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Original Research

Histopathologic and Immunohistochemical Analysis of Neurofibromas in a North-Western Nigerian Tertiary Hospital: A Ten-Year Retrospective Study.

***Zainab Ali Adamu[1], Mikhail Olayinka Buhari[2], Abdullahi Mohammed[1].**

[1]Department of Pathology, Ahmadu Bello University Teaching Hospital, Shika-Zaria, Kaduna State, Nigeria. [2]Department of Pathology, University of Ilorin Teaching Hospital, Ilorin-Kwara State, Nigeria.

Abstract

Background: Neurofibromas are the most common benign nerve sheath tumours occurring as solitary sporadic tumours or multiple Syndromic tumours associated with neurofibromatosis type 1(NF1). In Nigeria and West Africa, there is a paucity of literature and studies on neurofibromas. This study aims to analyse the histopathologic and immunohistochemical patterns of neurofibromas and determine the frequency, demographic and anatomic distributions.

Methodology: The study was a hospital-based retrospective study, and the study population constituted all surgical specimens submitted for histological examination to the Department of Pathology between 1st January 2010 to 31st December 2019 reported as neurofibroma. Records were retrieved from the archives and subjected to histopathologic and immunohistochemical analysis following standard protocols. Collated data was analysed, slides were reviewed, and results were presented in frequency distribution tables and statistical charts.

Results: A total of 125 cases were seen constituting 8.3% of all soft tissue tumours seen. Neurofibromas were more prevalent in females with a male-to-female ratio of 1:1.15. The age ranged between 2-70 years with a mean age of 25.38 years and the highest frequency of occurrence was in the second decade of life. The most frequent anatomic site of occurrence was the head and neck region. Most of the tumours 103 (82.4%) were sporadic while 22(17.6%) were Syndromic and associated with NF1. A malignant transformation of a pre-existing neurofibroma in an NF1 patient was seen. The most common histologic variant seen was the conventional variant. Ninety percent of these tumours showed SOX10 immunopositivity, 91% showed S100 immunopositivity and 95% showed CD34 immunopositivity. Calretinin expression was low showing 16%. No hot spots labeling index seen with Ki67 antibody.

Conclusion: Neurofibromas are more common in females in our environment and the most frequent anatomic site of involvement is the head and neck region.

Keywords: Neurofibroma; Females; Head & Neck; S100; CD34; Ki67; Calretinin; SOX10.

***Correspondence:** Dr Zainab Ali Adamu, Department of Pathology, Ahmadu Bello University Teaching Hospital (ABUTH), P.M.B.06, Shika-Zaria, Kaduna State, Nigeria. **Email:** superzeea@gmail.com; ORCID ID- https://orcid.org/0000-0003-0439-0627

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Quick Response Code:

Introduction

Neurofibromas are benign peripheral nerve sheath tumours, whose primary neoplastic cellular components are the Schwann cells, but they also have nonneoplastic peripheral nerve components, such as fibroblasts, CD34perineural cells, blood vessels, axons, and mast cells. [1,2,3,4]Neurofibromas are the most frequent benign neoplasms originating from the peripheral nerve sheath and occur as solitary (sporadic) or multiple (syndromic) tumours when associated with von Recklinghausen disease, better known as neurofibromatosis type 1 (NF1).[6,7,8,9,10] About 10% of neurofibromas are reported to be frequently associated with neurofibromatosis type 1.[4] Neurofibromas are associated with a disorder of the Ras (Rat Sarcoma) signal transduction pathway due to NF1 gene germline or somatic mutation located on chromosome 17q11.2 which has GAP(GTPase –activating protein) activity on Ras.[7,10,11] In a subset of NF1 patients, malignant transformation of a preexisting neurofibroma, typically a deep-seated plexiform lesion may occur. [1, 7, 10, 11] Studies on neurofibroma are sparse and uncommon in Nigeria and West Africa hence the need for this study which will bridge the gap in current knowledge, especially on immunohistochemistry. This is the first study of its kind in our centre. This study aimed to analyse the histopathologic and immunohistochemical patterns of neurofibromas at the institute from 1st January 2010 to 31st December 2019 and, also determine the frequency, demographic and anatomic distribution of these tumours. The results were further compared with other findings in published scholarly literature.

Methodology

Study Site/Area

The study was conducted at our institute which is a referral facility in the North-West zone of Nigeria. It is a tertiary hospital with a bed capacity of about 1000 and 2892 staff strength that is dedicated to the provision of healthcare services, teaching/training, and research. Patients with a myriad of diseases and disorders in the state and the northern regions are referred here for confirmation of diagnosis and/or treatment. The Department of Pathology (Morbid Anatomy) of our institute was founded in 1972 in northern Nigeria. This region is a semi-urban area with a temperature range of 15.3^0C to 36.2^0C and an annual rainfall that ranges from 0-816mm/month.

Study Design

The study was a hospital-based retrospective study.

Source Population

The source population constituted all biopsy specimens sent to the Department of Pathology during the study period.

The study population constituted all cases of neurofibroma diagnosed in the Department of Pathology between 1stJanuary 2010 to 31stDecember 2019.

Sample Size and Sampling

All cases of histologically diagnosed neurofibroma within the study period which strictly met the inclusion criteria were included.

A sample size was then calculated within the study population using Daniel's equation for sample size calculation and prevalence study. A study done by Guha D *et al* revealed a prevalence of 10-12% of all soft tissue neoplasms. [12]

Given the formula $n= z^2pq\div d^2$
Where z=1.96 q=1-p p=prevalence (upper limit of 12% taken) d=5 %(0.05)
$n= 1.96^2 \times 0.12 \times (1-0.12) \div (0.05)^2 = 3.8416 \times 0.12 \times 0.88 \div 0.0025 = 162$

Taking the proportion of neurofibromas,
Cases of neurofibroma ÷ Total number of cases x 162 =Proportion of neurofibroma.
Neurofibromas= 125÷202 x 162= 100 cases

Data collection tools and procedures
Records on surgical specimens submitted for histological examination to the Department of Pathology between 1st January 2010 to 31st December 2019 which were histologically diagnosed as neurofibroma were retrieved from the archives. The formalin-fixed, paraffin-embedded tissue blocks of these cases were retrieved and were remounted on a microtome and sections were made at 3 -5 microns each. Fresh haematoxylin and eosin-stained slides were produced for all the cases because the archived slides were broken and faded hence difficult to interpret. These new slides were reviewed for histologic diagnoses using the World Health Organisation Classification of Tumours of Soft Tissue and Bone 4th Edition.[7] Cases whose blocks and or slides could not be retrieved and specimens with inadequate materials for the tissue sections were excluded from the study. The clinical data, gross appearance and microscopic reports at initial diagnoses were also reviewed. The new haematoxylin and eosin-stained slides were reviewed and correlated with the archived slides serving as a verification procedure.

The indirect immunoperoxidase method using Bio SB (Bioscience IHC Technical Guide) Mouse/Rabbit PolyDetector 3, 3'-Di-amino-benzidine (DAB) horseradish peroxidase (HRP) Brown detector kit (Biotin-based detection system) was employed and immunohistochemical staining analyses were performed using the standard streptavidin-biotin-peroxidase method in 3-5-μm-thick tissue sections that had been obtained from formalin-fixed, paraffin-embedded blocks and mounted on positive charged adhesion microscopic slides (CITOGLAS^R). The protocol used for the immunohistochemical assay was that of the manufacturer's instructions. Sections were deparaffinized and prepared to minimize nonspecific signals by rehydration in water and antigen retrieval or unmasking of epitope was achieved by incubation in a citrate buffer of pH 6 for 60 minutes using the water bath method. Sections were then washed in water and then pretreated in hydrogen peroxide for 5 minutes to block or quench endogenous enzymes and secondary antibodies which may produce nonspecific binding. Sections were then washed in phosphate-buffered saline (PBS) and incubated in the primary antibodies in varying dilution factors for 60minutes at Room Temperature (RT).Sections were again washed in PBS and then incubated in the detection system (Bio SB Mouse/Rabbit PolyDetector DAB HRP Brown, USA detector kit) for 30minutes at RT. Sections were washed in PBS again and placed in Di-amino-benzidine (DAB) for 5 minutes to reveal the reaction end product and then finally washed again in PBS. Sections were then counterstained with Mayer Haematoxylin for 1 minute, washed in water, dehydrated, cleared with xylene, and mounted in Dibutylphthalate Polystyrene Xylene (DPX) and covered slipped. A total of five primary antibodies were used in this study and the primary antibodies used included; (a) S-100 protein [Solubility in 100%] (1:100,Bio SB,USA), (b) CD34[Cluster of Differentiation 34] (1:100,Bio SB,USA), (c) SOX10 [SRY (sex determining regionY)-box 10 protein] (1:100,Elabscience^R,USA), (d) Calretinin(1:100, Elabscience^R,USA) and (e) Ki67 [Antigen/Protein Keil 67] (1:100, GenomeMe, Canada). An automated slide stainer was used for the staining technique. Positive extrinsic and intrinsic control samples were used in each assay and were processed concurrently to ensure the reliability of the tests.

Special stains were also employed for some of the tumours; these stains include Masson Trichrome (MT) stain demonstrating the shredded carrot collagen bundles of neurofibroma and Toluidine blue stain to demonstrate mast cells in neurofibroma.

The histologic review of all the stained slides was carried out independently by two consultant pathologists using Leica DM750 LED Biological Microscope Series and photomicrographs were taken using Euromex CMEX-12f Pro 12 Megapixel Camera with a fast CMOS sensor (Complementary metal oxide semiconductor). Architectural, cytomorphological and immunohistochemical features of each individual case were noted. The antibodies employed, characterization and conditions for immunohistochemistry used in the study are shown in Tables 1 and 2.

Table 1: Immunohistochemical Markers Characterization.

Marker	**Localization**	**Extrinsic Control**	**Intrinsic Control**	**Primary Antibody**	**Species Reactivity**	**Isotype**
S100	Cytoplasmic& Nuclear	Schwannoma	Melanocytes	Mouse m	Human, dog, mouse	IgG2a
SOX10	Nuclear	Melanoma	Melanocytes	Rabbit p	Human, mouse, rabbit	IgG
CD34	Membranous	Fibroadenoma	Endothelium	Rabbit m	Human, mouse	IgG
CALRETININ	Cytoplasmic &Nuclear	Schwannoma	Adipocytes, mast cells	Rabbit p	Human, mouse, rabbit	IgG
Ki67	Nuclear	Pineoblastoma	Tonsils	Rabbit m	Human	

Abbreviation: m-monoclonal, p-polyclonal, IgG- Immunoglobin G, SOX10- SRY (sex determining regionY)-box 10 protein, S100- Solubility in 100%, CD34- Cluster of Differentiation 34, Ki67- Antigen/Protein Keil 67.

Table 2: Antibodies and Conditions for Immunohistochemistry used in study.

Antibody	Clone	Source	Dilution	Incubation Time	Detection System used
S100	4C4.9	Bio SB,USA	1:100	60mins at RT	BioSB DAB
SOX10	SOX10	Elabscience^{R},USA	1:100	60mins at RT	BioSB DAB
CD34	EP88	Bio SB,USA	1:100	60mins at RT	BioSB DAB
CALRETININ	CALB2	Elabscience^{R},USA	1:100	60mins at RT	BioSB DAB
Ki67	IHC167	GenomeMe,Ca	1:100	60mins at RT	BioSB DAB

Abbreviation: DAB- Diamino-benzidine; RT- Room Temperature, SOX10- SRY (sex determining region Y)-box 10 protein, S100- Solubility in 100%, CD34- Cluster of Differentiation 34, Ki67- Antigen/Protein Keil 67, BioSB-Bioscience IHC Technical Guide, IHC167-Immunohistochemistry167, EP88- Clone EP88, CALB2- Calbindin 2, USA- United States of American.

Immunohistochemical Staining intensity scoring systems.

Semi-quantitative scales of the staining intensity and extent adapted by Ji Y *et al* [9] were used and this is shown in Table 3. The cases were classified into the negative group (0) when the number of cells stained was less than 5% and into the positive group when the number of cells stained exceeded 5%. Immunore activity was evaluated as the staining intensities in the positive group (1, weak/mild; 2, moderate; 3, strong/intense). Positive results were expressed as the intensity (weak, moderate, or strong) and extent (focal/diffuse) of staining.

Table 3: Staining intensity for positive group

1 (+)	Weak/Mild
2 (+ +)	Moderate
3 (+ + +)	Strong /Intense

Staining for cellular neurofibroma with Ki67 antibody was not considered negative if the number of cells stained was<5% rather hotspot labeling index or diffuse staining was used.

Ki67 immunoexpression was evaluated using the Eye-10 method suggested by Kadivar *et al*. [13] Certain parameters were used and are defined as follows.

Ki67 Percentage Score: This is defined as the percentage of positively stained tumour cells among the total number of malignant cells assessed.

Ki67 Labeling Index: This is the percentage of cells with Ki67-positive nuclear immunostaining.

Hot Spots: These are defined as areas in which Ki67 staining was particularly higher relative to adjacent areas.

Based on these definitions, the Eye -10 method suggests: a percentage labeling index hot spots of <10% to be interpreted as a low-grade tumour, percentage labeling index hot spots of 10-20% to be interpreted as borderline tumour and >20% percentage labeling index hot spots to be interpreted as a high-grade tumour.

Data Analysis

Analysis of collated data was carried out using Statistical Program for Social Sciences (SPSS) version 20.0 for the different immunohistochemical subtypes, histologic subtypes and grades. The results were presented using frequency distribution tables and statistical charts were produced using Microsoft Excel 2020 version.

Ethical Consideration

Permission for the study: In conducting this study, ethical consideration was respected. Ethical clearance and consent to conduct the research was secured from the Health Research Committee and the Department of Pathology. The ethical clearance number is NHREC/10/12/2015 and the D.U.N.S number- is 954524802.

Anonymity and Confidentiality: The full information derived from this study is accessible to the investigators only.

Results

General Findings

In the study period of ten years, a total of 30,267 surgical specimens were received in the Department of Pathology. Out of these, 11,888 (39.3%) were diagnosed as neoplasms of which 1,502 were soft tissue tumours constituting 4.9% of all biopsies received. Of all the soft tissue tumours seen within the study period, 135 cases were diagnosed as neurofibromas of which 125 cases constituting 8.3% of all soft tissue tumours encountered and 12.1% of benign soft tissue tumours seen fulfilled the inclusion criteria.

Demographic Characteristics

Female preponderance was observed compared to males. Sixty-seven (53.6%) cases occurred in females while 58 (46.4%) cases occurred in males with a male-to-female ratio of 1: 1.15. The age ranged between 2 and 70 years with the highest frequency of occurrence in the 2nd decade of life and a mean age of 25.38 years. Out of the 125 cases of neurofibromas seen, 22 (17.6%) cases were clinically diagnosed as syndromic being associated with NF1 according to the diagnostic criteria while 103(82.4%) cases were sporadic. The most frequent anatomic site of occurrence was the head and neck region with 45 cases (73.8%) seen. Table 4 shows the demographic characteristics and anatomic sites of distribution.

Table 4: Demographic and Anatomic sites distribution of 125 Neurofibromas.

Characteristics	**Value**
Sex(M/F)	58/67 (1:1.15)
Age range with highest frequency	2nd decade (10-19 years)
Mean age (years)	25.38
Number of cases with NF1	22
Tumour Location	
Head and Neck	45(36%)
Upper limb	14(11.2%)
Trunk	19(15.2%)
Pelvis and Perineum	4(3.2%)
Gluteal Region	1(0.8%)
Lower Limb	19(15.2%)
Unusual Site (Sinonasal)	1(0.8%)
Generalized	22(17.6%)
TOTAL	125(100)

Figure 1 shows the age range frequency distribution and Figure 2 shows the anatomic sites of distribution**.**

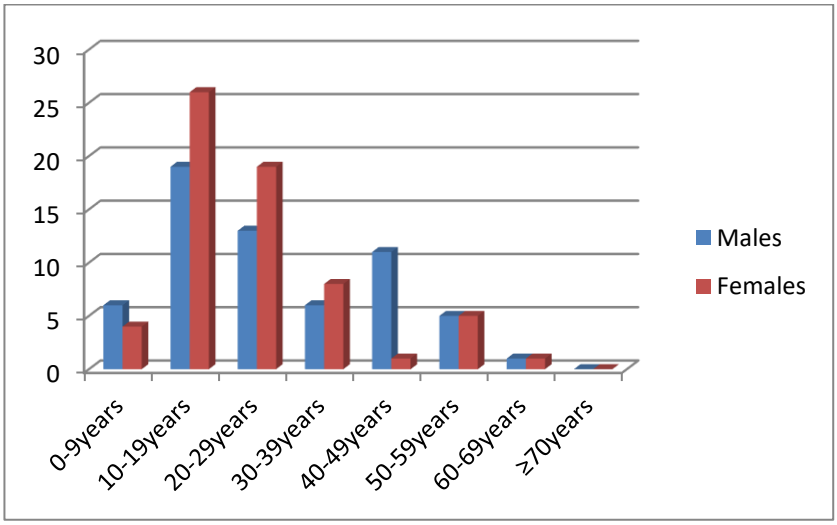


Figure 1: A combined bar chart showing age range and sex frequency distribution in both sexes.

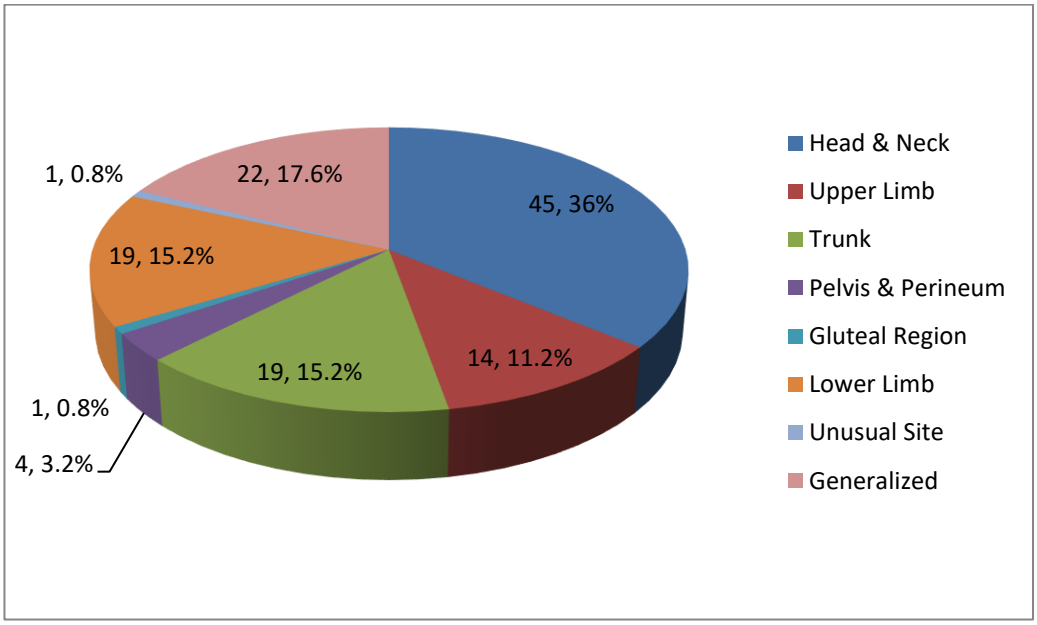


Figure 2: A pie chart showing frequency distribution of anatomic sitesof neurofibromas.

Morphologic Analyses

Morphologic analyses were done based on the World Health Organization (WHO) classification of soft tissue tumours 4th edition.[7]The most frequent histologic pattern seen was the diffuse pattern with 76(60.8%) cases and the most frequent histologic variant seen within the study period was the conventional variant constituting 87(69.6%) cases. Figures 3 and 4 show the histologic patterns and histologic variants encountered within the study period, respectively. The cell population ranged from mostly variable to invariable with 120(96.0%) cases showing a variable or mixed cellular population while 5(4.0%) cases showed a monotonous cellular population. These cases with the monotonous population were mainly the atypical variant of neurofibroma. Amongst the atypical variant, one out of the 11 cases studied fulfilled the diagnostic criteria of a low-grade malignant peripheral nerve sheath tumour (MPNSTs) rather than an atypical neurofibroma which occurs in patients with NF1.

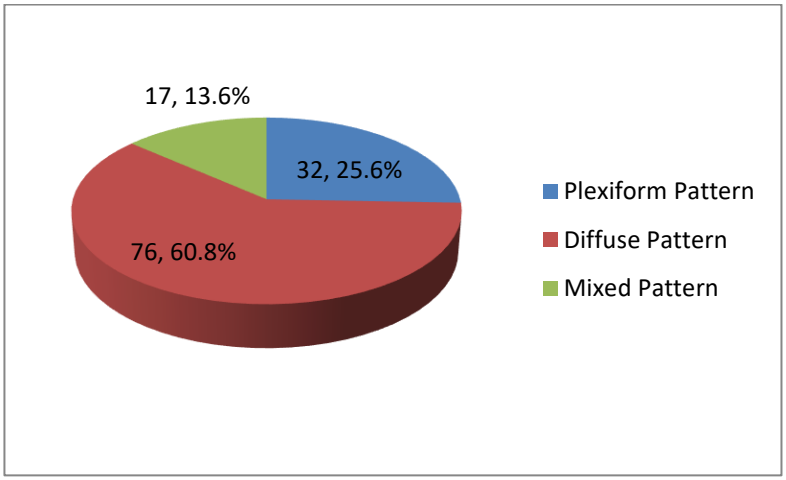


Figure 3: A pie chart showing the frequency distribution of different histologic patterns of neurofibromas.

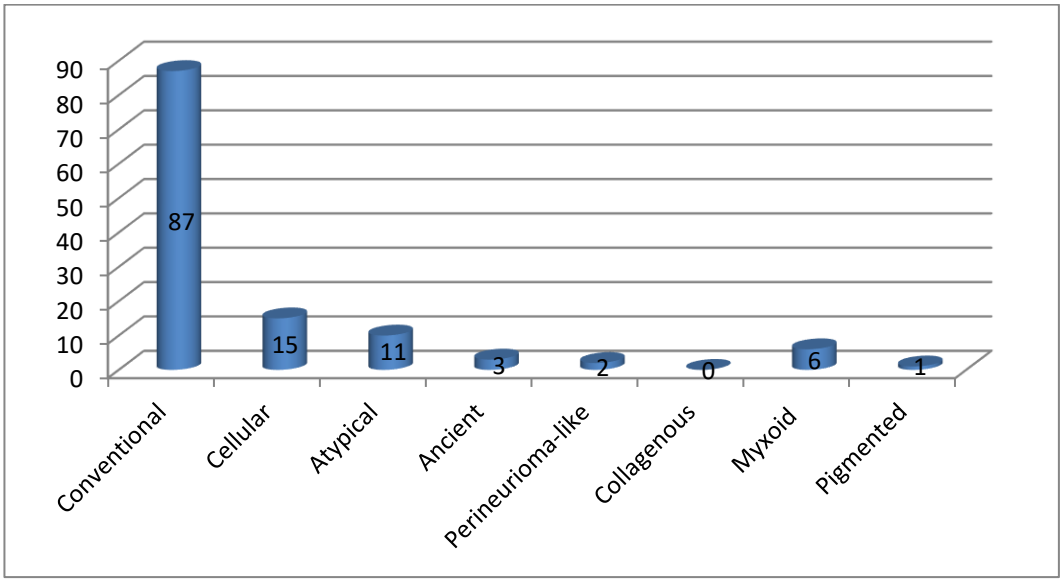
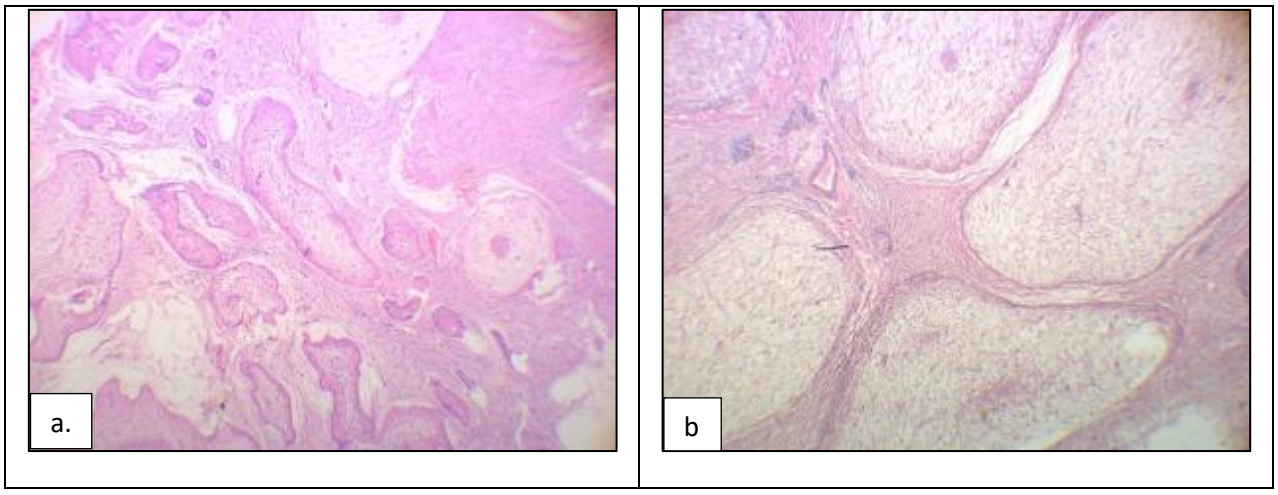


Figure 4: A stacked cylinder showing the frequency distribution of different histologic variants of neurofibromas

Nuclear features were predominantly bland and variable showing mainly fairly pleomorphic cells having mildly hyperchromatic nuclei with modest to scanty cytoplasm. The architectural, cytomorphological and stromal features of these tumours are illustrated in Plates 1(a-f), 2(a-b) and 3(a-c) and summarized in Tables 5, 6 and 7, respectively.



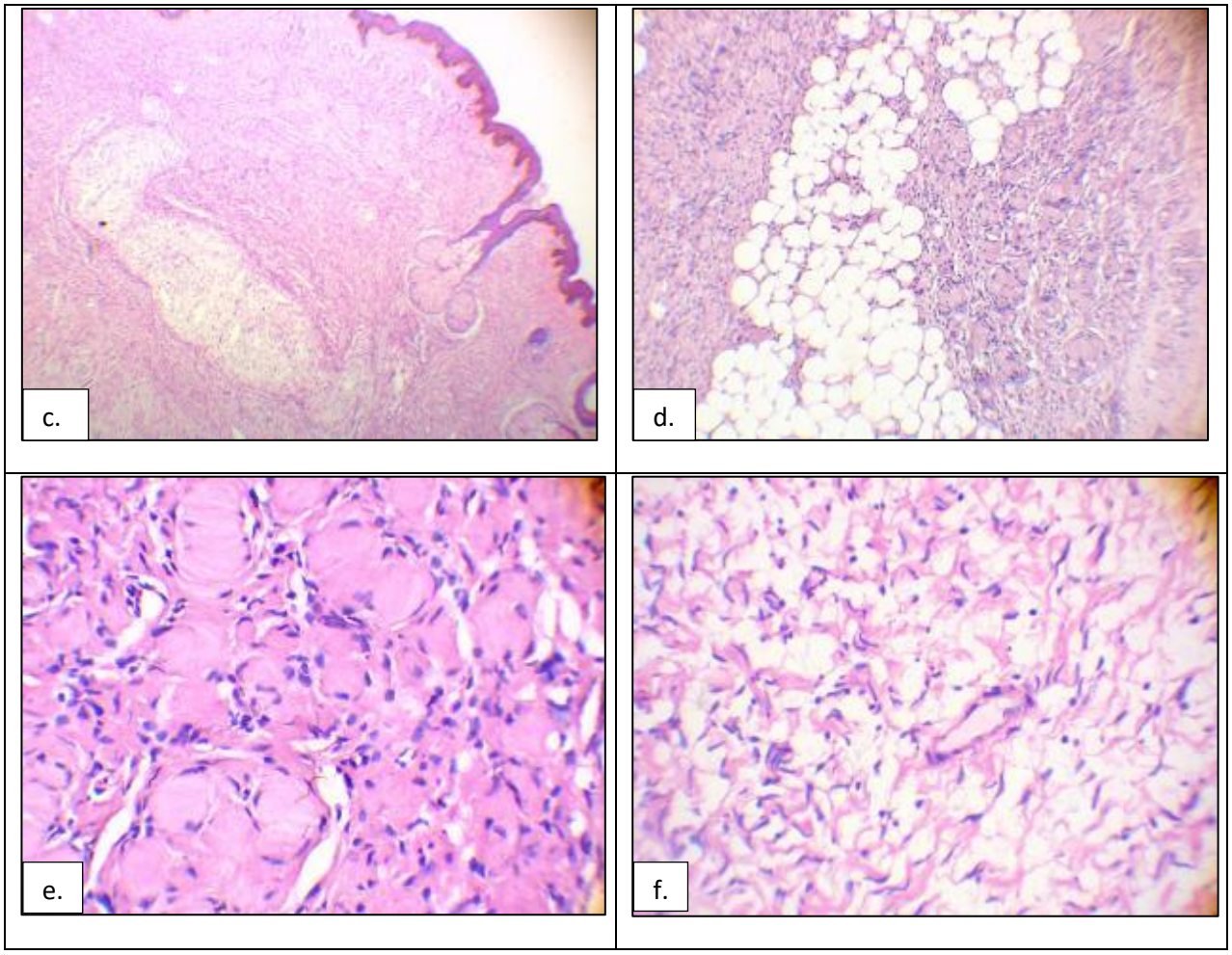


Plate 1(a-f) Legend: Histologic Patterns of Neurofibroma

(a) Photomicrograph showing plexiform pattern with nodules of proliferating nerve bundles, H&E x40. (b) Photomicrograph showing plexiform neurofibroma at a higher magnification, H&E x100. (c) Photomicrograph showing conventional neurofibroma with a plexiform nodule in the dermis, H&E x40. (d) Photomicrograph showing honeycomb pattern in diffuse neurofibroma and Wagner Meissner bodies on the right, H&E x 100.(e) Photomicrograph showing numerous Wagner Meissner bodies in neurofibroma H&E x400 (f) Photomicrograph showing heterogeneous population of cells in conventional neurofibroma, H&E x100.

Abbreviation- H&E- Haematoxylin and Eosin

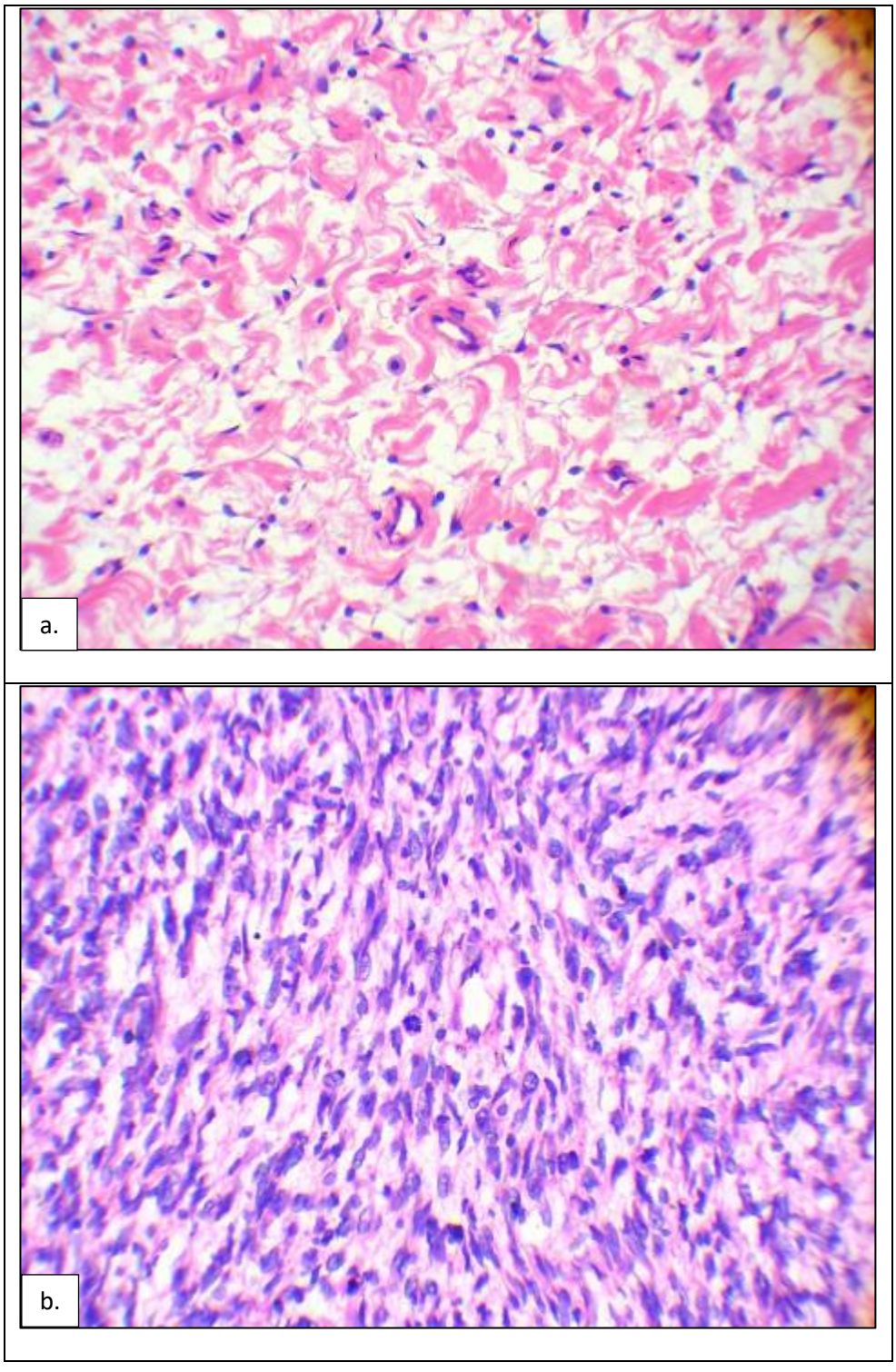


Plate 2(a-b) Legends: Histologic Patterns of Neurofibroma
(a) Photomicrograph showing a mast cell within ropey collagen bundles, H&E x400 (b) Photomicrograph showing star burst mitotic figure in an atypical neurofibroma, H&E x400.

Abbreviation- H&E- Haematoxylin and Eosin

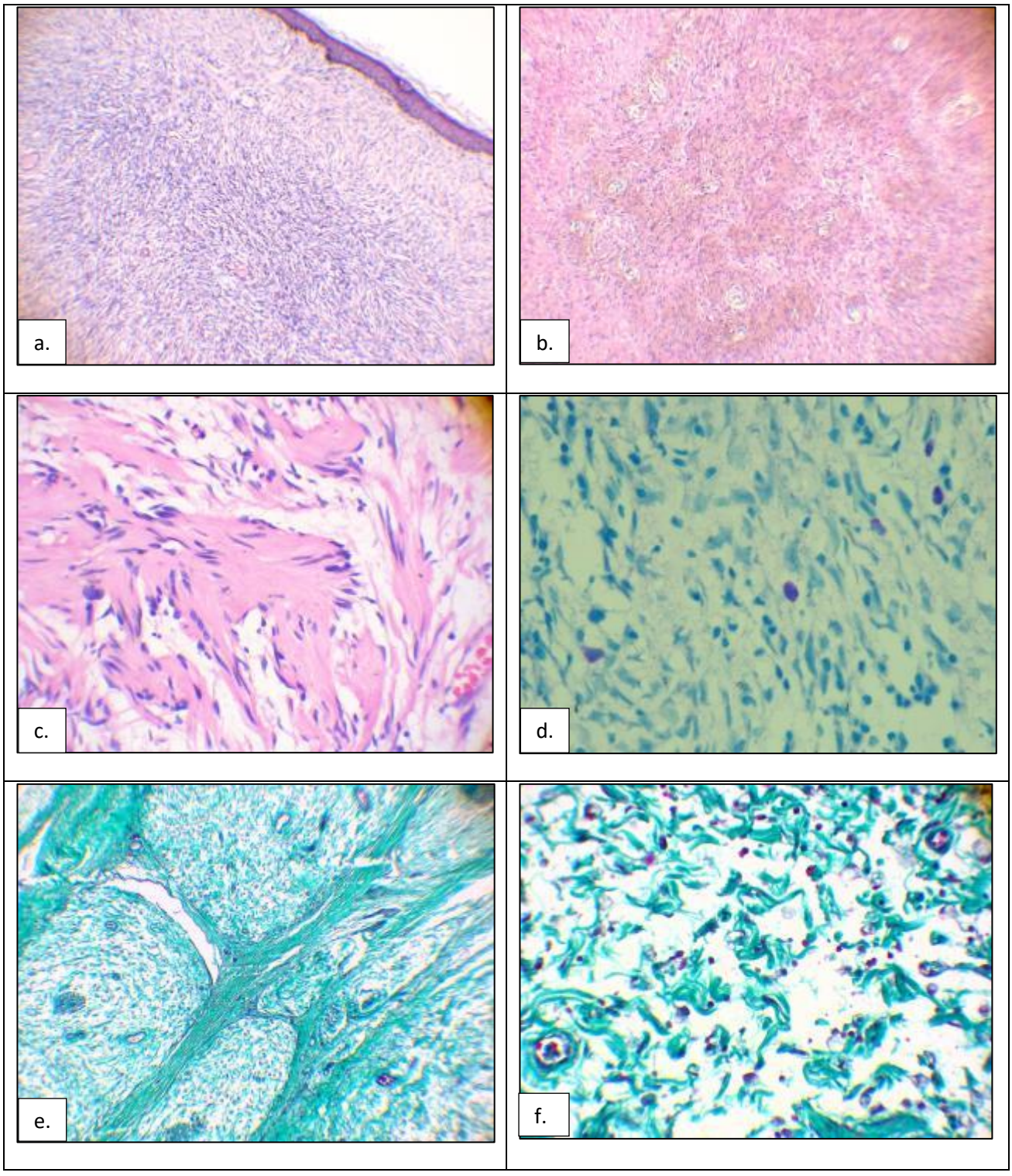


Plate 3 (a-f) Legends: Histologic Variants of Neurofibroma and Special Stains Employed

(a) Photomicrograph showing swirls and short sheaves in a diffuse neurofibroma, H&E x40. (b) Photomicrograph showing pigmented neurofibroma, H&E x100. (c)Photomicrograph showing collagenous neurofibroma, H&E x400.(d)Photomicrograph highlighting mast cells with the granules staining purplish, Toluidine blue stain x100. (e)& (f) Photomicrograph respectively highlighting ropey collagen bundles stained with malachite green, Masson Trichrome stain x100 and x400.

Abbreviation- H&E- Haematoxylin and Eosin

Table 5: Morphologic (Architectural) findings of 125 cases of Neurofibroma.

Architecture	*Number of Cases with the features (%)*
Organization Pattern	
Short Sheaves	99/125 (79.2%)
Long Sheaves	23/125 (18.4%)
Mixed Sheaves	3/125 (2.4%)
Shredded Carrot (Ropey collagen bundles)	
Present	
Few +	24/125 (19.2%)
Moderate ++	15/125 (12.0%)
Abundant +++	50/125 (40.0%)
Absent	36/125 (28.8%)
Wagner -Meissner corpuscles	
Present	
Few +	25/125 (20.0%)
Moderate ++	4/125 (3.2%)
Abundant +++	6/125 (4.8%)
Absent	90/125 (72.0%)
Honeycomb Pattern	
Present	31/125 (24.8%)
Absent	94/125 (75.2%)
Presence of Mast Cells	
Few +	60/125 (48.0%)
Moderate ++	41/125 (32.8%)
Abundant +++	24/125 (19.2%)

Abbreviations: + mild, ++ moderate, +++ abundant

Table 6: Morphologic (Cytomorphological)findings of 125 cases of Neurofibroma.

Cytological Features	*Number of Cases with the features (%)*
Cellularity	
paucicellular	9/125 (7.2%)
mild +	82/125 (65.6%)
moderate ++	27/125 (21.6%)
marked +++	7/125 (5.6%)
Predominant cell type	
Spindle (buckled,wavy,serpentine)	109/125 (87.2%)
spindle(elongate/oval)	16/125 (12.8%)
Nuclear features	
Nuclei	
variable	121/125 (96.8%)
monotonous	4/125 (3.2%)
Pleomorphism	
mild +	110/125 (88.0%)
moderate ++	14/125 (11.2%)
marked +++	1/125 (0.8%)
Hyperchromasia	
mild +	109/125 (87.2%)
moderate ++	16/125 (12.8%)
marked +++	0/125 (0.0%)
Random degenerative atypia or pleomorphism	
Present	15/125 (12.0%)
Absent	110/125 (88.0%)
Mitoses	
Absent	118/125 (94.4%)
Present <3/10hpf	6/125 (4.8%)
Present >3/10hpf	1/125 (0.8%)

Abbreviations: hpf-high power field, + mild, ++ moderate, +++ marked

Table 7: Morphologic (Stromal and secondary features) findings of 125 cases of Neurofibroma.

Stromal features	***Number of Cases with the features (%)***
Quality	
Loose fibrous	27/125 (21.6%)
Loose myxoid	8/125 (6.4%)
Dense fibrous	25/125 (20%)
Fibromyxoid	40/125 (32%)
Fibrillary predominantly	13/125 (10.4%)
Fibrohyalinized	12/125 (9.6%)
Quantity	
Scanty	37/125 (29.6%)
Moderate	44/125 (35.2%)
Abundant	44/125 (35.2%)
Necrosis	
Present	2/125 (1.6%)
Absent	123/125 (98.4%)
Secondary features	
Myxoid degeneration	9/125 (7.2%)
Hyaline degeneration	29/125 (23.2%)
Inflammation	5 (4.0%)
Nil	82/125 (65.6%)
Hyalinized vessel wall	
Present	9/125 (7.2%)
Absent	116/125 (92.8%)

Special staining techniques employed using toluidine blue highlighted the presence of mast cells in these tumours while the Masson Trichrome stain demonstrated ropey collagen bundles (shredded carrot appearance) in these tumours. Plate 3(e-f) illustrates the special staining technique features.

Immunohistochemical Findings

One hundred cases of neurofibroma out of the 125 cases were subjected to immunohistochemical analysis following sample size calculation. Antibodies employed were S100, CD34, Calretinin, SOX 10, and Ki 67(for cellular neurofibromas).

SOX10 antibody required nuclear immunopositivity for interpretation with melanocytes and Schwannoma serving as intrinsic and extrinsic controls, respectively. Of the hundred (100) cases stained with SOX10 antibody, ninety (90) cases representing 90% showed nuclear immunopositivity with varying degrees of staining intensity. Thirteen (13) cases out of the 90 cases (14.5%) showed strong staining intensity, 39 cases (43.3%) showed moderate staining intensity, and 38 cases (42.2%) showed mild staining intensity. Ten (10) cases representing 10% showed nuclear immunonegativity.

Calretinin antibody required both nuclear and cytoplasmic positivity for interpretation with mast cells and adipocytes serving as intrinsic control and Schwannoma serving as extrinsic control. Out of the hundred (100) cases stained with this antibody, only 16 cases (16%) showed immunopositivity while the remaining 84 cases (84%) were negative. Of the positive cases, strong staining intensity was not observed in any of the immunopositive tumours to this antibody. Two (2) cases representing (12.5%) out of the 16 immunopositive cases showed moderate staining intensity while the remaining 14 cases (87.5%) showed mild staining intensity.

S100 antibody required both nuclear and cytoplasmic immunopositivity for interpretation with melanocytes or adipocytes and Schwannoma serving as intrinsic and extrinsic controls, respectively. Ninety-one (91) cases, representing 91% of all neurofibromas showed immunopositivity while 9 cases (9%) were negative for this antibody. Of the 91 immunopositive cases, 18 cases (19.8%) showed strong staining intensity, 47 cases (51.6%) showed moderate staining intensity, and 26 cases (28.6%) showed mild staining intensity.

CD34 staining interpretation was membranous with vascular endothelium serving as intrinsic control and fibroadenoma serving as extrinsic control. It was observed that 95 cases (95%) were positive for this antibody with 22 cases (23.2%), 46 cases (48.4%), and 27 cases (28.4%) of these showing strong, moderate and mild staining intensity, respectively. Only five cases (5%) were negative for this antibody.

Fifteen of the cellular neurofibromas seen were stained with Ki67 antibody and no hot spots labeling index were seen indicating that none of these cellular neurofibromas expressed this antibody.

Plates 4 and 5 show photomicrographs of the immunohistochemical profile of the Neurofibromas using different antibodies mentioned. Tables 8, 9 and 10 summarize the immunohistochemical profiles.

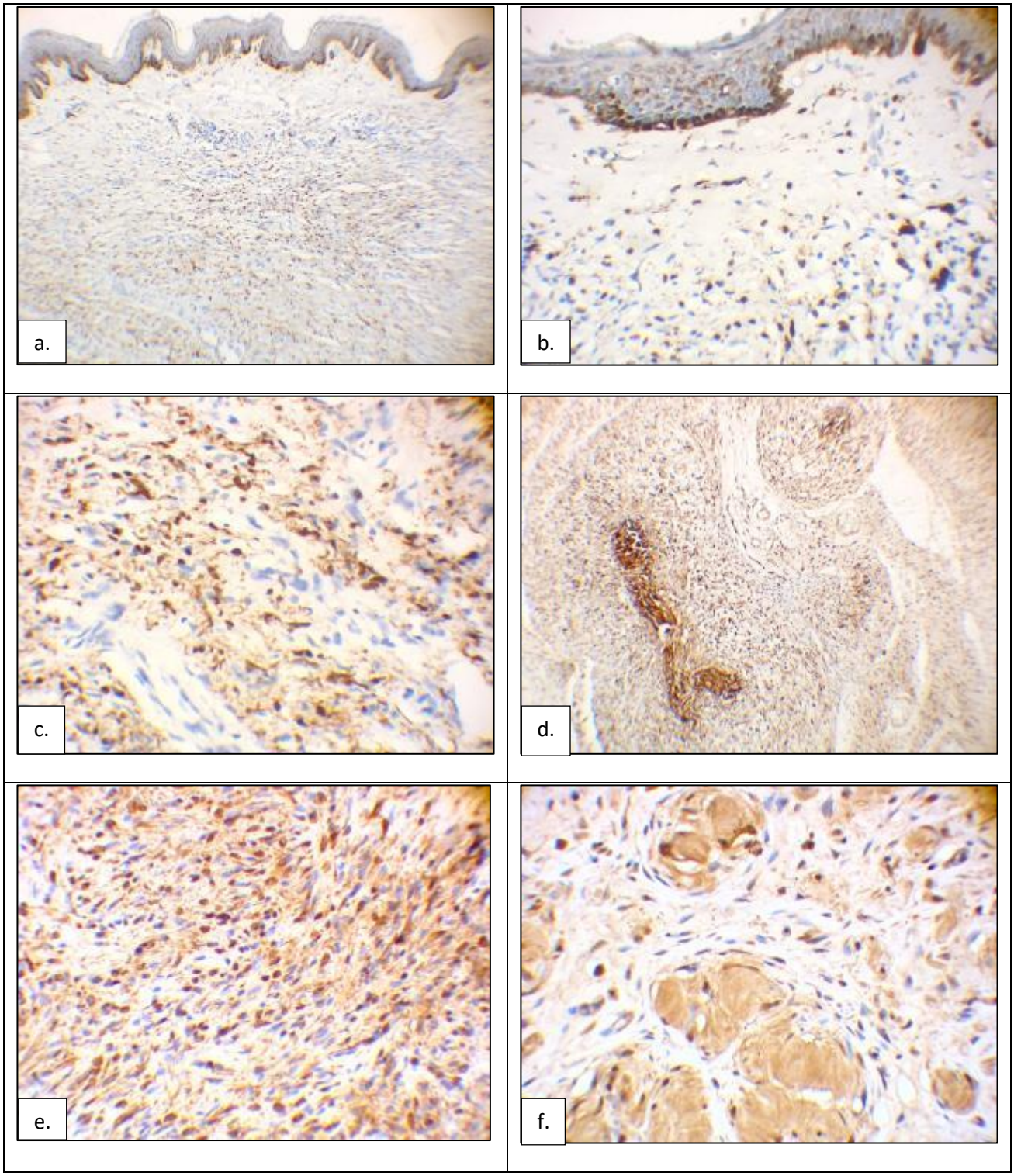


Plate 4(a–f) Legends: Neurofibroma Immunohistochemical Stains: S100 and SOX10 Antibodies

(a) Photomicrograph showing strong diffuse patchy positivity with melanocytes serving as intrinsic control, S100 stain PBM x40 (b) Photomicrograph showing antibody staining subpopulation of cells giving a patchy pattern, S100 stain PBM x400.(c) Photomicrograph showing staining of subpopulation of cells with strong (+++) diffuse patchy positivity, S100 PBM x100 (d) Photomicrograph showing strong (+++) patchy positivity in some cells, S100 PBM x400.(e)Photomicrograph showing antibody staining subpopulation of cells and shows diffuse strong (+++) patchy positivity,SOX10 PBM x400 (f) Photomicrograph showing antibody staining Wagner Meissner bodies, SOX10 PBM x400.

Abbreviation- PBM- Polymer Based Detection Method

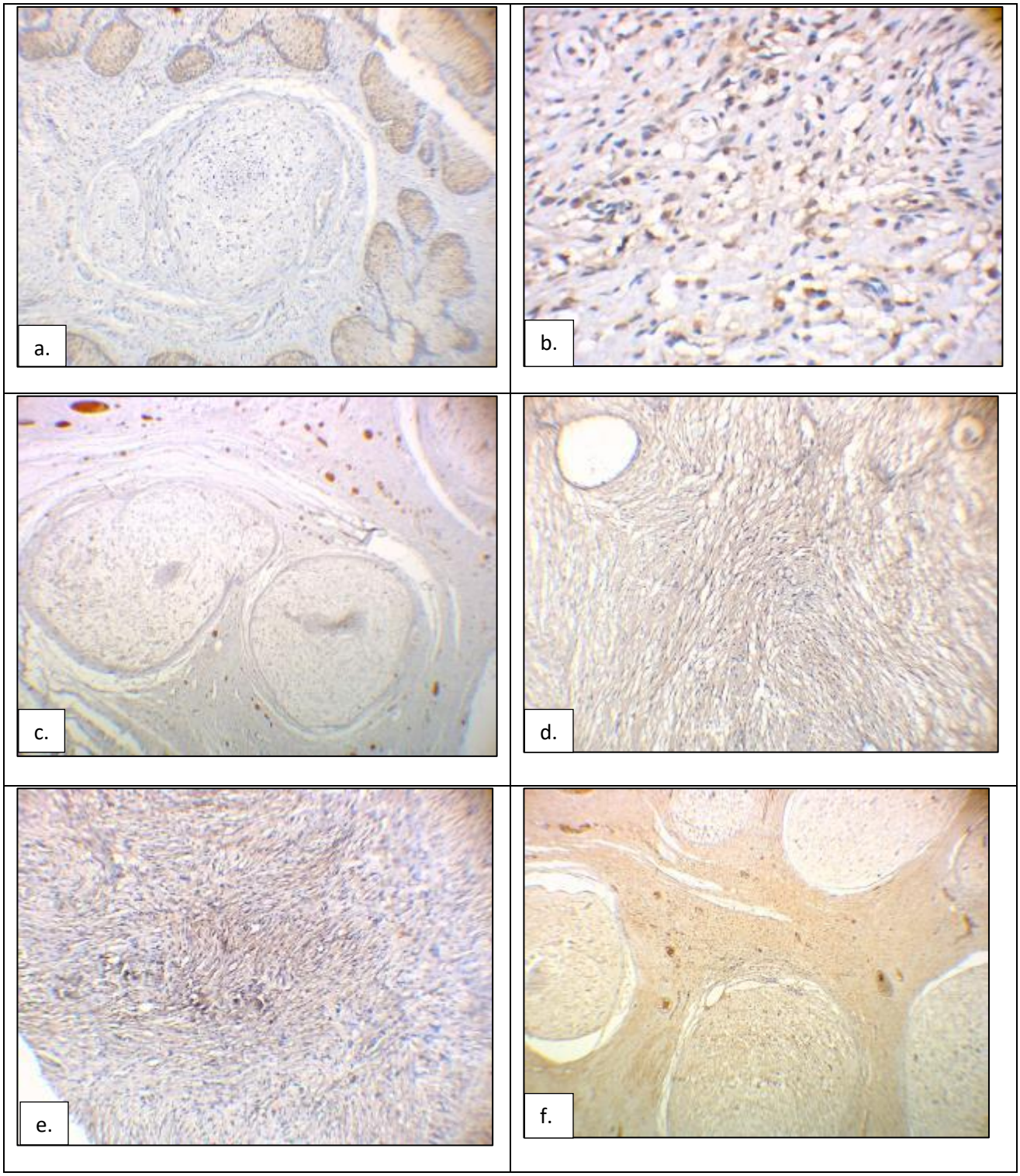


Plate 5(a-f) Legends: Neurofibroma Immunohistochemical Stains: Calretinin and CD34

(a) Photomicrograph showing Calretinin negative plexiform nodules and dermal appendage serving as intrinsic control, PBM x100.(b)Photomicrograph showing Calretinin highlighting mast cells staining their cytoplasm brownish, PBM x400.(c) Photomicrograph showing Calretinin negative nodule with few Schwann cells staining positive, PBM x100.(d) & (e) Photomicrograph showing CD34 staining both showing the fingerprint staining pattern in neurofibroma with endothelium as control, PBM x100.(f) Photomicrograph showing CD34 antibody staining showing diffuse moderate positivity, PBM x40.

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Table 8: Immunohistochemical Profile of 100 cases of Neurofibroma showing staining intensity.

	SOX10	Calretinin	S100	CD34	Ki67
Positivity	90/100(90%)	16/100(16%)	91/100(91%)	95/100(95%)	0
Strong intensity	13/90(14.5%)	0	18/91(19.8%)	22/95(23.2%)	-
Moderate intensity	39/90(43.3%)	2/16(12.5%)	47/91(51.6%)	46/95(48.4%)	-
Mild intensity	38/90(42.2%)	14/16(87.5%)	26/91(28.6%)	27/95(28.4%)	-
Negativity	10/90(10%)	84/100 (84%)	9/100(9%)	5/100(5%)	15/15(100%)

Abbreviation: SOX10- SRY (sex determining region Y)-box 10 protein, S100- Solubility in 100%, CD34- Cluster of Differentiation 34, Ki67- Antigen/Protein Keil 67.

Table 9: Immunohistochemical Profile of 100 cases of Neurofibroma showing the percentage of neoplastic cells stained.

Percentage (%)	SOX10	Calretinin	S100	CD34	Ki67
Positives	90/100(90%)	16/100(16%)	91/100(91%)	95/100(95%)	0
5-25%	54/100(54%)	13/100(13%)	37/100(37%)	20/100(20%)	0
>25-75%	36/100(36%)	3/100(3%)	54/100(54%)	52/100(52%)	0
>75%	0	0	0	23/100(23%)	0
Negatives					
<5%	10/100(10%)	84/100(84%)	9/100(9%)	5/100(5%)	15/15(100%)

Abbreviation: SOX10- SRY (sex determining region Y)-box 10 protein, S100- Solubility in 100%, CD34- Cluster of Differentiation 34, Ki67- Antigen/Protein Keil 67.

Table 10: Immunohistochemical Profile of 100 cases of Neurofibromas showing the extent of neoplastic cells stained.

Extent in Positives cases	SOX10	Calretinin	S100	CD34	Ki67
Diffuse	78/90(86.7%)	10/16(62.5%)	66/91(72.5%)	67/95(70.5%)	0
Focal	12/90(13.3%)	6/16(37.5%)	25/91(27.5%)	28/95(29.5%)	0

Abbreviation: SOX10- SRY (sex determining region Y)-box 10 protein, S100- Solubility in 100%, CD34- Cluster of Differentiation 34, Ki67- Antigen/Protein Keil 67.

Discussion

We present one of the largest single-institutionseries of neurofibromas. A total of 1,502 soft tissue tumours were diagnosed in our institute within the study period which represents 4.9% of all specimens received. Out of these, 125 cases were diagnosed as neurofibroma representing 8.3% of all soft tissue tumours encountered.

In the index study, the highest frequency of occurrence of neurofibroma occurred in the second decade of life with a mean age of 25.4 years and these tumours had a female predilection. This is in contrast to the study done by Ji Young P et al in South Korea who found a mean age of occurrence to be 33.6 years but with a similar female sex predilection and a male-to-female ratio of 1: 1.5.[9] Also, contrasting studies were done in Lagos, Nigeria and Benin City, Nigeria by Ademiluyi et al, and Onumu et al respectively showing male predominance of neurofibromas in their studies.[19, 20] Azeke AT et al, in Nigeria also found a male predilection in these tumours with a peak frequency of occurrence in the 4th decade of life.[21] This study found 22 cases which were Syndromic and associated with NF1. In Toronto, Guha D et al reported a much higher prevalence of 53% (26 of 49 cases) of neurofibromas associated with NF1, in their study.[12] Gabhane SK et al, in India, found a lower prevalence of 8.73% (11 cases) associated with NF1.[14] Nearly 100% of individuals with NF1 develop BPNSTs and in approximately 30-50% of them, atypical and plexiform neurofibromas were found.[14] Most neurofibromas are sporadic with some cases associated with NF1 and both sporadic and NF1-associated neurofibromas are associated with a deletion in the NF1 gene. In about 10% of NF1 patients, neurofibromas may undergo transformation to MPNSTs, which are highly aggressive. [7, 9, 10, 11, 14] In this study, only one case out of 22 with Syndromic neurofibromas (4.5%) transformed to MPNSTs. The most common site of occurrence of neurofibromas in this study was the head and neck region constituting (36%) of all neurofibromas anatomic site distribution. Similarly, Azeke et a,l in Nigeria also reported the head and neck region as the most frequent site of occurrence.[21] Ji Young P et al in South Korea also showed the head and neck region to be the frequent site of occurrence in their study. [9] Fewer neurofibromas may be located in the extremities, where they may be expected to be symptomatically or cosmetically noticeable at a smaller size in comparison with neurofibromas in a plexal location, which is consistent with other reports. [11] In this study, one neurofibroma was seen in the Sino-nasal region. The Sino-nasal region and the oral cavity are

not frequently involved in neurofibroma. In a Brazilian population, Fransciso et al reported eight oral neurofibromas, representing 0.2% of oral lesions diagnosed within a 16-year period. The buccal mucosa was the most frequent site and none of the cases was associated with NF1. Their study also revealed that solitary sporadic neurofibromas in the oral cavity are more common than NF1-associated neurofibromas.[8]

The index study found pseudo-capsulation in few cases only while most cases had neither capsule nor fair circumscription. These findings are in keeping with a review done by Gabhane SK, *et al*, in India who histologically found these architectural, cytomorphological and stroma features as the most frequent and demonstrated the presence of capsule in 14% of neurofibroma cases seen.[14] Gabhane SK *et al,* also demonstrated the presence of melanin pigments in three neurofibromas (5.55%) which is contrasting to our study which showed only one case (0.8%) of pigmented neurofibroma. Pigmented neurofibroma is a usual type of solitary neurofibroma that shows the presence of melanin pigment in the cytoplasm of Schwann cells. The proposed theories for the presence of melanin in this nerve sheath tumour are either phagocytosis by Schwann cells or the proliferation of two different cell types (Schwann cells and melanocytes). Embryologically, melanocytes and peripheral nerve elements have a common origin from the neural crest stem cells. These cells at their genomic level retain their capacity to produce pigment, which explains the presence of melanin in these nerve sheath tumours. [7,10,11,14] The diffuse histologic pattern was the most frequent pattern seen in the index study. This finding is in keeping with other studies which showed similar findings. [15, 16, 17] A Diffuse pattern is an infiltrative growth pattern composed of heterogeneous rounded to slightly fusiform cells that are uniformly distributed within a delicate collagenous background with sheets of laminated bodies resembling Wagner-Meissner corpuscles and honeycomb permeation appearance may be present or absent in this pattern.[14] The index study revealed that most of the cases had no secondary degenerative changes and necrosis was absent. The hyaline change was the most common degenerative change seen followed by myxoid. This finding contrasts with studies done by Chikkannaiah P *et al,* and Gabhane SK *et al,* in India, who found myxoid change as the most common secondary degenerative change followed by cystic change. [14,15] This study found the transformation of a solitary case of atypical neurofibroma to a low-grade MPNST in an NFI patient. This finding is in keeping with Gabhane SK *et al,* in India, who also reported a case of malignant transformation in pre-existing neurofibroma in patients with NF1.[2]

Immunohistochemical analysis using SOX10 antibody showed nuclear immunopositivity with varying staining intensity in 90% of these tumours. It was also observed that 16%, 95% and 91% of these tumours respectively showed immunopositivity to Calretinin, S100 and CD34 with varying staining intensity and distribution. The 15 cellular Neurofibromas stained with Ki67 were negative indicating that these tumours had a low proliferative index and are cellular neurofibromas and not low-grade MPNSTs. Neurofibromas are composed of a mixed population of cells and usually present a variable number of SOX10 and S100 positive cells that may localize to different areas of the same tumour. [10] This corroborates our findings of the variable extent of staining (diffuse or focal) for these cells. This finding is similar to studies done differently in a Brazilian population by Nascimento et al, and Franscisco A et al. [6, 8] Calretinin immunoexpression by these Neurofibromas was low showing 16% immunopositivity in all tumours stained and 84% negativity. This is in keeping with studies done in Canada by Fine SW, et al who revealed low expression of Calretinin by the 42 Neurofibromas studied in which 3 (7%) of 42 Neurofibromas were immunoreactive with Calretinin, with weak or moderate intensity in fewer than 25% of the tumour cells. [16] These results strongly suggest that Calretinin is a useful marker for differentiating Schwannomas from Neurofibromas. The low expression can be corroborated by the fact that even within a Neurofibroma, genetically distinct populations of Schwann cells exist and so Neurofibromas might originate from Calretinin-negative Schwann cells or Calretinin–positive Schwann cells. [16,17] Calretinin also labelled mast cells, which commonly were present in a scattered individual cell pattern easily differentiated from the clustered pattern of neoplastic spindle cells. Our results also demonstrated that

Calretinin is superior to S100 protein, the latter of which was positive in 95% of Schwannomas and 91% of Neurofibromas. Ji Young P et al in South Korea showed that CD34 was expressed by almost 2 times the number of neurofibromas (80.2%) as that of Schwannomas (42.6%).[9] This finding is in keeping with the CD34 immunostaining of 91% seen in this study. In this study, CD34 revealed a fingerprint staining pattern and the variable expression of CD34 immunostaining is like the study done by Franscisco A et al in Brazil. [8] Those differences in expression levels of CD34 have been attributed to the type of stroma, fibrous or myxoid and the age of the lesion. The CD34 staining pattern in neurofibromas is different from the pattern seen in Schwannomas.[2,3,4,17] In Canada, Mahmoud AK et al, in a study to determine whether CD34 expression in nerve sheath lesions was found in a unique cell population or in a subset of nerve sheath cells demonstrated that a distinct subpopulation of amoeboid CD34+ cells was seen consistently in every neurofibroma analyzed.[18] However, there was great variability in the proportions of immunoreactive cells from one tumour to another. This was also observed in the index study which showed immunopositivity to CD34.

Conclusion

Neurofibromas are common in our environment with a prevalence of 8.3% of all soft tissue tumours seen and 12.1% of all benign soft tissue tumours seen. These tumours were more prevalent in females and the highest age incidence was in the second decade of life. The commonest anatomic site of occurrence was the head and neck region. The use of special and immunohistochemical stains in the diagnostic approach of these tumours is paramount in the final definitive diagnosis and treatment.

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