

Tumours of Skin and Adnexa: A Histopathological Cross-Sectional Study

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Abstract

Aim: Pathologists and dermatologists face a major challenge in the diagnosis and treatment of skin and adnexal tumors due to limited clinical and pathological data. Adnexal tumors in old age tend to mimic malignant tumors. Histopathological examination is the mainstay for differentiating between benign and malignant tumors of the skin and adnexa because of the limited use of immunohistochemistry. Primary objective: To study the histopathological features of skin and adnexal tumors. Secondary - to classify various skin and adnexal tumors according to the World Health Organization classification and to study variations according to age, sex and site. A descriptive, cross-sectional study with statistical analysis.

Materials and Methods: This study was conducted in the pathology department of a tertiary care hospital in Central India. Total 190 samples were submitted. Collected data were analyzed by performing chi-square test using SPSS V.16 software.

Results: Out of 190 cases, 127 cases were diagnosed as benign and 63 cases were malignant tumors of the skin and adnexa; 42.1% cases were of keratinocytic origin. Malignant keratinocytic tumors were the most common. The study included 101 females and 89 males. The majority of cases (49%) occurred in the head and neck region. Neurofibroma is the most common benign skin tumor.

Conclusion: Skin tumors and their various histological types always create diagnostic difficulties. Histopathology is the gold standard that aids in the early diagnosis of skin and adnexal tumors, thus improving the prognosis.

Keywords: Skin, adnexa, tumours, histopathology

INTRODUCTION

A wide variety of benign and malignant skin tumors occur in clinical setting mentioned as “troubling tumors” by Cotton^{1,2} Their frequency varies due to different skin types, geographic location, occupational and sun exposure, skin protection awareness and its surveillance.³ Their frequency increases with age.⁴ In India, skin tumors constitute 1-2% of all cancers.⁵ They were classified into three categories involving the epidermis, dermis, and adnexa, respectively.⁶ Undifferentiated pluripotent stem cells, genetic influence, local vascularity, and the the microenvironment of the epidermis and dermis give rise to these tumors.⁷ Because the use of immunohistochemistry is limited, histopathological examination is essential for correct diagnosis and management.

MATERIALS AND METHODS

This study was a cross-sectional study conducted in the pathology department of a tertiary care center in Central India for two years. A total of 190 samples were submitted, which included skin, wedge, edge, and excisional biopsies. The patient’s clinical history was noted. Samples were processed routinely with hematoxylin and eosin staining. Special stains such as Periodic acid-Schiff, Masson’s Trichrome, and Masson-Fontana were used when required. Approval from the institutional ethics committee and informed consent from patients were obtained. Histopathologically confirmed diagnoses of skin and adnexal tumors were included.

Submission: 18-Sep-2023

Web Publication: 20-May-2024

Acceptance: 04-Jan-2024

Access this article online

Quick Response Code:



Website:

www.tjdonline.org

DOI:

10.4274/tjd.galenos.2024.96_23

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How to cite this article: Mirani VP, Helwatkar SB, Chawhan SM, Raja EA. Tumours of Skin and Adnexa: A Histopathological Cross-Sectional Study. Turk J Dermatol 2024;18(1):1-8.

Statistical analysis

Statistical analysis was performed using SPSS V.16 software. $P < 0.05$ was considered statistically significant.

RESULTS

In the present study, 190 cases of skin tumors were included, of which 127 and 63 cases were benign and malignant tumors, respectively. The benign to malignant tumor ratio is 2.03:1. Cases were classified according to the World Health Organization (WHO) classification of skin tumors (Table 1), showing that 80 (42.1%) cases were of keratinocytic origin followed by appendageal origin (30 cases, 15.8%). Malignant keratinocytic tumors were the most common, accounting for 53 (27.9%) cases, followed by 27 (14.2%) cases of benign keratinocytic and neural tumors. Out of 190 cases, 101 and 89 cases were females and males, respectively, with male to female ratio: 1:1.13. Head and neck region ($n = 94$, 49%) was most common site followed by extremities ($n = 42$, 22.6%), trunk ($n = 41$, 22.5%) and external genitalia ($n = 13$, 6.8%). Most commonly benign tumours were noted in 3rd decade (34 cases) and 4th decade (21 cases) and malignant tumours were in 6th and 7th decades accounting 14 and 16 cases, respectively.

In the present study, keratinocytic tumors comprised 27 (33.75%) benign and 53 (66.25%) malignant cases. Seborrheic keratosis was the most common benign tumor, followed by verruca vulgaris. Malignant keratinocytic tumors comprise 32 (60.4%) cases of squamous cell carcinoma and 21 (39.6%) cases of basal cell carcinoma (Table 2).

Of 32 cases of SCC, 21 cases were observed in males and 11 cases in females. External genitalia were the most common site of occurrence, accounting for 40.6% of cases, followed by the lower extremities. Most cases of SCC were noted in the 5th to 7th decade. Out of 21 cases of BCC, 13 cases were seen in males. 90% cases were seen in the head and neck, and the eyelid was the most common site. In the present study, melanocytic tumors comprised 18 benign and 6 malignant cases. Intradermal nevus and malignant melanoma were the most common benign and malignant melanocytic tumors, respectively (Table 3). Malignant melanoma showed equal distribution in males and females. Foot was the most common site. In the present study, appendageal tumors comprised 26 benign and 4 malignant cases. The majority of cases belong to sweat gland differentiation. Cyndroma and hidradenoma were the most common tumors encountered (Table 4). Benign appendageal tumor shows female predominance with male-to-female ratio of 1:1.3. Malignant appendageal tumors comprise 2 cases each trichilemmal carcinoma and sebaceous carcinoma with female predominance. The most common site was the head and neck region in both. In the present study, 27 (14.21%), 24 (12.6%) and 5 (2.6%) cases were of neural, vascular and smooth muscle origin, respectively. All were benign tumors. The most common neural tumor was neurofibroma ($n = 23$) followed by schwannoma. Vascular tumors comprising 14 cases of lobular capillary hemangioma followed by 03 cases each of lymphangioma and angiokeratoma, 02 cases each of cherry angioma and epitheloid hemangioma. Piloileiomyoma ($n = 4$) was the most common smooth muscle tumor followed by a single case of angioleiomyoma.

Table 1. Classification of skin tumors according to World Health Organization-2018

Tumours	Benign	Malignant	Total
Keratinocytic/epidermal	27 (21.25%)	53 (84.12%)	80 (42.1%)
Appendageal	26 (20.47%)	4 (6.3%)	30 (15.8%)
Neural	27 (21.25%)	00	27 (14.2%)
Vascular	24 (18.89%)	00	24 (12.6%)
Smooth muscle	5 (3.93%)	00	5 (2.7%)
Melanocytic	18 (14.17%)	6 (9.52%)	24 (12.6%)
Total	127 (66.84%)	63 (33.15%)	190 (100%)

$\chi^2=58.9$, $P < 0.001$ (highly significant)

Table 2. Spectrum and frequency of keratinocytic tumors

Benign tumours	Keratinocytic tumours			Malignant tumours	No of cases	%
	No of cases	%	No of cases			
Actinic keratosis	02	1.5	Basal cell carcinoma	21	33.3	
Seborrheic keratosis	12	9.4	Squamous cell carcinoma	32	50.7	
Verruca plana	01	0.7				
Verruca vulgaris	11	8.6				
Warty dyskeratoma	01	0.7				
Total	27	21.2		53	84.1	

Table 3. Spectrum and frequency of melanocytic tumors

Melanocytic tumours					
Benign tumours	No of cases	%	Malignant tumours	No of cases	%
Blue nevus	02	1.5	Malignant melanoma	06	9.5
Compound nevus	01	0.7			
Congenital melanocytic nevus	01	0.7			
Dermal nevus	02	1.5			
Halo nevus	01	0.7			
Intradermal nevus	10	7.8			
Lentiginous compound nevus	01	0.7			
Total	18	14.1		06	9.5

Table 4. Spectrum and frequency of appendageal tumors

Appendageal tumours					
Benign tumours	No of cases	%	Malignant tumours	No of cases	%
Hair follicle differentiation	7	5.5		02	3.1
Pilomatricoma	03	2.3	Trichilemmal carcinoma	02	3.1
Trichofolliculoma	01	0.7			
Trichoadenoma	01	0.7			
Trichoepithelioma	02	1.5			
Sweat gland differentiation	18	14.1			
Chondroid syringoma	02	1.5			
Cylindroma	04	3.1			
Eccrine poroma	03	2.3			
Hidradenoma	03	2.3			
Hidroadenoma	04	3.1			
Syringoma	02	1.5			
Sebaceous gland differentiation	01	0.7		02	3.1
Sebaceoma	01	0.7	Sebaceous carcinoma	02	3.1
Total	26	20.4		04	6.3

DISCUSSION

This study was conducted to assess the age, sex, and site-wise distribution of various skin and appendageal tumors and classified them according to the WHO classification. A total of 190 cases were studied, out of which 127 (67%) were benign and 63 (33%) were malignant, which is consistent with the study by Har-Shai *et al.*⁸ and Vaibhav *et al.*⁵ who noted 68.4% cases of benign and 31.6% cases of malignant tumors and 51.2% cases were malignant and 48.8% of cases were benign tumors, respectively. Out of 190 cases, 80 (42.1%) cases were of keratinocytic origin, 30 (15.8%) cases were of appendageal origin, 27 (14.2%) cases were of neural origin, 24 (12.6%) cases each of melanocytic and vascular origin, and 5 (2.7%) cases were of smooth muscle origin. Keratinocytic tumors were the most common tumors. These findings were comparable with the study by Vaibhav *et al.*,⁵ Kale and Bagate⁹, Sherpa and Raj KC.¹⁰ We noted 101 (53.1%) and 89 (46.9%) cases were in females and males, respectively, which was

comparable with the findings by Pappala *et al.*¹¹ In present study, the most common presentation was in the head and neck region (49.47%) followed by extremities (22.1%), trunk (21.57%), and external genitalia (6.84%), which were comparable with the study by Shrivastav *et al.*¹² and Uplaonkar *et al.*¹³ Skin tumors were presented in a wide age range varies from 1st decade to 9th decade in the present study. The majority of benign tumors were presented in the 3th and 4th decade, and the majority of malignant tumors were presented in the 6th and 7th decade. Similar observations noted by Gundalli *et al.*¹⁴ where 52.83% cases of benign tumors belonged to 3rd to 5th decade and 78.75% cases of malignant tumors belonged to 6th to 8th decade. Goel *et al.*¹⁵ observed that majority of benign tumors were in 3rd decade (26.9%) and 26.6% cases of malignant tumors were in 7th decade. In present study, majority of benign tumors were of keratinocytic origin (n = 27, 21.2%) and neural origin (n = 27, 21.2%) followed by appendageal tumors (n = 26, 20.4%), vascular tumors (n = 24, 18.8%), melanocytic tumors (n = 18, 14.1%) and smooth muscle origin

tumors (n = 5, 3.9%), which were comparable with the study done by Kale and Bagate⁹ and Sherpa and Raj KC.¹⁰ The majority of malignant tumors were of keratinocytic origin (84%) followed by melanocytic origin (9.5%) and appendageal origin (6%). Squamous cell carcinoma accounted for the majority of the cases (50.7%), which was also observed by Chakravorty and Dutta-Choudhuri,¹⁶ Budhraj et al.,¹⁷ Deo et al.,¹⁸ and Laishram et al.¹⁹ We noted that seborrheic keratosis was the most common benign keratinocytic tumor followed by verruca vulgaris. Goel et al.¹⁵ observed a majority of cases of verruca vulgaris followed by seborrheic keratosis. Polat et al.²⁰ observed a majority of cases of actinic keratosis followed by seborrheic keratosis. These variations in distribution could be due to geographical variation. We noted 12 cases of seborrheic keratosis with a male-to-female ratio of 1.4:1. Maximum cases were presented in 6th and 7th decades. Seven cases were located in the head and neck followed by the trunk region. Histology revealed acanthosis hyperkeratosis, papillomatosis, and parakeratosis with proliferation of basaloid cells and squamous cells and a keratin-filled horn cyst. Similar findings were observed by Bandyopadhyay et al.²¹ 11 cases of verruca vulgaris were encountered with female predominance, and the most common site was the head and neck and were presented between 21 to 70 years. Histology revealed acanthosis, hyperkeratosis, parakeratosis, and papillomatosis. Kilkenny et al.²² also found similar findings. Two cases of actinic keratosis were encountered; both were noted in males and on the back. Similar observations were studied by Roewert-Huber et al.²³ Histology revealed basaloid cells arranged in nodules with peripheral palisading and increased melanin pigmentation. A single case of verruca plana was encountered in a 16-year-old female over the trunk. Histology revealed hyperkeratosis and acanthosis with koilocytic changes in keratinocytes, which were consistent with observations by Pavithra et al.²⁴ Single case of warty dyskeratoma was noted in a 44-year-old female over the face. Histology showed suprabasilar clefting, acantholysis, dyskeratosis, and dysplastic changes, which were consistent with the findings of Harrist et al.²⁵ We noted that squamous cell carcinoma was the most common malignant keratinocytic tumor, accounting for 32 cases (50.7%), which was in accordance with the study by Budhraj et al.,¹⁷ Chakravorty and Dutta-Choudhuri,¹⁶ Deo et al.,¹⁸ and Laishram et al.,¹⁹ having an occurrence rate of 49.02%, 64.3%, 55.8%, 43.6% respectively. The majority of cases were males (21/32), compared to females (11/32), with the male to female ratio being 1.9:1. The most common site of SCC was the external genitalia, followed by the extremities, head and neck region, and trunk. Similar observations were noted by Budhraj et al.,¹⁷ Deo et al.,¹⁸ and Laishram et al.¹⁹ Majority of SCC cases were presented in 5th to 7th decade which was similar to the study by Kaur et al.²⁶ and Vaibhav et al.⁵ Histology of maximum

cases showed sheets and nests of malignant squamous cells that invaded deeper tissue. Cells having hyperchromatic, pleomorphic nuclei with moderate amounts of cytoplasm and keratin pearl formation (Figure 1). These findings were similar to those reported by Rupashree and Geethalakshmi²⁷ and Chakravorty and Dutta-Choudhuri.¹⁶ We noted 21 cases of basal cell carcinoma, of which 13 cases were in males and 8 cases were females. Maximum cases were seen in the head and neck region. The mean age of presentation was 61 years, and peak incidence was seen in 7th decade where the youngest case was seen in a 31-year-old female and the eldest one in a 92-year-old female. These were consistent with the findings by Solanki et al.,²⁸ Chakravorty and Dutta-Choudhuri,¹⁶ and Budhraj et al.¹⁷ Keratotic BCC was the most common type noted, whereas in Solanki et al.,²⁸ solid BCC was the most common type. Baruah et al.²⁹ and Adinarayan and Krishnamurthy³⁰ reported the nodular variant as the most common type. On histology, the majority of cases showed nests of basaloid cells with peripheral palisading and clefting artifacts (Figure 2). Marked melanosis was observed in three cases of pigmented BCC. One case of basosquamous BCC showed atypical squamous cells. We observed 24 cases of melanocytic tumors, including 18 benign and 6 malignant cases. Intradermal nevus (n = 10) was the most common benign tumor, followed by blue nevus (n = 2) and dermal nevus (n = 2). We observed 10 cases of intradermal nevus, of which 6 were seen in females. The majority of cases were located in the head and neck region followed by the trunk. Age of presentation varies between 1 and 50 years, with the youngest being an 8-year-old male over the trunk region. Histology revealed hyperkeratosis with increased basal cell pigmentation and dermis showing sheets and nest of nevus cells with intracytoplasmic melanin pigment. Two cases of blue nevus were encountered, both in females over the head and neck region. Histology showed proliferation of round,

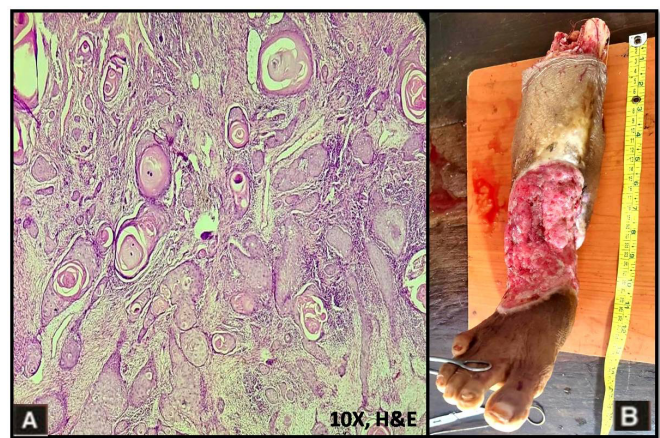


Figure 1. (A) Photomicrograph (x10; hematoxylin and eosin) of squamous cell carcinoma showing islands, nests, and sheets of malignant squamous cells with keratin pearls. (B) Gross: Photograph of the below-knee amputated specimen of SCC showing ulcerated growth

oval to spindle cells with melanin pigmentation, which was consistent with the findings of Rubinstein *et al.*³¹. We observed 2 cases of dermal nevus both in females, which occurred in the head and neck region. Histology revealed nevus cells with melanin pigment extending into the lower dermis and adnexal structures. A single case of compound nevus was seen in a 44-year-old male over the head and neck region. Histology showed nest of nevus cells throughout the dermis. A single case of halo nevus noted in a 13-year-old male over the upper extremity. Histology revealed a nest of nevus cells along with dense lymphocytic infiltrates in the dermis. Our observations were comparable with the findings observed in a study conducted by Shoko³² and Mooney *et al.*³³ We observed six cases of malignant melanoma showing equal incidence in males and females; however, in the study conducted by Sampat and Sirsat³⁴, Mukhopadhyay *et al.*,³⁵ and Katalinic *et al.*,³⁶ male predominance was observed. Maximum cases were seen in the extremities, which was in accordance with the study done by Sampat and Sirsat³⁴ and Mukhopadhyay *et al.*³⁵ The majority (83.3%) cases were seen on the foot region. Similar observations were noted by Sampat and Sirsat³⁴, Budhraj *et al.*¹⁷ and Ochicha *et al.*³⁷ in 54%, 83%, and 93% cases, respectively. Histology revealed nests and groups of polygonal cells having pleomorphic hyperchromatic nuclei with moderate amounts of cytoplasm, and intracellular melanin pigment deposition was also noted (Figure 3). In present study, 30 (15.8%) cases of appendageal tumors were noted, of which 26 and 4 cases were benign and malignant, respectively, which was consistent with the findings observed by Reddy *et al.*³⁸ and Vaishnav and Dharkar.³⁹ Appendageal tumors were differentiated into sweat gland tumors (69.2%),

hair follicle tumors (27%) and sebaceous gland tumors (3.8%), consistent with the findings of Nair⁴⁰ and Solanki *et al.*⁴¹ We observed 7 cases of benign hair follicular tumors, of which 5 were seen in females and 2 in males, which were consistent with the study done by Marrogi *et al.*⁴² Pilomatricoma and trichoepithelioma were the most common. Similar observations were made by Kartha *et al.*⁴³, and Solanki *et al.*⁴¹ Three cases of pilomatricoma were noted with female predominance and were presented between 11 and 40 years of age, commonly located in the head and neck. Histology revealed proliferation of basal cells, shadow cells, calcification, and keratinization, which were consistent with observations made by Solanki *et al.*⁴¹ Two cases of trichoepithelioma were found with equal sex incidence and located in the head and neck region. Histology revealed islands of basaloid cells with peripheral palisading and multiple keratinous horn cysts with papillary mesenchymal bodies (Figure 4). Similar findings were noted by Dissanayaka *et al.*⁴⁴ We observed 18 benign cases of sweat gland tumors. Hidradenoma and cylindroma were the most common. Four cases of cylindroma were noted, all were seen in females and located in the head and neck. The youngest case was presented



Figure 2. Photomicrograph (x10; hematoxylin and eosin) of basal cell carcinoma showing tumor arranged in islands of basaloid cells with peripheral palisading and clefting artefact inset - 3x2 cm blackish-colored skin lesion in the post-auricular region

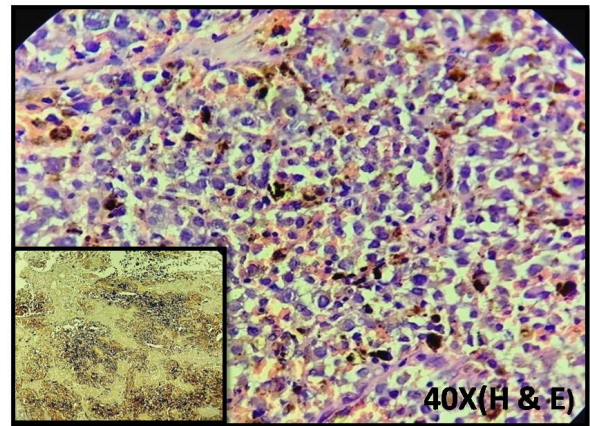


Figure 3. Photomicrograph (x40; hematoxylin and eosin) of malignant melanoma showing nests and groups of tumor cells with intracellular melanin pigment deposition. Inset - Masson-Fontana stain (x10) showing brown melanin pigment



Figure 4. (A) Photograph showing multiple skin-colored papules over the nose, forehead, and periorbital region. (B) Photomicrograph (x10, hematoxylin and eosin) of trichoepithelioma showing islands of basaloid cells with peripheral palisading and multiple keratinous horn cysts

in a 17-year-old female and the eldest was in a 76-year-old female. Histology revealed islands of basaloid cells with peripheral palisading separated by an acellular eosinophilic hyaline sheath and basaloid cells arranged in a jigsaw puzzle pattern (Figure 5). Similar observations were noted by Berke and Grant-Kels⁴⁵ four cases of hidradenoma were encountered with male predominance and commonly presented in 51-60 years with head and neck involvement. Similar observations were noted by Solanki *et al.*⁴¹ Histology revealed polygonal to cuboidal cells arranged in a lobular pattern with round to elongated nuclei with granular cytoplasm and few lumina lined by columnar to cuboidal cells. Two cases of chondroid syringoma were encountered with a male to female ratio 1:1. Histology showed a biphasic cell population with epithelial cells in the cord and nests and a nest of squamous cells with horn cysts and keratin formation. The stromal component showed a myxoid, chondroid, and hyaline matrix. Similar observations were noted by Berke and Grant-Kels⁴⁵ three cases of eccrine poroma were noted, presented in 41-80 years of age and located over the head and neck, lower extremities, and trunk. Histology revealed anastomosing bands of epithelial cells in the dermis and cuboidal cells with round nuclei and moderate cytoplasm. Similar findings were noted by Wankhade *et al.*⁴⁶ We noted a single case of sebaceoma in a 21-year-old male over the scalp. Histology revealed nests of basaloid cells and sebaceous cells. Similar observations were noted by Ahmed *et al.*⁴⁷, and David⁴⁸; we encountered four cases (6.3%) of malignant appendageal tumors comprising 2 cases each of hair follicle and sebaceous gland differentiation. We noted 2 cases of trichilemmal carcinoma, both located in the head and neck and in females. Histology revealed islands of basaloid cells with peripheral palisading and trichilemmal-type keratinization at certain sites (Figure 6). These findings were consistent with those of the study by Lee *et al.*⁴⁹ We noted 2 cases of sebaceous carcinoma, both in females and located on the head and neck, which were consistent with the observations by Wali and Al-Mujaini.⁵⁰ Histology revealed lobules of pleomorphic sebaceous cells separated by

fibrovascular stroma, polygonal cells with eosinophilic cytoplasm, pleomorphic nuclei with coarse chromatin, and prominent nucleoli. In the present study, 27 benign cases of neural tumors were noted, of which neurofibroma was the most common. Twenty-three cases of neurofibroma were encountered with female predominance and majority located in the head and neck followed by the trunk. The youngest case was seen in a 7-year-old male over the head and neck. Histology revealed a well-circumscribed mass with fascicular and palisading arrangement of spindle cells with a wavy elongated nucleus. Similar observations were noted by Ortonne *et al.*⁵¹ In the present study, 24 cases of benign vascular tumors were encountered, and lobular capillary haemangioma was the most common tumour. Fourteen cases of lobular capillary hemangioma were noted with female predominance and located in the head and neck followed by the trunk and upper extremities, commonly presented in 21-70

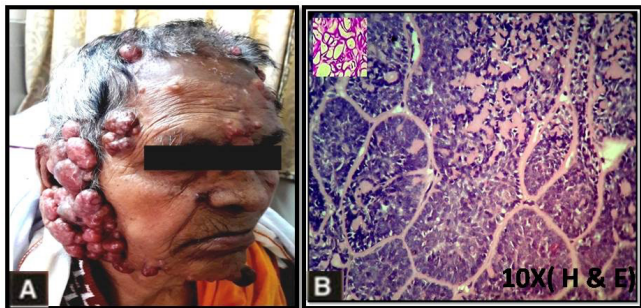


Figure 5. (A) Photograph showing multiple skin colored to erythematous nodules of varying size over the head and neck region. (B) Photomicrograph (x10, hematoxylin and eosin) of cylindroma showing islands of basaloid cells separated by a hyaline sheath in a jigsaw puzzle-like arrangement with hyaline droplets. Inset - PAS stain positivity of hyaline material

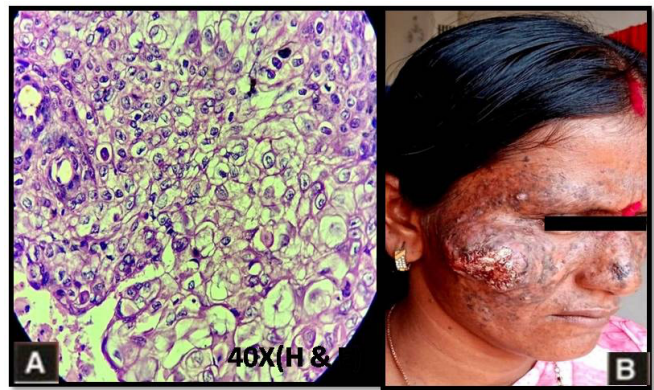


Figure 6. (A) Photomicrograph (x40; hematoxylin and eosin) of trichilemmal carcinoma showing tumor arranged in nest, lobules of clear cells with vacuolated cytoplasm, pleomorphic hyperchromatic nuclei, nuclear palisading, and mitosis with pilar-type keratinization. (B) Photograph showing ulcerative growth over face with rolled out margin

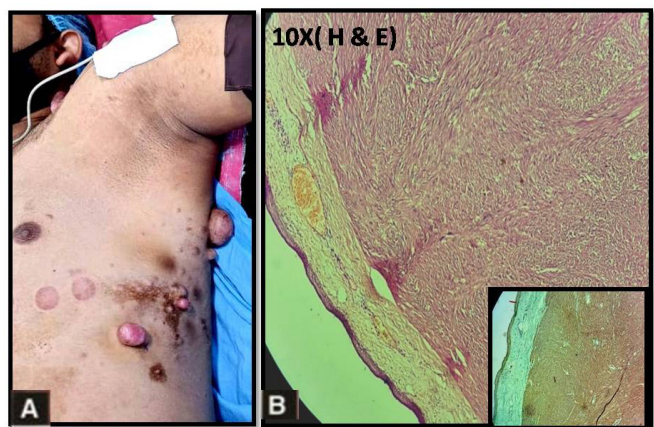


Figure 7. (A) Photograph showing multiple nodules over the abdomen and back. (B) Photomicrograph (x10, hematoxylin and eosin) of piloleiomyoma showing spindle cells arranged in bundles, fascicles, and whorls. Inset - Masson Trichrome stain (x10) showing red-colored smooth muscle fibers

years. Histology revealed lobules of variable-sized capillaries lined by plump endothelial cells. Similar findings were observed by Mills *et al.*⁵² We observed 5 cases of benign smooth muscle tumors, of which 4 cases were of piloleiomyoma with equal gender occurrence and commonly located at the trunk followed by the head and neck. Histology revealed a well-circumscribed mass comprising spindle cells arranged in bundles, fascicles, and whorls (Figure 7). Similar observations were noted by Albuquerque *et al.*⁵³

CONCLUSION

Skin is a complex and largest organ of the body with various lesions, including tumors from the epidermis, its appendages, and dermis. The varied presentation of skin tumors poses a diagnostic challenge to clinicians, and their distinctions into benign and malignant neoplasms are more difficult. Skin appendageal tumors are difficult to diagnose by clinicians and pathologists because of their similar clinical presentation and wide histomorphological differentiation along different lines in the same lesion. Histological study of skin tumors emphasizes the various patterns of skin tumors in a particular geographical location. Histopathological examination is the gold standard for the definite diagnosis of various skin tumors, which helps clinicians to improve the therapeutic approach and emphasize the importance of early diagnosis of malignant tumors owing to its prognostic implication and effective management.

Ethics

Ethics Committee Approval: Approval from the institutional ethics committee were obtained.

Informed Consent: Informed consent from patients were obtained.

Authorship Contributions

Concept: V.P.M., S.B.H., S.M.C., E.A.R., Design: V.P.M., S.B.H., S.M.C., E.A.R., Data Collection or Processing: V.P.M., S.B.H., S.M.C., E.A.R., Analysis or Interpretation: V.P.M., S.B.H., S.M.C., E.A.R., Literature Search: V.P.M., S.B.H., S.M.C., E.A.R., Writing: V.P.M., S.B.H., S.M.C., E.A.R.

Conflict of Interest: The authors declared that they have no conflict of interest.

Financial Disclosure: The authors declared that this study received no financial support.

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