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Original Article

Pathology Section

Role of Cytology for Diagnosis of Appendageal Tumours and Comparison with Histopathology: A Cross-sectional Study

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ABSTRACT

Introduction: Skin adnexal neoplasms are quite rare in routine pathology practice, yet the domain of Fine Needle Aspiration Cytology (FNAC) remains relatively unexplored. This current study explored different Adnexal Tumours (ATs) through FNAC, emphasising the efficacy of cytological diagnosis as a safe, simple, quick, and cost-effective tool with high sensitivity and specificity for diagnosing skin ATs.

Aim: To diagnose various appendageal tumours through cytological examination, confirm and categorise them (benign/malignant) via histopathological examination, and identify any cytohistological discrepancies.

Materials and Methods: This retrospective cross-sectional study was conducted over a five-year period (January 2016-December 2021) at the Department of Pathology, Nil Ratan Sircar Medical College and Hospital (NRSMCH) in Kolkata, West Bengal, India, following ethical approval. Patients underwent history and physical examination followed by FNAC. ATs were diagnosed cytologically in 78 out of 23,852 FNAC cases. The results were compared with histopathological diagnosis to assess concordance or discordance based on predefined criteria. Leishman-Giemsa (L&G) stain was used for cytological examination, and Haematoxylin and Eosin (H&E) stain for histopathology.

Results: Cytologically, ATs were diagnosed in 78 cases (0.33%), with the majority being benign 69 (88.46%). There was a female preponderance (49, 62.82%) with a male-to-female ratio of 1:1.7. Nodular Hidradenoma (NH) was the most common diagnosis (16/48=33.33%). Out of 59 biopsy-confirmed lesions, 55 showed cytohistological concordance (93.22%), with only four discordant cases (6.78%). The sensitivity, specificity, positive predictive value, and negative predictive value for detecting malignancy in this series were 70%, 97.90%, 87.50%, and 94.10%, respectively.

Conclusion: FNAC should be the primary choice for detecting skin ATs due to its safety, reliability, high predictive value, and low chance of discordance. Although occasional cytohistological discrepancies may occur, proper clinical correlation, aspiration from multiple sites, expert cytopathologists, and high-quality staining are essential to avoid misdiagnosis.

Keywords: Adnexal neoplasms, Cytohistological, Fine needle aspiration cytology

INTRODUCTION

Skin ATs encompass a broad spectrum of benign and malignant tumours that exhibit morphological differentiation towards one or more types of adnexal structures found in normal skin [1]. These tumours typically originate from undifferentiated pluripotent stem cells and eventually differentiate into specific tumours influenced by genetic alterations, local vascularity, and the microenvironment of the epidermis and dermis [1]. Most clinicians prefer excisional biopsy over FNAC for a definitive diagnosis as they are easily accessible, making skin ATs rarely encountered via FNAC. However, FNAC can be a primary choice for diagnosing skin ATs due to the absence of the need for anaesthesia. It can recognise subtypes, provide a low-cost diagnosis, aid in management, and benefit patients.

Despite the advantages, there are few case reports describing the cytomorphological features of skin ATs through FNAC as it is not widely practiced [2]. Bhadani PP et al., in their study, demonstrated that FNAC can help establish the epithelial nature of the lesion [3]. Daskalopoulou D et al., concluded from their study that certain constant cytomorphologic features can differentiate ATs from clinically and grossly similar-looking skin tumours. They also showed that cytological assessment is feasible for many rare skin lesions [4]. Dermal appendageal tumours encompass hundreds of neoplasms, both benign and malignant, showing differentiation towards various cutaneous adnexal structures [1,5]. These neoplasms are rare and typically present as subcutaneous swellings.

Although excisional biopsy is common for clinically suspected lesions, preoperative cytological evaluation can identify skin ATs on FNAC, enabling clinicians to manage patients promptly and follow-up on them. Cytological findings can aid in distinguishing these tumours from metastatic carcinomas and sarcomas, which are common differential diagnosis. Early recognition of ATs is crucial as they may serve as markers of certain syndromes associated with internal malignancies [6].

The present study was conducted to diagnose ATs through cytology. The study objectives were to diagnose and differentiate benign from malignant adnexal lesions through cytological examination, despite overlapping clinical features. The accuracy of cytological diagnosis was assessed in comparison to histopathology, detailing misdiagnosed cases with the hope of rectifying flaws for future improvements. Hence this study was conducted on ATs to determine the efficacy of FNAC and to establish the histological diagnosis with cytohistological association.

MATERIALS AND METHODS

The was a retrospective, cross-sectional, institution-based study conducted in the Department of Pathology at Nil Ratan Sircar Medical College and Hospital (NRSMCH) in Kolkata, West Bengal, India, over a period of five years (January 2016- December 2021). The study was approved by the Institutional Ethical Committee (IEC) and was assigned the IEC number: NRSMC/IEC/45/2022, dated 06.06.2022.

Inclusion criteria:

1) Patients presenting with palpable subcutaneous and dermal swellings for FNAC.

2) Histopathological examination and correlation of the cytologically diagnosed ATs.

Exclusion criteria:

1) Any non cutaneous swellings (e.g., lymph nodes, salivary glands, thyroid, intra-abdominal swellings, etc.).
2) Non palpable cutaneous swellings.
3) Patients unwilling to undergo FNAC despite being informed about the procedure.
4) Very hard cutaneous swellings (potentially calcified) for FNAC.
5) Patients who did not consent to FNAC/Biopsy.
6) FNAC smears showing bloody background, crushing artifacts, drying artifact, or paucicellularity.
7) Biopsy sections showing autolysed tissue.
8) History of any primary visceral malignancy and their metastasis.

The parameters evaluated were as follows: symptoms, age, sex, different sites of subcutaneous or dermal swellings, identification and categorisation of the adnexal neoplasms cytologically followed by histopathological examination, and lastly, to determine concordance/disconcordance between the cytological and histopathological diagnosis. Retrospective evaluation of cytological and histopathological findings from 23,852 cases in that period was conducted. A total of 23,852 cases underwent FNAC during that period, with only 1,579 cases (6.61%) presenting with subcutaneous or dermal lesions.

Cytologically, ATs were diagnosed in 78 cases (0.33%). After cytological examination, records of 19 cases (24.35%) were not found, leaving 59 patients (78-19=59) available for further study to assess the association between cytological and histopathological reports. L&G stain and Papanicolaou stains were used for cytological study, while H&E stain was used for histopathological examination.

In most cases, two pathologists signed off on both the cytology and biopsy reports. FNAC-diagnosed adnexal neoplasms were identified, and attempts were made to make a final categorical diagnosis.

STATISTICAL ANALYSIS

Statistical methods used in this study included quality control (for specificity and sensitivity) and probability tests (for positive predictive value and negative predictive value). Categorical variables were presented as numbers and percentages (%), while continuous variables were presented as mean±SD and range.

RESULTS

The FNAC-diagnosed adnexal lesions were included in the study. Further classification of lesions into benign or malignant was done based on cytology. Specific categorical diagnosis were attempted in possible cases. Subsequent histological evaluation was conducted in all cases, with the histopathological diagnosis regarded as confirmatory.

A total of 23,852 cases underwent FNAC during the study period, with only 1,579 cases (6.62%) presenting with subcutaneous or dermal lesions. Cytologically, ATs were diagnosed in 78 cases (0.32%), with the majority being benign (69/78=88.46%) and only 9 malignant cases (9/78=11.64%) [Table/Fig-1]. Of the total cyto-diagnosed ATs, In present study, female preponderance was noted (49 cases, 62.82%) compared to males (29 cases, 37.18%), with a ratio of 1:1.7. The majority of cases (37/78, 47.44%) were reported in the 21-40 years age group, with the head and neck region accounting for 53 cases (67.95%) [Table/Fig-2]. The mean age was 28.50±5.083 years.

Total cases	Subcutaneous/ dermal lesions	Adnexal Tumours (AT)		
		Benign	Malignant	Total
23852 (100%)	1579 (06.62%)	69/78 (88.46%)	09/78 (11.54%)	78 (00.33%)

[Table/Fig-1]: Incidence of Adnexal Tumours (AT).

After cytological examination, biopsy reports of 19 cases (24.36%) were not found in the biopsy record book, possibly missed on follow-up, leaving 59 patients available for further study. Initially, out of these 59 cases, cyto-diagnosed benign and malignant cases were 51 (86.44%) and 8 (13.56%), respectively. Upon thorough review by two pathologists, it was found that out of the 51 cases initially diagnosed as benign, 48 (94.11%) were confirmed as benign and 3 (5.88%) were malignant on histopathological examination. Specific cytodiagnosis was attempted in 48 benign cases initially [Table/Fig-3].

The NH was the most common diagnosis (16/48=33.33%) [Table/Fig-4], followed by Chondroid Syringoma (CS) (8/48=16.67%) [Table/Fig-5]. Cytologically, specific diagnosis were offered in only 3 (3/8=37.50%) malignant cases (two apocrine carcinoma and one trichilemmal carcinoma). The remaining malignant cases (5/8, 62.50%) were reported as malignant AT on cytology. After histological examination, 2 trichilemmal carcinoma, 2 apocrine carcinoma, 1 porocarcinoma, 1 sebaceous carcinoma, and 1 eccrine adenocarcinoma were found as malignant lesions. Out of 51 benign cases, 48 (94.11%) showed cytohistological correlation, with 3 (5.88%) false negative malignant lesions. In the malignant group, only 1 (12.50%) out of 8 cases was a false positive, while the remaining 7 (87.50%) cases yielded consistent reports [Table/Fig-6]. Ultimately, after thorough cytohistological correlations of ATs, the number of true benign and true malignant tumours were 49/59 (83.05%) and 10/59 (16.95%), respectively.

Cases showing cytological discrepancies were further evaluated to search for possible causes and potential remedies regarding cytological misdiagnosis. Histopathological correlation was attempted in all possible cases.

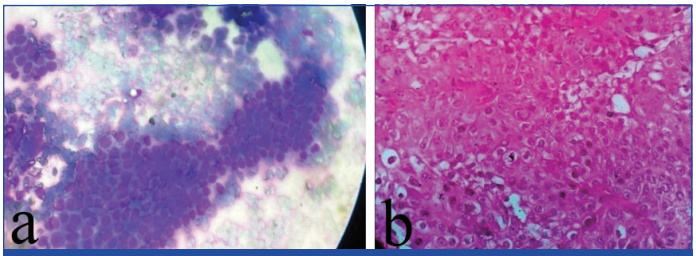
Cytologically, three out of 51 benign cases were reported as false negative malignancies (two cases as benign ATs, but the biopsy report indicated trichilemmal carcinoma in both cases [Table/Fig-7], and one case as an epidermoid cyst, but the biopsy report showed apocrine carcinoma) [Table/Fig-8]. One (01) case out of eight cases was dispatched as a false positive malignancy (reported as basal cell carcinoma, but the biopsy report revealed pilomatricoma) [Table/Fig-9]. Overall, 55 out of 59 biopsy-confirmed lesions showed exact cytohistological correlation (93.20%) with only four (04) (6.80%) discordant cases. Cytologically, the sensitivity, specificity, positive and negative predictive values for the detection of malignancy in the present series were 70.00%, 97.90%, 87.50%, and 94.10%, respectively.

Total Number of Adnexal Tumours (AT)	Sex		Age (years)				Site		
	Male	Female	0-20	21-40	41-60	>60	Head and Neck	Trunk	Extremities
78	29	49	08	37	23	10	53	09	16
100%	37.18%	62.82%	10.25%	47.44%	29.49%	12.82%	67.95%	11.54%	20.51%

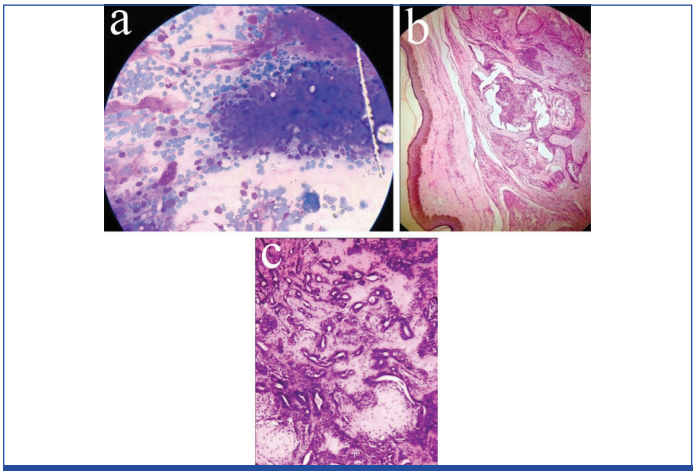
[Table/Fig-2]: Age-sex-site distribution of Adnexal Tumours (AT).

Cytologically diagnosed benign cases	Category of adnexal lesions (48) (94.12%) (Cytologically)	Interpretation on histology diagnosis
51	Nodular Hidradenoma (16)	Same
	Chondroid Syringoma (08)	Same
	Pilomatricoma (04)	Same
	Syringoma (02)	Same
	Spiroadenoma (02)	Same
	Cylindroma (02)	Same
	Proliferative Trichilemal tumour (03)	Same
	Spiroadenoma (02)	Same
	Syringo cyst adenoma Papilliferum (02)	Same
	Trichilemoma (02)	Same
	Hidradenoma Papilliferum (01)	Same
	Basaloid Follicular Hamartoma (01)	Same
	Benign Adnexal Tumour (AT) (02)	Trichilemmal carcinoma (02)
	Epidermoid cyst (01)	Apocrine carcinoma (01)

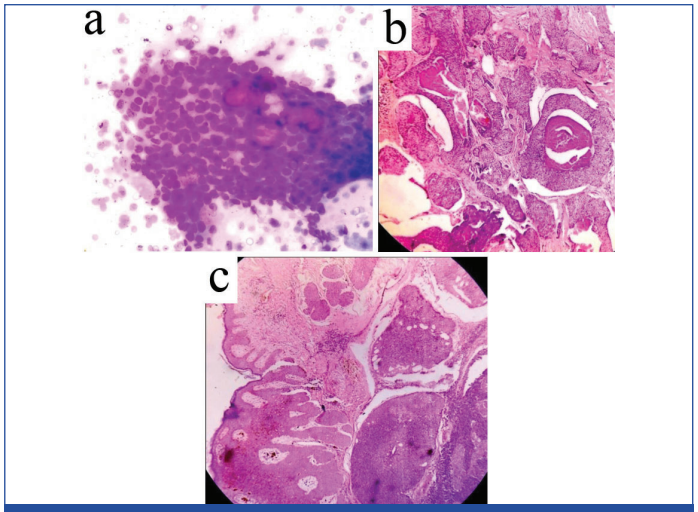
[Table/Fig-3: Cytology and histological categorisation of different Adnexal Tumours (AT).



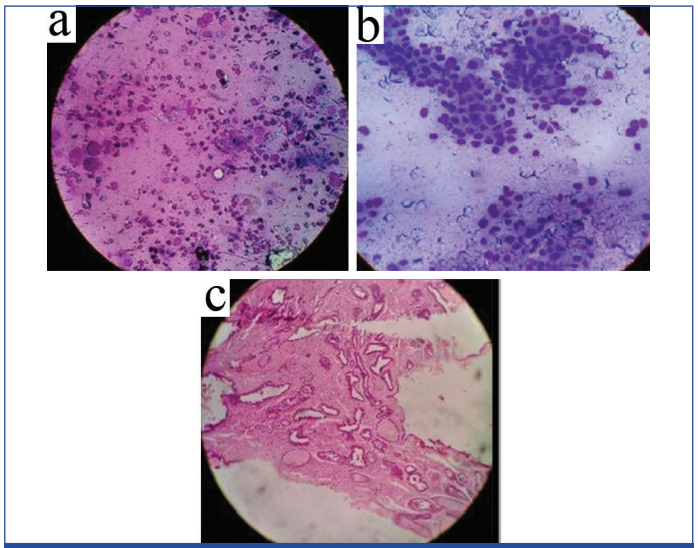
[Table/Fig-4]: a) FNAC of Nodular Hidradenoma (NH), (Leishman-Giemsa (L&G) stain, X40); b) Nodular Hidradenoma (NH) (Biopsy, Haematoxylin-Eosin stain- X40).



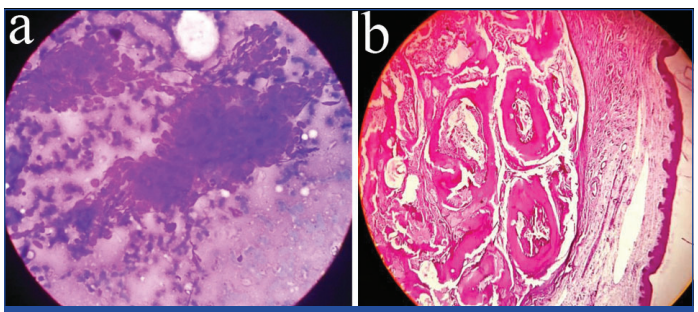
[Table/Fig-5]: a) Chondroid Syringoma (CS) (FNAC, Leishman-Giemsa (L&G) stain, X40); b,c) Chondroid Syringoma (CS) (Biopsy, Haematoxylin-Eosin stain- X40).



[Table/Fig-7]: a) FNAC of Trichilemmal carcinoma, Leishman-Giemsa stain, X40; b,c) Trichilemmal carcinoma (Biopsy images: Haematoxylin-Eosin stain, X40).



[Table/Fig-8]: a-c) Apocrine carcinoma, (a,b: FNAC, Leishman-Giemsa stain, X40); c) biopsy, haematoxylin-eosin stain-X40).



[Table/Fig-9]: a) Pilomatrixoma (FNAC, Leishman-Giemsa stain, X40); b) Pilomatrixoma, (Biopsy, Haematoxylin-Eosin stain-X40).

Total cytological Adnexal Tumours (AT) cases	Total number of cases undergoing histopathology	Category of cyto- diagnosis	Number of cases	Category of histo-diag-nosed cases		False positive malignancy	False negative malignancy
				Benign	Malignant		
78	59	Benign	51 (86.44%)	48 (94.12%)	03 (05.88%)	00	03 (05.88%)
		Malignant	08 (13.56%)	01 (12.50%)	07 (87.50%)	01 (12.50%)	00
Total benign ATS- 49/59 (83.05%) Total malignant ATS- 10/59 (16.95%)							

[Table/Fig-6]: Cytology-histological correlation of Adnexal Tumours (AT).

DISCUSSION

Skin ATs encompass a wide variety of tumours clinically presenting as asymptomatic papules or nodules. ATs are uncommon lesions, accounting for only a minor fraction (78, 0.33%) of total FNAC cases (23852). In this series, there were only 78 (4.94%) cytodiagnosed AT cases out of 1579 subcutaneous and dermal lesions aspirated. A comparable low incidence was reported by Samaila MO (3.90%) [7]. Female preponderance was noted (49 cases, 62.82%) compared to males (29 cases, 37.18%), at a ratio of 1:1.7 in the present study group. Similar observations were found by previous researchers, e.g., Saha A et al., (male patients= 10133, 34.78% and female patients= 17233 cases, 65.21%), Radhika K et al.,

(male-15, 42.85% and female-20, 57.14%), and Nair PS (male patients= 10, 30.30% and female patients= 23, 69.69%) [8-10]. Most of the cases (37, 47.40%) affected young adults (age range from 21-40 years, mean±SD for the age was 28.50±5.083 years), similar to other researchers like Samaila MO (median age- 33 years, mean±SD for the age was 30.30±4.081 years) [7]. In a large series reported by Samaila MO, the head and neck region were the most common site (24/52, 46.00%) of ATs, similar to this study (53/78, 67.95%) [7]. All the cutaneous swellings were non tender on clinical examination except spiradenoma, 03 cases (05.88%), similar to the study by Saha A et al., (Total benign ATs-50, spiradenoma-02 cases, 04.00%) [8]. Benign lesions (69/78, 88.46%) mostly outnumbered the malignant ATs in this study, similar to observations by Samalia M et al., (n=52 ATs, 46 benign tumours (88.46%), 06 (11.53%) malignant tumours) and Radhika K et al., (n=35 ATs, 27 (77.15%) benign and 08 (22.85%) malignant tumours) [7,9]. ATs are basically classified into four groups: tumours with differentiation toward hair follicles, sebaceous glands, eccrine glands, or apocrine glands [4].

In the present study, cytomorphologically this type of special classification was not used, but a possibility of the type of adnexal lesion, for example, cylindroma and pilomatricoma, was given wherever it was possible. Eccrine tumours like NH (16/51, 31.37%) and CS (08/51, 15.68%) [Table/Fig-3] were the two most commonly diagnosed ATs in this study. The cytological smear of NH showed three-dimensional papillary-like fronds and extracellular hyaline material [Table/Fig-4a]. The biopsy revealed a lobulated mass of variable-sized nests and nodules of epithelial cells within the upper dermis. No connection of the lesion with the overlying epidermis was noted [Table/Fig-4b]. Cytologically, the CS showed bland, monomorphic epithelial cells with round to oval nuclei embedded in a profuse magenta-coloured myxoid matrix [Table/Fig-5a]. The histological section showed epithelial cells arranged in tubules, ducts, and nests. There were outer cuboidal epithelial layers and an inner layer of columnar epithelium with eosinophilic cytoplasm. The tubules were filled with decapitation secretion. The stroma was chondro-myxoid [Table/Fig-5b,c]. Both NH and CS showed concordant correlations. Similar findings were also reported by Samaila MO (15/46, 32.60% and 08/46, 17.39%), Radhika K et al., (05/35, 14.20% and 01/35, 02.80%), respectively [7,9]. Whereas Nair PS found more cases of NH and pilomatrixoma (17/33, 51.55% and 12/33, 36.36%) respectively in their study, and the incidence of CS was less (01/33, 03.03%) than in this study [10]. Out of 59 biopsies correlated AT cases, only 04 cases (6.78%) showed cyto-histological discordant results. The rest of the 55 cases (93.20%) had FNAC reports compatible with histology (concordant), similar to the studies by Rege J et al., (n=12 ATs, 10/12, i.e., 83.30% of FNAC cases showed cyto-histological correlation) [11] and Dey P et al., (n=18 ATs, 16/18, i.e., 88.90% of FNAC cases showed cyto-histological correlation) [12]. Thus, FNAC can serve as a simple, cost-effective, quick diagnostic modality for preoperative assessment of ATs. According to Chand P et al., there was total correlation (concordant) between the FNAC diagnosis and final histopathological diagnosis in 08 cases (n=14, 57.10%), partial correlation in 04 cases (28.50%), and no correlation in only 02 cases (14.30%). The result was dissimilar to the present study, possibly due to the low number of AT cases included in their study [6]. Typing of ATs on cytology was possible in 82% of cases (9/11 cases) with a diagnostic accuracy of 88% (8/9 cases) by Arora A et al., [1]. In the present study, there were 03 false negative cases (3/51, 5.88%) and 01 false positive case (1/51, 12.50%) for malignancy.

Regarding the false positive malignant diagnosis, a 53-year-old female presented with an ulcerated lesion on the back of the neck. On aspiration, cellular smears showed clumps and groups of basaloid cells with nucleomegaly and nuclear atypia. A presumptive diagnosis of malignancy was made with a suggestion to exclude the possibility of basal cell carcinoma. However, on histology, the lesion was proven to be a pilomatrixoma [Table/Fig-9b]. Similar misdiagnosis have been reported in many series, such as Thapiyal N et al., and Greene RM et al., [13,14]. Cytologically, pilomatrixoma presents a variable mixture of different cellular and acellular components. Basaloid cells, ghost cells, and foreign body type giant cells are the three most important cytological findings [15-17]. Other accompanying components can include acellular materials, calcification, nucleated squamous cells, and inflammatory cells [17,18]. The possible cause of misdiagnosis in present study could be aspiration from marginal areas of the lesion. Aspirates from these areas, particularly if the lesions are relatively early, often appear hypercellular with nuclear atypia, mimicking malignancy. To avoid this potential trap, aspiration should be done from multiple sites, including the center of the lesions, to procure distinctive cellular and acellular components of this benign lesion [6,17]. Therefore, pilomatricoma should be considered in the differential diagnosis when primitive appearing cells are aspirated, especially in rapidly growing early lesions.

Among the three false negative cases (3/51, 5.88%), two (47 years old and 39 years old female patients) presented with slow-growing nodular lesions over the left cheek and below the right angle of the mandible, respectively. These were interpreted as benign adnexal lesions. Cytological smears were hypercellular and composed of a complex, syncytial sheet of squamoid epithelial cells without any keratin. The cells had moderate cytoplasm and vesicular nuclei. The cytological report suggested a benign adnexal lesion. However, the histopathological images showed a lobular proliferation of follicular epithelium in the dermis. The invasive lobules showed peripheral palisading of clear keratinocytes and centrally located trichilemmal type of keratinisation. Cytological atypia and mitotic figures were also noted [Table/Fig-7a]. Both lesions on subsequent histology were proven to be trichilemmal carcinoma [Table/Fig-7b,c]. Accurately diagnosing proliferating trichilemmal lesions on cytology is truly a challenging task. Cytohistological discordance regarding the diagnosis of malignancy has been previously reported by Folpe AL et al., [19]. They studied the cytohistological discrepancy in 5 cases of proliferating trichilemmal tumours [19]. The final confirmation depends on histopathological examination regarding cellularity, anaplasia, growth pattern, and mitotic activity [6,19].

A case of histologically confirmed axillary apocrine carcinoma was misinterpreted as an epidermoid cyst on preoperative cytological evaluation [Table/Fig-8a,b].

It was a slow-growing soft tissue swelling in the axillary region of a 29-year-old male with the presence of a discharging sinus. Aspirates revealed acellular material with foamy macrophages, inflammatory cells, and occasional degenerated cellular remnants. Based on the clinical features, a presumptive diagnosis of an epidermoid cyst was made. The histological section showed epithelial cells arranged in a solid and glandular architecture with infiltrative borders, mucin production, and consisted of atypical apocrine cells with eosinophilic granular cytoplasm, irregular nuclear membrane, and prominent nucleoli. Atypical mitosis was frequent [Table/Fig-8c]. The cause of misdiagnosis was the failure to aspirate cellular materials. Multiple aspirates, particularly from the periphery of the lesion, would have been helpful to aspirate atypical epithelial cell clusters in that situation. The cytological diagnosis of apocrine carcinoma is often difficult, and previous researchers have published erroneous results [20,21]. Since the axillary region contains multiple apocrine glands, it is the most common site for these apocrine tumours. Cytological suspicion for primary apocrine adenocarcinoma should be raised in the case of any axillary tumour, especially considering the poor prognosis and chances of early metastasis [20]. It should also be kept in mind that the axillary region retains many small lymph nodes, so metastasis of any malignancy from the chest and intrathoracic organs can affect these lymph nodes. The cytological picture of metastatic axillary lymph nodes closely mimics primary apocrine

carcinoma arising from the axilla, making it very challenging for the pathologist to differentiate these entities both cytologically and histopathologically [19]. Early recognition of apocrine tumours is important as they may serve as markers of certain syndromes associated with internal malignancy, such as trichilemmomas in Cowden syndrome and sebaceous tumours in Muir-Torre syndrome, Birt-Hogg-Dubé syndrome, and Brooke-Spiegler syndrome [22]. However, these types of cases were not found in this study after clinical correlation.

Limitation(s)

The present study had a few limitations, for example, follow-up of all 78 cases was not possible due to a dropout of 19 cases during the study period. Proper follow-up could have provided a better outcome. FNAC along with Fine Needle Aspiration Biopsy (FNAB) was not done simultaneously by the pathologist, which could have avoided the common biopsy procedure by the surgeons and saved time in reporting histopathological reports. The apocrine tumours were not classified into four groups: tumours with differentiation toward hair follicles, sebaceous glands, eccrine glands, or apocrine glands. Immunocytochemical or immunohistochemical analysis was not performed in discordant cases due to limited economic resources. Since the present study was single-centred and conducted in Kolkata, West Bengal, India, the results cannot be generalised to all regions.

CONCLUSION(S)

The ATs arising from dermal appendages are rare, presenting with histological diversity, with benign tumours being more common than malignant ones. While histopathological examination is confirmatory and more reliable for surgeons, proper cytological diagnosis is not possible without clinical correlation. FNAC plays a definite role in forming the initial diagnosis of AT, while histopathology, aided by immunomarkers, provides the final diagnosis. The study was able to accurately categorise over 90% (55/59 cases=93.20%) of the lesions on cytology compared to histopathology, indicating the need for further research in this slightly neglected field of cytopathology. It is expected that this will help future workers avoid potential misdiagnosis traps and ensure more purposeful utilisation of FNAC for diagnosing skin ATs. Additionally, FNAC in suspected malignant lesions can guide the surgeon regarding the extent of excision.

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