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# PRIMARY PANCREATIC LYMPHOMA DIAGNOSED BY ULTRASOUND GUIDED FINE NEEDLE ASPIRATION CYTOLOGY, FLOWCYTOMETRY AND IMMUNOHISTOCHEMISTRY : ANALYSIS OF 12 CASES

Pathology

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## ABSTRACT

**BACKGROUND:** Primary pancreatic lymphomas (PPL) are extremely rare neoplasm. Radiologicaly, PPL usually situated in the region of head of pancreas. An accurate cytopathologic diagnosis by USG guided fine-needle aspiration (USG-FNA) is indispensable, because the primary treatment is nonsurgical, based on a combination of chemotherapy and radiation therapy.

**METHODS:** Twelve cases of PPL were identified from the cytology division of pathology department over a 10 year period. All cases were diagnosed on USG-FNA performed under radiologic guidance.

**RESULTS:** The patients ranged in age from 14–75 years. The tumors varied in size from 2–10.2 cm, as evaluated on the radiologic scans (mean dimension of 8.0 cm). Abdominal pain was the most common presenting symptom (Nine patients) followed by jaundice (5case), acute pancreatiti(1case), Fever (1case), small bowel obstruction (1case), anorexia (2cases)and diarrhea (1case). The cytomorphologic features showed singly dispersed cells with round irregular nuclei, prominent nucleoli, mitoses, and scant to moderate cytoplasm. FC analysis in all seven cases demonstrated a monoclonal pattern of immunoglobulin light chain expression.The patients were treated with either chemotherapy alone or in conjunction with radiation therapy or stem cell transplantation.

**CONCLUSIONS:** PPL is a very rare neoplastic entity. USG-FNA with FC analysis appears to be highly accurate in the diagnosis of PPL and is the sole diagnostic modality used clinically.

## KEYWORDS

Pancreas, malignant lymphoma, cytopathology, fine-needle aspiration (FNA), flow cytometry (FC).

## INTRODUCTION

Primary pancreatic lymphomas (PPL) are extremely rare neoplasms. Radiologicaly, PPL usually situated in the region of head of pancreas. An accurate cytopathologic diagnosis by USG-FNA is indispensable, because the primary treatment is nonsurgical, based on a combination of chemotherapy and radiation therapy. USG-FNA is considered a safe, rapid, and easy procedure with high diagnostic accuracy, and can be applied when dealing with a variety of pathologic disorders in numerous organs and anatomic sites. Flow cytometry (FC) has significantly enhanced the diagnostic role of FNA, particularly in the case of hematolymphoid malignancies.With the aid of FC analysis, PPL can be accurately diagnosed by USG-FNA. These two simple investigational modalities are sufficient to provide a definitive diagnosis and obviate the need for invasive surgical procedures to obtain additional tissue. Moreover, the majority of patients with PPL can be managed without surgery and long-term disease remission reportedly can be obtained with chemotherapy alone. [1] The role of surgery in this setting is limited to the rare occasions when initial FNA and FC analysis are nondiagnostic. Twelve cases of PPL were identified, all cases were diagnosed on USG guided fine needle aspiration cytology under radiologic guidance with biopsy, flowcytometry and immunohistochemistry

## MATERIALS AND METHODS

Twelve cases of PPL were identified from the cyto-pathology files of Sanjay Gandhi Postgraduate Institute of Medical Sciences over a 10 year period (2006-2015). All twelve cases were diagnosed on cytopathology performed under the guidance of ultrasound or computed tomography (CT) using a 22 or 23 gauge Franseen needle. The smears were air-dried and stained with May grunnwald giemsa(MGG), or wet-fixed and stained with the Papanicolaou stain. In seven case, a separate vial of the needle rinses in Hanks balanced salt solution was submitted for immunophenotyping by three-color or four-color FC. The total cell count was determined manually using a hemocytometer for each antibody combination. Subsequently, the samples were suspended with the antibody starter. Antibodies against CD45, CD10, CD13, CD15, CD30, CD19, CD20, kappa, lambda, CD5, CD3, and CD56 were used in the screening panel. Additional antibodies were added in cases of lymphoma with a previously diagnosed unusual immunophenotype. In each case, CD45 gating was applied. All cases were finaly diagnosed on USG guided fine needle aspiration cytology under radiologic guidance with biopsy, flowcytometry and immunohistochemistry.

## RESULTS

### Clinical Findings

The clinical features of Twelve cases of PPL are summarized in **Table 1.** A strong male predominance (male to-female ratio of 8:4) was noted. The patients ranged in age from 14–75 years. The tumors varied in size from 2.5–10.2cm in greatest dimension, as evaluated on the radiologic scans (mean dimension of 6.4cm). Abdominal pain was the most common presenting symptom(Nine patients), followed by jaundice (five patients), acute pancreatitis and fever (1 each patient), small bowel obstruction (one patient),and diarrhea (one patient). Eleven cases were located in the head of the pancreas and only one tumor was noted in the body and tail region.

### Cytopathologic Findings

The smears from PPL showed variable degrees of cellularity, with the majority of the cases showed singly discohesive pattern. In most cases, the malignant lymphocytes were found to have large hyperchromatic nuclei (greater than three to four times the size of a mature lymphocytic nucleus) with single to multiple prominent nucleoli, marked anisonucleosis, prominent nucleoli, deep nuclear indentations, scant to barely discernible cytoplasm (**Fig 1a &1b**). Lympho-glandular bodies are seen in the background. In one case atypical lymphoid cells show irregular indented granular nuclei **(Fig 2a** and scant to moderate cytoplasm alongwith binucleated cells with prominent nucleoli and moderate cytoplasm .

### Immunohistochemical Findings

Immunohisochemistry (IHC) was performed in seven cases which was classified as large B-cell Non-hodgkin lymphoma with ,coexpression of CD19+ and CD20+ (**Fig 1c**) and T-Cell NHL in which case revealed a clonal population of T- lymphocytes with coexpression of CD3 **(Fig 2d)** and CD13, a phenotype that is consistent with a diagnosis of T-NHL. IHC for one case revealed a clonal population of lymphocytes with coexpression of CD15 and CD30 expression, confirmed a diagnosis of Hodgkin Lymphoma. IHC was not performed in 5 cases. **Flow-Cytometric Findings (Table 2)** was performed in seven cases which was classified as large B-cell lymphomas (LBCL), all of which had Ig light chain restriction and CD20 expression. FC data concerning

another case revealed CD19, CD20 expression and kappa light chain restriction. FC in one case revealed a clonal population of T-lymphocytes with coexpression of

CD3 and CD13 **(Fig 2b)**, a phenotype that is consistent with a diagnosis of T-NHL. Lastly, FC for one case revealed a clonal population of lymphocytes with coexpression of CD15 and CD30 expression, a phenotype that is consistent with a diagnosis of Hodgkin Lymphoma.

**Histopathological Findings:**

Histopathology was performed in 7 cases which was classified as large B-cell NHL **(Fig 1d)**, T-Cell lymphoma,(T-NHL) and HL cases. B-cell NHL showed CD19+, CD20+ expression and kappa light chain restriction. FC in one case revealed a clonal population of T-lymphocytes with coexpression of CD3 and CD13, a phenotype that is consistent with a diagnosis of T-NHL. Lastly, FC for one case revealed a clonal population of lymphocytes with coexpression of CD15 and CD30 expression, a phenotype that is consistent with a diagnosis of Hodgkin Lymphomas. In one case , mainly necrosis alongwith few atypical lymphoid cells are seen. Normal pancreatic acini are also seen. **(Fig 2c)**

**TABLE 1: Clinicopathologic Details of PPL (n=12)**

| Case No | Age(yrs)/ Sex | Location in pancreas | Tumor Size (cm) | Presenting Symptoms |
|---|---|---|---|---|
| **1** | 72/F | HOP | 8.0 | Pain in abdomen, jaundice |
| **2** | 50/F | HOP | 9.2 | Pain in abdomen, No jaundice |
| **3** | 15/M | HOP | 5.5 | Pain in abdomen |
| **4** | 46/M | HOP | 9.4 | Pain in abdomen, jaundice |
| **5** | 37/M | HOP | 10.2 | Pain in abdomen, diarrhea |
| **6** | 14/M | HOP | 2.5 | Fever, mass in epigastrium,and jaudince, |
| **7** | 62/M | HOP | 4.0 | Anorexia, pain abdomen |
| **8** | 34/F | Body & Tail | 6 | Pain in abdomen, jaundice |
| **9** | 15/M | HOP | 6.5 | Fever, mass in epigastrium |
| **10** | 75/F | HOP | 7.5 | Small bowel problem, Pain abdomen |
| **11** | 61/M | HOP | 4 | Pain in abdomen, jaundice |
| **12** | 54/M | HOP | 5.0 | Acute pancreatitis, Anorexia |

**TABLE 2: Cytological Diagnosis, Flowcytometric Analysis and Treatment of PPL (n=12)**

| Case No | Cytological diagnosis | Final Cyto-histological diagnosis with IHC | Flow-cytometric analysis | Treatments |
|---|---|---|---|---|
| **1** | NHL | NHL | NA | NA |
| **2** | Positive for Atypical Lymphoid cells | Large B-NHL | Cd19+, CD20+ | Chemotherapy |
| **3** | NHL | NHL | NA | NA |
| **4** | NHL | Large B-NHL | CD19+, CD20+ | Chemotherapy |
| **5** | NHL | T-NHL | Positive CD13(60%) Cytoplasmic,CD3(88%) | Chemotherapy, RT |
| **6** | Malignant Lymphoma | HL | CD15+.CD30+ | Chemotherapy |
| **7** | NHL | NHL | NA | NA |
| **8** | NHL | Large B-NHL | Lymphocytes CD20+ | Chemotherapy |
| **9** | Malignant Lymphoma | Large B-NHL | CD19+ , CD20+ | Chemotherapy |
| **10** | NHL | Large B-NHL | CD20+ CD19+ | Chemotherapy |
| **11** | Positive for Atypical Lymphoid cells | Necrosis, Few Atypical Lymphoid cells | NA | NA |
| **12** | NHL | NHL | NA | NA |

HL= Hodgkin Lymphoma ,NHL = NonHodgkin Lymphoma , NA=Not available

## DISCUSSION

PPL are extremely rare (comprising of 0.5% of pancreatic tumors[2] and can existing as an isolated mass mimicking pancreatic carcinoma. However, unlike carcinomas, PPL are potentially treatable. Secondary invasion of the pancreas from contiguous, retroperitoneal lymph node disease is the predominant mode of involvement. In one study,[3] seven patients with PPL each initially presented with a pancreatic mass. There were 8 males and 4 females ranging in age from 14–75 years (mean age, 75 years). All patients reported clinical symptoms of epigastric pain, jaundice, anorexia, or early satiety.Abdominal CT scan was accurate in identifying and localizing the pancreatic mass in all patients. The dimension of the pancreatic mass ranged from 2.5–10.2 cm (mean, 6.4cm) and the mass was located in the head of the pancreas in 11 patients. Progressive painless jaundice associated with an aggressive pancreatic mass and a lack of significant lymphadenopathy creates diagnostic confusion with a primary ductal carcinoma.[4] Pancreatic lymphoma is often described as a large, homogeneous mass with extrapancreatic extension, with or without associated lymphadenopathy. Less common presentations are masses in the body or tail of the pancreas, or, more rarely, diffuse involvement of the entire organ.[5] Clinically, the differential diagnosis of primary pancreatic lymphoma from pancreatic carcinoma is most often extremely difficult, particularly when these masses are associated with an elevated CA 19-9 level.[6] In another study of 19 cases of PPL comprised of 13 men and 6 women, abdominal pain was the most common presenting symptom.[7] The tumors were located most commonly in the pancreatic head (12 cases), with the remainder noted either in the tail or the body of the pancreas, and ranged in size from 4–17 cm. The majority of patients with PPL can be managed without surgery and long-term disease remission can be obtained with chemotherapy alone.[1] The role of surgery in this setting is limited to rare occasions when FNA plus FC are nondiagnostic, and tissue diagnosis therefore is required. In one study of 10 patients with PPL, the mean survival was 13 months for patients who received chemotherapy alone ($n$= 2), 22 months for patients treated with radiation therapy only ($n$ = 5), and 26 months for patients receiving combined radiation therapy and chemotherapy($n$=3).[8] Based on cytomorphology, the main differential diagnoses of PPL are secondary lymphomas, pancreatic endocrine neoplasms (PEN), acinar cell carcinoma, and florid chronic pancreatitis. Imaging studies help to differentiate PPL (which are either organ confined or further involve the immediate peripancreatic lymph nodes) from secondary lymphomas. PEN, another mimicker of lymphomas, could be identified by their distinctive cytomorpholgy, immunoperoxidase characteristics, and negative FC analysis. PEN usually express CD56 and are negative for lymphoma markers, findings that could be evaluated by both FC and immunoperoxidase analysis. PEN demonstrate relatively small uniform neoplastic cells with a well large, demonstrated kappa immunoglobulin light chain restriction, and were found to coexpress CD20, CD10, and CD19. Preserved rim of granular-appearing cytoplasm. Nuclei are most often eccentrically placed within the cell cytoplasm with finely granular and evenly distributed chromatin. Rarely, a predominant population of tumor cells with naked nuclei is observed simulating NHL. Less often, acinar cell carcinoma can have a predominant population of discohesive single cells and may resemble an NHL. However, closer examination reveals fragile, basophilic, granular cytoplasm. Nuclei often have prominent nucleoli and the slide background shows distinctive finely vacuolated or foamy material derived from tumor cell cytoplasm. FC is extremely sensitive in the detection of antigen expression and identifies small clonal populations.[9] FC analysis distinguishes lymphomas from chronic pancreatitis through the detection of clonality based on surface Ig light chain expression studies. In lymphomas, Ig light chain expression is usually restricted to either kappa or lambda, whereas inflammatory processes reveal a mixed expression of kappa and lambda light chains[10]. FC also has limited capability in classifying lymphomas into different, well recognized subcategories. This is made possible by studies of surface marker expression and is best applicable for low-grade lymphomas. In the current study, we evaluated the role of FNA and FC in the diagnosis of PPL. All twelve cases of PPL in the current study were diagnosed accurately and subclassified based on cytomorphology, IHC and FC data. The data from the current study suggested that FNA coupled with FC analysisis highly accurate in the diagnosis of PPL. An accurate FNA diagnosis of PPL is critical for timely, nonsurgical management and obviates the need for an exploratory laparotomy.

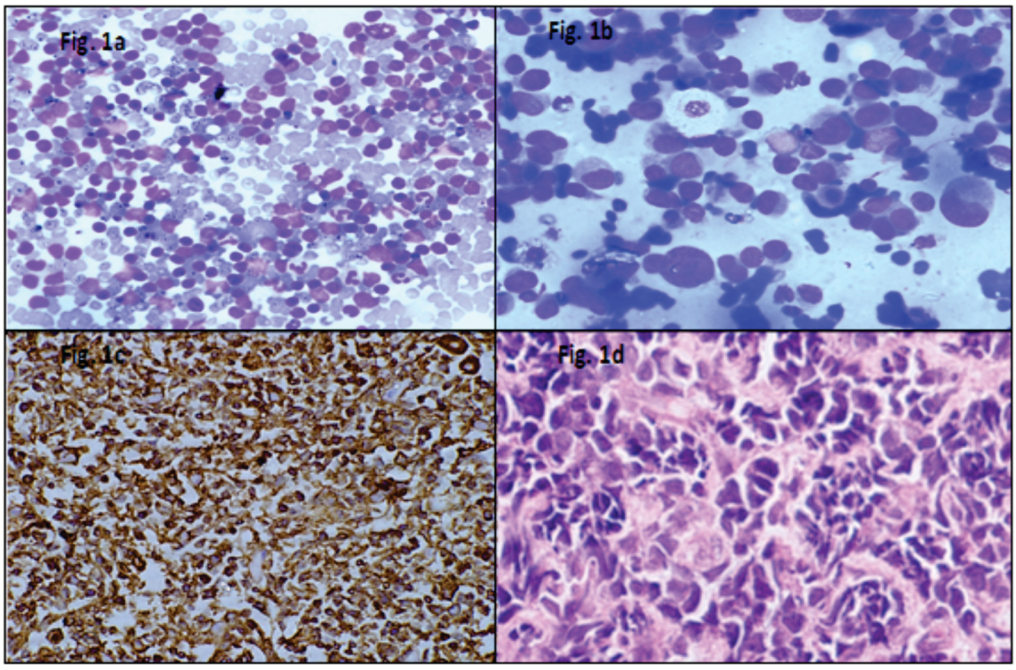


**Figure 1a.** Fine-needle aspiration cytology smears show medium to large atypical lymphoid cells (Magnification X 200).**Figure 1b.** Fine-needle aspiration cytology smears showed medium to large atypical lymphoid cells (Magnification X 400).**Figure 1c.** CD20 immunostaining is positive in atypical large lymphoid cells (Magnification x 400). **Figure 1d.** Histopathology Section shows sheets of large atypical lymphoid cells (Magnification x 400)

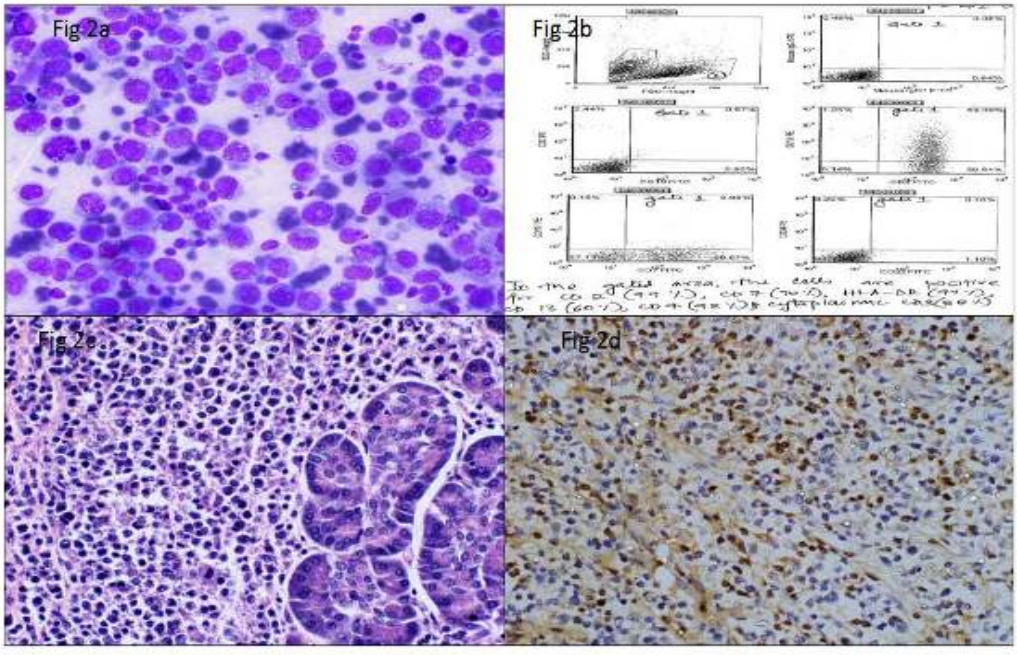


**Figure 2a.** Fine-needle aspiration cytology smears medium to large atypical lymphoid cells with indented nuclei (Magnification X 400).**Figure 2b.** Flowcytometry analysis showed CD13, CD4 and CD3 in Gated Areas. **Figure 2c.** Histopathology Section shows sheets of atypical lymphoid cells with indented hyperchromatic nuclei, condensed chromatin and scant to moderate cytoplasm. Normal Pancreatic duct and acini are also seen. **Figure 2d.** CD3 Immunostaining is positive in atypical lymphoid cells.

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