Fine Needle Aspiration Cytology of Enlarged Lymph Nodes and Gender Differences

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ABSTRACT

Objective: To observe patterns in the pathological findings of lymph-node FNAC. **Design:** Exploratory and Cross-sectional examination of medical records. **Place & Duration of Study:** Department of Histopathology of Sheikh Zayed Hospital, Lahore, from 1992 to 1995 (four years). **Patients & Methods:** A total of 548 fine needle aspiration cytologies were performed on patients presenting with enlarged lymph nodes. Two histopathologists evaluated each slide to confirm the findings. Descriptive analysis of the FNAC results was conducted and efficacy of the procedure was estimated. Male to female ratios were calculated and chi-square test was applied. **Results:** Eighty-eight percent of the FNACs yielded a positive result on the first attempt. Infectious and cancerous FNACs averaged 42.36% and 32.09%, respectively. Granulomatous lesions were most frequently due to tuberculosis. Men were twice as likely as women to have cancer detected by an FNAC, χ^2 24, (p 0.05). Poorly differentiated cancer was three times as likely to be found in males than females, χ^2 8.02. Male: female ratio for metastatic lesions was 2:1, χ^2 11.12 (p 0.05). **Conclusions:** In this study, infectious diseases appeared to present more frequently than cancerous lesions, as is observed in underdeveloped countries. This study complements other studies and opens new research questions, regarding gender differences in the prevalence of cancer found in enlarged lymph nodes, as cancers including the metastatic, detected on FNAC were more common in males than females.

Key Words: Lymphadenopathy, FNAC Malignant.

INTRODUCTION

Arabian physician Abul Qasim (1013-1107 AD) described the use of needle puncture as a differential diagnostic tool for thyroid goiters[1]. Since the middle of nineteenth century, needle aspiration cytology and microscopy has been gaining ground as a diagnostic modality. With the arrival of microtome and paraffin embedding, however, this use of cytology declined[2-4]. There was a brief rise of interest in cytological techniques in late 1920s and early 1930s with the introduction of Pap smear in 1928, and use of 18-gauge needle for aspiration cytology of head and neck lesions[5,6]. After the second world war, the interest in fine needle aspiration as a primary diagnostic tool declined and did not regain popularity until recently in the 1980s and today its position as a fast and reliable diagnostic method is well established[7].

FNA biopsy can be defined as the removal of a sample of cells, using a fine needle, from a suspicious mass for diagnostic purposes. A fine needle 22-gauge or smaller diameter, is used in this procedure. With the fine needle, complications are minimal or rare and a cytological rather than histological specimen is obtained. A fine needle, filled with cells, is usually sufficient to make half a dozen cellular smears for diagnosis[8]. Beveled needles work better than flat cutting tip needles[9]. A plain glass slide is needed to prepare a smear.

Fine needle aspiration cytology is being employed for almost all superficial organs of the body, as it is a safe and quick procedure requiring little in the way of equipment. For deep-seated masses and organs, however, an ultrasound or CT guided procedure may be performed, which in some cases prevents exploratory surgery or assists in planning surgical procedures. FNAC is being

regularly employed to aid in medical diagnoses of lymphadenopathies and cancer grading. Location of adenopathy, size, texture and tenderness, are important in differential diagnosis of lymph node involvement. Generalized lymphadenopathy, i.e. three or more anatomic regions, implies systemic infection or lymphoma. Tender lymph nodes are usually benign, where as matted and immobile nodes are indicative of metastatic disease. History and clinical examination, coupled with histopathology can yield highly accurate diagnosis of any type of lymph node enlargement.

Ninety-five percent of cells found in a normal lymph node FNA biopsy, are lymphocytes. Lymphohistiocytic aggregates are a found in three-quarters of benign lymph node FNA biopsy specimens. Chronic lymphadenitis or reactive hyperplasia is the single most common diagnosis rendered in FNAC. Combined use of tuberculin skin test and FNAC are complementary and efficient in the diagnosis of tuberculosis[10]. The highest diagnostic accuracy with FNA biopsy is in the diagnosis of metastatic carcinoma[11]. The type of tumor can be determined and primary site of origin be suggested. In case of malignant lymphomas, FNAC is supplemented with excisional biopsy, as the histological pattern may sometimes be difficult to distinguish the benign from the malignant[12]. FNAC alone can usually diagnose high-grade Non-Hodgkin's lymphoma and most cases of Hodgkin's disease, but difficulty arises in diagnosing low-grade malignancy[13].

In our study, we carried out FNACs to evaluate etiologies encountered in clinical lymphadenopathy. This study adds to the fine needle aspiration cytology studies carried out in the region[14,15]. We reiterate with this study that FNA biopsy is a reliable complementary diagnostic procedure in low resource settings and raise some questions about the gender-based distribution of cancerous lesions in lymph nodes, proposing exploratory studies in cancer epidemiology.

PATIENTS AND METHODS

A total of 548 fine needle aspiration cytologies were performed between 1992-1995 by the Department of Histopathology, both in the Cytology Outpatient Department and as a bedside procedure on the patients admitted in the various surgical and medical wards of Shaikh Zayed Hospital, Lahore. The patients were considered eligible for FNA cytology based on the clinical judgment of the referring physician within the hospital. No specific selection criteria were applied for the purpose of the study. Patients presented with lymph nodes that were typically enlarged, superficial and located in the cervical, axillary and the inguinal region. For deep-seated lesions ultrasound guidance was used with the assistance of the radiologist.

After taking a brief history and clinical examination, the patient was explained the procedure. Local anesthetic was avoided with the patient's consent wherever possible. The skin was cleaned with cotton spirit swab. Typically 10 or 20cc syringes were used with needles of gauge 22 or 23. For aspiration of superficial lymph nodes, the mass was immobilized with one hand and needle of the syringe was carefully and swiftly introduced into the mass perpendicular to it. Suction was applied after entering the mass and maintained throughout the procedure. With the needle still inside the mass, a sawing back and forth motion was applied with a frequency of 2 per second. When the material was seen in the hub of the needle, suction was halted and after releasing the negative pressure the needle was gently withdrawn from the mass.

The needle was then detached from the syringe, which was then filled with air. The needle was reattached. The bevel of the needle was placed directly on the glass slide and the aspirated material expressed using the air filled syringe to express material from the needle on to the slide in one drop. A spreader slide was gently lowered across over the droplet and the material was spread to the edges. The spreader slide was then gently pulled straight back in one smooth motion down the length of the diagnostic slide. Some of the slides were air dried for Giemsa staining and some were immediately fixed in 95% alcohol for Papanicolaou stain. A Z-N stain was also used where required. Specimens were carefully observed under the microscope and findings on each slide were documented as part of patient record and reported to the referring physician. Each slide was evaluated by two histopathologists,

to concur and confirm the findings. Inconclusive diagnosis on the first FNACs were followed up with either repeat FNACs or excision biopsies, in order to report a definite diagnosis.

Descriptive analysis of the FNAC was conducted, and efficacy of the procedure based on the positive yield on first attempt was estimated. Results were tabulated. Male to female ratios were calculated for the various lymph node pathologies detected. Two-celled chi-square tests were applied to the male: female distributions of the FNACs findings.

RESULTS

Selection of the patients was not specifically for the purpose of the study rather the selection was dependent upon the physicians referring the patients with suspect pathology for FNAC of lymph nodes, to the Pathology Department. All patients with lymph node enlargement who received an FNAC over a period of four years were included in this study. Out of the 548 FNACs performed, microscopic examination of 66 were initially determined to be inconclusive for a definitive diagnosis and thus required either a repeat fine needle aspiration procedure or an excisional biopsy. Approximately 88% of the FNACs yielded a positive result on the first attempt, i.e. 482 biopsies.

The year-by-year, broad diagnostic categories of the FNACs are given in Table 1. There was annual variation between granulomas, lymphomas and metastatic lesions found on FNA biopsies. The metastatic lesions were 15-26% of the FNACs and granulomatous lesions varied between 28-43% of the FNACs throughout the years. The FNA biopsies with conclusive diagnosis of 'reactive' (lymphoid follicular hyperplasia) were relatively stable between 11-18% through out the four years. Excluding the 'inconclusive' and the 'reactive' cytology reports at first attempt, the cumulative four-years' infectious and cancerous FNACs averaged 42.36% and 32.09%, respectively. Lymphoid follicular hyperplasia (reactive picture) was found to be common in less than 30 years of age.

Gender based differences in broad diagnostic categories are shown in Table 2. Two-celled chi-

square estimates for the male: female ratios were carried out to estimate the level of statistical significance. More males presented for FNACs than females with a ratio of 1.32:1. This ratio was significant at p value of 0.05, with χ^2 value of 10.26.

For the infectious lesions (granulomatous and abscess-related) the χ^2 value was 2.086, which was not significant, suggesting that there was no gender difference between the infectious processes presenting with enlarged lymph nodes requiring FNACs. Granulomatous lesions were most frequently due to tuberculosis. The male: female ratio for the inconclusive FNACs on first attempt was 1.75: 1. It was also tested for the null hypothesis of 1:1 ratio, resulting in a χ^2 value of 4.5 with Yates correction. It was a very minute difference, and null hypothesis was hard to reject. For the cancerous lesions, however, the study suggested that males were twice as likely as females to have cancer detected by an FNAC, with the χ^2 value of 24 with Yates correction. This was statistically significant at p value of 0.05. The Lymphomas were twice as common in males than females with enlarged lymph nodes presenting for FNAC (Yates corrected $\chi^2 = 8.48$).

Table 3 shows the detail of the metastatic lesion found in the enlarged lymph nodes cases that underwent FNACs. Although, the number of oat cell carcinoma cases was small in males, no female cases presented for FNACs with oat cell carcinoma over a period of four years. Poorly differentiated cancer was three times as likely to be found in males than females (χ^2 8.02, Yates corrected), Squamous cell carcinoma was twice as likely (χ^2 2.90), and Adenocarcinoma was 1.5 times more likely to be found in males than females (χ^2 1.6) who presented for FNA biopsies. Except for the poorly differentiated carcinoma the differences were not statistically significant. However, excluding the metastatic lesions detected in low numbers from the calculation, the male: female ratio for metastatic lesions (66 vs. 32) was 2:1, with statistically significant χ^2 value of 11.12, for a two-celled test. The average age for patients with metastatic lesions, with the exceptions of Teratomas, Thyroid carcinomas and Sarcomas, was between 50-60 years for males and females.

Table 1: Year based distribution of diagnostic categories (n = 548)

Diagnosis	1992	1993	1994	1995	Total	Percentage
Granulomatous	30	64	70	44	208	37.99
Metastatic	28	25	33	26	112	20.43
Reactive	14	18	21	21	74	13.50
Non-Hodgkin's Lymphoma	16	13	6	5	40	7.29
Hodgkin's Lymphoma	1	7	5	4	17	3.10
Abscess	3	5	14	2	24	4.37
Leukemia	4	3	0	0	7	1.27
Inconclusive*	13	25	14	14	66	12.05
Total	109	160	163	116	548	100

*Inconclusive diagnosis on the first FNACs were followed up with either Repeat FNACs or Excision Biopsies.

Table 2: Gender based distribution of diagnosis categories (n = 548)

Diagnosis	Male	Female	Ratio M:F
Granulomatous	92	116	0.8:1
Abscess	13	11	1.2:1
Reactive	44	30	1.33:1
Non-Hodgkin's Lymphoma	28	12	2.3:1
Hodgkin's Lymphoma	12	5	2.4:1
Metastatic	77	35	2.2:1
Leukemia	4	3	1.33:1
Inconclusive	42	24	1.75:1
Infection related*	105	127	0.83:1
Malignancy related**	121	55	2.2:1
Total	312	236	1.32:1

*Infection related includes granulomatous and abscesses only
**Malignancy related lesions include Non-Hodgkin's Lymphoma, Hodgkin's Lymphoma, Metastases and Leukemias.

Table 3: Types of metastatic lesions found in lymph nodes (n = 112)

Lesion	Cases	Male	Female
Poorly differentiated carcinoma	36	27	9
Squamous cell carcinoma	22	15	7
Adenocarcinoma	40	24	16
Oat cell carcinoma	7	7	0
Teratoma	3	1	2
Sarcoma	2	1	1
Papillary carcinoma (Thyroid)	2	2	0
Total	112	77	35

DISCUSSION

There has been a desire in recent medical history to find out the most non-invasive method for the diagnosis of disease. In the cases of lumps and bumps the classical approach, and that with the greatest yield, has always been histological examination. The technique of FNAC today is an

accepted and established procedure, which has made its place as a vital tool in diagnostic modalities. The first recorded utilization was in 1833, when, at St. Bartholomew's Hospital, London, an aspiration was performed on a liver mass of a 62 year old[16]. The technique was not reported much until Guthrie published in 1921, his account using a 21 gauge needle, similar to the needles used today[17]. During the past two decades there has been a dramatic rise in its use. Now FNAC is considered acceptable and essential in the diagnosis and treatment of conditions such as the breast cancer[18]. According to Das et al, the FNAC should be the first line of morphological investigation of lymphadenopathy[19].

Lymph node enlargement can be clinically insignificant or critical. FNAC is instrumental not only in determining whether the mass is in fact an enlarged lymph node and not anything else, mimicking an enlarged lymph node, varying from salivary gland to skeletal structures[20], but also assists in determining the benign or malignant nature of the enlargement. It is not always feasible to excise every enlarged lymph node therefore FNAC can be helpful in choosing the best lymph node to excise.

In our study FNACs were performed on the superficial lymph node groups such as cervical, submandibular, supraclavicular, axillary and inguinal lymph nodes. Ultrasound guidance was used to perform the FNAC of the abdominal lymph nodes. Ahuja et al have used ultrasound guidance for cervical lymph nodes aspirations[21]. Although the patients were not selected for the study by a random process, the sample size approximated reasonably, for the assumption that it can be representative of a larger population of enlarged lymph node cases. The efficacy of FNACs was estimated to be 88%, defined here as the number of biopsies that yielded a positive result on the first attempt out of the total number of biopsies performed. Twelve percent FNACs required repeat procedure or excisional biopsy for a confirmatory diagnosis. This ranks favorably with other studies[22]. Excisional biopsies were specially carried out when the initial cytological examination revealed mononuclear cells mimicking Reed Sternberg Cells suggestive of Hodgkin's Lymphoma, an approach that is well established[23].

In the developed countries the prevalence of cancer is higher than the granulomatous disease found in enlarged lymph nodes[24], whereas in our study granulomatous disease was found to be more frequently occurring than the cancerous lesions, a phenomenon observed in similar studies carried out in underdeveloped countries[25].

Slightly more FNACs were carried out on males than females. This could be because of more males being referred with lymphadenopathy for FNAC. It can be speculated that such referral pattern is either confounded by lack of access to care for females to tertiary care hospitals or lymphadenopathy is more common among males. This difference may have a minute affect on the differences in male to female ratios, observed in cancerous lesions. However, it can be argued that since there was no gender difference in the males and females referred for possible infectious lesions, it is likely that difference in cancerous lesions especially metastatic carcinomas diagnosed on FNACs is not due to preferential referral pattern or access to care. Similarly there was no statistically significant difference noted between the males and the females, for lymphoid follicular hyperplasia and the biopsies rendered inconclusive on first attempt. With 12% FNACs requiring repeat or another diagnostic procedure, suggests that it may be even harder for the referring physicians to distinguish by clinical examination alone, between a cancerous lesion and an infectious lesion, prior to referring, to significantly alter the results. Nevertheless, this study emphasizes the need of further research into the use of FNACs and lymphandenopathies, with robust study designs, minimizing the influence of confounders.

Our experience of FNACs over a period of four years, included in this study, has endorsed for the authors, the advantages of the procedure that other studies have time and again documented, such as simplicity, accuracy, being fast, and economical. It has the best safety record of any method of procuring tissue for a morphologic diagnosis[26]. Increased recurrence of tumor has been associated with cervical lymph node incisional biopsy[27] and not with FNAC. Simplicity of FNAC makes it a natural extension of the physical examination, facilitating early diagnosis. FNAC leaves no scar, posing a

cosmetic problem. Another plus is that viable cells are obtained, on which special testing such as cytochemical studies or tissue culture etc can be performed. FNAC can be used to mark metastatic lymph nodes, as prognostic indicator for follow-up care. FNA biopsy can be particularly useful in post-mortem examinations as well where a full autopsy is not permitted. Complications from FNAC are unlikely, even if the wrong target is hit (28). The risk for needle tract seeding, using fine needles, is extremely rare, and has been estimated to be less than 0.0045%[29]. In our study, no complications worth reporting were experienced by the patients, except for pain and some minor bleeding.

In hospitals where FNA biopsy service is available, the turnaround time between receiving a call, carrying out the procedure and delivering the result is typically less than an hour. FNAC carried out in outpatient, at a very low cost, allows for selective hospitalization. This cost-saving feature is useful in low budget healthcare settings such as in Pakistan. Fine needle aspiration cytology has been carried out regularly in Pakistan, over the past decade (14). The best results are obtained when a single physician sees the patient, performs the FNAC procedure and interprets the smear, preferably the pathologist[30]. Clinical information is critical for the cytopathologist to arrive at the final diagnosis. FNA biopsy is a link in the chain of diagnostic tests and procedures. In Pakistan where tuberculosis is endemic, FNAC is a cost-effective and reliable tool in comparison with excisional biopsy in cases of cervical lymphadenopathy, with sensitivity of 95% and specificity of 100%[15]. Our evaluation of relationship between FNAC and lymphadenopathy complements these studies and opens new research questions to explore more thoroughly, particularly regarding gender-based prevalence of malignancy.

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