Section

1

FNAC of Head and Neck Paragangliomas

DEFINITION, HISTOLOGY, PHYSIOLOGY AND ANATOMICAL DISTRIBUTION OF PARAGANGLIA

Paragangliomas are tumours of the paraganglia. Paraganglia are anatomically dispersed neuroendocrine organs associated with autonomic nervous system derived from neural crest.[1,2] Paraganglia are composed of neuroendocrine cells, sustentacular cells, connective tissue cells, capillaries, myelinated and unmyelinated nerve fibres and intrinsic neurons and commonly contain mast cells.[3,4]

The neuroendocrine cells have been referred to by various names like granule containing cells, chromaffin cells and chromaffin-like cells in sympathetic paraganglia and chief cells, glomus cells and type 1 cells in parasympathetic paraganglia. In H&E sections neuroendocrine cells appear as polygonal with amphophilic or basophilic cytoplasm and small spherical or ovoid pale staining nuclei arranged in cords and clusters designated as "Zellballen" surrounded partially by sustentacular cells. Sustentacular cells are flattened with less conspicuous cytoplasm and more deeply basophilic nuclei with coarse clumped chromatin. These appear to be glial cells possibly related to non-myelin forming Schwann cells in peripheral nervous system. These cells are inconspicuous in H&E sections and are highlighted on immunostaining.[4]

Endocrine cells are confirmed by neuroendocrine markers like chromogranin and synaptophysin and catecholamine biosynthetic enzymes. Sustentacular cells are S-100 protein immune-reactive.

For physiologic and pathological purposes, paraganglia comprise of two groups associated with either sympathetic (adrenal/extra-adrenal) or parasympathetic nerves. These share a common cellular origin but differ in clinicopathologic standoff attributed to type, timing and intensity of physiologic signals. Both secrete catecholamines and a variety of peptides but differ in type and quantity of catecholamine synthesis and secretion.[4]

The pathologic lesions of sympathetic paraganglia comprise of tumours of neuroendocrine lineage (paragangliomas) and neuronal lineage (neuroblastoma, ganglioneuroblastoma and ganglioneuroma). Pathological lesions of parasympathetic paraganglia comprise of hyperplasia and neuroendocrine neoplasms (paragangliomas).[4,5]

Sympathetic paraganglia comprise of adrenal medulla and extra-adrenal paraganglia. Extra-adrenal sysmpathetic paraganglia extend from neck to pelvis and are distributed along the pre-vertebral and para-vertebral sympathetic chains and along sympathetic nerve branches that innervate the organs of the pelvis and retroperitoneum[4] (Fig. 1). Sympathetic paraganglia are not known by individual names except those located in adrenal medulla and organs of Zuckerkandl located at the origin of inferior mesenteric artery,

Parasympathetic paraganglia are distributed along the cranial and thoracic branches of glossopharyngeal and vagus nerves (Fig. 2). Carotid body paraganglia are the only parasympathetic paraganglia visible macroscopically. The other parasympathetic paraganglia are highly variable in number and location and microscopic structures.[4] The parasympathetic paraganglia are named according to anatomic location and include carotid body paraganglia, tympanic paraganglia, jugular paraganglia, vagal paraganglia, laryngeal paraganglia, subclavian and aortico-pulmonary paraganglia. Carotid body and tympanic paraganglia are related to glossopharyngeal nerve. The jugular, vagal, laryngeal, subclavian and aortico-pulmonary paraganglia are related to vagus nerve.

Sympathoadrenal Paraganglia

Sympathoadrenal paraganglia comprise of:

1. **Adrenal medulla**
2. **Extra-adrenal paraganglia:** Are distributed along prevertebral and paravertebral sympathetic chain from neck to pelvis (with the exception of lower spinal/cauda equina paraganglia) and comprise of the following:
    a. ***Organ of Zuckerkandl:*** Near origin of inferior mesenteric artery from aorta. These are functional sympathetic ganglia with adrenal chromaffin like cells *in utero* reduced to vestiges after birth.
    b. ***Thoracic sympathetic paraganglia:*** Are present along prevertebral sympathetic chain in posterior mediastinum

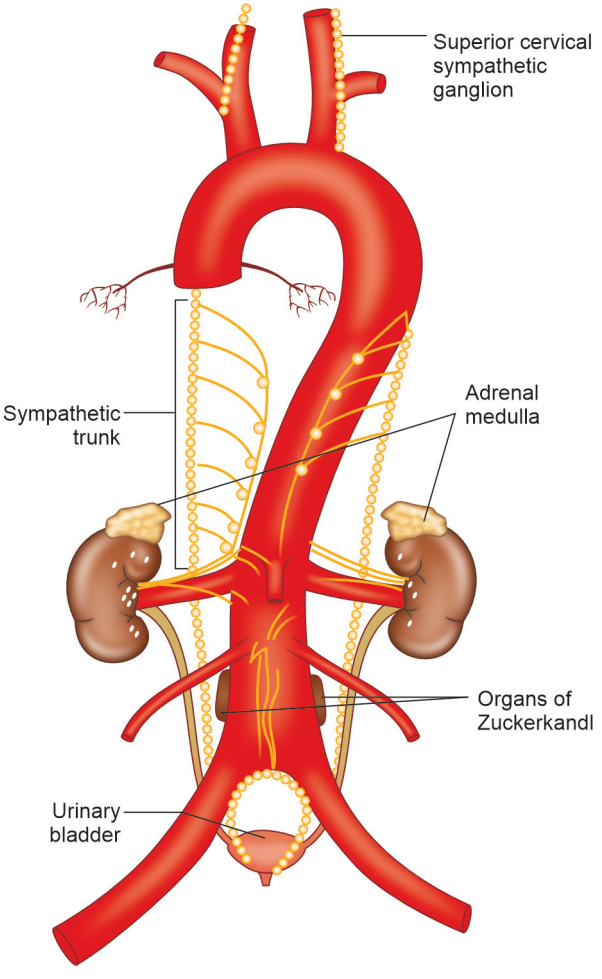


Fig. 1: Anatomical distribution of sympathoadrenal paraganglia
Adapted from Mills, Stacey E. Histology for Pathologists. United States of America: Wolters Kluwer Health, Inc.; 2012, *with permission*

c. ***Superior cervical sympathetic ganglion paraganglia:*** Located high up in the neck above carotid bodies

d. ***Urinary bladder paraganglia:*** Located in the muscle coat of urinary bladder.

Parasympathetic Paraganglia

Parasympathetic paraganglia are located in the head and neck and anterior and middle thoracic cavity. They are given specific names according to anatomical location and include the following:

a. ***Carotid body paraganglia:*** One on either side of neck, arise on medial side of common carotid artery bifurcation
b. ***Jugulo-tympanic paraganglia:*** Are 0–12 in number with an average of 2.8 on either side, located in middle ear related to glossopharyngeal nerve and vagus nerve.
c. ***Vagal paraganglia:*** Are seen on either side in relation to vagal trunk at or above the level of ganglion nodosum or near the jugular ganglia.
d. ***Laryngeal paraganglia:*** comprising of a superior and an inferior pair. The superior pair is seen in anterior third of false vocal chords. The inferior pair is inconstant in location and occurs either between cricoid and thyroid cartilage or below cricoid cartilage.
e. ***Subclavian paraganglia:*** Located in the subclavian artery
f. ***Aorticopulmonary paraganglia:*** Located at the base of these major vessels.

The paraganglia with the exception of carotid body paraganglia are microscopic collections of neuroendocrine cell clusters. Carotid body paraganglia are the largest and only paraganglia visible to the naked eye, which together weigh approximately 30 mg at low altitudes and are much larger (hypertrophic) at higher altitudes.

Paragangliomas are categorized into two broad categories:

1. Sympatho-adrenal paragangliomas
2. Parasympathetic paragangliomas

Paragangliomas usually arise from the anatomical locations of sympathetic and parasympathetic paraganglia mentioned above and less often outside these well-known sites.[4]

Parasympathetic head and neck paragangliomas are rare at low altitudes, attested by the observation of 69 tumours from 1937 to 1975 on more than 600,000 cases of the Department of Surgical Pathology, Memorial Sloan Kettering Cancer Center with incidence of 0.012%.[6] These constitute approximately 1–3% of all paragangliomas.[7] Their prevalence is much higher at high altitudes.[8–10] This is particularly true of carotid body paragangliomas.[7] High prevalence of carotid

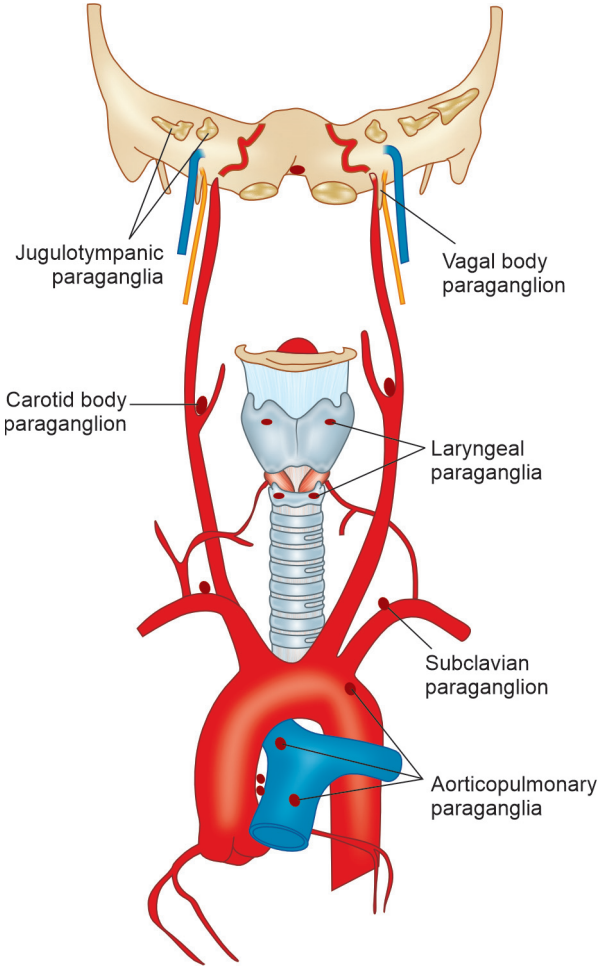


Fig. 2: Anatomical distribution of parasympathetic paraganglia
Adapted *from* Fletcher, Christopher DM Diagnostic Histopathology of Tumors. United States of America: Elsevier Science and Technology Journals; 2013, *with permission*.

body paragangliomas (CBPs) is attributed to their chemo-receptor function.[11] The chemo-tactic stimuli include chronic hypoxemia with low pO_2, low pH or high pCO_2. Carotid body hyperplasia is seen at high altitudes[11] and, in cardio-pulmonary disorders.[12] Vagal body paraganglioma has also been reported in cardio-pulmonary disorders.[13] Carotid body at high altitudes is believed to progress

in a sequence of chronic hypoxemia, chief cell hyperplasia and neoplasia.

Parasympathetic head and neck paragangliomas differ at low and high altitudes in many respects. The low altitude tumours are rare, grow slowly and show a wide age range with median age of 50 years being rare in children and at some sites show slight female predilection. A 10–50% of paragangliomas at low altitudes are hereditary or familial. The low altitude tumours are comparatively more aggressive than high altitude paragangliomas and 3–12% are malignant.[14] The malignancy is defined by regional and/or distant spread to regional lymph nodes, lungs, liver, bone and other non-paraganglionic tissues.

The high altitude head and neck paragangliomas on the other hand show much higher prevalence, wide age range, high female predilection, rare bilaterality or multicentricity, lack of familiarity and aggression.[8–10] No molecular studies on SDH genes are available for high altitude HNPs. Both low and high altitude HNPs are generally biochmically inert and only 1–3% are functional and secrete catecholamines.

Up to one third of patients of pheochromocytomas/extra-adrenal paragangliomas carry germline mutations in one of ten genes the most common being MEN2 A and B, RET, VHL, NF1 and SDH.[4,15] Some head and neck parasympathetic paragangliomas are associated with germ line mutations in succinic dehydrogenase (SDH) gene sub-units such as SDHD, SDHB and SDHC[16] and rarely SDHA and SDHAF2. Carotid body paragangliomas (CBPs) are 5.8 times more likely to have familial predisposition than other paragangliomas.[17] 10% sporadic and 38% of familial CBPs are bilateral.[18] Multicentricity is reported to occur in 80% of familial paragangliomas and in 10–20% of sporadic paragangliomas.[19]

DNA ploidy of low altitude sporadic and hereditary HNPs have not helped to predict growth rate or malignancy.[20] No such studies are available on high altitude HNPs.

Carotid body paragangliomas (CBPs) arise from tunica adventitia of posteromedial aspect of the common carotid artery just above carotid bifurcation.[4,5] These tumours are mobile from side to side but not vertically (Fontainne's sign). Bruit may be present in these tumours. 1–3% of low altitude tumours are supposed to be functioning. Carotid body paragangliomas are supposed to be

chemoreceptors as well as baroreceptors. The CBP is the most common head and neck parasympathetic paraganglioma both at high and low altitudes. These tumours grow slowly as a painless submandibular bulge or pharyngeal mass with variable tonsillar deviation. A small percentage of tumours can result in hoarseness, vocal cord palsy, dysphagia and Horner's syndrome.

Jugulo-tympanic paragangliomas (JTPs) are the second most common head and neck paragangliomas, often lumped together because of close anatomical association in the middle ear. These paraganglia are distributed along auricular branch of the vagus nerve (Arnold's nerve) and the tympanic branch of the glossopharyngeal nerve (Jacobson's nerve) in the middle ear over promontory, wall and mastoid canaliculus[21] and in Jugular bulb. Majority (85%) of JTPs arise in Jugular bulb associated with Arnold's nerve and 12% from Jacobsson's nerve.[22] Tumours rarely (3%) arise in external auditory canal.[22] These tumours can present with tinnitus, hearing loss, ear discharge, haemorrhage, pain, facial nerve deformities and vertigo.[22] On otoscopy a reddish mass is seen behind an intact tympanic membrane. They can also present as polyp in the external auditory canal or as mass in the base of skull.[5]

Vagal paraganglia are located within or adjacent to the vagal trunk in or near the ganglion nodosum or jugular ganglia.[23] Vagal paragangliomas are third most common and rare (<5%) of head and neck paragangliomas. These present as parapharyngeal masses or extend up into oropharynx or base of skull. Some patients may be associated with hoarseness and dysphasia from vagal nerve palsy.[23,24]

Normal larynx contains two pairs of paraganglia, superior and inferior. The superior pair is localized to the upper anterior third of the false cords. The inferior pair is more variably situated either between thyroid and cricoid cartilage or just below the cricoid cartilage.[25] Laryngeal paragangliomas (LPs) are rare and present as hoarseness and dysphagia.[26]

Aortico-pulmonary paraganglia occur on ventral and dorsal aspect of these vessels in the base of the heart.[27] Aortico-pulmonary paragangliomas (APPs) are rare and can present with hoarseness, dysphagia or discomfort and rarely with superior vena cava syndrome.[28]

Paragangliomas are diagnosed by a number of techniques (Table 1).

Table 1: Diagnosis of paragangliomas	
Catecholamine estimation: Epinephrine/non-epinephrine and their metabolites in the plasma and urine	
Imaging	Doppler ultrasound, contrast enhanced CT scan,
	MRI, MRA, carotid angiography
	Scintiscan with 18-fluoro dopa, PET and I^{131} MIBG.
Incisional biopsy	
FNAC	

Head and neck paragangliomas are diagnosed by carotid angiography, CT scan, MRI. FNAC in experienced hands can be a reliable, safe and non-invasive diagnostic procedure.

MATERIAL AND METHODS

The author presents preoperative FNAC diagnosis of 171 head and neck paragangliomas from 168 patients. The first case of the series was reported by the author from the Department of Pathology, Government Medical College (GMC), Srinagar in the year 1988 from a 50-year-old male. The remaining 167 cases of head and neck paragangliomas including three cases of bilateral tumours were reported by the author from FNACs conducted personally at Dr Khan's Diagnostic Lab and Research Center. The total number of FNACs conducted and reported by the author in the Pathology department of GMC Srinagar (May 1984 to June 2002) and at Dr Khan's Lab (1985 to Dec 2016) sum up to more than 100,000.

FNAC was conducted as an outpatient procedure (Table 2). 22 gauge sterilised disposable needles used initially with 10 ml or 20 ml were replaced by 24 gauge needles subsequently. Syringe-holder was an option. Gentle suction was applied and aspiration stopped as soon as the blood appeared in the hub of the needle and material quickly ejected on multiple slides placed on a sheet of white paper. Excess of blood was sucked with thin cotton swab placed at one end of the slightly tilted slides and smears were drawn quickly. This is the most crucial step for final outcome of the results. The needle puncture site was covered with sterilised cotton swab with firm pressure for a few minutes followed by a cover of medicated dressing. No immediate or remote complications were recorded.

All but five cases had adequate material in smears for diagnosis on first FNAC. Out of the remaining five cases, four cases were

Table 2: Technique/staining	
Outpatient procedure	
Supine position with extended neck	
Alcohol (70%) swabs, medicated dressing	
10 ml and 20 ml disposable plastic syringes, syringe holder (optional)	
24 gauge disposable needles, grease free glass slides.	
Staining	MGG stain/Giemsa stain on dry smears
	Pap stain, H&E on wet smears.
	Immunostains, chromogranin, cytokeratin, S-100 (cytospin, cell blocks).

Note: MGG/Giemsa stain was used in this study

diagnosed in the second attempt and the fifth case in the third attempt. The interval between the first and repeat FNACs varied between three months to more than one year.

Four cases out of those patients who were not subjected to surgery, either because of refusal by the patients to undergo surgery or due to some medical contraindication, were re-aspirated after periods ranging from 1 to 8 years on the request of concerned clinicians. There was no change in smear cytomorphology between the first FNAC and the repeat FNAC as will be illustrated by one case here, (58F, LCBP) aspirated first in 1991 and re-aspirated in 1999.

RESULTS AND FOLLOW- UP

177 paragangliomas from 174 patients were subjected to aspiration from various anatomical sites over a period of more than 32 years (Table 3) in more than 100,000 FNACs conducted and reported by the author. These included 171 HNPs from 168 patients. These were all high altitude paragangliomas. 162 patients were from Kashmir province of JandK state residing at an altitude of 1585 meters and above. Out of the remaining 6 cases one patient was from Kargil District (altitude ~ 2676 metres) of Ladakh province and 5 patients were from the hilly regions of Jammu province including 3 from Banihal, 1 from Doda and 1 from Poonch.

The tumours included 167 CBPs from 164 patients including three bilateral tumours, two JTPs and two VPs (Table 4). The clinical data of these tumours is summarised in (Tables 5 to 7; Charts 1 and 2) and the photographs of some patients of this study

Table 3: FNAC diagnosis of paragangliomas 1988–2016		
Site	**No. of patients**	**No. of tumours**
1. Head and neck	168	171
2. Retroperitoneal (including 1 metastatic case)	5	5
3. Urinary bladder	1	1
Total	174	177

Table 4: Anatomical location of head and neck paragangliomas		
Anatomical location	**No. of patients**	**No. of tumours**
1. Carotid body (CBPs)	164	(3 bilateral cases) 167
2. Jugulo-tympanic (JTPs)	2	2
3. Vagal (VPs)	2	2
Total	168	171

Table 5: Clinical data of head and neck paragangliomas	
1. Age (range)	11–90 years
2. Sex	F : M : : 142 : 26
3. Side	Right –81
	Left –84
	Bilateral –3 cases (6 tumours) (30/F, 40/F, 75/M)
4. Sporadic/non-familial	100% (all the cases)
5. Multicentricity	1
6. Clinical presentation	i. Slowly growing painless nodule or submandibular bulge, with tonsillar bulge in one case of 1–15 years duration –CBPs, VP. One case of CBP (90F) had non-Hodgkin's lymphoma in maxilla.
	ii. Tinnitus, aural discharge and aural polyp –one case (JTP)
	iii. Tinnitus, deep pharyngeal bulge, aural polyp and cranial nerve palsies—another case of JTP.

Table 6: Age frequency of head and neck paragangliomas	
Age (years)	**No. of patients**
1–10	0
11–20	7
21–30	24
31–40	28
41–50	44
51–60	37
61–70	17
71–80	9
81–90	2
91–100	0
TTL	168

Chart 1: Age frequency of head and neck paragangliomas—graphical representation

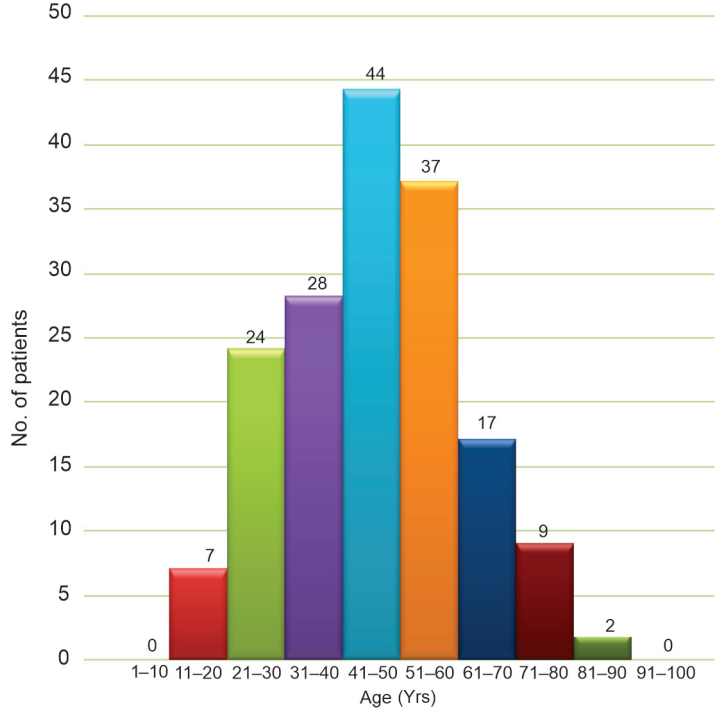
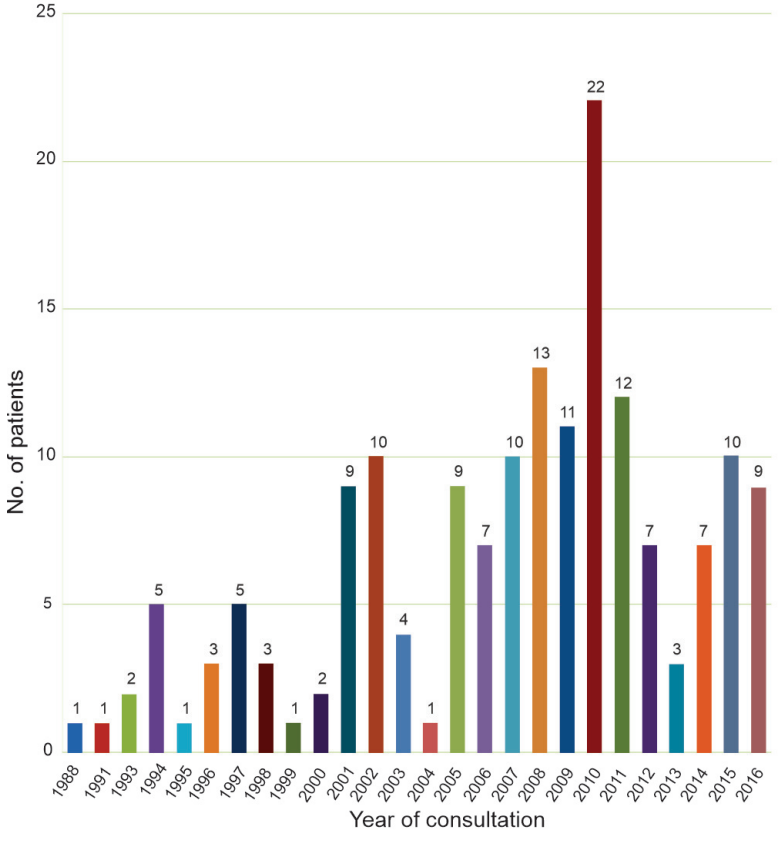


Table 7: Year wise distribution of patients of head and neck paragangliomas

Year	No. of patients
1988	1
1991	1
1993	2
1994	5
1995	1
1996	3
1997	5
1998	3
1999	1
2000	2
2001	9
2002	10
2003	4
2004	1
2005	9
2006	7
2007	10
2008	13
2009	11
2010	22
2011	12
2012	7
2013	3
2014	7
2015	10
2016	9
TTL	168

Chart 2: Year wise distribution of patients of head and neck para—gangliomas—graphical representation



are shown in Figs 3 to 38. One patient (90 F), in addition to CBP (Fig. 54), had swelling in the left maxilla which on FNAC proved to be non-Hodgkin's lymphoma large cell (Fig. 55) confirmed by trucut biopsy, histology and immunohistochemistry.

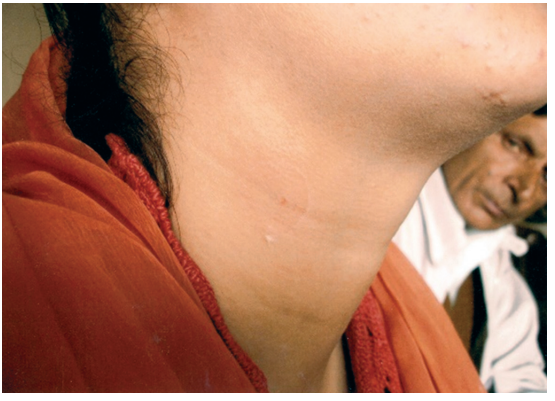


Fig. 3

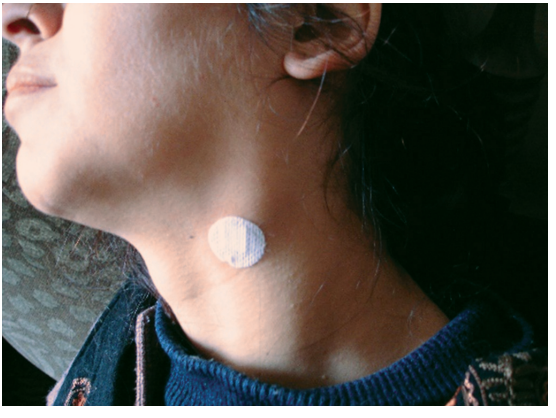


Fig. 4

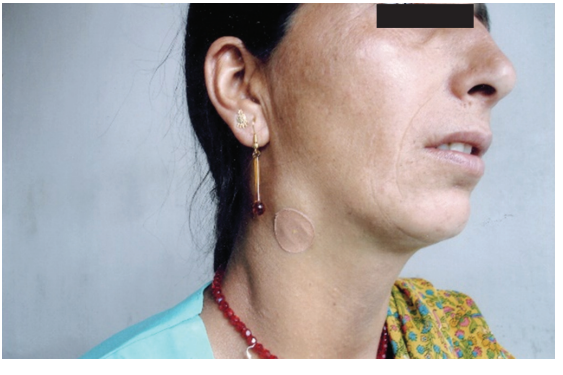
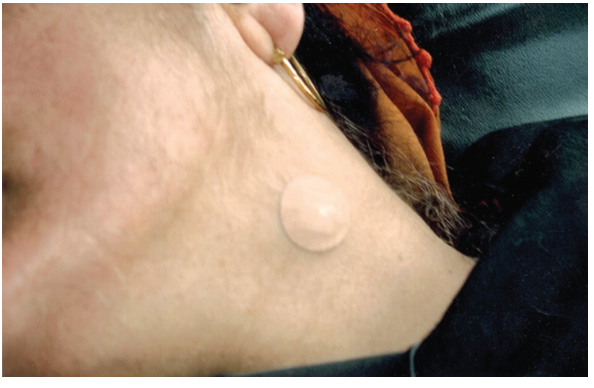


Fig. 5

Fig. 6



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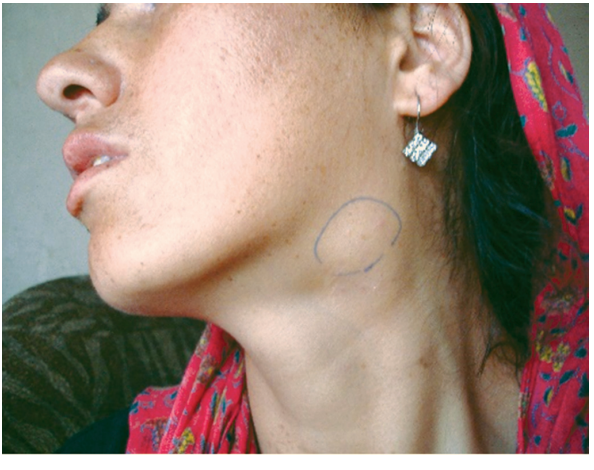


Fig. 8

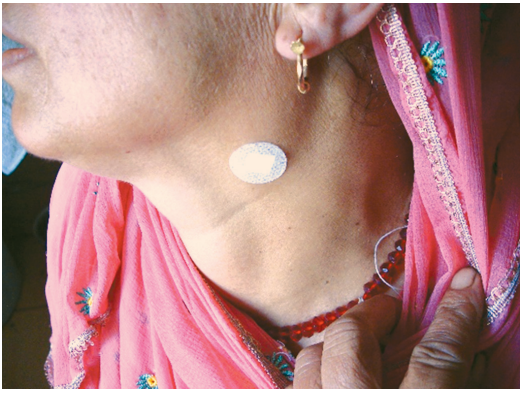


Fig. 9

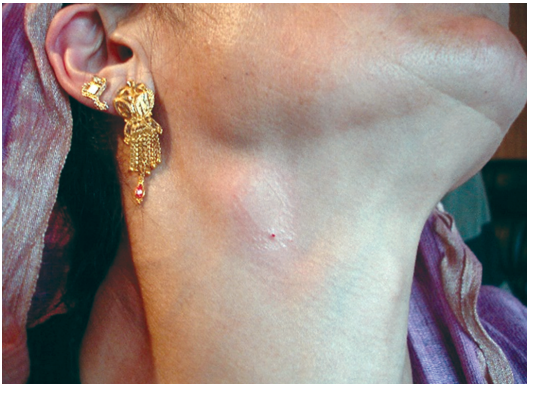
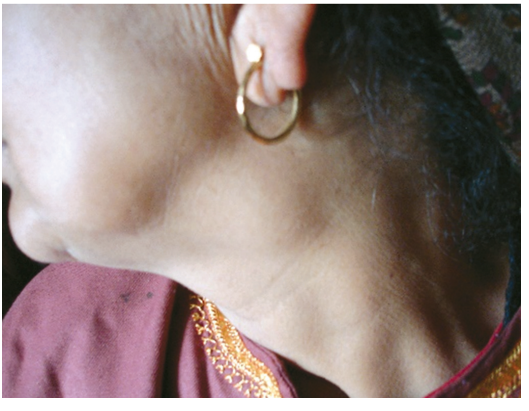


Fig. 10



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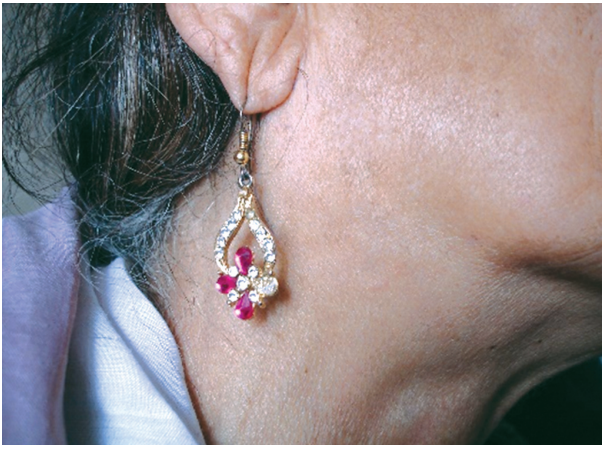

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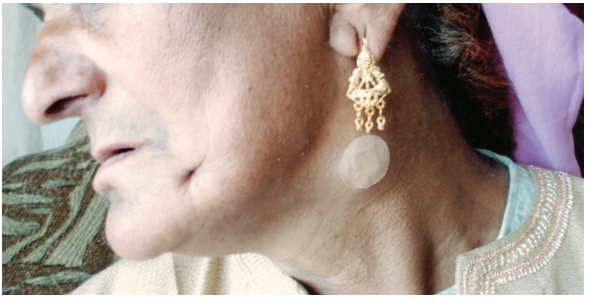
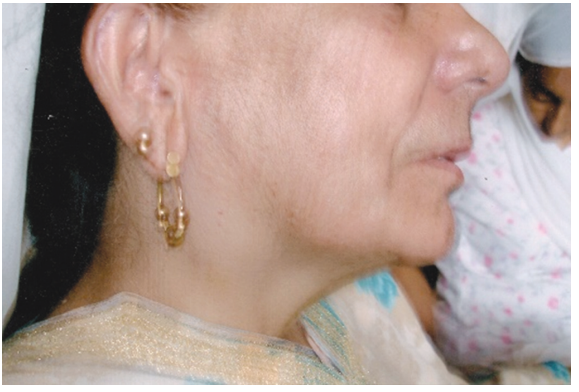


Fig. 13

Fig. 14



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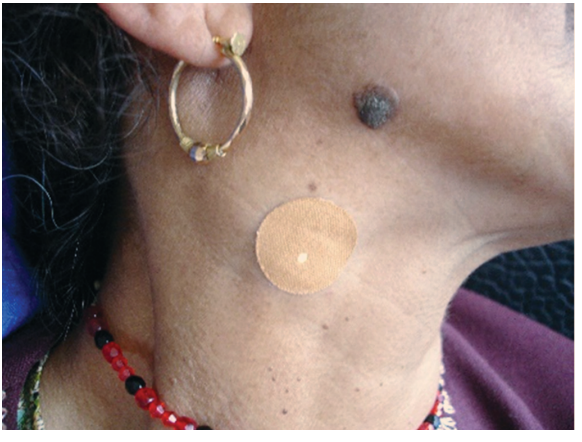
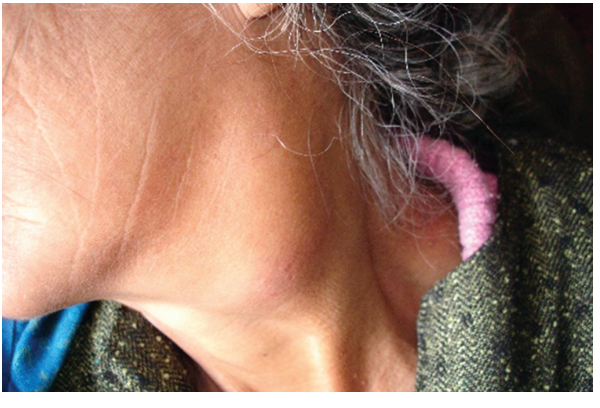


Fig. 16



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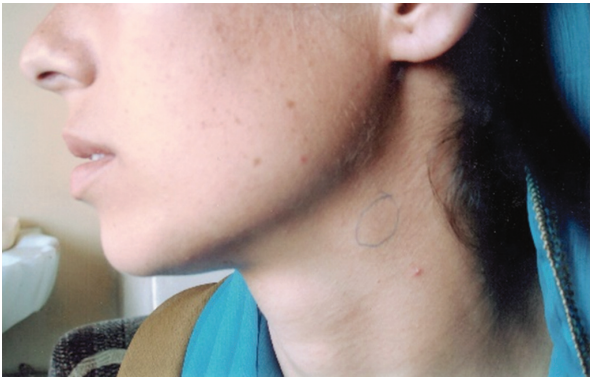

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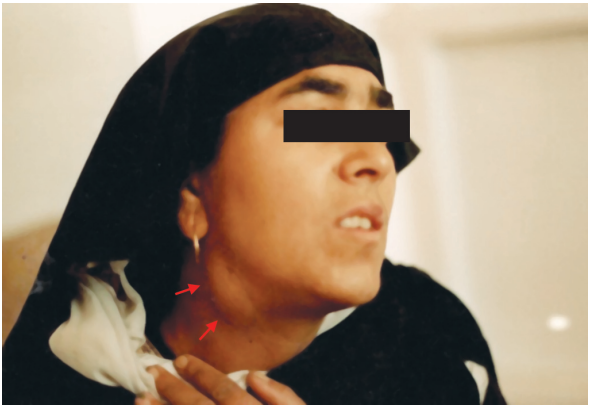

Fig. 19

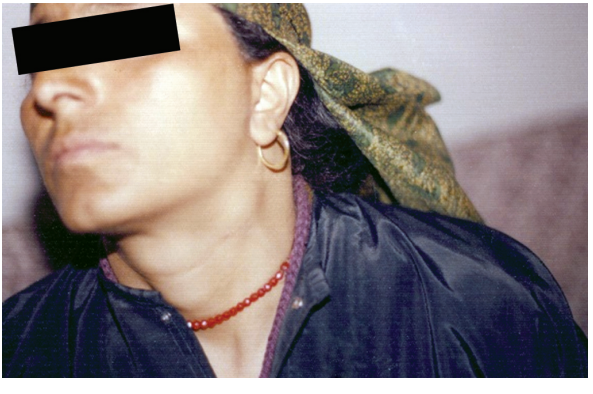

Fig. 20

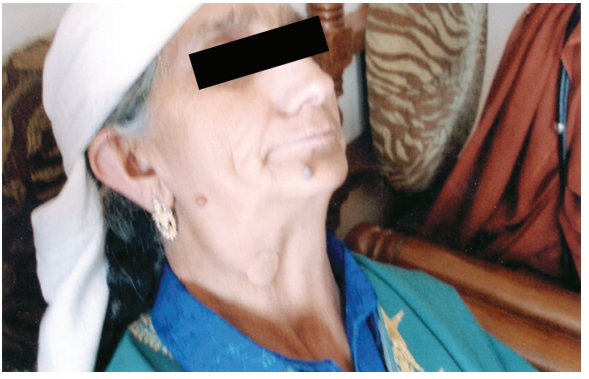

Fig. 21

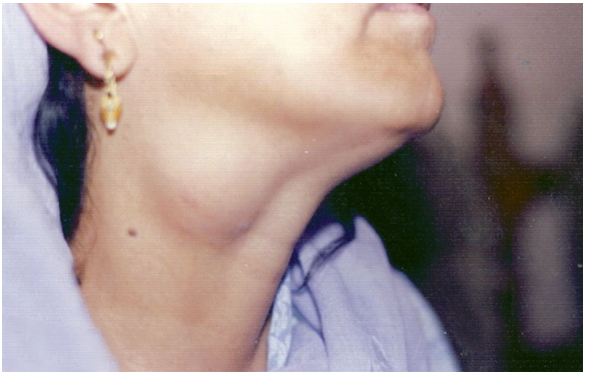

Fig. 22

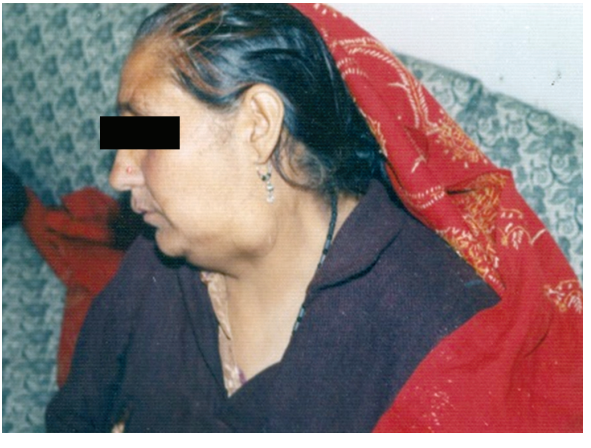

Fig. 23

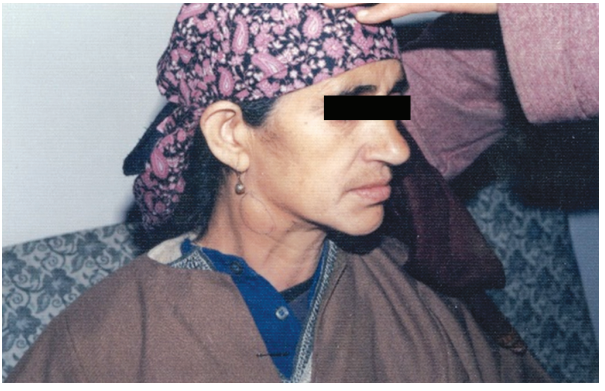

Fig. 24

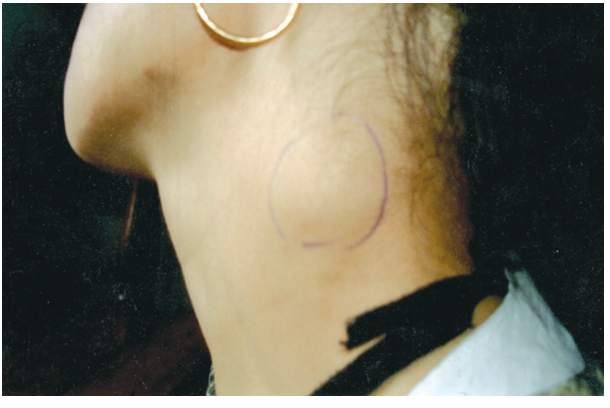

Fig. 25

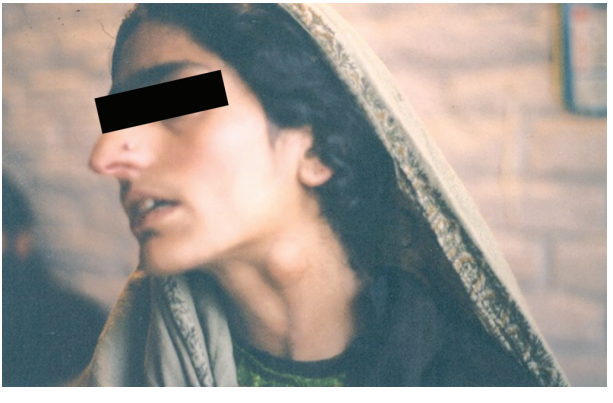

Fig. 26

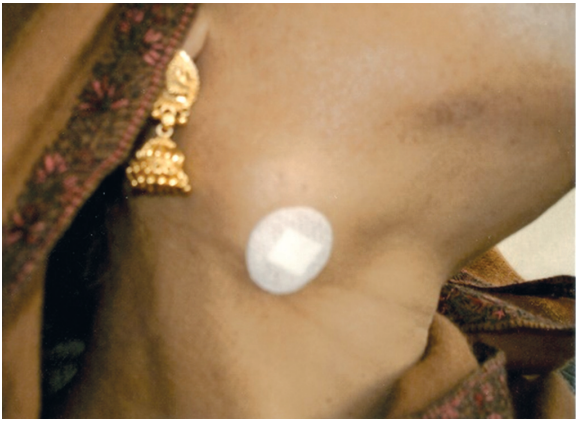

Fig. 27

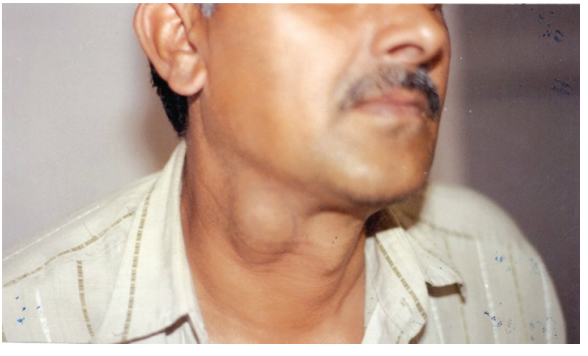

Fig. 28

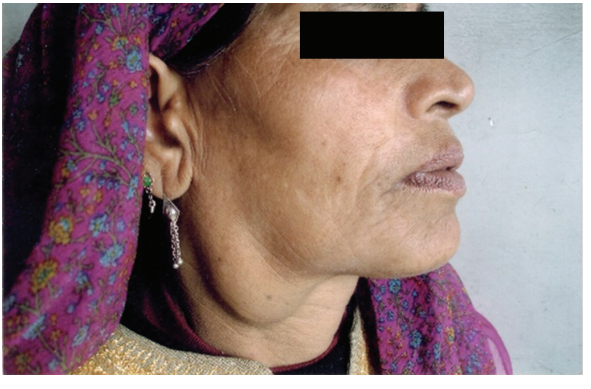

Fig. 29

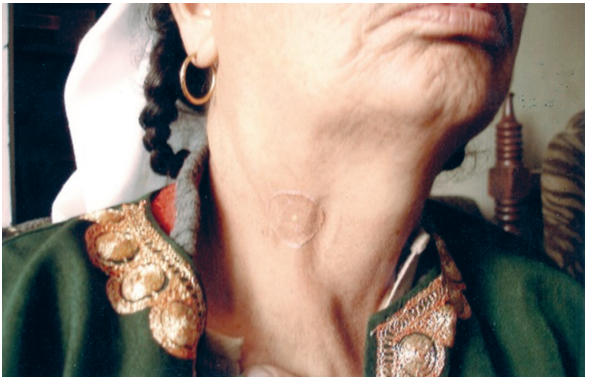

Fig. 30

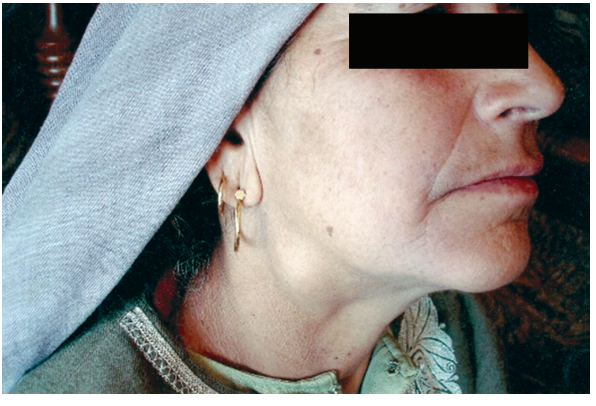

Fig. 31

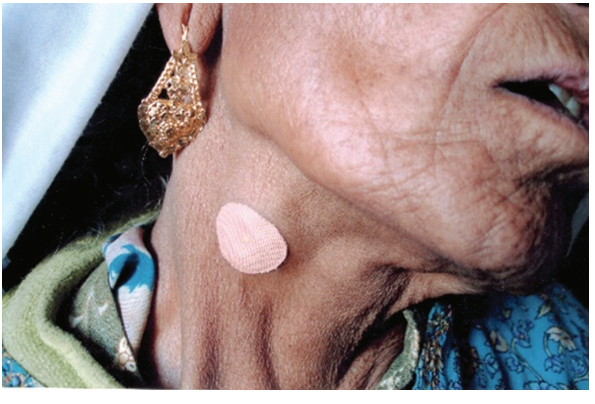

Fig. 32

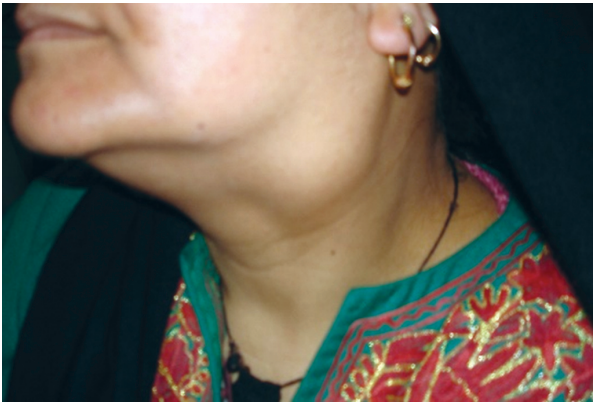

Fig. 33

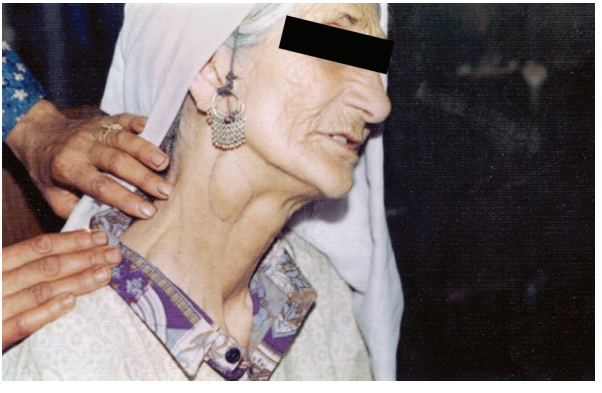

Fig. 34

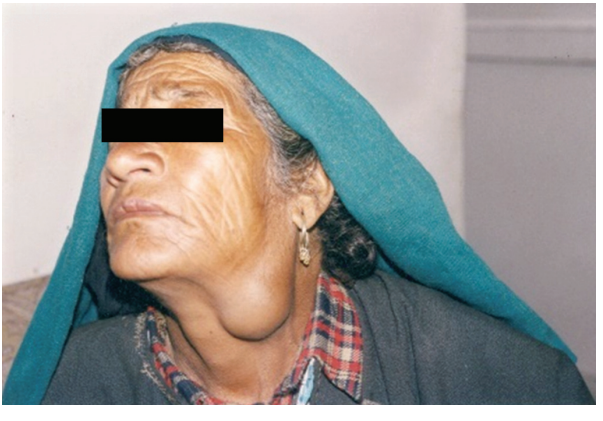

Fig. 35

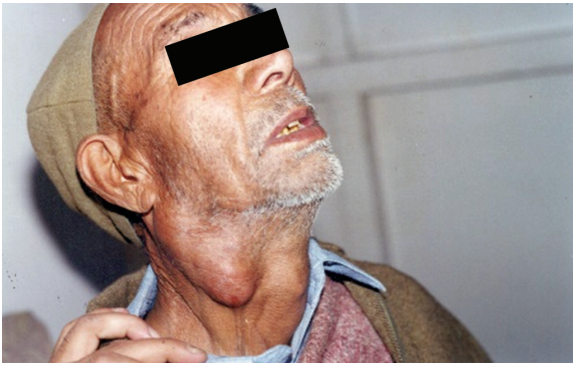

Fig. 36

Fig. 37

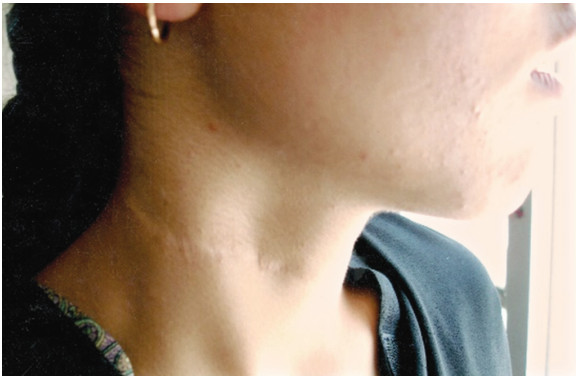

Fig. 38

Figs 3 to 38: Carotid body paragangliomas in patients of varying age in both sexes predominantly females, show tumours barely appreciable as in Fig. 3 to large tumours as in Figs 35 and 36

The salient features include a wide age range of 11–90 years and high predominance of 84.5% in females. All were apparently sporadic and non-functional. However, no molecular studies were done on various genes associated with familial paragangliomas. 109 cases of CBPs and 2 cases of VPs (111 patients) underwent surgical excision. Incisional biopsy was also available from aural polyp from 1 case of JTP. Histopathology confirmed FNAC diagnosis of paraganglioma in all these cases. Surgeries on 54 patients were conducted by ENT surgeons of SMHS Hospital, 51 patients were operated by Cardiovascular Thoracic Surgeons of SKIMS, Soura, 3 patients were operated by ENT surgeons of SKIMS Medical College and Hospital, Bemina and 3 patients were operated at New Delhi-2 at Rajiv Gandhi Cancer Hospital and 1 case in St Stephen's hospital.

There was 1 patient of malignant CBP with Horner's syndrome at the time of presentation who was not operated upon.

In a brief follow up of cases one patient (11 F) who was diagnosed with left CBP and in the year 2005 and operated upon, showed local recurrence and regional lymph node metastasis, three years after surgery in 2008 (Fig. 38). Another patient (21 M, CBP) reported with local recurrence without regional lymph node metastasis, 5½ years after surgery. Diagnosis of recurrence was confirmed by FNAC and repeat surgery was performed successfully in both these patients. Another patient (40F, JTP) was found unfit for surgery and died two years after diagnosis by local aggression and invasion of base of brain.

There was one case of surgery related mortality.

FNAC FINDINGS

Paragangliomas are highly vascular tumours and therefore yield bloody aspirates with dilution of tumour cells and hence negative results. To overcome this, clinical suspicion from anatomical location is most important. This is particularly true in case of carotid body paragangliomas with definite anatomical location. In jugulotympanic and vagal paragangliomas, imaging is highly contributory by raising suspicion of a paragaglioma. Carotid body paragangliomas, apart from their location on posteromedial aspect of common carotid artery bifurcation, can also be suspected from their mobility from side to side but not vertically. Bruit can also be present sometimes. These swellings are usually long standing and

asymptomatic. The author did not have any imaging support prior to FNAC except in one case. The haemorrhagic aspirate with anatomical site should alert the cytopathologist to keep the possibility of paraganglioma in mind. Use of 24 gauge sharp needles with delicate suction is must. Suction should be stopped as soon as blood enters the hub of the needle so that minimal blood is aspirated to avoid dilution of tumour cells. The aspirate is quickly ejected on to multiple slides. The slides are then tilted with cotton whisps at one end of the slide to absorb excess of blood and the smears are quickly drawn. This is a crucial step for ultimate success. Subsequent staining and microscopic diagnosis then becomes fruitful. Thus the technique of aspiration is crucial for the final outcome

Smears yield scanty to abundant cellular material (Figs 39 to 59), depending upon the dilution of tumour cells by blood. But even in scanty cellular smears diagnosis can be made confidently with experience and by reference to cytopathology text books. The smears in this series also showed varying cellularity from scanty to very high. Tumour cells were arranged in loose clusters, cords, acini and individually dispersed. The cytoplasm was generally moderate in amount, basophilic, finely granular or pale and ill-defined wispy. The nuclei were round, ovoid and occasionally spindled with fine chromatin and regular nuclear membranes. Some deeply stained nuclei were also seen. Some smears are monomorphic (Fig. 49) whereas others show marked nuclear pleomorphism (Fig. 54). Acinar differentiation was striking in some tumours (Figs 40 to 42, 46, 47). Some smears had sclerotic pattern with entrapment of tumour cells in dense connective tissue (Figs 44 and 45). Nuclear vacuoles were occasionally seen (Fig. 53). Malignant tumour diathesis was absent. The smear pattern of three aggressive tumours (2 CBPs and JTP) did not differ from other non-aggressive tumours.

Figures 39 to 59: FNAC smears of HNPs' MGG stained high power views. The basic cytomorphology of smears from different paragangliomas is identical with minor variation in cellularity and nuclear pleomorphism At least four patients were re-aspirated after being diagnosed with paragangliomas on FNAC after an interval of three months to eight years on advice of concerned physicians since they were on follow up without undergoing surgery. The basic smear pattern remained unchanged as will be exemplified by one patient 58F with left carotid body paraganglioma, who presented

with submandibular swelling of approximately 2 cm diameter in 1991 (FNAC Fig. 39) and re-aspirated in 1999 when the tumour had enlarged to 8 cm (FNAC Fig. 40). The smears of 1991 and 1999 do not show any appreciable change in cytomorphology. The patient could not be subjected to surgery in 1991 because of some medical contraindication.

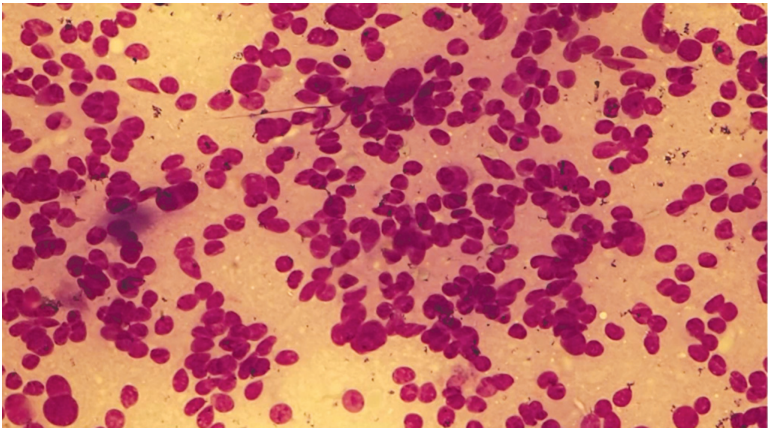

Fig. 39: FNAC smear from 58F left carotid body tumour is highly cellular with loose clusters of cells, cords of cells, acini and single cells. FNA was done in 1991. MGG HP

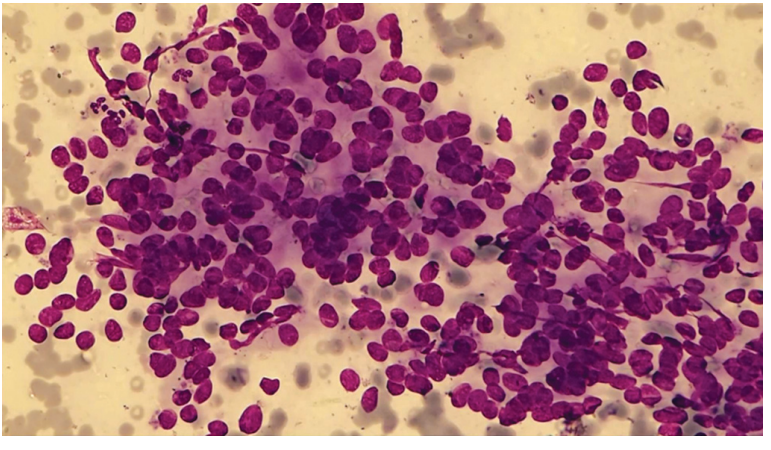

Fig. 40: FNAC smear from 1999 from case shown in Fig. 39. The smear pattern is basically same. MGG HP

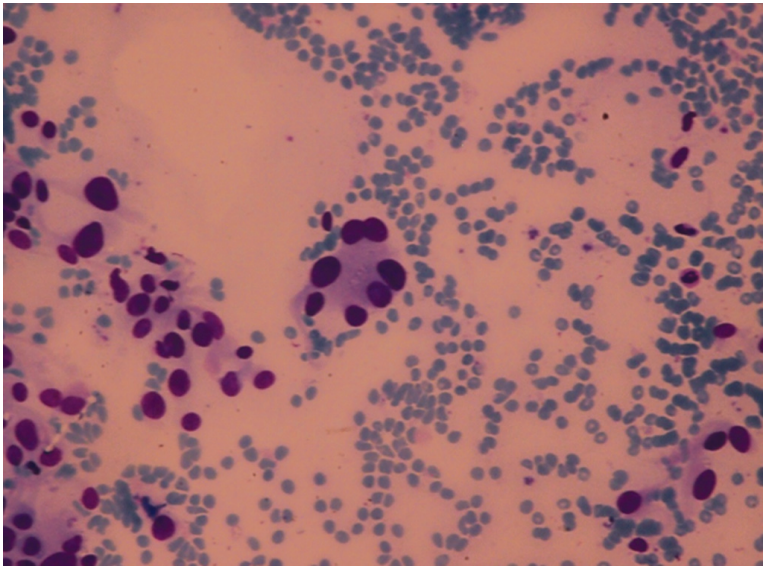

Fig. 41: FNAC smear from carotid body paraganglioma of relatively low cellularity with considerable nuclear variation of tumour cells, round to ovoid, ill-defined basophilic cytoplasm in loose clusters and occasional acinar pattern as well as single cells. MGG HP.

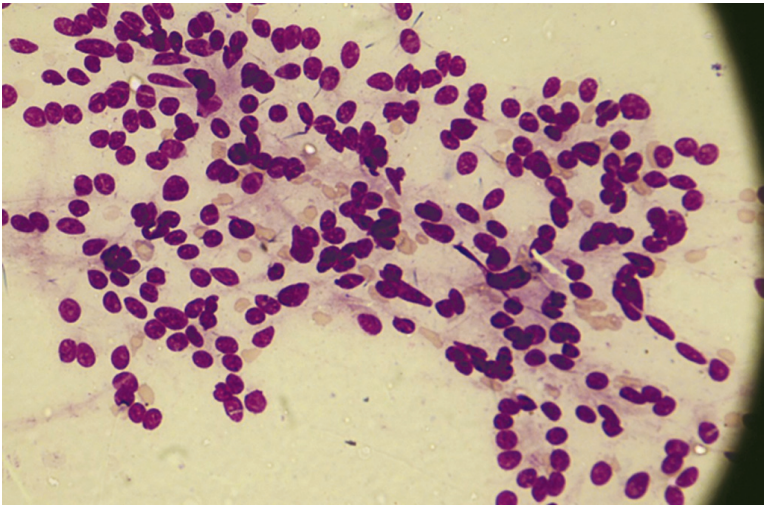

Fig. 42: FNAC smear from carotid body paraganglioma shows loose clusters and cords of tumour cells with occasional acini and many single cells. MGG HP.

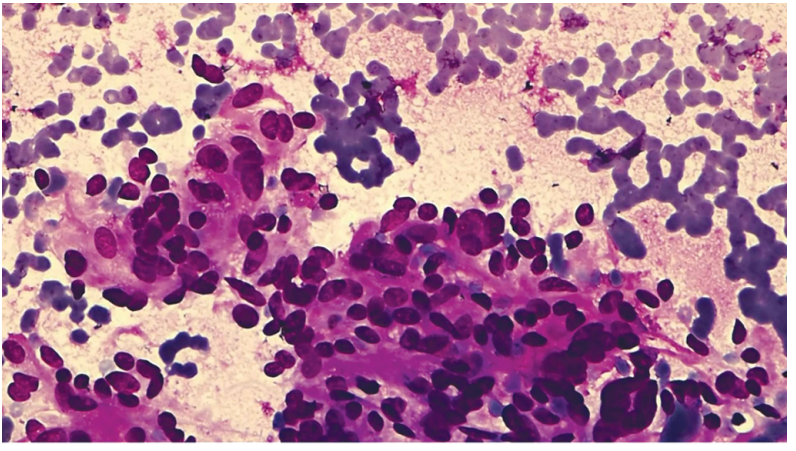

Fig. 43: FNAC smear from carotid body paraganglioma with clusters of round to spindled tumour cells with overlapping nuclei and ill-defined basophilic cytoplasm forming a syncytium. MGG HP

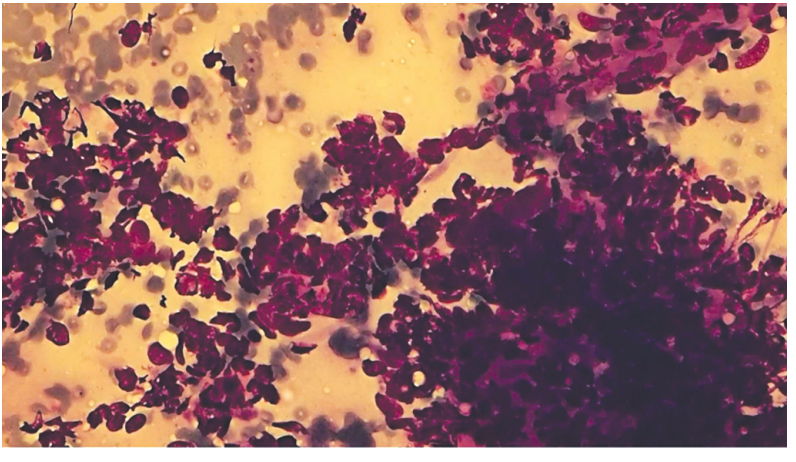

Fig. 44: FNAC smear from sclerotic carotid body paraganglioma shows clusters of overlapping crushed cells with only occasional dispersed single cells. Diagnosis of paraganglioma from these smears can be considered only in haemorrhagic smears with anatomical background of carotid body. MGG HP

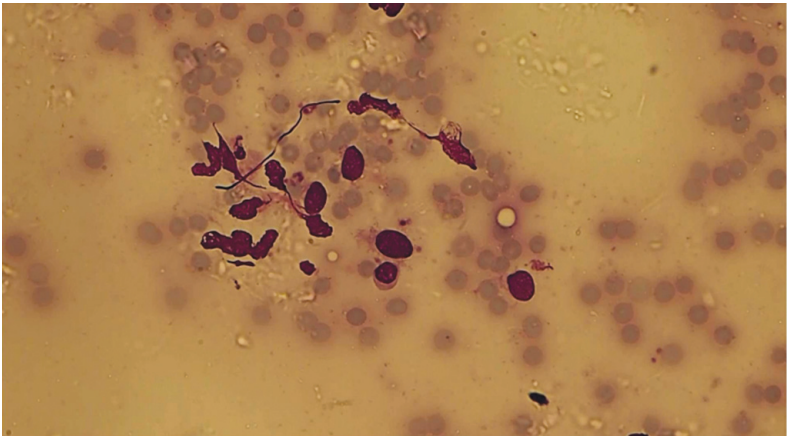

Fig. 45: FNAC smear from the case shown in Fig. 44 shows singly dispersed cells with suggestion of paraganglioma. MGG HP. The histology of this case is sown in Fig. 77

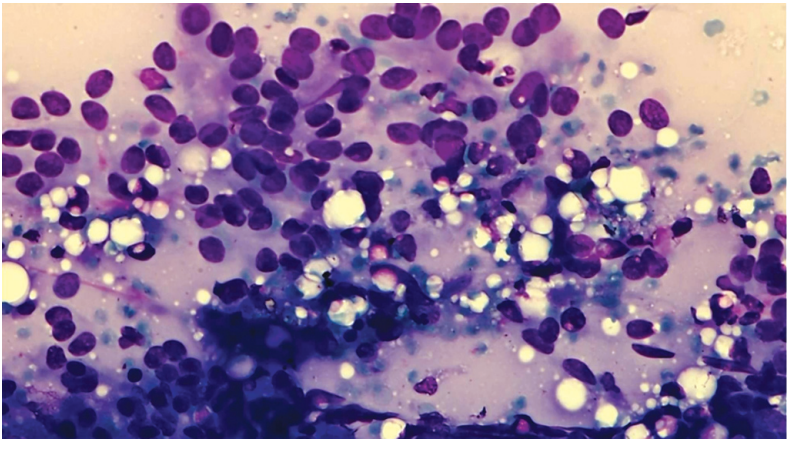

Fig. 46: FNAC smear from carotid body paraganglioma shows tumour cells in acinar arrangement at the top and loose aggregates and single cells elsewhere. MGG HP

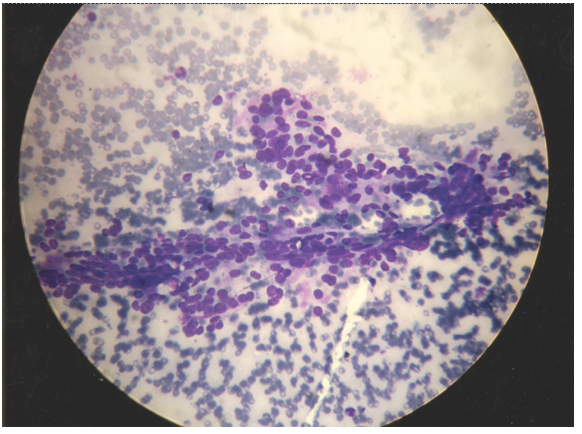

Fig. 47: FNAC smear from Jugular paraganglioma in 40F. This patient presented in 1999 with parapharyngeal mass and cranial nerve palsy. A small polyp was also detected in the external auditory canal. Aspiration was done from parapharyngeal mass and diagnosed as paraganglioma from the smear shown above. The smear shows cords and loose clusters of monomorphic tumour cells with occasional acini. Histology of incisional biopsy from aural polyp is shown in Fig. 78 and Fig. 79. The patient died two years after diagnosis from invasion of base of brain. She could not be subjected to surgery because of some medical contraindication at the time of presentation in the year 1999. The smears are bland with no malignant tumour diathesis. MGG HP.

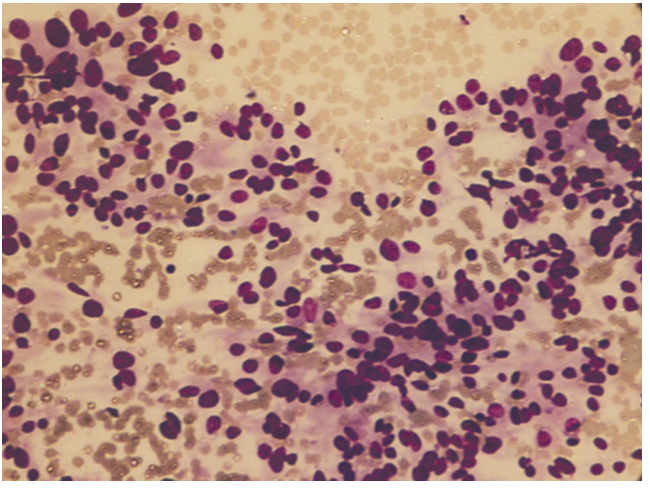

Fig. 48: FNAC smear from a case of carotid body paraganglioma with moderate anisokaryosis. The tumour cells are arranged in cords and dispersed cells with occasional acini. MGG HP

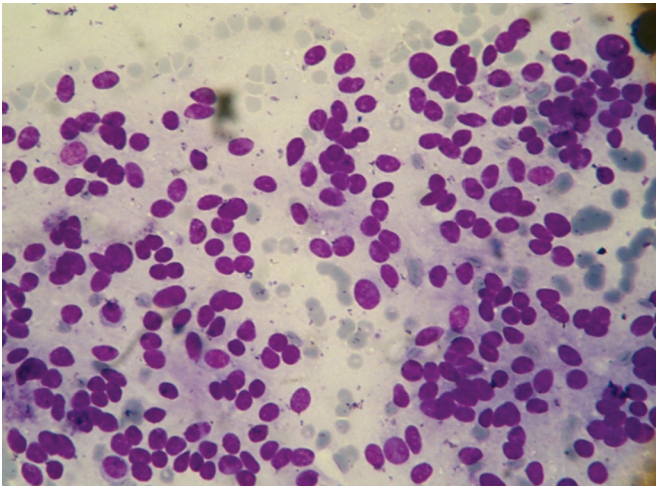

Fig. 49: FNAC smear of RCBP of 11F diagnosed in 2005 on FNAC. The tumour cells are monomorphic with mild anisokaryosis, arranged in loose clusters, cords and are singly dispersed.MGG HP.

There was a relapse with local recurrence of the tumour and regional lymph node metastasis three years after surgery in 2008. The FNAC smears of lymph node metastasis are shown in Figs. 50 and 51.

The scar of previous surgery is visible on right side of the neck as shown in Fig. 38.

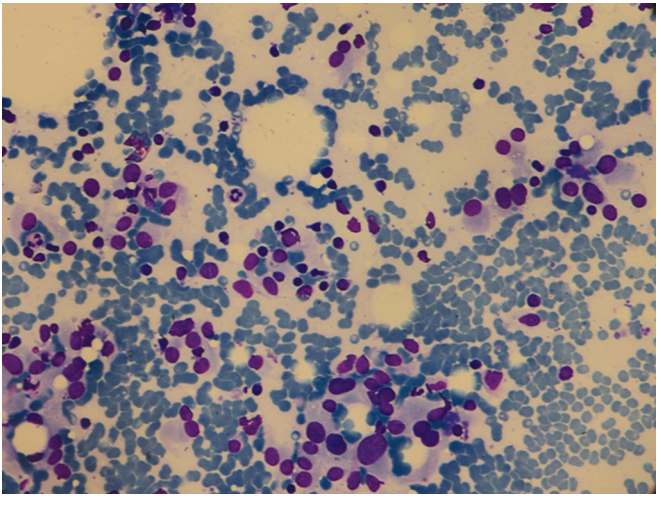

Fig. 50: FNAC smear shows metastasis of paraganglioma in regional lymph node with clusters of tumour cells with dispersed lymphocytes in the background. MGG HP.

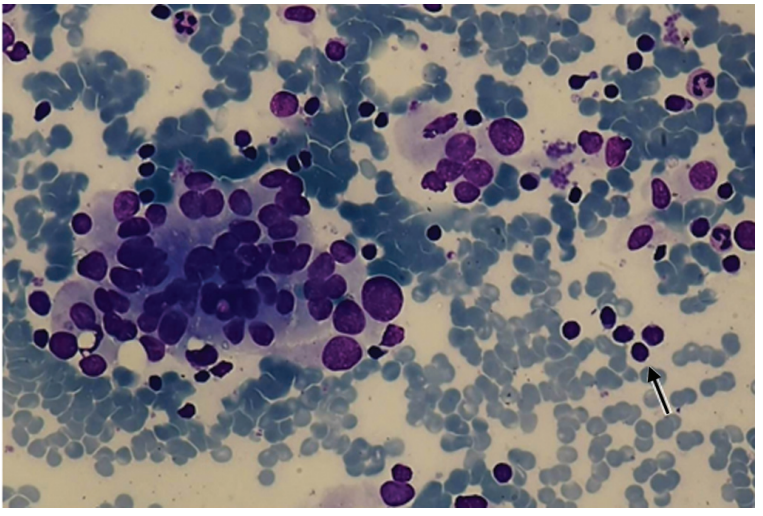

Fig. 51: Higher power view of FNAC smear show in Fig. 50. Lymphocytes are much smaller than tumour cells and marked by the arrow.

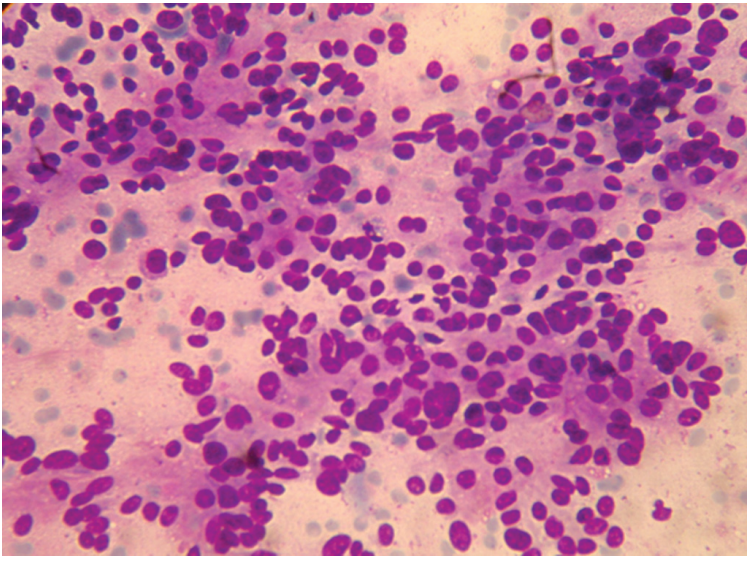

Fig. 52: FNAC smear of carotid body paraganglioma shows a cellular smear of monomorphic tumour cells in acinar pattern and single cells. Malignant tumour diathesis is absent. MGG HP.

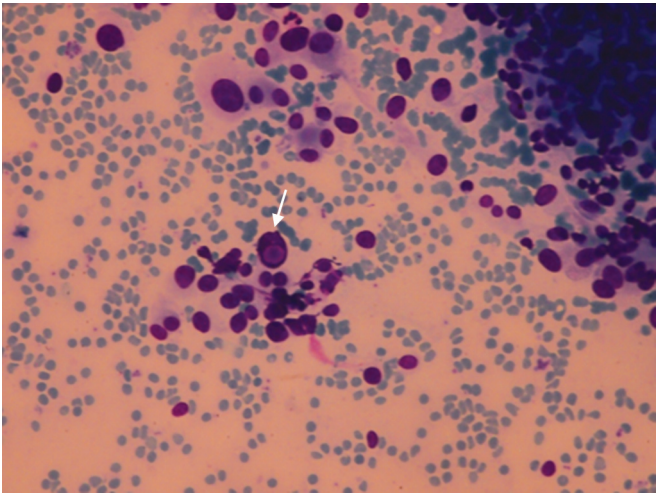

Fig. 53: FNAC smear of carotid body paraganglioma with usual cytomorphology and intranuclear vacuolation in one cell in the middle of the picture.

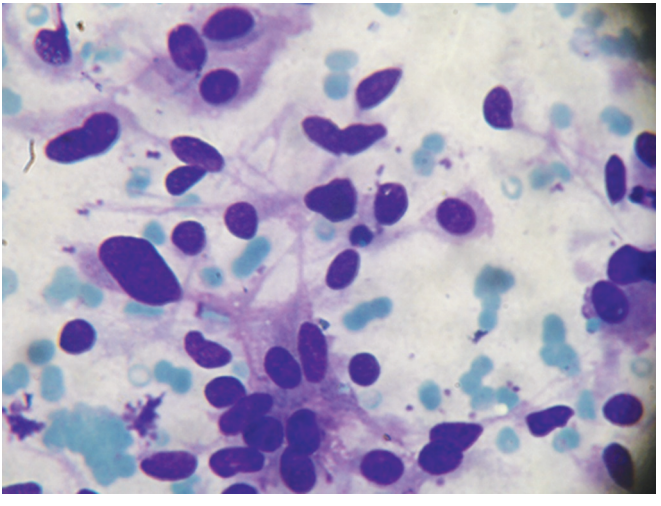

Fig. 54: FNAC smear from left carotid body tumour of 90F under higher magnification, MGG stained, shows marked pleomorphism of tumour cells with ill-defined basophilic cytoplasm in loose clusters and single cell with clean background. Malignant tumour diathesis is absent. The anatomical site with bloody aspirate combined with cytomorphology is the key to diagnosis of paraganglioma. This patient also had swelling of the left maxilla, which on FNAC proved to be non-Hodgkin's lymphoma.

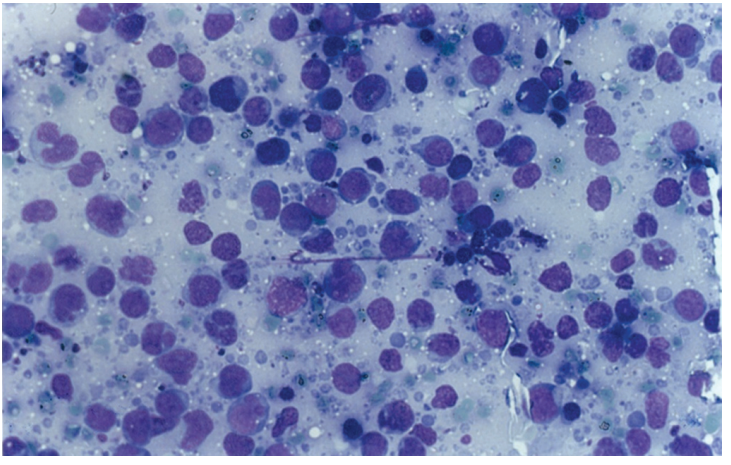

Fig. 55: Non-Hodgkin's lymphoma large cell from patient from case shown in Fig. 54. The FNAC smear shows lymphoid cells dispersed with many lymphoglandular bodies. MGG HP.

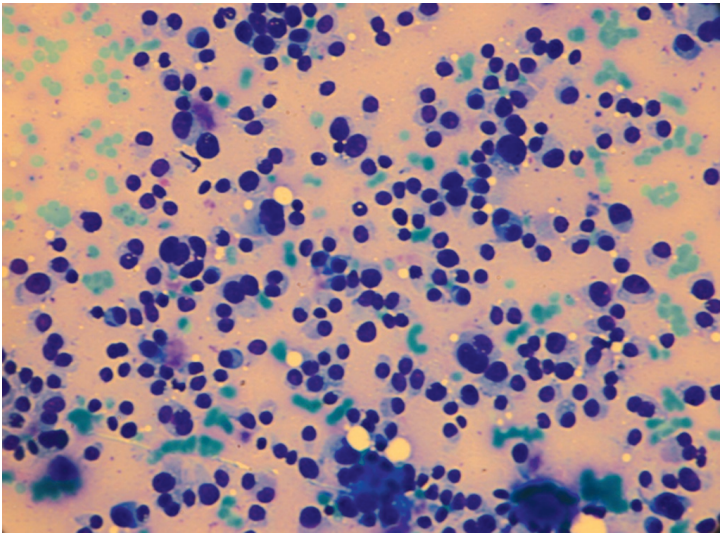

Fig. 56: FNAC smear from medullary carcinoma thyroid, metastatic to cervical node, can mimic paraganglioma. Polyhedral tumour cells widely dispersed with occasional small loose clusters show plasmacytoid morphology with one or more nuclei, moderate basophilic cytoplasm and well defined cell borders. The nuclear chromatin is uniform, bland and granular typical of neuroendocrine tumours shared with paragangliomas. MGG HP.

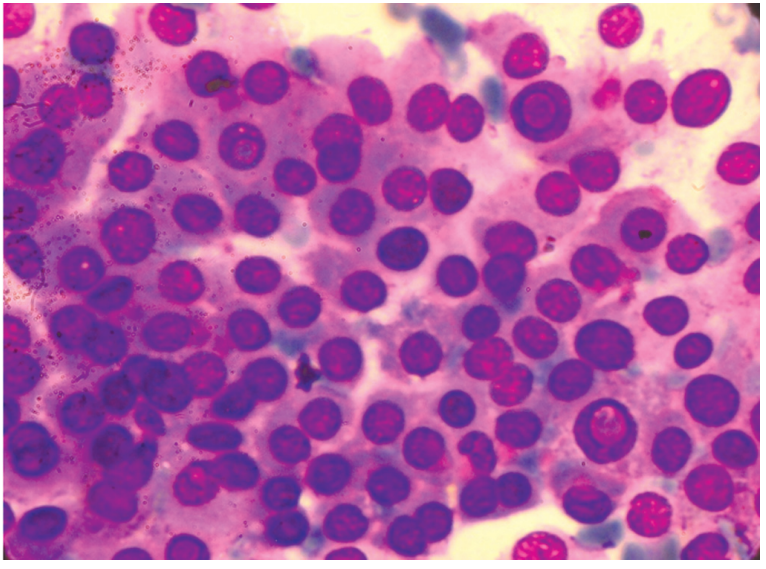

Fig. 57: FNAC of papillary carcinoma thyroid metastatic to cervical node shows sheets of tumour cells with moderate, well defined cytoplasm, intranuclear vacuolations and dispersed chromatin. MGG HP

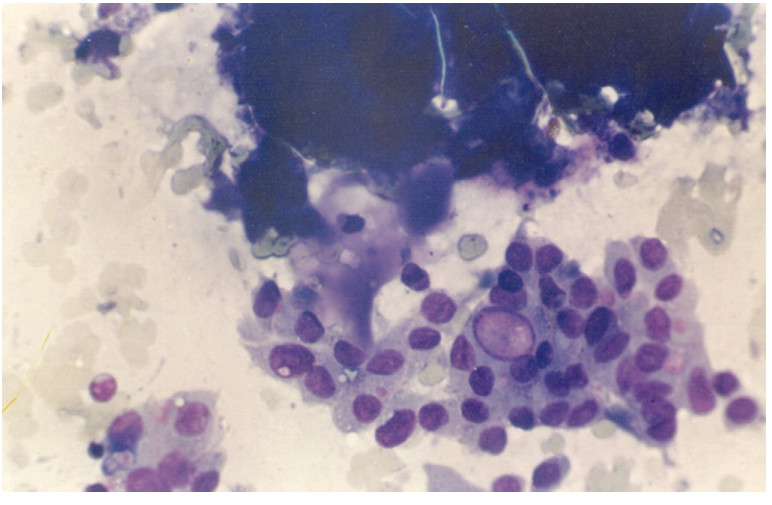

Fig. 58: FNAC of papillary carcinoma metastatic to cervical node. This smear shows clusters of monomorphic cells with moderate cytoplasm and a tumour cell with large intra-nuclear vacuolation and abundant colloid. MGG HP.

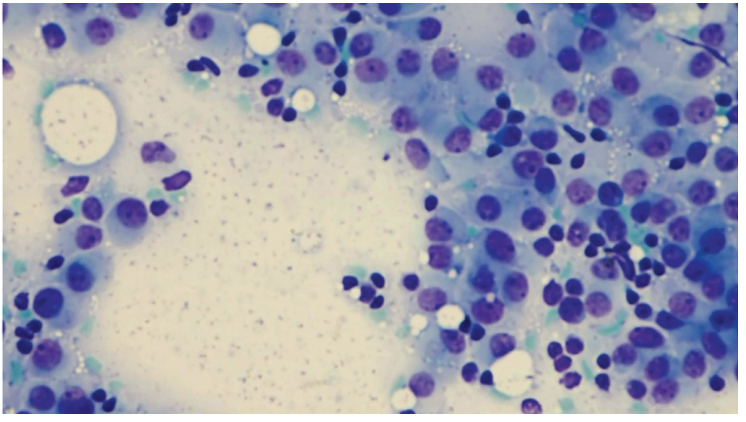

Fig. 59: FNAC smear of poorly differentiated adenocarcinoma metastatic to cervical lymph node. Loosely packed tumour cells show coarser chromatin with prominent nucleoli and well-defined moderate basophilic cytoplasm in contrast to bland nuclei with ill-defined wispy cytoplasm of paragangliomas. Residual lymphoid cells are present in the background of tumour cells. MGG HP.

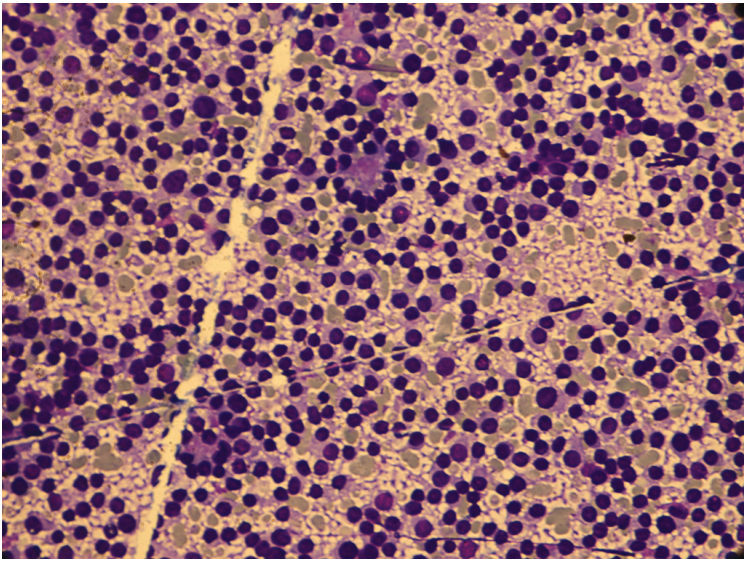

Fig. 59a: Extra-skeletal Ewing's sarcoma/ PNET. Shows small round cells with high N/C ratio and scanty ill-defined cytoplasm, dispersed, in loose clusters and a rosette in a lacy background. MGG HP.

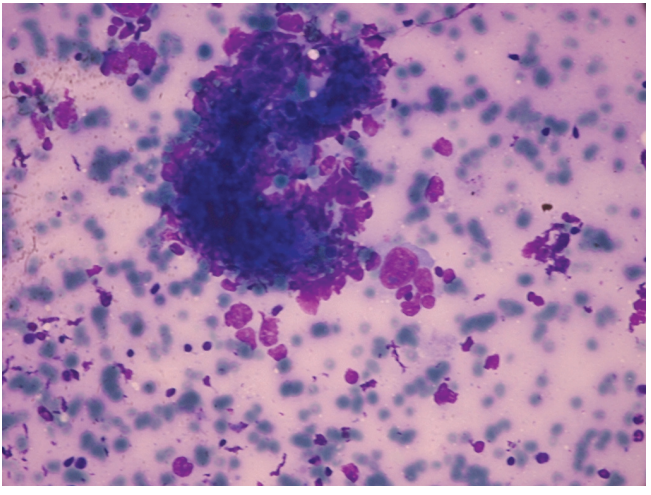

Fig. 59b: Alveolar rhabdomyosarcoma. Micrograph shows a tumour fragment and dispersed tumour cells. Tumour cells show marked variation in size and shape from small undifferentiated round cells with round nuclei and scanty cytoplasm to large cells with irregular hyperchromatic nuclei, irregular nuclei membranes and abundant cytoplasm. The tumour cells were immunoreactive to desmin and myogenin. MGG HP.

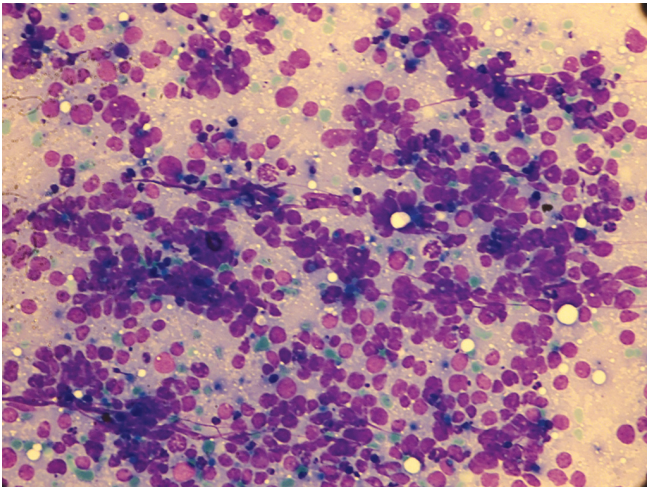

Fig. 59c: Metastatic small cell carcinoma to cervical lymph node. Shows small round undifferentiated tumour cells in loose clusters and dispersed. Tumour cells have coarse granular chromatin and scanty cytoplasm. Necrotic tumour cell debri is also present MGG HP.

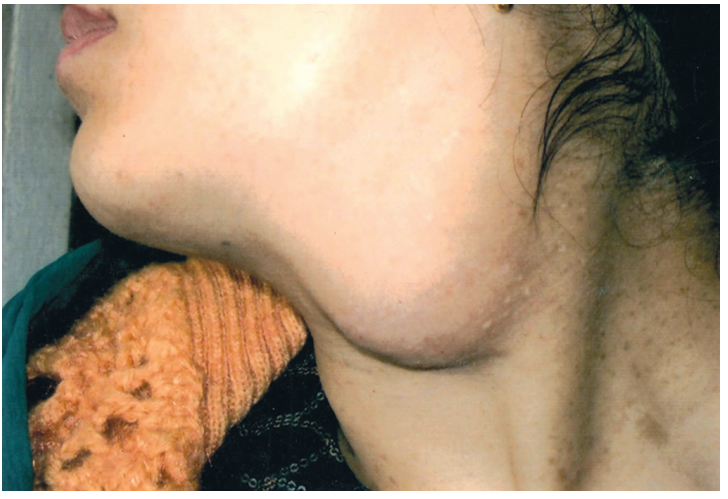

Fig. 60: Shows a patient with left vagal tumour (Schwannoma) which clinically mimics paraganglioma.

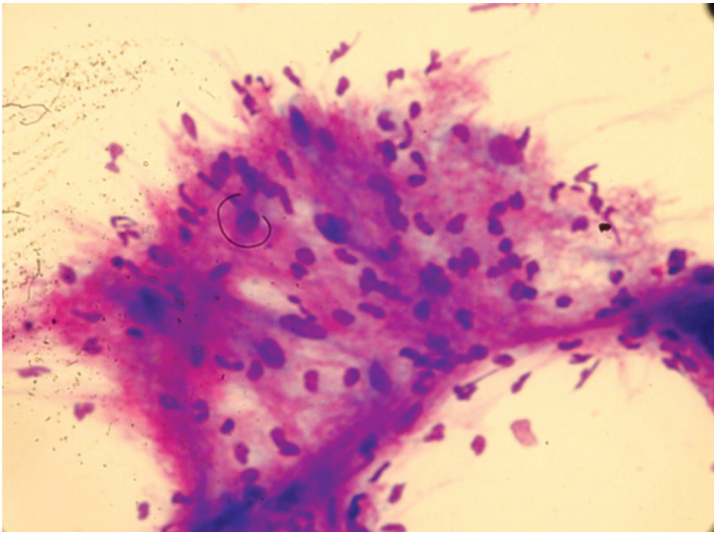

Fig. 61a: Aspiration from the tumour shown in Fig. 60, yielded bloody aspirate which further exaggerated the impression of paraganglioma. The FNAC smears (MGG stained) shown in this slide shows scanty material composed of bland spindle cells adherent to connective tissue stroma, which rules out paraganglioma. The subsequent histology of the tumour confirmed the diagnosis of Schwannoma. MGG HP.

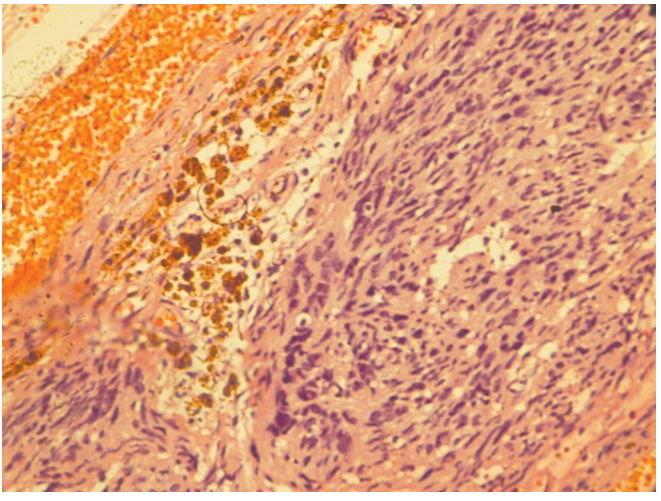

Fig. 61b: Histology of schwannoma of the FNAC smear shown in Fig. 61a, H&E HP.

PATHOLOGY

Histopathology of the excised specimens of HNPs diagnosed on FNAC by the author was mostly reported by pathologists in Departments of Pathology, Govt. Medical College (GMC), Srinagar; Sher-I- Kashmir Institute of Medical Sciences, Soura, Srinagar (SKIIMS). Three cases were operated on and reported by Pathologists at Rajiv Gandhi Cancer Institute and Research Centre, New Delhi (2 cases) and St. Stephen's Hospital, New Delhi (1 case).

The author had the opportunity of examining histology of 18 cases of head and neck paragangliomas including 10 cases of jugulo-tympaninc and 8 cases of carotid body paragangliomas. 6 of these cases were preceded by FNAC diagnosis. These specimens reported earlier on HandE sections were immunostained with chromogranin and S-100 protein during compilation of this data for publication. All the cases were chromogranin and S-100 protein reactive. The description, macroscopic and microscopic, given here is based on the tumours examined by the author.

Macroscopic Appearances

Tumours were well capsulated and measured between 2 cm to 8.5 cm in maximum diameter (Fig. 61). External surface was smooth. The cut surface was soft to firm brownish with focal sclerosis in some tumours (Figs 62 to 64).

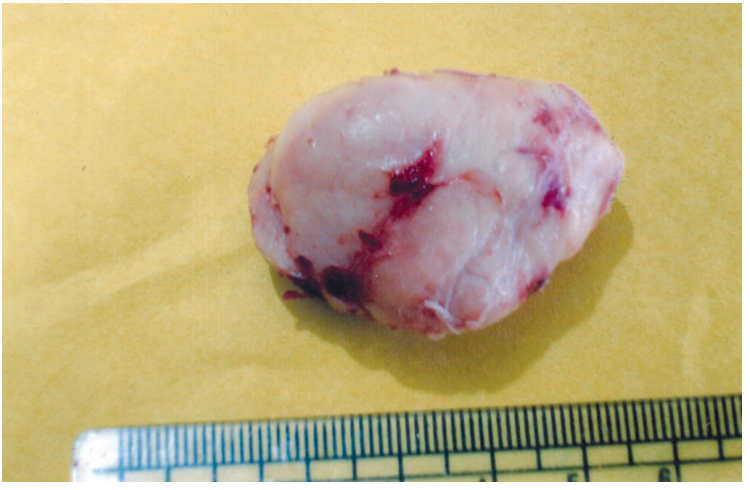

Fig. 62: Surgically excised specimen of carotid body paraganglioma. It is a well capsulated tumour with smooth surface devoid of any adhesions.

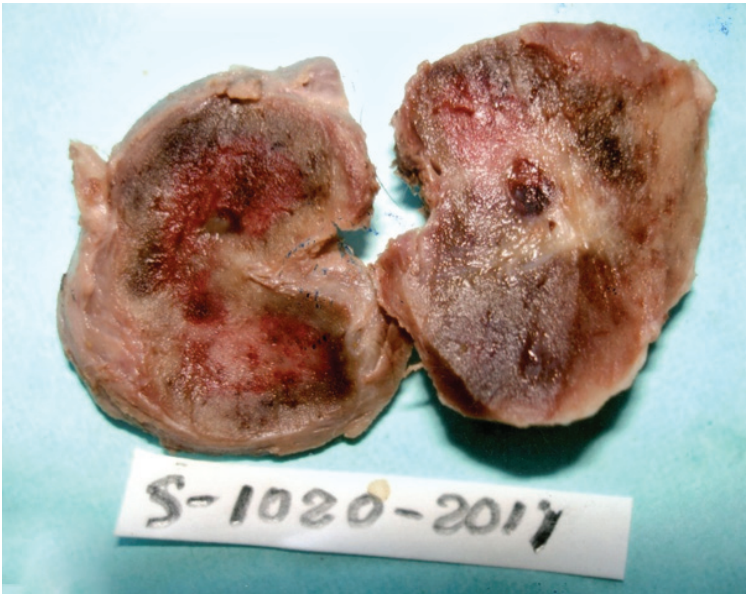

Fig. 63: Cut surface of the tumour in Fig. 61 with haemorrhagic brown appearance. The tumour was firm in consistency.

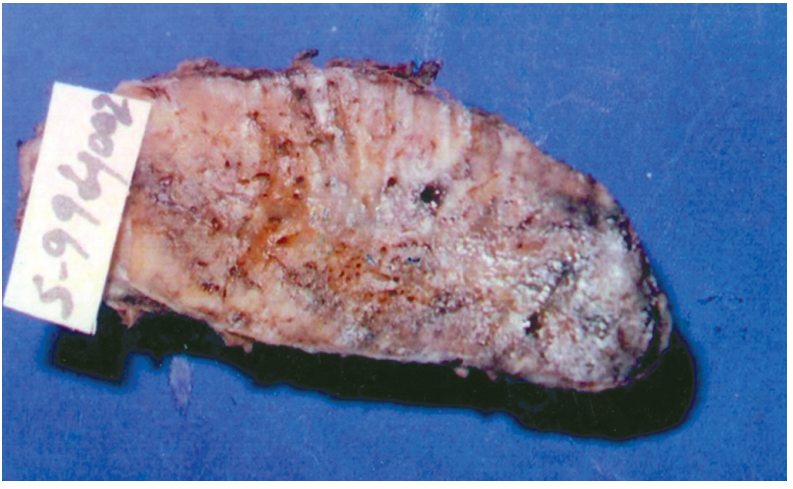

Fig. 64: Another carotid body tumour cut surface

Microscopic Appearances

Tumours showed residual hypertrophic paraganglionic tissue outside the capsule in some tumours (Figs 65 and 66). A capsule of varying thickness was present (Figs 65, 67 and 68). A typical nesting or "Zellballen" pattern with rich capillary stromal network was observed in some tumours (Figs 68 and 69) where as others showed marked vascularity of the stroma (Figs 70 to 73). The latter cases have been designated sometimes as "angiomatous" paragangliomas. Fig. 80 shows a higher power view of the tumour cell nest to highlight details of neuroendocrine cells. Tumour cells are polyhedral with moderate basophilic granular cytoplasm, round to ovoid or spindled nuclei with fine chromatin and thin nuclear membranes. Some nuclei are deeply stained, consistent with degenerate/ancient change (Fig. 80 arrows). One tumour showed abundant stromal sclerosis reducing the tumour nests to cell cords (Fig. 77). Jugulotympanic tumours showed sparse cellularity and less prominent cell nests (Figs 78 and 79). Nuclear pleomorphism was present in some tumours but mitotic figures and tumour necrosis were not observed in any tumour. Diagnosis of paraganglioma was obvious in haematoxylin and eosin sections. The nesting pattern can be highlighted by reticulin stain. Immunohistochemistry was done in some cases for academic purposes only (Figs 82 to 84).

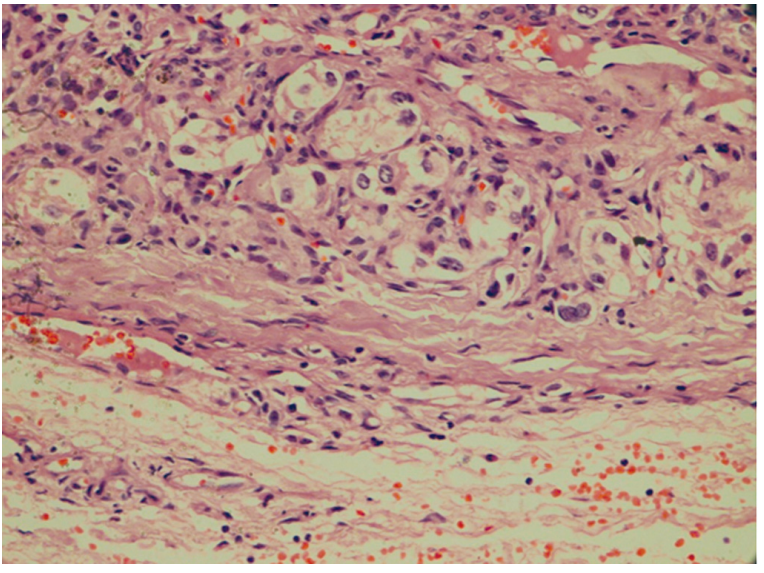

Fig. 65: Microscopic section of carotid body paraganglioma. It has a thin capsule. The residual paraganglionic tissue is seen outside the capsule. H&E HP.

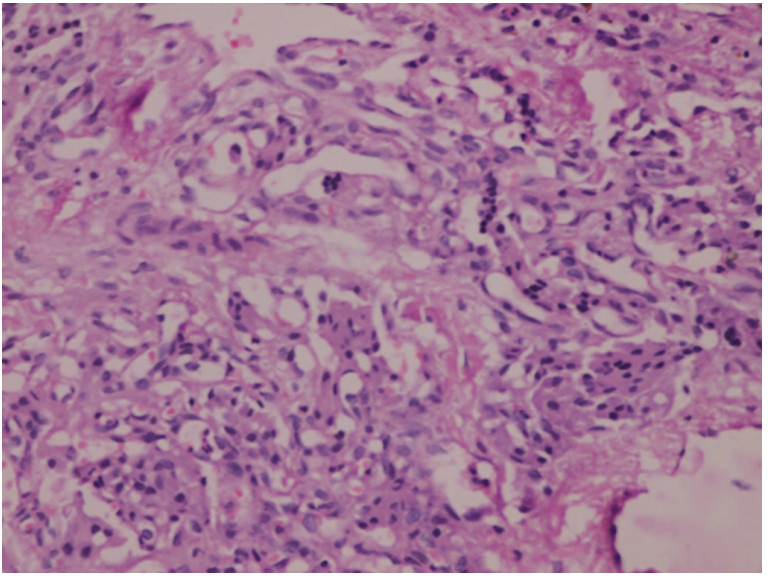

Fig. 66: Residual hypertrophic paraganglionic tissue outside the capsule of another high altitude carotid body paraganglioma. H&E HP.

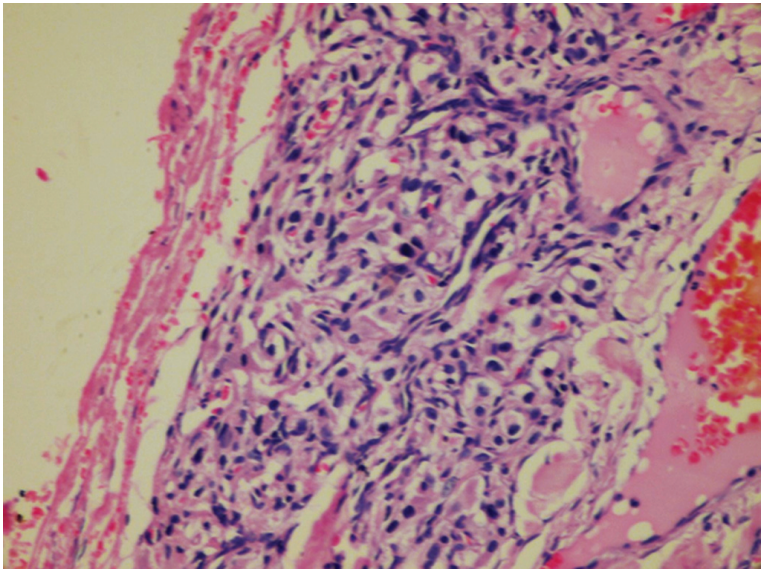

Fig. 67: Carotid body paraganglioma with a thin fibrous capsule. H&E HP.

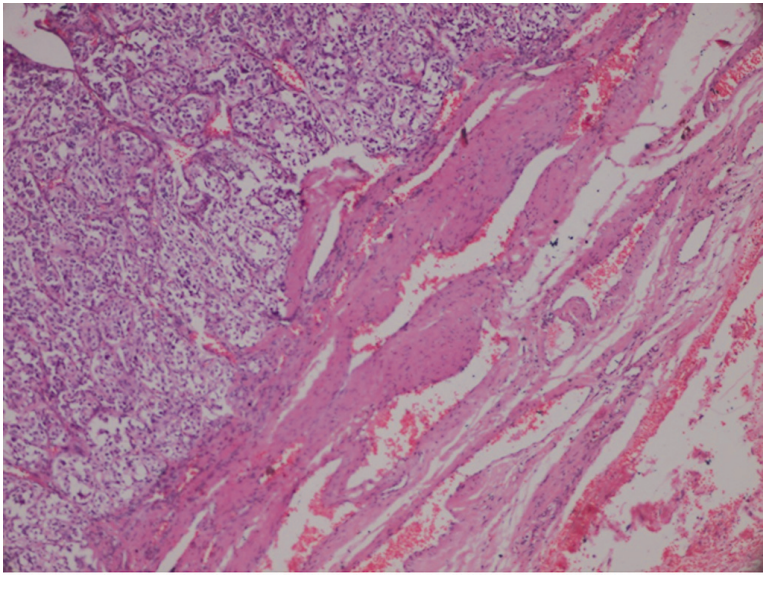

Fig. 68: carotid body paraganglioma with a thick fibrous capsule. H&E LP.

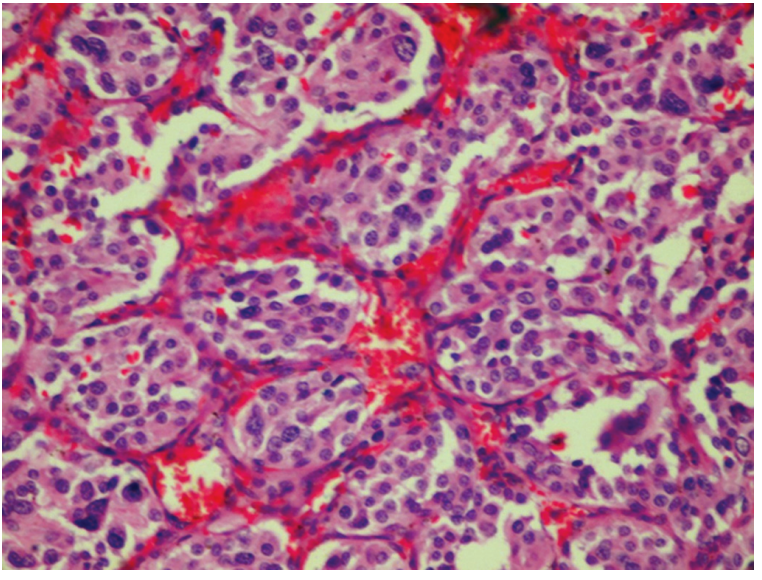

Fig. 69: Microscopic section of a carotid body paraganglioma with typical cell nesting "Zellballen" arrangement and vascular stroma. H&E HP.

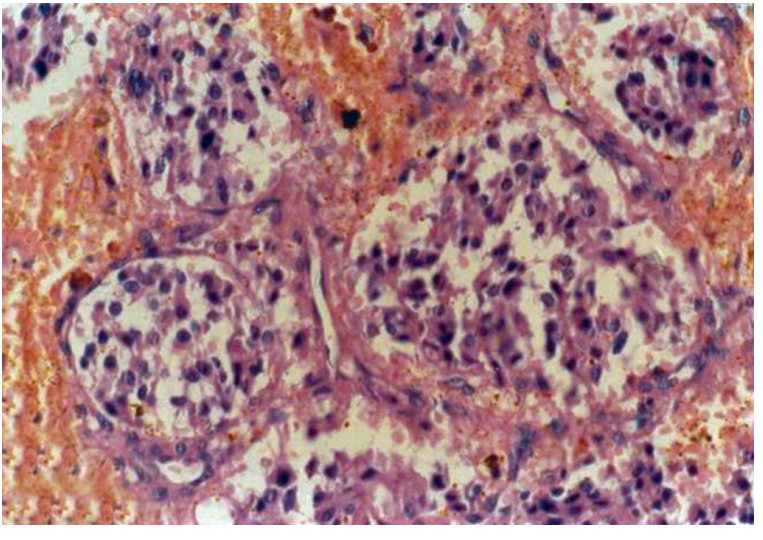

Fig. 70: Microscopic section of paraganglioma wih marked stromal vascularity. The tumour cell nests show glomeruloid appearance. H&E HP.

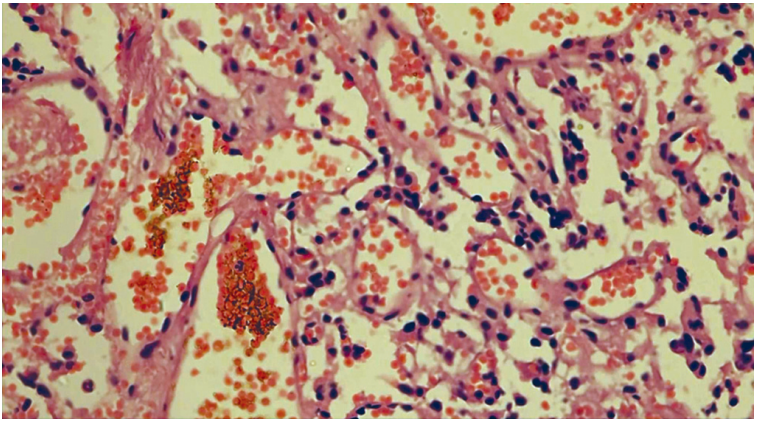

Fig. 71: Microscopic section of carotid body paraganglioma with marked stromal vascularity and inconspicuous tumour cells outlining these vessels. A tumour like this will need immunohistochemistry for confirmation of paraganglioma. H&E HP

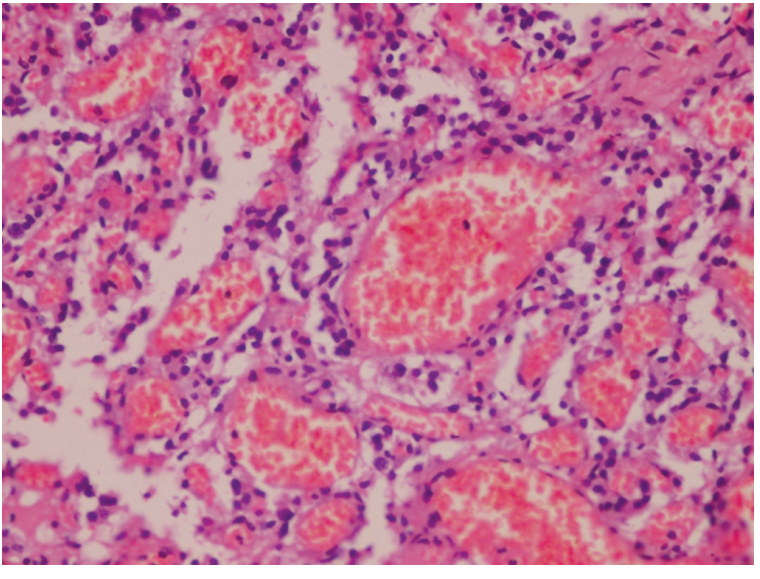

Fig. 72: Microscopic section of carotid body paraganglioma with marked stromal vascularity. H&E HP.

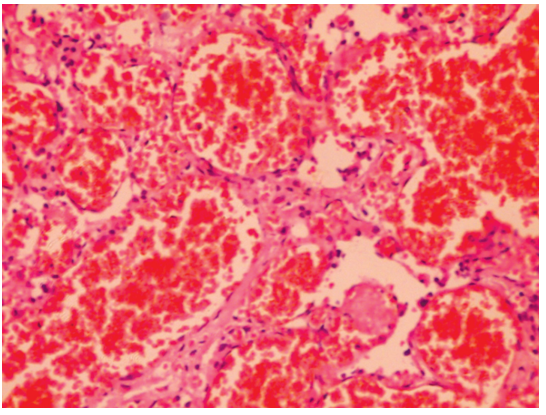

Fig. 73: Microscopic section of carotid body paraganglioma with marked stromal vascularity. H&E HP.

Figures 71 to 73 show carotid body paragangliomas with marked stromal vascularity mimicking hemangioma so called "angiomatous" paragangliomas. The pathologists inexperienced with paragangliomas, can misinterpret these as hemangiomas. It also reflects the problems encountered in FNAC diagnosis of such paragangliomas. However, aspirations form more than one site done carefully, with 24G or 25G needles, can yield aspirates from more cellular areas to clench the diagnosis as was done by the author in all these cases.

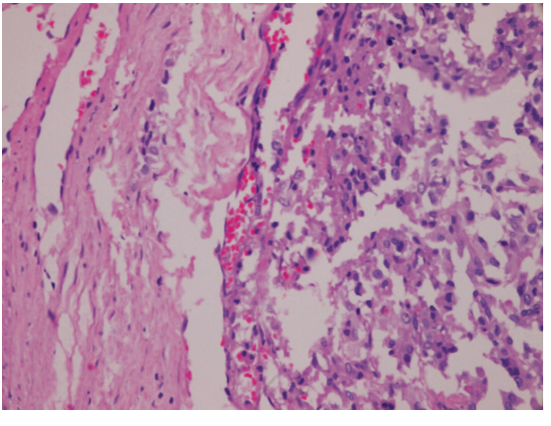

Fig. 74: Histology of carotid body paraganglioma with a thick fibrous capsule and inconspicuous tumour cell nests separated by empty spaces which represent the stromal vessels emptied of bloodduring processing. H&E HP.

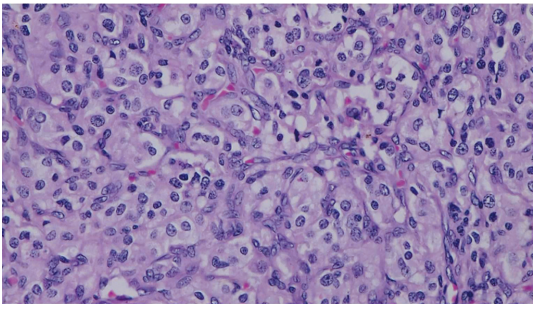

Fig. 75: Histology of carotid body paraganglioma with cell nests and inconspicuous stromal vascularity. H&E HP.

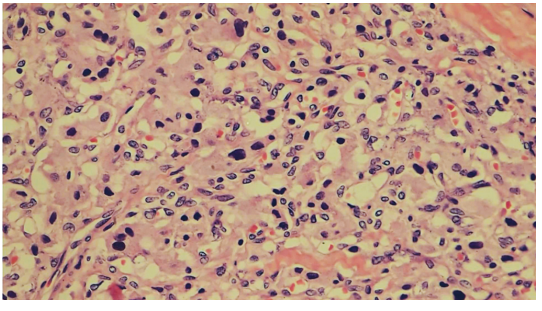

Fig. 76: Histology of carotid body paraganglioma with vague cell nests and inconspicuous stromal vascularity. H&E HP.

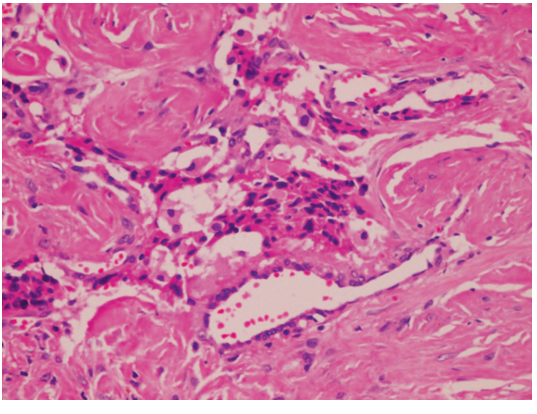

Fig. 77: Histology of sclerotic carotid body paraganglioma shows abundant collageneous stroma with few vessels and scanty tumour cell component compressed into cords. The FNAC smear for this is shown in Figs. 44 and 45. Other areas from this tumour showed typical histology of carotid body paraganglioma. H&E HP.

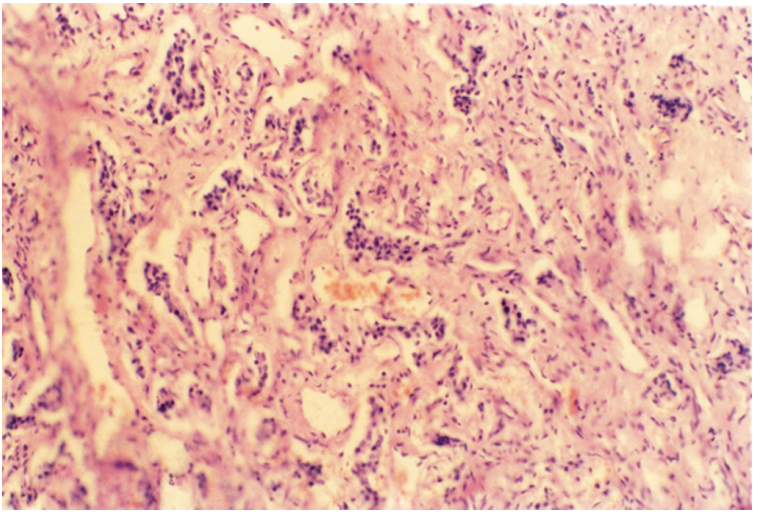

Fig. 78: Microscopic sections from incisional biopsy of aural polyp in the external auditory canal of Jugular paraganglioma in a 40F, shows widely scattered cell nests in abundant collagenous stroma with empty vascular channels. This is typical histology pattern of jugular paraganglioma reported in the literature and observed by the author. This is in total contrast to carotid body paragangliomas invariably showing typical unmistakable nesting. H&E LP.

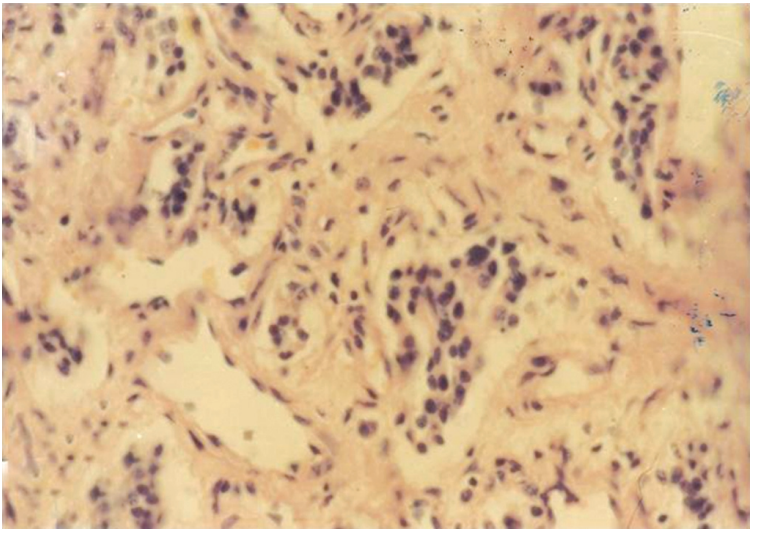

Fig. 79: Higher magnification of tumour shown in Fig. 78. H&E HP.

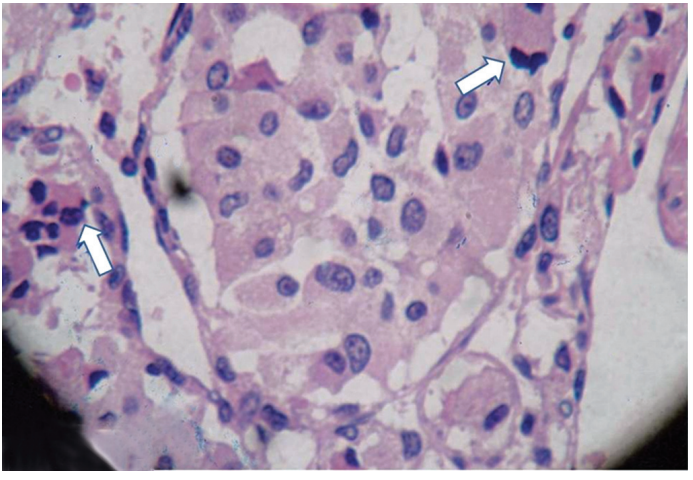

Fig. 80: H&E stained section in higher magnification of carotid body paragangliomas to highlight details of tumour cells. Chief cells in nests are surrounded by thin walled capillaries in the stroma. Tumour cells are polyhedral with bland nuclei and abundant amphophilic cytoplasm. Also seen are deeply stained degenerate nuclei, marked by arrows, similar to the degenerate/ancient nuclei of schwannoma and leiomyoma of uterus. The tumour is conspicuous by the absence of cell necrosis and mitotic figures. The sustentacular cells cannot be made out confidently in H&E sections.

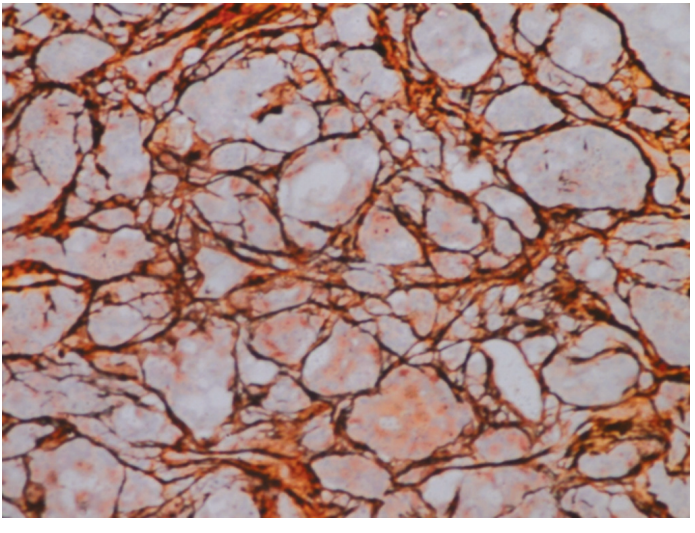

Fig. 81: Reticulin staining of carotid body paraganglioma to highlight nesting pattern of the tumour.

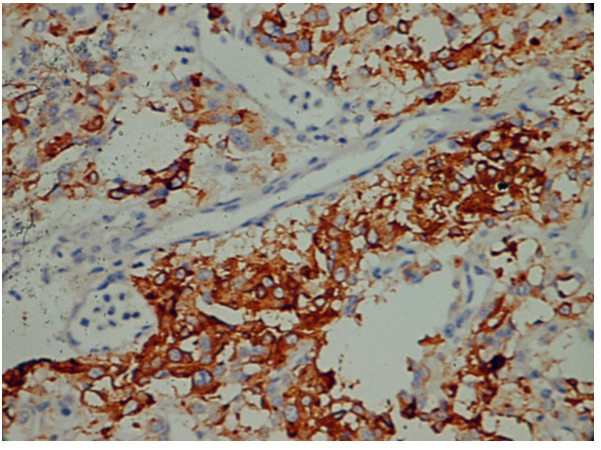

Fig. 82

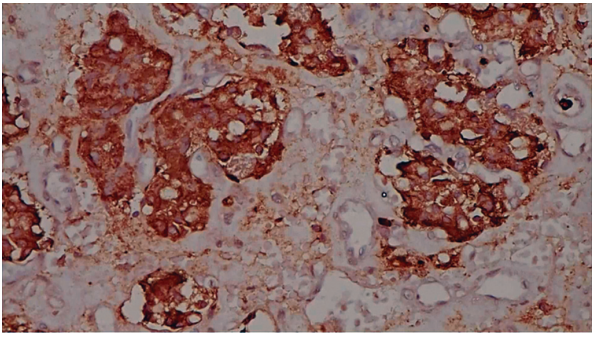

Fig. 83

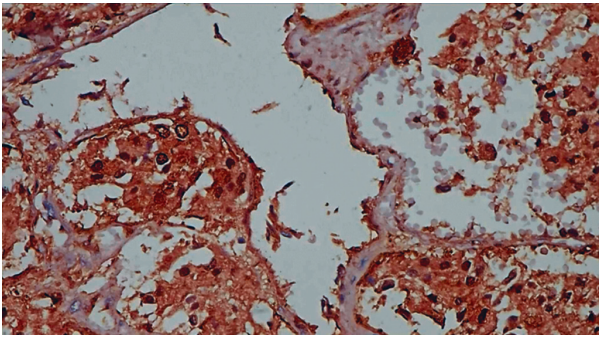

Fig. 84

Figs 82 to 84: Chromogranin staining of carotid body paragangliomas shows immune-reactive chief cells

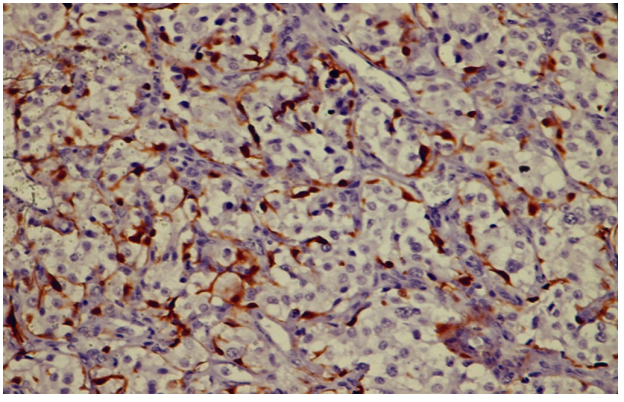

Fig. 85: Carotid body paraganglioma S-100 protein immunostain, shows S-100 positive sustantacular cells at the periphery of cell nests.

DISCUSSION

Head and neck paragangliomas are diagnosed by selective angiography and by imaging techniques like CT and MRI. Angiography is a gold standard, but invasive technique with potential for complications. FNAC of CBP as a preoperative diagnostic procedure was advocated by Conley[29] in 1956. Rarity of these tumours at low altitude coupled with high vascularity has resulted in high false negativity of 30–54% and even higher.[30,34,43] FNAC has been accepted as a preoperative diagnostic procedure for head and neck paragangliomas (HNPs) by most cyto-pathologists. However, a few[9,30] are against the use of FNAC as pre- operative diagnostic procedure. Preoperative FNAC diagnosis was made on 171 paragangliomas from 168 patients, from head and neck and non-head and neck sites, in this study over a period spanning more than 32 years (May 1984 - 31 Dec 2016) on more than 100,000 FNACs conducted and reported by the author (Table 4). There were 171 head and neck paragangliomas, 5 retroperitoneal and 1 urinary bladder paraganglioma. In the head and neck category 171 tumours included 167 CBPs from 164 patients with three bilateral tumours, 2 JTPs and 2 VPs. These are all high altitude head and neck paragangliomas. The four largest series of FNAC on low altitude HNPs are those of Engzell et al.[30], Lack et al.[6], Fleming et al.[42], Kapila et al.[43] and Mondal.[44] Engzell et al.[30] in retrospective analysis of 13 CBPs made correct diagnosis in 7 cases and incurred 1 mortality related to FNAC procedure. Lack et al conducted FNAC on 19 head and neck paragangliomas including 15 patients of CBP and 4 patients

of VPs and diagnosis was suggested in 6 out of 15 CBPs and 3 out of 4 VPs with overall correct diagnosis in 47.36% of cases. Overall the false negative rate has been high in all these studies. The author has earlier reported a series of 29 cases of high altitude HNPs, with correct preoperative FNAC diagnosis in all the cases.[10]

The smear patterns of head and neck paragangliomas have been discussed above (Figs 39 to 59). Before making a diagnosis of paraganglioma a number of other possibilities are to be considered and to be ruled out on cytomorpholgy while bearing in mind that head and neck paragangliomas occur over a wide age range. Various tumours in neck regionthat need to be ruled out on morphology include metastatic medullary carcinoma and metastatic papillary carcinoma of thyroid, metastatic adenocarcinoma poorly differentiated, non-Hodgkin's lymphoma, Ewing's sarcoma/peripheral neuroectodermal tumour, rhabdomyosarcoma and metastatic small cell carcinoma. The thyroid papillary and medullary carcinomas share the nuclear characteristics with paragangliomas but overall cell arrangement and cytoplasmic characteristics are different from the latter tumour (Figs 56 to 58). Metastatic adenocarcinoma show malignant nuclei with irregular chromatin clumps and irregular dense nuclear membrane and variable haemorrhage and necrotic debris in the background (Fig. 59).

Non-Hodgkin's lymphoma shows proliferation of monotonous non-cohesive round cells with lymphoglandular bodies in the background (Fig. 55). Ewing's sarcoma/PNET (Fig. 59a) show population of small cells with deeply stained dark nuclei and larger pale cells with round to oval nuclei having fine chromatin and pale vacuolated fragile wispy ill-defined cytoplasm. The background is often lacy or tigroid representing glycogen released from disruption of tumour cells. Rhabdomyosarcomas (Fig. 59b) show wide variation in tumour cells and malignant tumour diathesis. Tumour cells vary from small undifferentiated round and spindled cells with hyperchromatic nuclei and scanty cytoplasm to more differentiating rhabdomyoblasts, larger in size with more abundant pink cytoplasm and eccentric hyperchromatic nuclei, many mitotic figures and necrotic/haemorrhagic background. Metastatic small cell carcinomas (Fig. 59c) show small undifferentiated tumour cells in loose clusters and individually dispersed. Tumour cells show high nucleocytoplasmic ratio with scanty ill-defined cytoplasm with coarse granular chromatin, nuclear moulding, many mitotic figures

and karyorrhectic debris. Schwannoma has occasionally been reported in some studies to mimic paraganglioma[30] (Figs 60 and 61).

Paragangliomas in contrast to these tumours show mild to marked anisokaryosis but nuclei are bland with regular chromatin without irregular chromatin clumping or dense irregular nuclear membranes. Background is typically clean without malignant tumour diathesis.

The author has diagnosed these tumours on cytomorphology alone without help of immunocytochemistry owing to high prevalence of head and neck paragangliomas in this high altitude region of Kashmir province including a few cases from other hilly regions of the state. This may not be possible for cytopathologists practising at low altitudes who rarely come across these tumours because of their rarity at low altitudes. At low altitudes carotid body paraganglioma is the one likely to be subjected to FNAC. A few suggestions that can be helpful are as follows:

Carotid body paraganglioma patients are of wide age range with painless swelling usually of long duration at carotid bifurcation. Aspirates are haemorrhagic. Suction is to be stopped as soon as blood appears in the needle hub. The next step would be to smear the aspirate quickly on multiple slides. Smearing of aspirate material is most crucial for final outcome of results and once achieved, subsequent staining and interpretation become easy. Success in smearing the bloody aspirates has helped the author to make easily interpretable smears, as is evident from the figures seen in this atlas, and identify rare cases like metastatic paragaglioma to sternum and paragaglioma urinary bladder (see sections 2 and 3 of this atlas).

Once paraganglioma is suspected in smears by cytopathologists, who may rarely encounter carotid body paragangliomas in the head and neck region or elsewhere, confirmation can be achieved by applying immunostains like chromogranin, S-100P and cytokeratin directly on smears.

Paragangliomas have two types of cells

i. Endocrine/chief cells which are stained with immunostains like chromogranin, synaptophysin and NSE and are non-reactive to cytokeratin. This is in contrast to other neuroendocrine tumours which react with pan neuroendocrine markers and low molecular weight cytokeratin
ii. Sustentacular cells which are stained with immunostain S-100P

SUMMARY AND CONCLUSION

This is a preoperative FNAC report of 171 head and neck paragangliomas from 168 patients diagnosed over a period of more than 32 years (May 1984 - 31 Dec 2016), recorded on more than 100,000 FNACs conducted and reported by the author. The head and neck paragangliomas numbered 171 tumours from 168 patients with a wide age range (11–90 years), high female sex predilection (142/168 patients - 84.5%) and without significant side predilection (84 left, 81 right and 3 bilateral CBPs). These were sporadic, non-hereditary, rarely multicentric (one case) and non-functional paragangliomas. There was one case of malignant CBP at the time of diagnosis. In the operated group a brief follow up showed local recurrence in one case (21 M, CBP) and local recurrence with regional lymph node metastasis in another case (11 F, CBP). In the un-operated group one patient (40 F, JTP) died of invasion of base of brain two years after diagnosis. Out of 111 patients subjected to surgery, there was one surgery related mortality. There were no false-positive cases in this study.

This study supports the dictum of high prevalence of head and neck paragangliomas at high altitude. The basic stimulus being chronic hypoxemia leading to chief cell hyperplasia and neoplasia.

There were 164 cases of carotid body paragangliomas out of a total of 168 head and neck paragangliomas. If we include other cases of head and neck paragangliomas reported by the author during this period without prior FNAC, which included 8 cases of jugulo-tympanic paragangliomas and 2 cases of carotid body paragangliomas; then the total number of head and neck paragangliomas becomes 178 cases. This is explained by high prevalence of carotid body paragangliomas at higher altitudes. CBP is also the most common head and neck paraganglioma at low altitudes but comprises only 60% of the tumours. This reflects higher prevalence of carotid body paragangliomas at high altitudes and also shows that jugulo-tympanic paraganglioma is the second and vagal paraganglioma the third common paraganglioma at high altitudes.

This is the largest study of preoperative FNAC diagnosis of head and neck paragangliomas in literature. It also highlights the role of FNAC in preoperative diagnosis of HNPs in experienced hands. The anatomical background in well smeared and well stained haemorrhagic smears shows a fairly characteristic cytomorphology to suggest the diagnosis.

FNAC in all these cases was conducted as an office/OPD procedure without any FNAC related complications.

This atlas was expected to be published in late 2019 but due to COVID-19 it got delayed till this date. The Author takes this opportunity to add another 25 cases of carotid body paragangliomas diagnosed on FNAC over last 6 years (2017–2022) as given under:

2017	7 cases	35F, 50F, 45F, 65F, 36F, 45F, 24F
2018	2 cases	62M, 47F
2019	6 cases	50F, 57F, 21F, 50F, 37F, 65F
2020	3 cases	34F, 55F, 40F
2021	5 cases	46F, 50F, 26F, 65F, 70F
2022	2 cases	50F, 70F