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

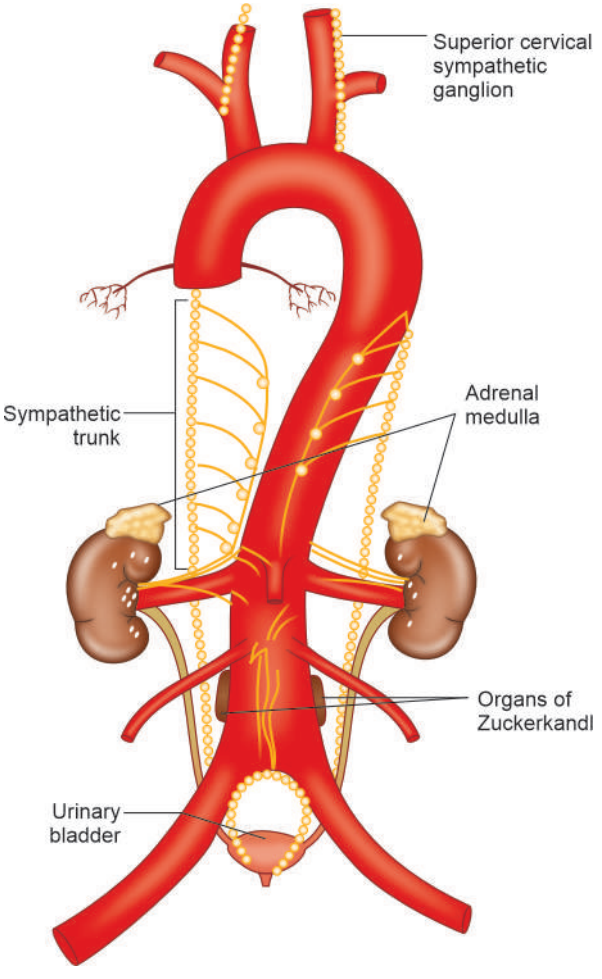


Fig. 1: Anatomical distribution of sympathoadrenal paraganglia Adapted from Mills, Stacey E. Histology for Pathologists. United States of America: Wolters Kluwer Health, Inc.; 2012, *with permission*

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

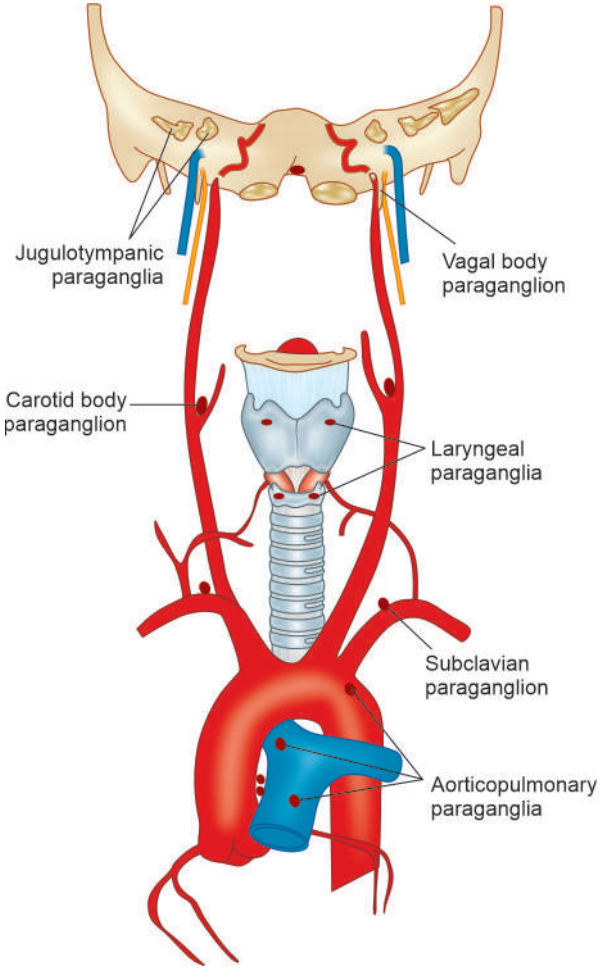


Fig. 2: Anatomical distribution of parasympathetic paraganglia
Adapted *from* Fletcher, Christopher DM Diagnostic Histopathology of Tumors. United States of America: Elsevier Science and Technology Journals; 2013, *with permission*.

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

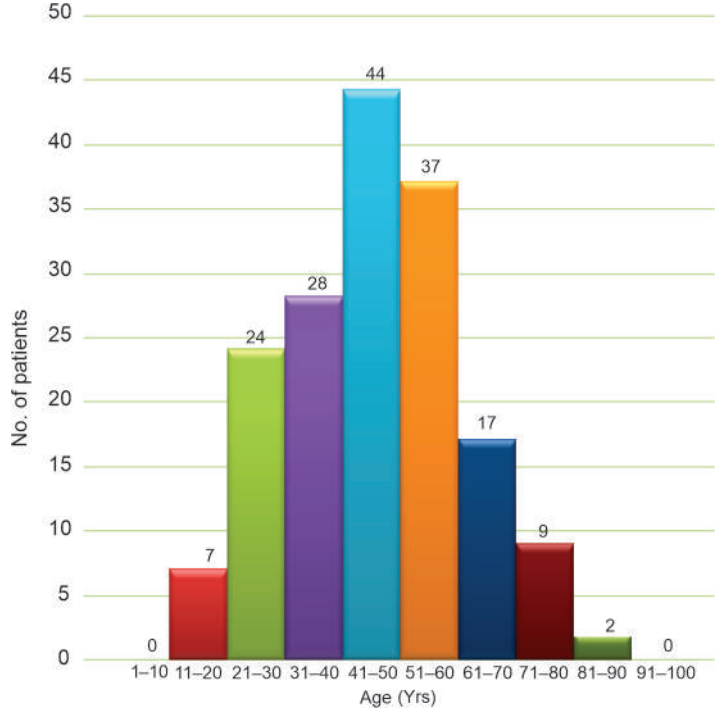
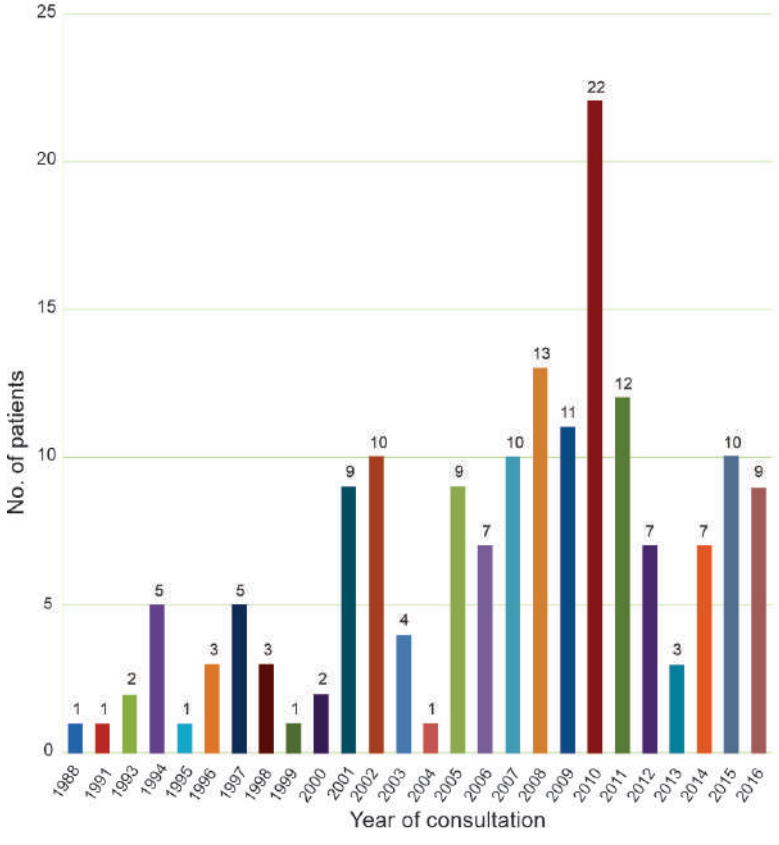


Table 7: Year wise distribution of patients of head and neck paragangliomas

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.

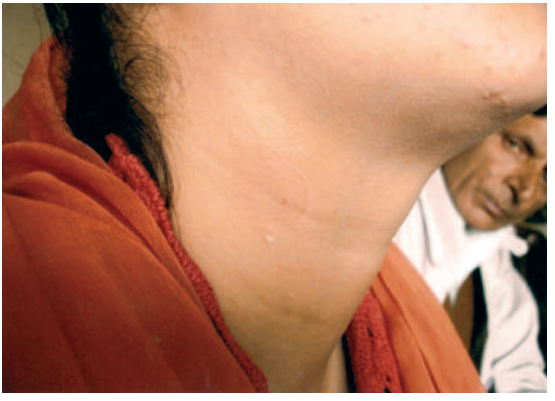


Fig. 3

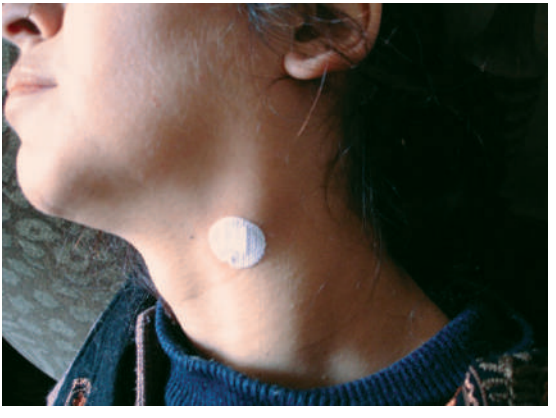


Fig. 4

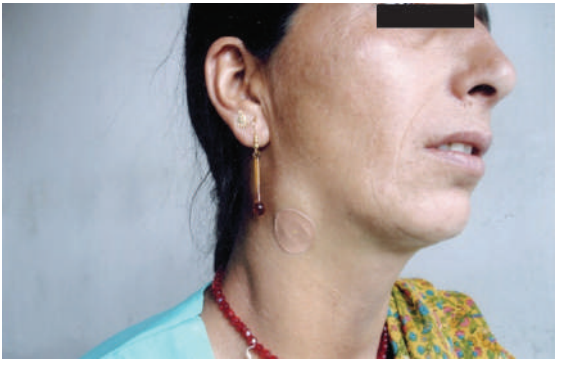


Fig. 5

Fig. 6

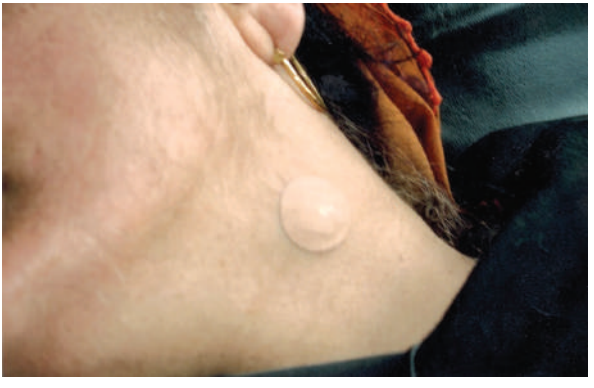


Fig. 7

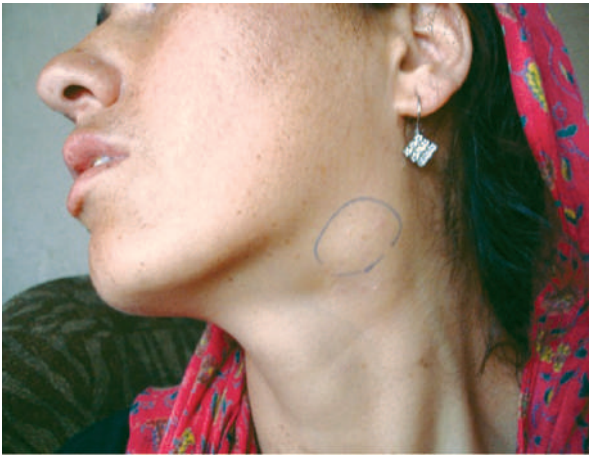


Fig. 8

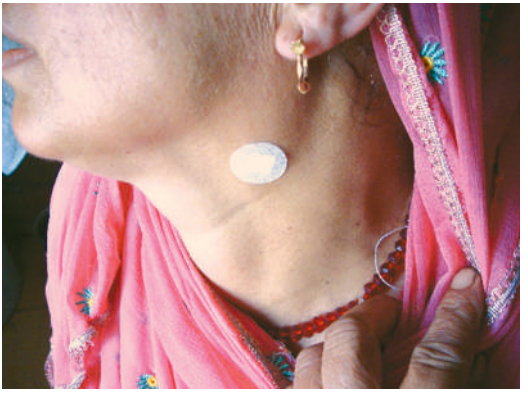


Fig. 9

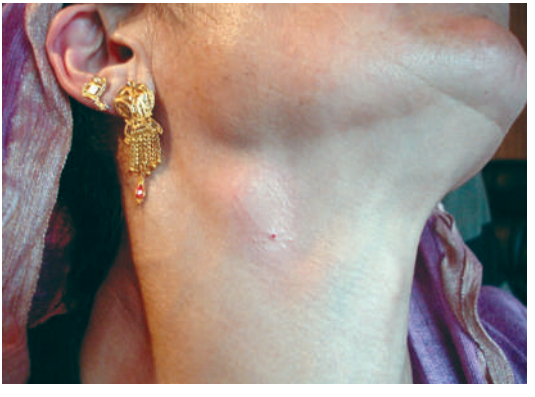


Fig. 10

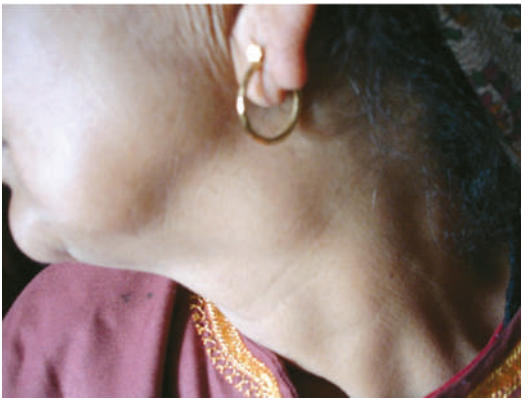


Fig. 11

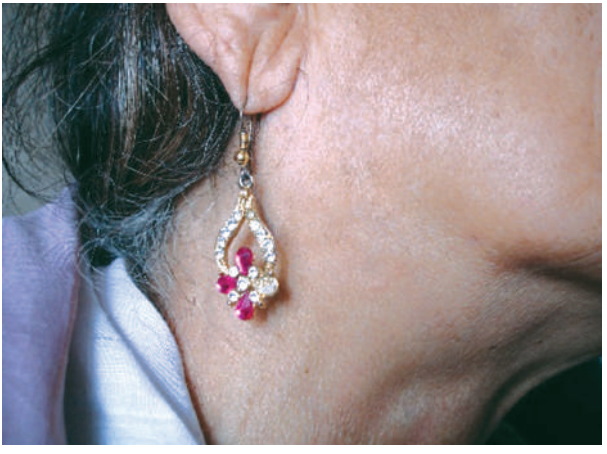
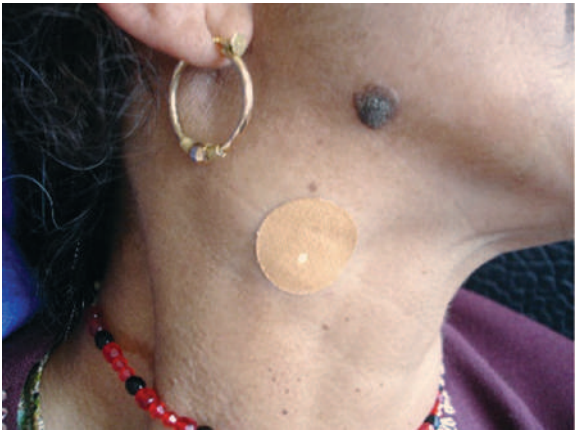


Fig. 12

Fig. 13

Fig. 14

Fig. 15



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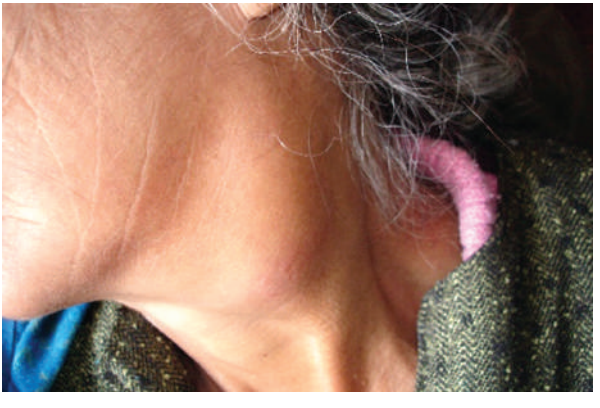

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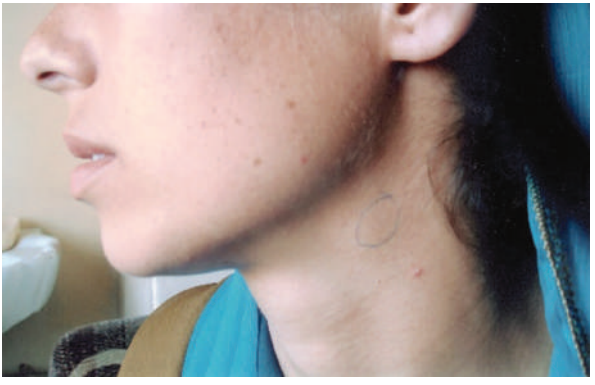


Fig. 18

Fig. 19

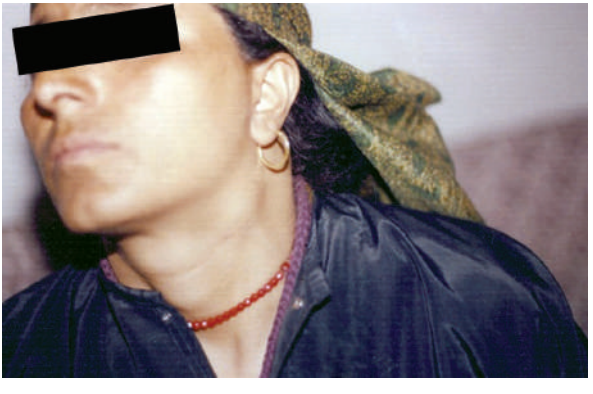


Fig. 20

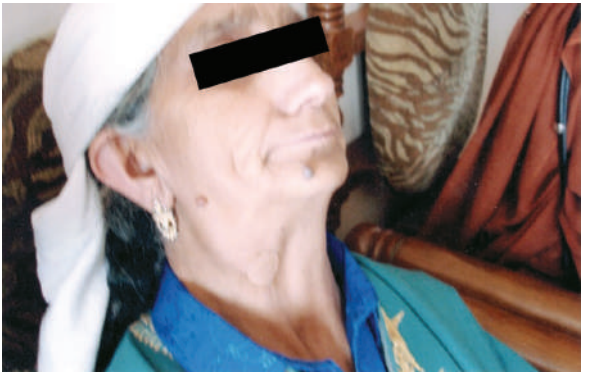
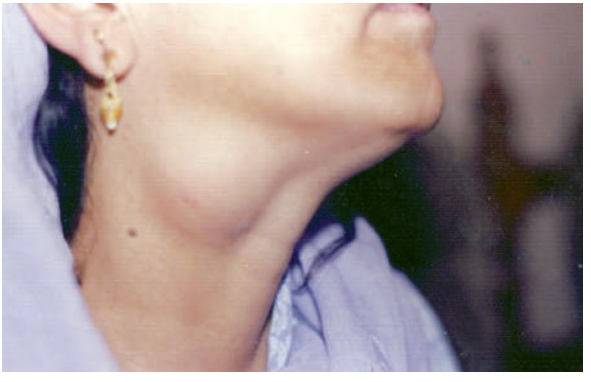


Fig. 21



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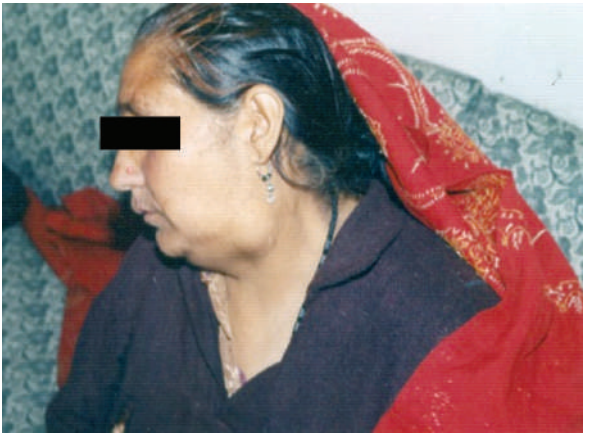
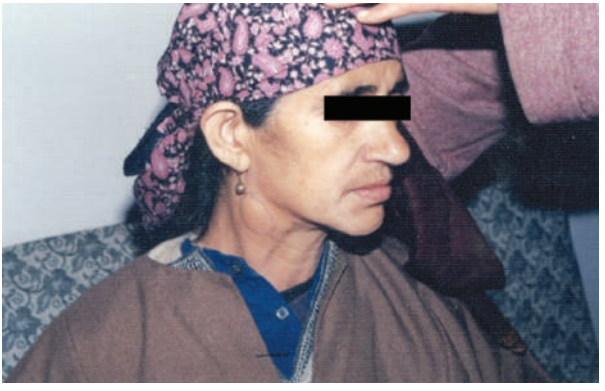


Fig. 23



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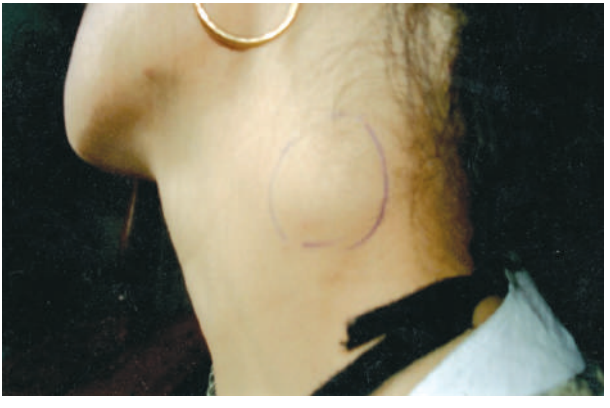

Fig. 25

Fig. 26

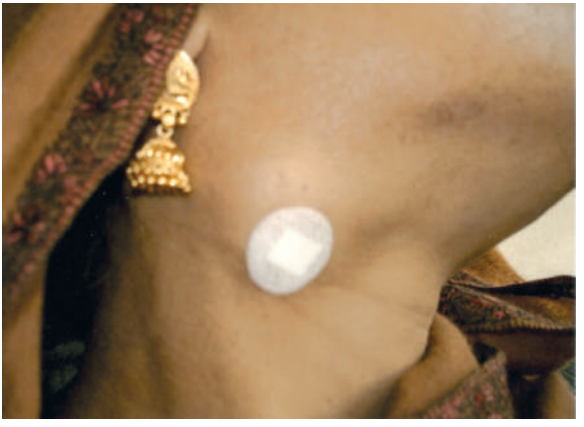

Fig. 27

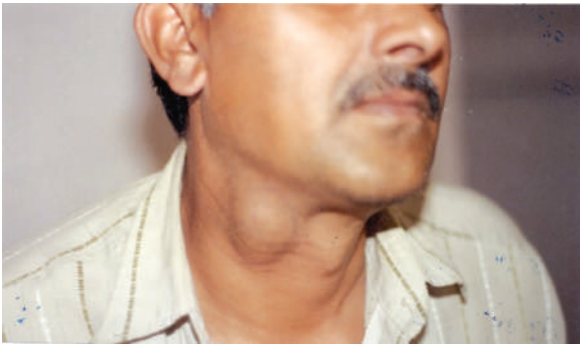

Fig. 28

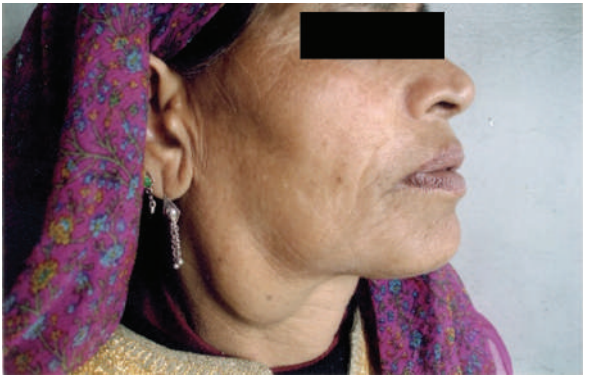

Fig. 29

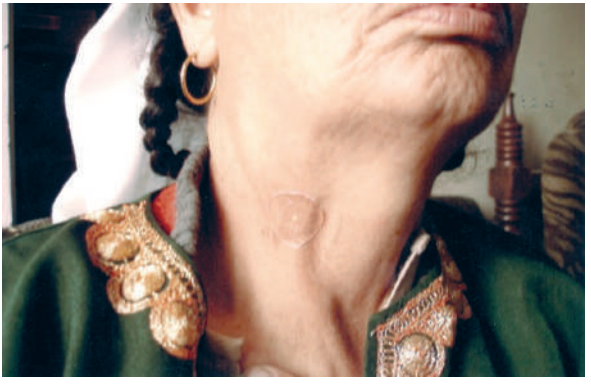

Fig. 30

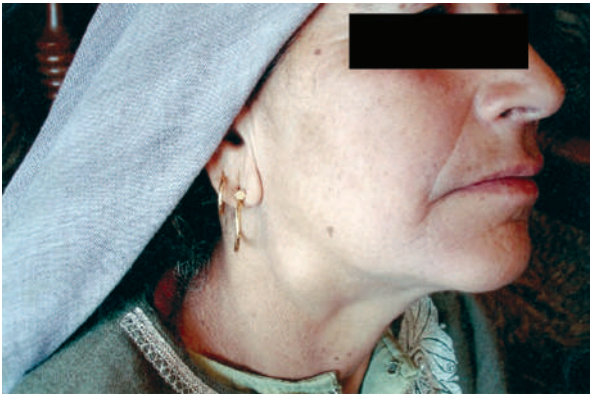

Fig. 31

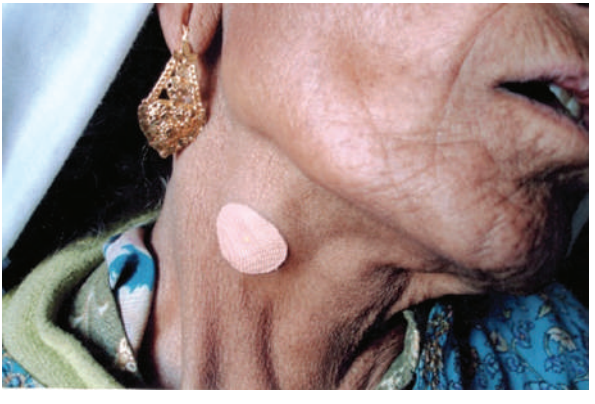

Fig. 32

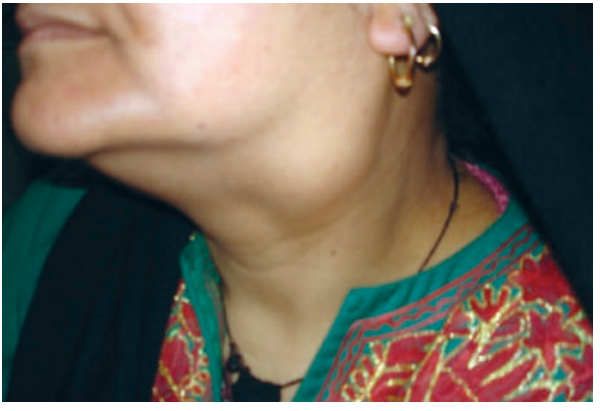

Fig. 33

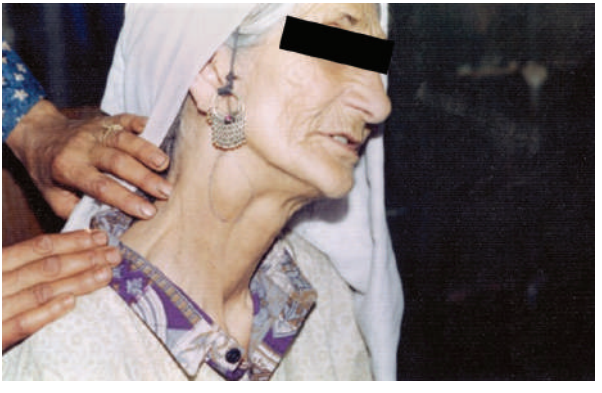

Fig. 34

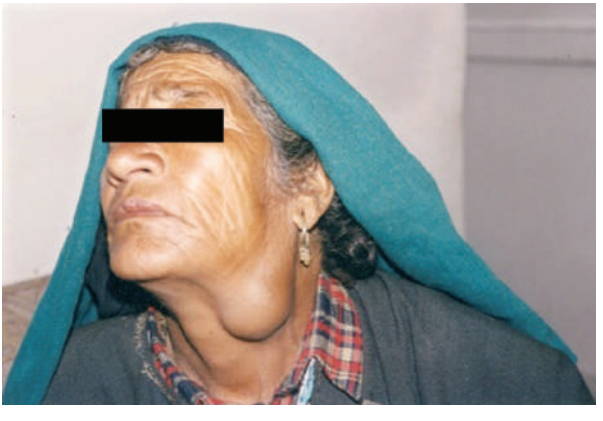

Fig. 35