><strong>54.5 %</strong></td>
<td><strong>45.5 %</strong></td>
<td><strong>100.0 %</strong></td>
</tr>
</tbody>
</table>

FIGURE 1: PHOTOMICROGRAPH OF LYMPH NODE SHOWED A CASEATING GRANULOMATOUS CHRONIC INFLAMMATION COMPRISING CENTRAL AREAS OF NECROSIS RIMMED BY MULTINUCLEATED GIANT CELLS CONSISTENT WITH TUBERCULOUS LYMPHADENITIS X 10 MAGNIFICATION.
FIGURE II: PHOTOMICROGRAPH OF LYMPH NODE SHOWED A CASEATING GRANULOMATOUS CHRONIC INFLAMMATION COMPRISING CENTRAL AREAS OF NECROSIS RIMMED BY MULTINUCLEATED GIANT CELLS CONSISTENT WITH TUBERCULOUS LYMPHADENITIS X 40 MAGNIFICATION.

FIGURE III: PHOTOMICROGRAPH OF LYMPH NODE SHOWED VARIABLY SIZED LYMPHOID FOLLICLES WITH PROMINENT GERMINAL CENTER CONTAINING TINGIBLE-BODY MACROPHAGES CONSISTENT WITH LYMPHOID (FOLLICULAR) HYPERPLASIA X 10 MAGNIFICATION.
FIGURE IV: PHOTOMICROGRAPH OF LYMPH NODE SHOWED INTERFOLLICULAR STROMA WITHIN WHICH ARE NUMEROUS DILATED SINUSES CONTAINING HISTIOCYTES CONSISTENT WITH SINUS HISTIOCYTOSIS X 10 MAGNIFICATION.

30 years in 52 (78.8 %) cases. Similar observations in which children and adolescents were most commonly vulnerable to inflammatory lymphadenitis were found in other studies (1,2,5-7,12,17). These findings are further corroborated by a study in South India where younger population was more commonly affected by inflammatory lymphadenitis (12). The dominance of inflammatory lymphadenitis in younger age group could be adduced to the fact that lymph node poses as one of the 2nd line of defense immune system against foreign bodies including infections. In addition, children are more vulnerable to infections of the upper aero-digestive system because of their poor oral hygiene, indiscriminatory attitude towards ingestion and inhalation of foreign bodies innocently and developing immune system (2,5,12).

In index study, males were more commonly affected than females with a male to female ratio of 1.2:1. This finding compares relatively with observations in some studies within and outside Nigeria (1,2,5-7,10,12). In contrast to most studies, a slight female preponderance of inflammatory lesion was found in Owerri and Lagos, Nigeria (7,17). This discordant finding may be a mere coincidence or the fact that a male preponderance may also be related to the propensity of male adolescents to engage in some evil social vices including cigarette and Indian helms smoking which have tendency to reduce immunity.

In the index study, cervical lymph node was most biopsied (57.6 %), followed by axillary lymph node (19.7%), inguinal lymph node (11.7%), as well as supraclavicular and auricular lymph nodes (6.1 % each) in that order of frequency. This finding compares relatively with findings of other studies. In two cities in India (Mangalore and Chandigarh), majority of lymph nodes biopsied were from cervical lymph nodes, followed by axillary lymph nodes and inguinal lymph nodes in that order of frequency (1,2). In Jos, North-Central Nigeria, the cervical lymph node was the...
commonest site affected (54.1%), followed by mesenteric (22.3%), axillary (13.2%) and inguinal lymph nodes (10.3%) (8). In Owerri, South-East Nigeria, cervical lymph nodes were the most frequently biopsied, followed by the axillary and supraclavicular lymph nodes (17). Contrary to most studies, Ojo et al in Ilorin, North-Central Nigeria reported that axillary lymph node was the most commonly biopsied for localized inflammatory lymphadenopathy (38.0%), this was closely followed by cervical (32%), inguinal (8%), and submandibular lymph nodes (8%) (9).

From the foregoing, it is obvious that cervical lymph node is most frequently biopsied in cases of superficial lymphadenopathies. The supportive hypothesis may be that the aero-digestive orifices have been known to be easily accessible route of transmission of some infective lesions including respiratory tract infection, pharyngitis, and periodontal disease, in which cervical lymph nodes predominantly receive lymphatic flow from these inflamed regions (16). On the other hand, supraclavicular lymph nodes on the right side of the neck are more susceptible to malignancy of mediastinum, lungs or esophagus whereas left supraclavicular (Virchow’s) lymph node receives lymphatic flow from the thorax and abdomen, and may signal pathology in the testes, ovaries, kidneys, pancreas, prostate, stomach or gallbladder (16, 12).

There are many documented reports from studies on inflammatory lymphadenopathies within and outside Nigeria (1-17). Shahraz et al described five distinct patterns of inflammatory lymphadenopathy which include follicular hyperplasia, paracortical hyperplasia, sinus hyperplasia, granulomatous inflammation, and acute lymphadenitis (15). On the other hand, Woodhouse et al grouped pathologies of lymph node enlargement into acute lymphadenitis, chronic lymphadenitis, reticuloses and lipodystrophies, primary lymphadenopathies, secondary lymphadenopathies and differential diagnosis of specific cases of lymph node enlargement (3).

The pattern of inflammatory lymphadenopathies observed in our study was comparable to that of other studies within and outside Nigeria (5,12). Tuberculosis was more preponderant comprising 45.5 % of inflammatory peripheral lymphadenopathy, which is also consistent with findings of similar studies (5,8-10,13,17). In our study, tuberculous lymphadenitis was more common in male patients aged below 30 years with a maximum frequency in the 11-20 years age group and presented mostly with cervical lymphadenopathy. This finding agrees with similar observations in other studies (1,3,5,8-10,12,13,15,17). In contrast to most studies, female dominance was observed in tuberculous lymphadenitis from studies conducted in Owerri, South-East Nigeria and Kathmandu, Nepal (13,17). The incidence of tuberculous lymphadenitis varies with geographic variation and immunological status of the patients with high percentage being recorded in areas where there is occurrence of low socioeconomic status and poor standard of living (1,10,13). In addition, recent upsurge in incidence of HIV infection has contributed greatly to the re-emergence of tuberculosis in most developing countries including Nigeria (1,3,4,6,15-17). The preponderance of tuberculosis in first three decades of life in most studies is consistent with the fact that primary tuberculosis, the commonest type in childhood and adolescence chiefly involves lymph nodes (5).

In the index study, all tuberculosis cases were screened for HIV and acid-fast bacilli (AFB), 6/30 (20%) of them were HIV positive and one case (3.3%) was positive for AFB. Two of five cases of generalized lymphadenopathy (40.0%) were HIV positive. This finding compares relatively with a similar study in Owerri, South East Nigeria where acid-fast bacilli and HIV were demonstrated in 30.0% and 35.0% of tuberculous lymphadenitis respectively; they also reported that generalized lymphadenopathy was HIV positive in 66.6% (17). Furthermore, a study in Chandigarh, India showed that 15.3% of...
tuberculous lymphadenitis was positive for acid-fast bacilli (AFB). Similar to the index study, a study conducted in Kano, North-West North Nigeria showed that tuberculous lymphadenitis was negative for acid-fast bacilli (AFB) which pointed to the fact that histological demonstration of AFB by Ziehl-Neelsen stain in tuberculosis is notorious for its poor sensitivity (5,18). These aforementioned findings affirmed co-existence of tuberculosis and HIV infection which has been widely reported by most studies (1,15-17).

Reactive (lymphadenitis) hyperplasia (mostly follicular and sinus histiocytosis) was second most common lesion in the index study (27.3 %) with male children and adolescents as well as cervical lymph node being affected mostly; this finding agrees with similar observations in other studies (1,5,9,10,12,13). This however differed from other studies within and outside Nigeria in which reactive changes was ranked the leading inflammatory lesion with predilection for cervical lymph nodes (2,6,7,12,13,17). In contrast to most studies, Freidig et al in 22.2 % reported sarcoidosis to be the leading inflammatory lesion of the lymph node and Mbata et al reported female dominance for reactive hyperplasia (11,15).

The preponderance of reactive lymphadenopathy in children could be explained by repeated exposure to minor exogenous stimuli at the instance of developing immune system (2,10,12). In addition, the lymph node hyperplasia appears to be a consequence of variety of pathological processes; an important factor being that of upsurge in incidence of HIV infection (17).

Another author emphasized the role of some infective agents in the etiology of reactive hyperplasia; these included cat-scratch disease with predilection for cervical or axillary adenopathy, infectious mononucleosis and some sexually transmitted diseases have predilection for cervical and inguinal adenopathies respectively (16).

Besides tuberculosis and reactive hyperplasia, varying proportions of other inflammatory lymphadenopathy have been reported in many studies which compare relatively with findings from the index study (1-17). These include reticuloses, Castleman disease, rheumatoid arthritis, systemic lupus erythematosus, and amyloidosis. Of note in the index study is HIV - associated lymphadenopathy which accounted for 7.6 % of all inflammatory non-specific lymphadenitis which is higher than 1.0 % recorded in a study conducted in South India (14). HIV infection is frequently associated with generalized non-tender lymphadenopathy with predilection for cervical, axillary, and occipital lymph nodes initially (15).

Aside from malignancy of lymph node, inflammatory lymphadenopathies including tuberculous lymphadenitis, HIV – associated lymphadenopathy, systemic lupus erythematosus, rheumatoid arthritis, and sarcoidosis require urgent attention ranging from prompt diagnosis and treatment to forestall graven mortality and morbidity associated with diseases (15). Besides, a need to provide modern diagnostic laboratory equipment for immunohistochemistry, cytogenetics and molecular diagnostic techniques like lymphocyte receptor gene rearrangements by the governments at various levels and corporate organizations, there is also an urgent need to sharpen the skills of health personnels on these modern diagnostic laboratory equipment to increase their diagnostic efficiency and accuracy, as reported in other studies (9,17).

CONCLUSION

Tuberculous lymphadenitis and reactive hyperplasia were the two leading inflammatory lesions of the peripheral lymph nodes in our environment with children and cervical lymph node being more favourably affected, similar to what were reported in other studies.

LIMITATIONS

This study is limited by non-availability of more sophisticated modern diagnostic
facilities including immunohistochemistry, cytogenetics and molecular diagnostic techniques.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
No conflict of interest declared.

FINANCIAL SUPPORT
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