The 2004 WHO classification of endocrine tumors restricts the term ‘Pheochromocytoma’ (PHEO) to define a tumor arising from catecholamine producing chromaffin cells in the adrenal medulla which is also called ‘intra-adrenal’ paraganglioma (PGL). Closely related tumors of extra-adrenal sympathetic and parasympathetic paraganglia are classified as extra-adrenal PGL. Over the last decade, major advances have been made in understanding the nature of this disease which has translated into drastic improvements in patient care. This review focuses on recent advances in the management of PHEO/PGL.

BIOCHEMICAL DIAGNOSIS

The first step in the evaluation of a suspected case of PHEO/PGL is biochemical confirmation of catecholamine excess status. In contrast to PHEO and sympathetic PGL (mainly in abdomen, pelvis and thorax), those arising from parasympathetic tissue (mainly in the head and neck, referred as HNPGL) rarely produce significant amounts of catecholamines.

Currently, the commonly used tests for biochemical diagnosis of catecholamine excess status in India are 24-hour urinary Vanillyl Mandelic Acid (VMA), 24-hour urinary total metanephrines and serum catecholamines. However, these tests are limited by their poor sensitivity (Table 1) and can lead to misdiagnosis.

Technical advances and improved understanding of catecholamine metabolism have led to the discovery of better biochemical tests with optimum diagnostic sensitivity. It is now clear that catecholamines are metabolized within chromaffin cells to metanephrines (i.e. norepinephrine to normetanephrine and epinephrine to metanephrine). This intratumoral process occurs independently of catecholamine release, which can occur intermittently or at low rates. Consistent with these concepts, studies have confirmed that measurements of fractionated metanephrines (i.e. normetanephrine and metanephrine measured separately) in urine or plasma provide superior diagnostic sensitivity to measurements of the parent catecholamines.

In 2002, a large study by Lenders et al unquestionably proved that measurement of plasma free metanephrines (PFMN) by HPLC is the best test with both high sensitivity (98%) as well as specificity (89%). Although 24-hour urinary fractionated metanephrines test had a similar sensitivity (97%), it had lesser specificity (75%).

It is important to note that the accuracy of the test is dependent on appropriate collection and storage of the sample for the test. The blood sample should be collected from the patient being in resting supine state for half an hour. It should be stored at 2-8°C until centrifuged to separate the plasma.

| Table 1: Sensitivity and Specificity of traditionally used biochemical tests for PHEO/PGL |
|-----------------------------------------------|---------------|---------------|
| Test                                          | Sensitivity   | Specificity   |
| 24 hr urinary VMA                             | 77%           | 93%           |
| 24 hr urinary total metanephrines             | 64%           | 95%           |
| Serum catecholamines                          | 83%           | 81%           |
within two hours after blood collection. The plasma sample can be stored at 2-8°C for up to 6 hours until it is assayed, for longer periods (up to 6 months) at -20°C.

False positive results may be obtained due to inappropriate sample collections. In addition, drugs like tricyclic antidepressants and phenoxybenzamine can lead to false positive elevation of PFMN and hence, should be avoided. It is more common to encounter false positive results when tested in patients with low risk of PHEO. In these patients, false positives can be minimized by increasing the cut off by two to four folds. In borderline cases, clonidine suppression test may be used to rule out a false positive test.

In addition to its high accuracy, PFMN test has other advantages. PFMN values are not affected by factors like diet, age and renal function status. As opposed to the 24 hr sample collection for urinary metanephrines, which is a tedious process for the patient with a risk for unreliability due to incomplete collection, PFMN tests is relatively easier. However, the analysis still remains difficult due to low concentration of free metanephrines in plasma and emphasizes the use of advanced techniques for correct estimations. Furthermore, the availability of the test is limited.

PFMN are best estimated by HPLC or LC-MS/MS methods. Other simple methods like enzyme immunoassay (EIA) have also demonstrated similar performance. Recently, we have also shown high specificity (94%) and sensitivity (94%) of PFMN by EIA. This test has recently become available in India and is currently the best test available for biochemical diagnosis of PHEO in the Indian scenario.

Biochemical phenotype may also be useful to predict the site and syndromic association, thereby helping in genetic testing. Predominantly epinephrine (metanephrine) secreting tumors are almost always located in adrenal glands and are commonly encountered with multiple endocrine neoplasia (MEN)-2 syndromes. Only norepinephrine (normetanephrine) secreting tumors arise either from adrenal medulla or other sympathetic ganglia which are located in the pelvis, abdomen and thorax along the paravertebral area. They are usually associated with von Hippel Lindau (VHL) and SDH (Succinate dehydrogenase) related syndromes. More recently, biochemical phenotype also been shown to predict malignancy. Isolated or co-secretion of dopamine (best assessed with plasma or urinary methoxytyramine) is associated with higher risk of malignancy.

**DIAGNOSTIC LOCALIZATION**

Localization of PHEO should be attempted using at least two imaging modalities. Anatomical imaging studies [computed tomography (CT) or magnetic resonance imaging (MRI)] should be combined with functional (nuclear medicine) imaging studies for optimal results. Functional imaging helps to confirm the primary tumor and locate multiple tumors or metastatic foci.

CT and MRI localize PHEO with high sensitivity, but have less than optimal specificity. As these imaging modalities are currently less expensive, less time consuming and more readily available than functional imaging studies, they should be used as first line imaging modalities. In addition, they should always be carried out over the abdomen first, because PHEO are mostly situated within the adrenal medulla followed by extra-adrenal abdominal ganglia.

Unlike previously used ionic contrast, the currently used nonionic contrast does not pose a significant risk of a hypertensive crisis. With elimination of this risk, contrast enhanced CT may be preferred over MRI due to lower cost of the latter. However, MRI has the advantages of high sensitivity in detecting adrenal disease (93–100%) and extra-adrenal disease especially intra-cardiac, juxta-cardiac and juxta-vascular PHEO, better specificity, lack of exposure to ionizing radiation, optional use of contrast agent, and better delineation of vascular invasion.

Metaiodobenzylguanidine (MIBG) scan is considered the gold standard test for functional imaging of PHEO. MIBG is an aralkylguanidine that resembles norepinephrine. Radioactive labeling is performed with the iodine isotopes 131I and 123I. Like norepinephrine, MIBG is taken into sympatho-medullary tissues by a noradrenergic transporter system and into intra cytoplasmic vesicles through a vesicular monoamine transporter (VMAT) system. MIBG is thus accumulated within adrenergic tissues. 123I-MIBG scintigraphy has better sensitivity (83–100%) than 131I-MIBG scintigraphy (77–90%) but both the scans have similar specificity (95–100%).

With the introduction of PET tracers like 18F-FDG, 18F-FDOPA and 18F-FDA, major revolution has occurred in the field of functional imaging of PHEO. Although one study has shown almost similar sensitivity of all scans (18F-FDOPA: 81%, 123I-MIBG: 77%, 18F-FDA: 77%, 18F-FDG PET/CT: 78%) for non-metastatic PHEO, another study has shown better sensitivity of 18F-FDOPA (90%) than 123I-MIBG (65%). For adrenal and extra-adrenal abdominal tumors, 123I-MIBG has similar sensitivity (94%) as that of 18F-FDOPA (97%). However, for thoracic and cervical tumors 123I-MIBG has lower sensitivity (15%) than 18F-FDOPA (100%). For metastatic PHEO, 18F-FDA (76%) and 18F-FDG PET/CT (74%) had better sensitivity than 123I-MIBG (57%) and 18F-FDOPA (45%).

Among the above mentioned scans, currently, only 131I-MIBG and 18F-FDG PET/CT are commonly available in India (18F-FDOPA is available in select centers like AIIMS, New Delhi). 18F-FDG is a nonspecific tracer. Hence, 131I-MIBG is still the functional imaging of choice in India. Whenever
malignancy is suspected, 18F-FDG PET/CT may be considered as an additional investigation.

**MANAGEMENT**

Appropriate α-blockade (10-14 days) still remains the gold standard of preoperative management and is often combined with β-blockade especially if there is α-blocker induced tachycardia. Calcium channel blockers have also been found to be as effective as α-blockers for preoperative medical management of PHEO. Volume expansion is recommended both before and after surgery.

Excision of both adrenal glands in patients with bilateral PHEO leads to a state of primary adrenal insufficiency which needs lifelong replacement of glucocorticoids and mineralocorticoids. It also poses the risk of adrenal crises which may be life threatening. An important advance in the field of pheochromocytoma management is use of surgical sparing adrenalectomy (partial adrenalectomy) for bilateral PHEO. In the hands of experienced surgeons, it effectively preserves adrenal function in more than 80% patients. However, there is a concern of increased recurrence rate with partial adrenalectomy.

Even after many advances in the field of PHEO, the results of treatment of malignant PHEO still remains dismal. 131I-MIBG therapy is the most widely studied nonsurgical therapy for malignant PHEO and is considered the most useful treatment for patients with unresectable tumors. Clearly, 131I-MIBG therapy is not curative, but some evidence suggests a high-dose regimen can result in sustained complete remission in a small number of patients. Its high side effect profile at higher doses and low improvement in overall survival make it less than ideal, but it remains the best nonsurgical option to date. The most widely used systemic chemotherapy regimen for malignant pheochromocytoma is CVD therapy. It has no proven survival benefit and its use is mostly reserved for patients with unresectable disease who are symptomatic from catecholamine excess and had no response to 131I-MIBG therapy. With dismal results of 131I-MIBG therapy and chemotherapy, there is a constant search for new drugs that are effective for the treatment of malignant PHEO. Tyrosine kinase inhibitors like Sunitinib and m-TOR inhibitors like Everolimus have been tried with minimal success.

**GENETIC TESTING**

Genetic testing plays an important role in the management of PHEO/PGL. In a given PHEO patient, it not only helps to predict the phenotype of PHEO (site, number, recurrence and malignancy) but also predicts associated tumors (for e.g. medullary thyroid carcinoma (MTC) and parathyroid adenoma in germ line mutations of RET proto-oncogene). It is also essential for accurate genetic counseling of family members.

PHEO/PGL commonly occurs in association with VHL, MEN-2, Neurofibromatosis-1 (NF-1) and Familial PGL syndromes 1-4. VHL occurs due to mutations in VHL gene and is associated with hemangioblastomas of the central nervous system and retina, renal cysts and clear-cell renal cell carcinoma, pancreatic cysts and cystadenomas, endolymphatic sac tumors, papillary cystadenomas of the epididymis (males) and round ligament (females). PHEO occurs in 10-15% of VHL patients and only in type 2 disease. In VHL-2C, PHEO is the only manifestation of the disease. In a patient with FHL gene mutation, screening for all components of VHL should start by five years of age except for hemangioblastomas of CNS for which screening should be started by 10 years of age.

MEN-2 syndromes occur due to mutations in RET proto-oncogene. PHEO occurs in 50% of patients with these syndromes. MEN-2A is characterized by MTC and parathyroid adenoma/hyperplasia whereas MEN 2B is characterized by MTC, mucosal neuromas of the lips, tongue, and eyelids, medullated corneal nerve fibers, distinctive facies with enlarged lips, megacolon/ganglioneuromatosis of the GI tract and marfanoid body habitus. Specific genotype-phenotype correlations have also been established in MEN2A and MEN2B syndromes, which guide patient management. RET proto-oncogene has six hot spots for mutations: exons 10, 11, and 13-16. Codon 918 and 883 (ATA risk level D) are commonly mutated in MEN2B, while codon 634 and 630 (ATA risk level C) in MEN 2A. These four mutations are characterized by early onset of medullary thyroid carcinoma and PHEO, which mandates early screening for these tumors. For mutations in codon 918 and 883, MTC screening should be started as soon as possible and prophylactic thyroidectomy should be carried out before one year of age. For mutations codon 634 and 630, MTC screening should be started by 3-5 years of age and prophylactic thyroidectomy should be carried out before five years of age. For all these four mutations, PHEO screening should be started by 8 years of age with plasma free metanephrines.

Usually NF-1 is diagnosed clinically and not screened genetically owing to the large size of the gene and the absence of hotspots. Neurofibromatosis is diagnosed in a patient if he/ she has two or more of the following features.

1. Six or more café au lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
2. Two or more neurofibromas of any type or one plexiform neurofibroma
3. Freckling in the axillary or inguinal regions (Crowe’s sign)
4. Optic glioma
5. Two or more Lisch nodules (iris hamartomas)
6. A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex with or without pseudoarthrosis

7. A first-degree relative (parent, sibling, or offspring) with NF1 by the above criteria

PHEO is seen in less than 5% of NF-1 patients. PHEO screening has to be considered only if these patients develop hypertension.

Familial PGL syndromes 1-4 occur due to mutations in SDHD, SDHAF-2, SDHC and SDHB, respectively. In PGL syndromes 1, 3 and 4, PHEO/PGL may be associated with gastrointestinal stromal cell tumor (Carney-Stratakis dyad). For PGL syndrome 4 PHEO screening should be started by 5 years of age whereas for other PGL syndromes PHEO screening should be started by 10 years of age. Recently, mutations in new genes like SDHA, TMEM 127, MAX, KIF1B and PDH 2 have also been implicated in the etiology of PHEO/PGL.

Earlier only 10% of PHEO/PGL patients were thought to have hereditary basis. This, ‘Rule of Ten’ was challenged in 2002 when Neumann et al., reported germ line mutations (VHL, RET, SDHB, SDHC, and SDHD) in 24% of apparently sporadic PHEO/PGL patients. Other studies have also shown germ line mutations in more than a quarter of PHEO/PGL patients from French, Spanish, Italian and German cohorts which have completely eliminated the “Rule of Ten’.

Characteristics of PHEO/PGL such as age of presentation, location and number, rate of malignancy and recurrence vary according to the gene involved. Table 2 summarizes PHEO/PGL features associated with six familial syndromes compared with sporadic tumors. Patients with VHL syndrome present at a younger age. PHEO/PGL almost exclusively arise from adrenal medulla in MEN2 an NF-1 whereas extra-adrenal tumors commonly occur in VHL and PGL syndromes. Head and neck PGL are particularly common in PGL 1-3 syndromes whereas malignant tumors most commonly occur in PGL 4 syndrome.

Recent studies have also suggested a role for genetic information in choosing the appropriate nuclear imaging for PHEO/PGLs. In non-SDHB patients with metastatic disease, ¹⁸F-DOPA has the best sensitivity (93%), followed by ¹⁸F-FDA (76%), ¹²³I-MIBG (59%), and ¹⁸F-FDG (62%). However, in SDHB patients with metastatic PGL, ¹⁸F-DOPA (20%) has lowest sensitivity whereas ¹⁸F-FDA (82%) and ¹⁸F-FDG (83%) have a higher sensitivity. Hence, for non-SDHB patients with metastatic disease, ¹⁸F-DOPA should be used for functional imaging but not for SDHB patients with metastatic PGL where ¹⁸F-FDA and ¹⁸F-FDG should be used.

The first international symposium on PHEO recommends genetic screening of all PHEO/PGL patients. However, it may not be cost-effective to screen all PHEO/PGL patients for all genes. Hence, there is a constant effort to reduce the cost of genetic screening by prioritizing genetic testing. Factors like history of syndromic components in the patient or family members, biochemical phenotype, site and number of PHEO/PGL have been used to prioritize genetic testing. The suggested algorithm for gene prioritization using this information is depicted in Figure 1.

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### Table 2: Characteristics of PHEO/PGL-associated syndromes compared with sporadic tumors

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>MEN 2</th>
<th>VHL</th>
<th>PGL 1</th>
<th>PGL 3</th>
<th>PGL 4</th>
<th>NF 1</th>
<th>Sporadic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene name</td>
<td>RET</td>
<td>VHL</td>
<td>SDHD</td>
<td>SDHC</td>
<td>SDHB</td>
<td>NF1</td>
<td>No</td>
</tr>
<tr>
<td>Age in years at diagnosis (median and range)</td>
<td>36 (21–57)</td>
<td>22 (5–67)</td>
<td>27 (5–65)</td>
<td>46 (13–73)</td>
<td>41 (12–66)</td>
<td>34 (14–61)</td>
<td>46 (4–84)</td>
</tr>
<tr>
<td>Multifocal tumors</td>
<td>59%</td>
<td>56%</td>
<td>55%</td>
<td>9%</td>
<td>11%</td>
<td>20%</td>
<td>6%</td>
</tr>
<tr>
<td>Adrenal tumors</td>
<td>97%</td>
<td>92%</td>
<td>86%</td>
<td>0</td>
<td>43%</td>
<td>100%</td>
<td>93%</td>
</tr>
<tr>
<td>Extraadrenal abdominal tumors</td>
<td>3%</td>
<td>17%</td>
<td>59%</td>
<td>0</td>
<td>62%</td>
<td>0</td>
<td>8%</td>
</tr>
<tr>
<td>Thoracic tumors</td>
<td>0</td>
<td>5%</td>
<td>27%</td>
<td>0</td>
<td>11%</td>
<td>0</td>
<td>8%</td>
</tr>
<tr>
<td>Head and neck tumors</td>
<td>0</td>
<td>0</td>
<td>41%</td>
<td>100%</td>
<td>8%</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>Malignant</td>
<td>3%</td>
<td>4%</td>
<td>0%</td>
<td>0</td>
<td>32%</td>
<td>12%</td>
<td>4%</td>
</tr>
<tr>
<td>Family history</td>
<td>59%</td>
<td>47%</td>
<td>18%</td>
<td>0</td>
<td>22%</td>
<td>16%</td>
<td>0</td>
</tr>
</tbody>
</table>
Although, presence of syndromic components in the patient or family members predicts the gene involved with 100% specificity, other clues have less discrimination power. Recently, SDHB immunostaining has been found to be useful in distinguishing SDH related tumors from non SDH related tumors with almost 100% accuracy. SDHB, SDHC and SDHD genetic testing is indicated only in patients with SDHB-negative tumors. The grade of SDHB immunostaining may also help to differentiate SDHD related tumors from SDHB related ones. Overall, SDHB immunostaining can be used as an effective test to reduce the cost of genetic testing and is now being used as the major branching point for gene prioritization.

In conclusion, the management of PHEO/PGL has undergone several developments in the Indian scenario. Biochemical diagnosis can be best achieved by evaluating PFMNs by EIA. Localization should be done by at least two modalities: anatomical imaging (CT/MRI scan) and functional imaging (MIBG/18F-FDG-PET). The choice of the imaging strategy can be guided by the biochemical phenotype. Given the hereditary nature of the disease, special attention should be paid while recording family history, where at least the first degree relatives should be investigated for the disease. Genetic testing can play an important role in the management of patient and family members and is recommended.

REFERENCES