Background

Fine needle aspiration cytology (FNAC) is a very helpful technique in diagnosis of benign and malignant lesions of lymph node [1-4]. Many authors also claim that FNAC can accurately diagnose Hodgkin's and non-Hodgkin's lymphoma (NHL)[5,6]. However there is a wide variation of diagnostic sensitivity and specificity of FNAC in non-Hodgkin's lymphoma [5-8]. The role of cytology in primary diagnosis and sub-classification of non-Hodgkin's lymphoma is controversial [9-12]. After the introduction of REAL/WHO classification, there is much difference in the cytologist's approach of lymphoma diagnosis and classification. WHO and REAL classification emphasized immense importance on the cytomorphology and immunophenotype of lymphoma for accurate sub classification [13,14]. In this present study we have analyzed the role of flow cytometric immunotyping as an adjunct to FNAC for diagnosis and sub-classification of NHL according to WHO classification.

Materials and methods

This study is of five years duration from the year 2000 January to 2004 December. Only cases suggested or confirmed as NHL by FNAC were selected. FNAC smears were prepared for May Grunwald Giemsa (MGG) and Haematoxyline and Eosin stain in each case. The May Grunwald Giemsa smears were studied immediately. A second pass of the needle was done and material was collected in citrate buffer for flow cytometric immunophenotyping (FCI). The specimen was immediately processed and a complete panel of antibodies was used for immunophenotyping. Both cytologic findings and FCI data were interpreted together to diagnose and sub-classify NHL according to WHO classification as far as possible. Wherever possible the final histological diagnosis was correlated with FNAC and FCI diagnosis.

Specimen preparation

FNAC material was collected in citrate buffer solution and immediately transferred to flow cytometry laboratory. The sample was washed in phosphate buffer solution three times for 5 minutes at 2000 revolutions per minute. The supernatant fluid was discarded and the deposits of cells were studied for cell viability and count. After that, the suspension was divided into multiple tubes depending on the adequacy of the cell. Samples were then incubated for 15 minutes in dark with 5 μl of antibody solution tagged with Fluorescein isothyocyanate (FITC) or Phycoerythrin (PE). The following antibodies were used: CD3, CD2, CD 4, CD5, CD8, CD7, CD10, CD19, CD20, CD23, CD45, κ and λ. (Becton Dicikinson, San Jose, CA, USA). After incubation, red blood cells were lysed by a lysing solution (Becton Dicikinson, USA, Catalogue no 349202) for 15 minutes and then washed by a cell wash solution (Becton Dicikinson, USA, Catalogue no 349524). After centrifuging, the supernatant solution was discarded and 500 μl cell fixative was added (Becton Dicikinson, Catalogue no 340181). Flow cytometry analysis was then performed in Becton Dicikinson flow cytometer (San Jose, CA, USA) by dual-color analysis technique. Gating was done using forward and side scatter and also by the help of Cell Quest software (San Jose, CA, USA). We analyzed a minimum number of 10,000 events for each cell marker.

Results

There were total 48 cases included in this study and in all these cases FNAC confirmed or suggested the diagnosis of NHL. Age of the patients ranged from 6 year to 78 years. There were 34 male and 14 female patients. Twenty-nine cases were first time investigated in our FNAC clinic for primary diagnosis and 19 cases were recurrent NHL. In 36 cases, the masses were palpable and FNAC was done without any radiological guidance (table 1). In 12 cases masses were non-palpable and FNAC was done with the help of Ultrasonographic guidance. FNAC yielded adequate material in all these deep-seated cases. The cases were classified on FNAC as predominant small cells (12), mixed small and large cells (5) and large cells (26) [table 2]. In five cases a suggestion of NHL was offered on FNAC material and these cases were labeled as NHL not otherwise specified (NHL-NOS). Flow cytometry could be performed in 45 cases (93.8%) and in rest of the three cases the material was inadequate because of scanty blood mixed aspirate. There were total 40 cases of B-NHL. These cases showed predominant CD19 and CD20 positive cell population. Light chain restriction (figure 1) could be demonstrated in 30 out of 40 cases of B-NHL (75%). There were 15 each cases of κ and λ light chain restriction. In 10 cases there was no demonstrable light chain restriction on FCI. However cytology smears and predominant population of CD19 cells indicated a NHL of B cell origin.

The diagnostic distribution of cases on the basis of FCI is highlighted in table 2. With the help of combined FCI and

Table 1: Anatomic distribution of aspirated lesions

Sites	Number of patients
Palpable	
Cervical lymph node	19
Inguinal lymph node	3
Axillary lymph node	8
Submandibular lymph node	3
Supraclavicular lymph node	2
Skin	1
Ultra Sonogram guided	
Abdominal lymph node	6
Bowel wall	2
Mediastinum	3
Testis	1