Pathology Section

Utility of Abdominal Fat Pad Aspiration in the Early Diagnosis of Systemic Amyloidosis: A Cross-sectional Study

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ABSTRACT

Introduction: Abdominal Subcutaneous Fat Pad aspiration (AFPA) is a quick, sensitive, and specific screening and diagnostic method for detecting amyloid deposits. This procedure can be performed in an outpatient setting. The present study aimed to demonstrate amyloid deposits in subcutaneous fat pad aspirates on Congo Red stained slides using polarised microscopy.

Aim: To evaluate the diagnostic accuracy of abdominal fat pad aspiration cytology as a screening procedure for systemic amyloidosis.

Materials and Methods: A cross-sectional study was conducted over a three-year period (January 2019 to July 2021) at SDM College of Medical Sciences and Hospital, Sattur, Dharwad. Fat pad aspirate slides stained with Congo Red stain were independently reviewed by two cytopathologists. Slides were examined under a polarising microscope and interpreted as either positive or negative for amyloid deposits based on the presence or absence of apple green birefringence. The data were evaluated using Microsoft Excel and Statistical Package for the Social Sciences (SPSS) software version 20.0.

Results: The present study included 54 abdominal fat pad aspirates. The ages ranged between 29 and 80 years, with a mean age of presentation of 59 years. Among all AFPA samples, 35 (64.8%) were positive for amyloid, 16 (29.6%) were negative, and three (5.5%) were inconclusive for interpretation. Follow-up deep/visceral biopsies, such as kidney and bone marrow biopsies, were performed in 31 (57.4%) cases, and approximately half of these cases showed amyloid deposition on deep biopsy. AFPA demonstrated a sensitivity, specificity, positive predictive value, and diagnostic accuracy of 94%, 92%, 94%, and 93.3%, respectively, considering visceral biopsies including bone marrow and/or kidney as the gold standard.

Conclusion: Abdominal fat pad aspiration is a quick, minimally invasive, outpatient-based procedure that helps in the early diagnosis of systemic amyloidosis before patients clinically present with complications.

INTRODUCTION

Amyloidosis is a heterogeneous group of disorders with symptoms manifested due to the deposition of misfolded insoluble proteins in various organs and tissues [1,2]. These fibrils have a characteristic β-pleated sheet configuration in Congo Red stained smears, which gives apple green birefringence under the polarising microscope. Amyloid fibrils can be formed by a wide variety of proteins, and the different forms of amyloidosis are categorised according to the amyloidogenic protein and the distribution of amyloid deposits, which can be either localised or systemic [3]. Primary Amyloidosis (AL) is the most common form of systemic amyloidosis in the United States [4]. Light chain amyloidosis is characterised by clonal production of Ig light chains with subsequent multi-organ amyloid deposition and dysfunction [5]. The disease usually presents with subtle signs and symptoms, with frequent clinical features mimicking cardiac, renal, and neurological diseases [6]. The timely diagnosis of amyloidosis before systemic manifestation is crucial to provide appropriate therapy and prevent irreversible organ failure [7,8].

Earlier, less invasive biopsies from the rectum and gingiva were proposed in the 1960s to demonstrate amyloid in visceral tissues. This technique served as an alternative to invasive deeper biopsies from other organs like the kidney and bone marrow [9]. However, they are invasive procedures with significant life-threatening complications [5,10].

Westermark P and Stenkvist B introduced abdominal fat pad aspiration cytology as an alternative to biopsy [5]. AFPA is a quick, sensitive, and specific screening as well as a diagnostic method for detecting amyloid deposits. This method has high specificity, a positive predictive value (~100%), and variable sensitivity depending on the chemical nature of amyloid and the adequacy of the fat pad aspirate. On the other hand, abdominal fat pad excision biopsy yields adequate material for an exact diagnosis, including ancillary studies like electron microscopy [11,12]. However, it is slightly more invasive, technically challenging, and time-consuming than AFPA, which is a simple, minimally invasive, and quick procedure that can be performed in an outpatient setting. Hence, the present study was undertaken to evaluate the diagnostic efficacy of abdominal fat pad aspiration cytology as a screening procedure for systemic amyloidosis.

MATERIALS AND METHODS

The present cross-sectional study was conducted over a period of three years (January 2019 to July 2021) at SDM College of Medical Sciences and Hospital, Sattur, Dharwad. Data for the study were archived from the filed cytology slides and medical records. All slides of abdominal fat pad aspirates received between January 2019 and July 2021 were retrieved and analysed. Ethical clearance was obtained from the Institutional Ethics Review Board with IEC number (SDMIEC/2021/103).

Inclusion and Exclusion criteria: Smears with at least two adipose tissue fragments in cytology were considered adequate [13] and were included in the study. A total of 54 cases with adequate AFPA were analysed. Samples from already diagnosed cases of systemic

amyloidosis and inadequate aspirate smears were excluded from the study.

In all 54 cases, abdominal fat was aspirated using a 21 Gauge needle connected to a 20 mL syringe. The aspirate was then smeared on glass slides and fixed in 95% isopropyl alcohol [Table/Fig-1] [13]. These smears were stained with 0.55 glycine-buffered Congo Red at pH 10.0 and counter stained with haematoxylin. The Congo Red-stained fat pad aspirate smears were examined independently by two cytopathologists under a polarising microscope and interpreted as either positive or negative for amyloid deposits based on the presence or absence of apple green birefringence with a kappa score of 0.94 [14].

[Table/Fig-1]: Abdominal fat pad aspiration procedure.

Very small or isolated deposits with orange-yellow shades of birefringence were considered negative. Biochemical parameters such as serum creatinine, total protein, serum calcium, and serum electrophoresis findings were correlated with cytology results in addition to relevant visceral organ biopsy findings wherever possible.

STATISTICAL ANALYSIS

Statistical analysis, including sensitivity, specificity, and positive predictive values for the true disease status, were evaluated based on the presence of amyloid deposition in the histopathological biopsy from another site. This analysis was performed using Microsoft Excel and SPSS software version 20.0.

RESULTS

A total of 54 cases of abdominal fat pad aspirates were performed during the study period, which comprised nearly 1% of all Fine Needle Aspiration Cytologys (FNACs) performed. Among the 54 cases, 32 (59.3%) were females and 22 (41%) were males, with a ratio of 1.4:1. The age ranged between 29-80 years, with a mean age of presentation of 59 years. The majority of patients were in the 61-70 years age group.

The clinical diagnosis of the patients based on clinical parameters and laboratory investigations were tabulated [Table/Fig-2,3].

Among all AFPA cases, 35 (64.8%) were positive for amyloid [Table/Fig-4], 16 (29.6%) were negative, and three (5.5%) were inconclusive for interpretation. The majority of cases with amyloid deposition in the abdominal fat pad aspirates were of multiple myeloma (17 cases), followed by chronic kidney disease (8 cases), dermatomyositis (2 cases), rheumatoid arthritis (1 case), and others (7 cases), including anaemia, pancytopaenia, immune thrombocytopenic

purpura, myelodysplastic syndrome, myelofibrosis, and acute respiratory distress syndrome.

Clinical diagnosis	Total number of patients	Percentage (%)
Multiple myeloma	28	51.8%
Plasma cell dyscrasias	02	3.7%
Primary Amyloidosis (AL)	01	1.8%
Chronic kidney disease	12	22.2%
Dermatomyositis and rheumatoid arthritis	03	5.5%
Miscellaneous	08	14.8%
Total	54	100%

[Table/Fig-2]: Clinical diagnosis of patients who underwent AFPA.

Laboratory parameters	Number of patients
Rise in serum creatinine (≥1 mg/dL)	31
Urine protein (2+ to 4+ in dipstick)	10
24 hoururine protein excretion (>500 mg/day)	13
Anaemia (<11g% for females, <12 g% for males)	28

[Table/Fig-3]: Important laboratory profiles of patients who underwent AFPA.
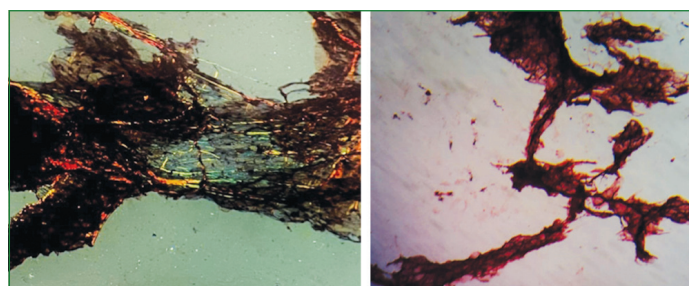
[Table/Fig-4]: Abdominal fat pad aspirate under polarised microscopy.
{Congo Red stain depicting apple green birefringence when examined under polariser (in A), negative (in B) original magnification x400}

In 31 (57.4%) cases, further deep/visceral biopsies were taken, including those of the kidney and bone marrow and in the remaining 4 cases, deep biopsy was not available. Of these, half of the cases showed amyloid deposition. Cytological testing was positive in one case (3.2%), but a bone marrow biopsy did not reveal any amyloid accumulation. This was considered a false positive for the calculation of diagnostic accuracy. Among the total 31 cases, 13 (41.9%) were diagnosed as negative on cytological examination for amyloid deposition. Of these, 12 cases did not show any amyloid deposition on deep biopsy, and one case (3.2%) which was given as negative on aspiration cytology, but showed amyloid deposition in the deep biopsy, which was considered a false negative in cytology.

Out of 31 cases, in one case, bone marrow biopsy yielded scant material. So total samples with visceral biopsies used for calculation of sensitivity, specificity and PPV were 30 cases. Using the deep tissue biopsy as the gold standard for diagnosing systemic amyloidosis, sensitivity, specificity, positive predictive value, and negative predictive values for the subcutaneous abdominal fat pad aspiration were calculated and tabulated in [Table/Fig-5], with a diagnostic accuracy of 93.33%.

| Cytopathology | Histopathology* | | Predictive values |
	Positive	Negative	
Positive (n-17)	16	1	PPV-94%
Negative (n-13)	1	12	NPV-92%
Sensitivity and specificity	Sensitivity- 94%	Specificity- 92%	

[Table/Fig-5]: Concordance between cytopathology and biopsy findings in thirty cases.
*histopathology was done in 31 cases. One case upon bone marrow biopsy did not yield any material, so total cases were 30

DISCUSSION

Systemic amyloidosis is a chronic and highly variable disease characterised by the extracellular deposition of beta-pleated amyloid fibrils. Patients with systemic amyloidosis often present with nonspecific symptoms and signs that can overlap with other diseases, making the diagnosis challenging [13]. Currently, approximately 30 amyloid precursor proteins have been discovered [15]. The deposition of amyloid in deeper tissues such as the kidney, gastrointestinal system, heart, liver, and skin can lead to multiple organ failure [16]. Traditionally, the gold standard for diagnosing amyloidosis involves the detection of amyloid deposition in deeper tissues such as the kidney, bone marrow, rectum, and stomach. However, these procedures are invasive. As a result, abdominal fat pad aspiration cytology has emerged as a less invasive alternative for detecting amyloid deposition [13]. In cases where there is clinical suspicion of amyloidosis, abdominal fat pad aspiration can help avoid more invasive biopsies of the affected organs [13].

In the present study, the mean age of the patients was 59 years, which was comparable to the studies conducted by Dhingra S et al., and Halloush RA et al., with mean ages of 48.12 years and 67 years, respectively [13,17]. The age range in our study was 29-80 years, which is similar to the age ranges reported in the studies by Halloush RA et al., (40-88 years) and Dhingra S et al., (20-88 years). The authors observed a female preponderance in the present study, similar to the findings of Dhingra S et al., where there were 126 men and 171 women, whereas Halloush RA et al., reported an equal gender distribution of 19 women and 19 men [13,17].

Multiple myeloma was found to be the most common underlying cause (51.8% of cases) in our study. However, in a study by Ansari-Lari MA and Ali SZ, monoclonal gammopathy of undetermined significance was the most common (34%) [18]. In the study by Dhingra S et al., rheumatoid arthritis (46.7%) was the most common clinical presentation [13].

Congo Red stain was performed on deep biopsies of 30 follow-up cases in our study, and the sensitivity and specificity for AFPA were calculated to be 94% and 92%, respectively. These findings were comparable to other studies as shown in [Table/Fig-6] [11,13,18,19].

Authors	Year of study	Place of study	Sensitivity (in %)	Specificity (in %)
Dhingra S et al., [13]	2007	Lucknow	78%	93%
Ansari-Lari MA and Ali SZ [18]	2004	Maryland (USA)	75%	92%
Guy CD et al., [11]	2001	North Carolina (USA)	58%	100%
Masouyé I [19]	1997	Geneva, Switzerland	82%	100%
Present study	2023	Dharwad	94%	92%

[Table/Fig-6]: Comparison of sensitivity and specificity of AFPA with different studies [11,13,18,19].

The literature reviewed has shown that many positive abdominal fat pad aspirates may indicate subclinical amyloidosis and may not show amyloid deposition in visceral biopsies even after a longer duration of follow-up, such as 14 years [18]. It is important to note that a negative abdominal fat pad aspiration does not rule out systemic amyloidosis, as false negative reports can occur due to factors such as improper staining, operator skill, extent of organ involvement, inadequate material, and improper use of a polarising microscope [8,13,20]. These factors can affect the sensitivity and specificity of the test [8,13]. In this study, the specificity was found to be 92%, which is comparable to the studies conducted by Dhingra S et al., and Ansari-Lari MA and Ali SZ, with specificities of 93% and 92%, respectively [13,18]. It is important to note that false positive amyloid on fat pad aspiration can occur due to collagen, which also exhibits yellow birefringence [21].

Limitation(s)

A study conducted on a larger sample size with improved follow-up and deep biopsy performed on all positive abdominal fat pad aspirates would enhance the sensitivity of the diagnostic test.

CONCLUSION(S)

The incorporation of the AFPA technique into routine cytology practice is highly recommended. AFPA is a quick, safe, and minimally invasive outpatient-based procedure. The accurate diagnosis is facilitated by a thorough microscopic examination of Congo Red stained smears under polarising microscopy. This technique not only demonstrates high sensitivity and diagnostic accuracy but also enables clinicians to make early diagnoses of systemic amyloidosis, thereby avoiding invasive deep biopsies in some cases and ensuring optimal treatment.

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