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EDITORIAL

Dear esteemed readers of TurkJEM Family,

Summer has come and for this issue's editorial I would like to focus on WHO 2018 Global Report on Diabetes report. Report covers global and country wide findings encouraging us to focus global and Turkey’s findings for comparison. It is estimated that there are 422 million people around the world living with diabetes in 2014. Global prevalence has reached to 8.5 percent for adult population. During the last decade diabetes caused 1.5 million deaths. Higher blood glucose caused an additional 2.2 million deaths adding to 3.7 million deaths around the world.

In the case of Turkey, death due to diabetes is in 2017 is 3310 for males and 5001 for females. For age specific analysis, number of deaths among males is 31.11% for ages 30-69 and 68.89% for ages 70+. For female's, number of deaths are 31.11% for ages 30-69 and 68.89% for ages 70+.

Looking at number of deaths attributable to high blood glucose is 12760 for males and 13500 for females. For age specific analysis death among males is 33% for ages 30-69 and 67% for age group 70+. For female's, is 24% for ages 30-69 and 76% for ages 70+.

From these findings one clearly can see that both diabetes and deaths attributable to high blood glucose is increasing for elderly population. Thus aging population will further deteriorate prevalence of diabetes and high blood pressure. On other striking finding is female population is more vulnerable to diabetes and to high blood pressure compared to male population. Steps to take starts with early diagnosis, effective Initial Surgical Approach in Patients with Diabetic Nephropathy", “LEPR Deficiency: Prevalence and Importance of a Novel Mutation and Significant Genetic Variants, Usually Underestimated”. "Complications of Stump Healing Among Diabetic Population” within the review category. As case report we have four publications: "Could Retroperitoneal Ganglioneuroma be a Dopamine Secreting Ganglioneuroma?", “Diagnostic Evaluation and Characterization of Von Hippel-Lindau (VHL) Syndrome by Functional Imaging (68Ga-DOTANOC, 99mTc-HYNIC-TOC, and 131I-MIBG)", “Two Cases of Hypothyroidism with TSH Receptor Gene D727E Polymorphism that Converted to Hyperthyroidism after Many Years”, “Successful Preoperative Treatment with Plasmapheresis of Patients with Hyperthyroidism” and an invited review on "The Diagnosis of Neuroendocrine Tumours: An Endocrine Perspective".

I would like to express my sincere thanks to the contributors on their academic and scientific devotion on behalf of the TurkJEM family.

I hope more manageable days for diabetes and high blood pressure is nor too low. With this edition we also have a supplement printed edition of abstracts from the 40th Turkish National Endocrinology and Metabolism Congress which was held in Antalya Turkey on 9-13 May 2018. I wish you all a pleasant and productive summer.

With my best regards,

Nilgün Başkal MD
Editor-in-Chief
Hemithyroidectomy Seems to be a Reasonable Initial Surgical Approach in Patients with Cytological Bethesda Category III Thyroid Nodules: An Institutional Experience

Önemi Belirsiz Atipi/Önemi Belirsiz Foliküler Lezyon Tiroid Nodüllerinde İlk Cerrahi Yaklaşım Olarak Hemitiroidektomi Mantıklı Gibi Görünenktedir


Abstract

Objective: The atypia of undetermined significance or follicular lesion of undetermined significance is a heterogeneous category of Bethesda system. This study aimed to evaluate the malignancy rates in the initial operation and contralateral thyroid lobe after completion thyroidectomy in patients with atypia of undetermined significance/FLUS thyroid nodules who underwent hemithyroidectomy.

Material and Methods: We reviewed the medical records of 47 (7 male, 40 female; mean age 40.3±13.3 years) patients with cytological atypia of undetermined significance/FLUS thyroid nodules (total 48 nodules).

Results: The preoperative cytology was evaluated as atypia of undetermined significance in 32 (66.7%) nodules and follicular lesion of undetermined significance in 16 (33.3%) nodules. The histopathology was reported as benign in 34 (72.3%) patients and malignant in 13 (27.7%) patients. Out of these 13 patients, complementary thyroidectomy was performed in 11 (23.4%) patients, of which 9 (81.8%) patients had benign histopathology and 2 (18.2%) had malignant histopathology.

Conclusion: Lobectomy seems to be a reasonable initial surgical approach in patients with atypia of undetermined significance/follicular lesion of undetermined significance thyroid nodules in cytology.

Keywords: Bethesda category III; atypia or follicular lesion of undetermined significance; malignancy; hemithyroidectomy; completion thyroidectomy

Keywords: Önemi belirsiz atipi/önemi belirsiz foliküler lezyon Bethesda sisteminin heterojen bir kategorisidir. Bu çalışmada, sitolojide önemi belirsiz atipi/önemi belirsiz foliküler lezyon nodülü olduğu için hemitiroidektomi yapılan hastalardaki başlangıç malignite oranının, tamamlayıcı tirotektomi ihtiyacı, karşı lobdaki malignite oranının belirlenmeye çalışılması amaçlanmıştır.

Anahtar kelimeler: Bethesda kategori III; önemi belirsiz atipi/önemi belirsiz foliküler lezyon; malignite; hemitiroidektomi; tamamlayıcı tirotektomi

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Introduction
The fine-needle aspiration biopsy (FNAB) is the gold standard method for differentiation of malignancy in thyroid nodules. National Cancer Institute developed "Bethesda system for reporting thyroid cytopathology" to create a common language for and ensure standardization in the assessment of thyroid nodule cytology (1). There are six groups in the Bethesda system: nondiagnostic or unsatisfactory; benign; atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS); follicular neoplasm or suspicious for a follicular neoplasm; suspicious for malignancy (typically papillary cancer); and malignant. The AUS/FLUS constitutes 15% to 30% of all cytology samples and these nodules carry a malignancy risk of 5% to 15% (2). Although it has a low predictive value, male patients and those with advanced age and nodule size of greater than 4 cm have an increased risk of malignancy (3-5). Cytologically, if limited surgery is preferred in patients with an undetermined solitary nodule, lobectomy is recommended as the initial surgical method. This decision can be changed depending on the clinical and ultrasonographic features, patient’s preference, and molecular tests (6). Total thyroidectomy is recommended if the undetermined nodule is large (>4 cm), sonographically suspicious, and carcinoma-specific mutation positive and if the patient has a family history of thyroid cancer and/or self-history of head and neck radiotherapy. Completion thyroidectomy is not always necessary in patients with intrathyroidal papillary thyroid cancer and low-risk follicular thyroid cancer (6). The surgical risk of completion thyroidectomy performed following lobectomy is close or similar to that of total thyroidectomy (7). This study aimed to determine the adequacy of hemithyroidectomy as an initial surgical method in patients diagnosed with AUS/FLUS and identify the malignancy risk in the initial operation and contralateral malignancy rate after completion thyroidectomy.

Materials and Methods
The medical records of 474 patients who underwent either total thyroidectomy or hemithyroidectomy because of AUS/FLUS cytological results from January 2009 to November 2015 were retrospectively reviewed. Furthermore, 47 patients whose cytology results showed AUS/FLUS and who underwent hemithyroidectomy were enrolled in the study. The demographic characteristics of the patients include age and gender. In laboratory evaluations, thyroid stimulating hormone (normal range: 0.4-4.0 uIU/mL), free thyroxine (normal range: 0.61-1.12 ng/dL), free triiodothyronine (normal range: 1.57-4.71 pg/mL), anti-thyroid peroxidase (anti-TPO) antibody (normal range: <10 IU/mL), and anti-thyroglobulin (anti-TG) antibody (normal range: <30 IU/mL) levels were evaluated. The presence of euthyroidism, hyperthyroidism, and hypothyroidism was evaluated according to the relevant laboratory tests and drug usage information such as levothyroxine, propylthiouracil, and methimazole. The histopathology of these patients was reviewed. The initial malignancy rate was estimated after hemithyroidectomy, and furthermore, the indications of completion thyroidectomy and contralateral malignancy rate were identified. In the light of these data, the selection of hemithyroidectomy was questioned for patients whose histopathology results were benign or showed microcarcinoma and who were in the low-risk group.

Ultrasound
Esaote color Doppler US (model 796FDII; MAG Technology Co. Ltd., Yung-Ho City, Taipei, Taiwan) and superficial probe (model LA52313-4, 5.5-7.8 MHz) were used for ultrasonographic evaluation. The ultrasonographic characteristics examined were nodule count, nodule diameter (mm), nodule localization (right lobe or left lobe), nodule component (cystic, solid, and mixed), echogenicity (isoechoic, hypoechoic, iso-hypoechoic, heterogeneous, anechoic, and hyperechoic), margin (regular or irregular), calcifications (microcalcification, macrocalcification, and peripheral macrocalcification), presence of halo, and shape (anteroposterior (AP)/transverse (T) ratio). The suspicious ultrasound findings were hypochogenicity, presence of microcalcification, irregular margin, absence of halo, and appearance of taller than wide (increased AP/T ratio) shape (6).

Fine-Needle Aspiration Biopsy
Ultrasound-guided biopsy (Logic Pro 200 GE and 7.5 MHz probe; Kyunggigo, Korea) was performed for all solid thyroid nodules equal to or larger than 10 mm and smaller than 10 mm but had highly suspicious ultrasound findings using a 27-gauge needle and 20-mL syringe by an experienced endocrinologist. All smears were air-dried.

Cytology
Smears were stained using May-Grunwald Giemsa stain and evaluated by experienced pathologists. The Bethesda system was used for
cytological classification. The patients diagnosed with Bethesda category III (AUS/FLUS) were included in the study (2).

Histopathology
The histopathology of carcinoma, carcinoma variant, carcinoma foci count, and carcinoma diameter (mm) was given in the histopathological data. The patients with a tumor size of <10 mm (microcarcinoma) and papillary carcinoma of follicular, oncocytic, and clear cell variants were considered the low-risk group (6). In this study, the low-risk group refers to patients who do not need completion thyroidectomy following hemithyroidectomy as per the recommendations. The patients with tumor size of ≥10 mm, papillary carcinoma with the tall cell, columnar cell, insular, solid, diffuse sclerosing variants of papillary cancer, and invasive follicular cancer were considered the high-risk group (6).

Statistical Analysis
All statistical analyses were performed using SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA). Descriptive analyses were presented using mean ± standard deviation (SD) for normally distributed variables, median and range (min-max) for non-normally distributed variables, and the number of cases (%) for nominal variables. The approval of the local ethics committee was obtained for this study.

Results
A total of 47 patients whose cytopathology results were AUS/FLUS and who underwent hemithyroidectomy were examined in this study. Out of these patients, 7 (14.9%) were male and 40 (85.1%) were female. The mean age was found to be 40.3±13.3 SD (range: 17-72 years; Table 1). According to the preoperative thyroid function tests, 38 (80.8%) patients had euthyroidism, 1 (2.1%) had hyperthyroidism, and 8 (17%) had hypothyroidism. The number of anti-TPO or anti-TG positive patients was 19 (40.4%), whereas the number of anti-TPO and anti-TG negative patients was 28 (59.6%). The mean nodule count was 1.6±1.2 SD (range: 1-8). A total of 28 (59.6%) patients had a solitary nodule and 19 (40.4%) had multinodular thyroid disease. Among these 19 patients, 3 (6.3%) had bilateral multinodular thyroid disease and 16 (34.1%) had unilateral multinodular thyroid disease. A total of 48 AUS/FLUS nodules were obtained from 47 patients. The AUS nodules were observed in 32 (66.7%) patients, and the FLUS nodules were present in 16 (33.3%) patients (Table 2). The mean diameter of these nodules was 20.7±11.3 mm (range: 6.6-51.2 mm). In terms of ultrasonographic characteristics, 23 (47.9%) AUS/FLUS nodules were located in the right lobe and 25 (52.1%) were located in the left lobe. The nodule margin was regular in 31 (64.6%) and irregular in 17 (35.4%) nodules. Halo was present in 24 (50%) and absent in remaining 24 (50%) nodules. Peripheral macrocalcification was present in 5 (10.4%) and absent in 43 (89.6%) nodules. Microcalcification was present in 12 (25%) and absent in 36 (75%) nodules. Regarding the nodule component, 45 (93.8%) nodules were solid, 1 (2.1%) was mixed, and 2 (4.2%) were cystic. In terms of nodule echogenicity, 19 (39.6%) nodules were isoechoic, 6 (12.5%) were hypoechoic, 21 (43.8%) were iso-hypoechoic, and 2 (4.2%) were anechoic. According to the histopathology results of 47 patients diagnosed with AUS /FLUS who underwent hemithyroidectomy, 34 patients (72.3%) were reported to have benign nodules and 13 (27.7%) were reported to have malignant nodules. Out of the 13 patients diagnosed with the malignant...
nodule, 11 patients (23.4%) underwent completion thyroidectomy and 1 underwent hemithyroidectomy. Furthermore, one patient dropped out of postoperative follow-up (Table 3). Furthermore, the histopathology results revealed that 12 patients (92.3%) with malignant nodule had papillary carcinoma and 1 (7.7%) had Hurthle cell carcinoma. In terms of papillary cancer variants, seven patients (53.8%) were in the classical papillary variant group, 3 (23.1%) in the follicular variant group, 1 (7.7%) in the solid variant group, and 1 (7.7%) in the encapsulated follicular variant group. Out of 13 patients with the malignant nodule, 9 (19.2%) showed high-risk characteristics and 4 (8.5%) showed low-risk characteristics. However, all patients in the high-risk and low-risk groups underwent completion thyroidectomy, as patients in the low-risk group desire to be comfortable in the follow-up period. According to the histopathology of the contralateral lobe, nine nodules (81.8%) were benign and two (18.2%) were malignant. The mean carcinoma foci count was 1.6±1.5 (1-6), and ten patients (76.9%) had a single focus, 1 (7.7%) had two foci, one (7.7%) had four foci, and one (7.7%) had six foci. Evaluating the patients with multiple foci separately, the patient diagnosed with six foci was found to have a solitary nodule in the preoperative ultrasonographic evaluation and the long axis of the nodule was 37 mm. The patient’s pathology was papillary carcinoma follicular variant, and the primary tumor diameter was 30 mm. The remaining cancer foci were millimetric. This patient underwent completion thyroidectomy, and no malignancy was detected in the contralateral lobe. The patient diagnosed with four foci was found to have four nodules (one in the right lobe and three in the left lobe) in the preoperative ultrasonographic evaluation. The histopathology of the single AUS nodule was benign. The tumor was identified as incidental and showed bilaterality, i.e., the histopathology of contralateral lobe was malignant. The tumor histopathology was papillary carcinoma classical variant, and the tumor diameter was 1 to 5 mm in the left lobe and 3 to 6 mm in the right lobe. The patient diagnosed with two foci was found to have a single nodule in the preoperative ultrasonographic evaluation, and the long axis of the nodule was 6.6 mm. The carcinoma diameter was 3 mm and the histopathology was papillary carcinoma classical variant. This patient underwent completion thyroidectomy, and malignancy was detected in the contralateral lobe (papillary cancer, 1 mm). Among the patients diagnosed with one focus and no contralateral malignancy, seven patients were found to have a single nodule and three were found to have two nodules in the preoperative ultrasonographic evaluation. The nodules of these patients were in the same lobe. After hemithyroidectomy and completion thyroidectomy, the mean diameter of dominant carcinoma (n=15) was 13.8±11.5 mm (1-40 mm).

Discussion

The American Association of Clinical Endocrinologists, the American Thyroid Association, and the European Thyroid Association recommend surgical treatment for patients with recurrent AUS/FLUS

| Table 2. Ultrasonographic features of nodules in AUS/FLUS patients. |
|-------------------------------|------------------|------------------|
| Cytology (n=48)               | FLUS             | 16 (33.3%)       |
|                               | AUS              | 32 (66.7%)       |
| Nodule localization (n=48)    | Right lobe       | 23 (47.9%)       |
|                               | Left lobe        | 25 (52.1%)       |
| Nodule diameter (n=48)        | Mean±SD          | 20.7±11.3 mm     |
|                               | Range            | 6.6-51.2 mm      |
| Component (n=48)              | Cystic           | 2 (4.2%)         |
|                               | Solid            | 45 (93.8%)       |
|                               | Mixed            | 1 (2.1%)         |
| Echogenicity (n=48)           | Isoechoic        | 19 (39.6%)       |
|                               | Hypoechoic       | 6 (12.5%)        |
|                               | Iso-hypoechoic   | 21 (43.8%)       |
|                               | Anechoic         | 2 (4.2%)         |
| Border regularity (n=48)      | Regular          | 31 (64.6%)       |
|                               | Irregular        | 17 (35.4%)       |
| Peripheral macrocalcification (n=48) | Present | 5 (10.4%)         |
|                               | Absent           | 43 (89.6%)       |
| Microcalcification (n=48)     | Present          | 12 (25%)         |
|                               | Absent           | 36 (75%)         |
| Halo (n=48)                   | Present          | 24 (50%)         |
|                               | Absent           | 24 (50%)         |

AUS: Atypia of undetermined significance; FLUS: Follicular lesion of undetermined significance.
The malignancy rate of 5% to 15% in these patients leads to different approaches in determining the operation size (2). In addition, a biopsy is performed again and some patients do not undergo surgery because of the benign nodule, which leads to a relative increase in the malignancy rate in operated patients (9). In previous studies, the malignancy rate was between 14.4% and 45.7% for patients with AUS/FLUS nodules (10-14). The differences between the rates are assumed to be caused by ultrasonographic and clinical findings. In this study, the malignancy rate was 27.7%. In a recent study, we reported the malignancy rate as 23.4% (of the 449 operated nodules, 105 were malignant (13). This rate is similar to that found in the study conducted by Kuru et al. (22.8%) (14). The malignancy rate was reported to be 28.3% by Layfield et al. (10), 25% by Renshaw et al. (11), 45.7% by PE Vanderlaan et al. (9), and 14.4% by Bongiovanni M (12).

In fact, another factor to be considered in this subject is the application of hemithyroidectomy in patients with a low expectation of malignancy or patients with malignancy but with a satisfactory expectation of prognosis. As this study only included patients who underwent hemithyroidectomy, the malignancy rate was compared only within this group. Ryu et al. conducted a study with 51 patients who underwent hemithyroidectomy because of AUS. In addition, 36 patients had benign nodules and 15 had malignant nodules, which indicate a malignancy rate (29%) similar to that found in this study (15). However, studies including a large patient population are required on this subject. Despite the time period of five years, the number of patients in this study was low, which is because of the fact that total thyroidectomy is the preferred surgical method in our institution, Endocrinology and Metabolism Department of Ankara Yildirim Beyazit University. Total thyroidectomy is preferred because of patient and surgeon desire to avoid a possible second surgery because of malignancy risk, ultrasonographic tumor size, and multinodular thyroid presence. Therefore, the majority of patients who underwent hemithyroidectomy in our clinic (57.4%) had a solitary thyroid nodule.

In this study, 23.4% of the patients underwent completion thyroidectomy. In fact, when the need for completion thyroidectomy is considered rela-

<table>
<thead>
<tr>
<th>Table 3. Pathologic characteristics of patients at first surgery and completion thyroidectomy.</th>
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<tbody>
<tr>
<td><strong>Histopathology (n=47)</strong></td>
</tr>
<tr>
<td>Benign</td>
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<tr>
<td>Malignant</td>
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<tr>
<td>Low risk</td>
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<td>High risk</td>
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<tr>
<td><strong>Histopathology at completion thyroidectomy (n=11)</strong></td>
</tr>
<tr>
<td>Benign</td>
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<tr>
<td>Malignant</td>
</tr>
<tr>
<td>Benign + low risk</td>
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<tr>
<td><strong>Histopathology of carcinoma after hemithyroidectomy and completion thyroidectomy (n=15)</strong></td>
</tr>
<tr>
<td>Papillary cancer</td>
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<tr>
<td>Hurthle cell carcinoma</td>
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<tr>
<td><strong>Carcinoma focus number</strong></td>
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<td>Mean±SD</td>
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<td>Range</td>
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<tr>
<td><strong>Carcinoma focus distribution</strong></td>
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<tr>
<td>1</td>
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<td>3</td>
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<td>&gt;4</td>
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<tr>
<td><strong>Carcinoma diameter (n=15)</strong></td>
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<td>Mean±SD</td>
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</table>
tive to the risk groups, the high-risk group constitutes 19.2% of all patients. The difference came from the microcarcinomas with 5 to 9 mm tumour diameter. The completion thyroidectomy is preferred in these patients, considering there may be difficulties related to malignancy follow-up (inability to effectively use laboratory and imaging techniques such as whole-body iodine scan, thyroglobulin, and anti-thyroglobulin), risk of recurrence, or possibility of incidental tumor. The rate of completion thyroidectomy was approximately 11.7% in a study by Ryu et al. (15). In the study with a similar number of patients conducted by Dobrinja et al., the completion thyroidectomy rate was reported to be 10.5% (16). When considered relative to 11 patients who underwent completion thyroidectomy, the contralateral malignancy rate was 18%. Badr Ibrahim et al. found the contralateral malignancy rate to be 48% in patients who underwent hemithyroidectomy (17). In addition, the contralateral malignancy rate was reported to be 44% by Pacini et al. and 36% by Kim et al. (18,19).

Among the patients who underwent hemithyroidectomy, the patients with benign pathology (72.3%) should be considered separately. Hospitalization duration and comorbidities (hypoparathyroidism, vocal cord paralysis, permanent hypothyroidism, and high-dose levothyroxine needs) of these patients may be lower than that of patients who underwent total thyroidectomy (20,21). The frozen section procedure and transition to total thyroidectomy as a result of an intra-operative decision are recommended for patients in the benign group, constituting the majority of patients. A second surgery may be avoided for patients diagnosed with malignant nodules in the frozen section procedure. The frozen section procedure was used for 58 patients with AUS/FLUS in a study conducted by SPosillico et al. and resulted in a definitive benign or malignant diagnosis in 37 (64%) patients. In the latter study, however 32 patients had a benign nodule and the operation was limited to hemithyroidectomy, four patients underwent total thyroidectomy because of malignancy. Furthermore they reported that the frozen section procedure did not produce any results for 36% of the patients, which is a limitation of the procedure (22).

This study has some limitations. The number of patients who underwent hemithyroidectomy is relatively low in our clinic because total thyroidectomy is preferred, which is the major limitation of this study.

Conclusions

In patients with AUS/FLUS, lobectomy is appropriate initially, as 80.8% of the patients are in the benign and low-risk cancer group after surgery. It should be considered that the frozen section procedure may produce more positive results in terms of long-term morbidity in patients with malignant nodules when applied by experienced pathologists. In addition, long-term retrospective studies that evaluate the results of hemithyroidectomy and total thyroidectomy in these patients are necessary. Recurrence and contralateral malignancy rates in patients who underwent hemithyroidectomy should be evaluated using larger patient populations.

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Conflict of Interest: No conflicts of interest between the authors and/ or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Bekir Çakır, Cevdet Aydın; Design: Muhammet Cüneyt Bilginer, Reyhan Ersoy; Control/Supervision: Oya Topaloğlu, Didem Özdemir, Cevdet Aydın; Data Collection and/or Processing: Cevdet Aydın, Reyhan Ersoy, Bekir Çakır; Analysis and/or Interpretation: Muhammet Cüneyt Bilginer, Gürkan Dumlul, Hayriye Dogan; Literature Review: Bekir Çakır, Reyhan Ersoy; Writing the Article: Didem Özdemir, Muhammet Cüneyt Bilginer; Critical Review: Didem Özdemir, Oya Topaloğlu; References and Fundings: Cevdet Aydın, Bekir Çakır; Materials: Muhammet Cüneyt Bilginer, Gürkan Dumlul, Hayriye Dogan.

References


Analysis of Risk Factors of Neck Nodal Metastasis in Patients with Papillary Thyroid Microcarcinoma

Objective: The aim of this study was to evaluate the risk factors of neck nodal metastasis on papillary thyroid microcarcinoma patients.

Material and Methods: About 123 patients diagnosed with thyroid papillary microcarcinoma, who had undergone total thyroidectomy, between January 2012 and December 2014, were analyzed retrospectively for neck nodal metastasis and distant metastasis risk factors. Laboratory and surgical data were collected for these patients. Other factors such as gender, age, levels of anti-thyroid peroxidase and anti-thyroglobulin antibodies, thyroid stimulating hormone, level of thyroglobulin on thyroid stimulating hormone suppression, size of tumor, presence of capsular invasion, extrathyroidal extension, metastasis of central neck lymph node, vascular invasion, multifocality, bilateral involvement, size of preoperative node, duration of the disease, radioactive iodine therapy, metastasis of lateral neck lymph node and histopathological type were investigated.

Results: Among the cases that were studied, there were 104 (84.6%) females and 19 (15.4%) males. The average age was 48.60±12.05 years and the mean tumor size was 6.06±2.63 mm. In patients with lateral neck lymph node metastasis and capsular invasion, the thyroglobulin levels after thyroid stimulating hormone suppression were higher. The rate of total neck lymph node metastasis was found to be higher in patients with capsular invasion. Furthermore, the Anti-thyroid peroxidase levels were higher in thyroid papillary microcarcinoma patients who did not have lateral lymph node metastasis.

Conclusion: The thyroglobulin levels after thyroid stimulating hormone suppression and capsular invasion are important risk factors for neck nodal metastasis, in papillary thyroid microcarcinoma patients.

Keywords: Thyroid papillary microcarcinoma; TSH; capsular invasion; lateral neck lymph nodal metastasis

Amaç: Bu çalışmada, papiller tiroid mikrokarsinomlu hastalardaki boynun lenf nodu metastazlarının risk faktörlerinin incelenmesi amaçlanmıştır.


Bulgular: Vakaların 104 (%84,6)’arı kadın, 19 (%15,4)’ari erkek hastalardan oluşmaktadır. Ortalama yaş 48,60±12,05 yıl’dır. Hastalarımızın tümör çapi ortalamada 6,06±2.63 mm’dir. Lateral boynun lenf nodu metastazı ve kapsüler invazyonu olan hastalarındaki tiroid stimüle edici hormon süpersyonuda iken tirotglobulin değerleri daha yüksektir. Kapsüler invazyonu olan hastalarındaki total boynun lenf nodu metastazı oranı daha yüksektir bulunmuştur. Anti-tiroid peroksidaz antikoru değerinin, lateral boynun lenf nodu metastazı olmayan Tiroid papiller mikrokarsinomlu hastalarda daha yüksektir olduğu belirlenmiştir.

Sonuç: Papiller tiroid mikrokarsinomlu hastalarda, tiroid stimüle edici hormon süpersyonuda iken tirotglobulin değerleri ve kapsüler invazyon boynun lenf nodu metastazı için önemli birer risk faktörü oluştururduklar.

Anahtar kelimeler: Tiroid papiller mikrokarsinom; TSH; kapsüler invazyon; lateral boynun lenf nodu metastazı
Introduction

Malignant neoplasms of the thyroid gland constitute nearly one percent of all known cancers. They are the most common tumors that arise from endocrine organs and are usually encountered in both young and middle-aged adults. Approximately 122,000 new cases are diagnosed every year (1), among which, the most common type of thyroid carcinoma is papillary carcinoma (2). The prevalence of this carcinoma varies based on both environmental and genetic factors. Thyroid papillary microcarcinoma is classified as a subgroup of thyroid papillary cancers, with largest tumor size of one centimeter or smaller. Though some researchers have reported that this size can also be about one and a half centimeter (3).

In 2004, the classification by the World Health Organisation (WHO), narrowed down this definition, to include the incidental tumors that were smaller than one centimeter and exclude the small-sized thyroid papillary microcarcinoma with clinical signs (1,4).

Although thyroid papillary microcarcinoma, which is the most common form of thyroid papillary cancers, is clinically silent, the patients of this type rarely present with lymph node metastasis and distant metastasis (1, 4-6).

In our study, we retrospectively analyzed 123 patients having thyroid papillary microcarcinoma for the risk factors of neck nodal metastasis and distant metastasis.

Material and Methods

A total of 123 patients with microcarcinoma of thyroid papillary, who had undergone total thyroidectomy at our clinic, between January 2012 and December 2014, were retrospectively analyzed for the risk factors of neck nodal metastasis and distant metastasis. Only the patients showing pathological findings of thyroid papillary microcarcinoma were included in this descriptive study. The exclusion criteria were, age younger than 18 years, pregnancy, and tumor with a diameter exceeding one centimeter.

The patient’s laboratory and surgical data were collected for this study. Factors like gender, age, levels of Anti-Thyroid Peroxidase (Anti-TPO) and anti-thyroglobulin (Anti-Tg) antibodies, Thyroid Stimulating Hormone (TSH), level of thyroglobulin after TSH suppression, tumor size, presence of capsular invasion, extrathyroidal extension, metastasis of central neck lymph node, vascular invasion, multifocality, bilateral involvement, pre-operative node size, duration of the disease, RAI (radioactive iodine therapy), metastasis of lateral neck lymph node and histopathological type were investigated.

Dependent and Independent Variables

The dependent variables in this study were lymph node metastasis, thyroglobulin level after TSH suppression, Anti-TPO values and Anti-TG values and the independent variables were a capsular invasion, age, sex and RAI therapy.

Data Pooling and Statistical Analysis

Data summary is represented in numbers and percentages for qualitative variables and arithmetic mean and standard deviations for quantitative variables (in case of normal dispersion). The groups were evaluated using the Likelihood Ratio tests and Logistic Regression analysis. The Mann-Whitney U and Wilcoxon W test for variance were employed when no difference between the groups in terms of quantitative variables was found. Independent variables were tested with the help of T-test, Pearson’s Chi-Square Test and Fisher’s Exact Test, when assumptions were made. A p-value <0.05 was considered to be statistically significant.

Results

A total of 123 patients having thyroid papillary microcarcinoma were analyzed retrospectively to identify the risk factors for neck nodal metastasis and distant metastasis. Among the patients studied, 104 (84.6%) were female and 19 (15.4%) were male, with an F/M ratio of 5.49 and mean age of 48.6±12.05 years. From the whole group, 11.4% had metastasis of total neck lymph node while 88.6% did not have any metastasis of total neck lymph node. The median value of TSH was 0.09 mIU/mL (0.02-3.09) and the mean value of thyroglobulin after TSH suppression was 8.56±40.52 ng/mL. The mean level of anti-TPO antibody was 21.26±40.53 IU/mL. The mean tumor size in our study group was found to be 6.06±2.63 mm and the median duration of the disease was 32 (20.75-38) months. The mean anti-Tg value was observed to be 30.13±49.76 IU/mL (Table 1).

The study of the relationship between the presence of total neck lymph node metastasis and capsular invasion revealed that, in patients with capsular invasion, the rate of total neck lymph node metastasis was 37.5%, in contrast to the rate of 6.6% among those without capsular invasion. Therefore, a statistically significant rela-
A relationship was found between the presence of capsular invasion and metastasis to the neck lymph nodes \((p=0.020)\) (Table 2).

The relationship between the level of thyroglobulin after TSH suppression and the presence of capsular invasion was also examined. It was found that the mean level of thyroglobulin after TSH suppression was 27.10±72.36 ng/mL in the group with capsular invasion while in the group without capsular invasion it was 1.80±7.62 ng/mL. Thus, the level of thyroglobulin after TSH suppression was higher in patients with capsular invasion, indicating the presence of a significant relationship between the level of thyroglobulin values after TSH suppression and capsule invasion \((z=-2.379, p=0.017)\) (Table 2).

Anti-TPO values and the presence of lateral neck lymph node metastasis in patients were compared. The mean Anti-TPO value in patients with and without lateral neck lymph node metastasis was 10.52±8.26 IU/mL and 22.48±42.53 IU/mL, respectively. The Anti-TPO values were higher in patients who did not have lateral lymph node metastasis, suggesting that there existed a significant relationship between the presence of lateral neck lymph node metastasis and Anti-TPO values \((z=-2.510, p=0.012)\) (Table 2).

The levels of thyroglobulin after TSH suppression were evaluated in relation to the presence of lateral neck lymph node metastasis. It was found that the median thyroglobulin value on TSH suppression in the group with and without lateral neck lymph node metastasis was 0.59 (0.04-18.59) ng/mL and 0.04 (0.04-0.04) ng/mL, respectively. Thus, a statistically significant relationship was seen between the levels of the thyroglobulin after TSH suppression and the presence of lateral neck lymph node metastasis \((z=-3.022, p=0.003)\) (Table 2).

The logistic regression analysis indicated that gender, age, Anti-TPO, TSH, and additionally RAI (radioactive iodine therapy) variables were not risk factors for total neck lymph node metastasis in patients with microcarcinoma of papillary thyroid, moreover, statistically significant relationship could not be determined with respect to the other parameters (Table 3).

Variable(s) entered on step 1: Gender, Age, Anti-TPO, TSH, TSH Suppressed Tg, RAI therapy.
Data presented as mean±SD, median (IQR) or n (%).
*p<0.05, **p<0.01, considered statistically significant.

<table>
<thead>
<tr>
<th>Thyroid papillary microcarcinoma patients (n=123)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>The mean age of patients of the group with central lymph node metastasis (years)</td>
<td>51.71±3.67</td>
</tr>
<tr>
<td>The mean tumor size of the group with central neck lymph node metastasis (mm)</td>
<td>5.42±2.50</td>
</tr>
<tr>
<td>The number of male patients of the group with central neck lymph node metastasis [n(%)]</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>The number of female patients of the group with central neck lymph node metastasis [n(%)]</td>
<td>7 (71.4%)</td>
</tr>
<tr>
<td>The mean anti-Tg values in patients of the group with lateral neck lymph node metastasis (IU/mL)</td>
<td>21.87±15.13</td>
</tr>
<tr>
<td>The mean age of patients of the group with lateral neck lymph node metastasis (years)</td>
<td>45.08±3.61</td>
</tr>
<tr>
<td>The mean anti-TPO values in patients with lateral neck lymph node metastasis (IU/mL)</td>
<td>10.52±8.26</td>
</tr>
<tr>
<td>The mean TSH values in patients with lateral neck lymph node metastasis (mIU/mL)</td>
<td>0.49±0.21</td>
</tr>
<tr>
<td>The median thyroglobulin value on TSH suppression with lateral neck lymph node metastasis (ng/mL)</td>
<td>0.59 (0.04-18.59)</td>
</tr>
<tr>
<td>The mean tumor size of the group with lateral neck lymph node metastasis (mm)</td>
<td>6.27±2.50</td>
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<tr>
<td>The number of male patients of the group with lateral neck lymph node metastasis [n(%)]</td>
<td>2 (10.5%)</td>
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<tr>
<td>The number of female patients of the group with lateral neck lymph node metastasis [n(%)]</td>
<td>10 (9.6%)</td>
</tr>
<tr>
<td>The mean age of patients with capsular invasion (years)</td>
<td>47.25±9.70</td>
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<tr>
<td>The mean anti-Tg values in patients with capsular invasion (mL)</td>
<td>49.26±21.89</td>
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<tr>
<td>The number of lateral neck lymph node metastasis in patients with capsular invasion [n(%)]</td>
<td>2 (18.2%)</td>
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<tr>
<td>The number of total neck lymph node metastasis in patients with capsular invasion [n(%)]</td>
<td>6 (37.5%)</td>
</tr>
<tr>
<td>The mean anti-TPO values in patients with capsular invasion (mIU/mL)</td>
<td>17.27±4.31</td>
</tr>
<tr>
<td>The mean TSH values in patients with capsular invasion (mIU/mL)</td>
<td>20.02±9.12</td>
</tr>
<tr>
<td>The mean thyroglobulin value on TSH suppression in the group with capsular invasion (ng/mL)</td>
<td>27.10±32.36</td>
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<tr>
<td>The mean tumor size of the group with capsular invasion (mm)</td>
<td>7.09±2.23</td>
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<td>The number of male patients of the group with capsular invasion [n(%)]</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>The number of female patients of the group with capsular invasion [n(%)]</td>
<td>15 (14.6%)</td>
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<tr>
<td>The number of central neck lymph node metastasis in patients with capsular invasion [n(%)]</td>
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<td>The mean age of patients with total lymph node metastasis (years)</td>
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<td>The mean thyroglobulin value on TSH suppression with total neck lymph node metastasis (ng/mL)</td>
<td>31.07±88.04</td>
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<td>The mean tumor size of the group with total neck lymph node metastasis (mm)</td>
<td>6.46±0.73</td>
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<td>The number of male patients of the group with total neck lymph node metastasis [n(%)]</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>The number of female patients of the group with total neck lymph node metastasis [n(%)]</td>
<td>13 (12.5%)</td>
</tr>
</tbody>
</table>
Dependent variables in this study were lymph node metastasis, the values of thyroglobulin after TSH suppression, Anti-TPO, and Anti-TG values while the independent variables were a capsular invasion, age, sex and RAI therapy.

Discussion
The medical records of 123 patients, diagnosed with papillary thyroid microcarcinoma, were evaluated retrospectively. The number of male and female patients was 19 and 104, respectively, resulting in an F/M ratio of 5.49. In a multicentre study in Denmark by Rossing et al. in 2012, there were 282 females out of 340 patients (7), while a study conducted in Spain by Mantinan et al. in 2012, reported, 71 females out of 95 patients (8). All these findings are consistent with our results.

Most of the patients in this study were young and their age distribution was found to be similar to that of other studies conducted in Europe (7,8).

The average age of the patients (calculated in mean) with total neck lymph node metastasis was 50.21±11.90 years when compared to the mean age of 54 years in the study of Lin et al. (9).

The gender distribution of patients with respect to the total neck lymph node metastasis was examined and it was found that out of the 19 male patients, only one (5.3%) had total neck lymph node metastasis, while the remaining 18 (94.7%) did not have total neck lymph node metastasis at all. Similarly, out of the 104 female patients, 13 (12.5%) had total neck lymph node metastasis, while the remaining 91 (87.5%) did not show total neck lymph node metastasis. Moreover, no significant relationship was observed between the two groups in terms of gender and total neck lymph node metastasis (p=0.690). A study conducted in China that was published in the year 2016, reported the presence of a significant relation between total neck lymph node metastasis and male gender (10). Our results were incompatible with this literature, probably due to the small number of patients that were examined.

During our analysis, we detected total neck lymph node metastasis in 14 (11.4%) patients and no total neck lymph node metastasis in 109 (88.6%) patients. This was in contrast to the study conducted by the Mayo Clinic where total neck lymph node metastasis was found in 98% of the patients (11).

The mean tumor size, of thyroid papillary microcarcinoma, in our patients, was 6.06±2.63 mm when compared to the average tumor size of 7 mm as reported by a multi-center study in the US, that was published in the year 2012 (12).

The association between patient age and the presence of capsular invasion were evaluated, and the mean ages of patients with and without capsular invasion were found to be 47.25±9.70 years and 48.66±12.35 years, respectively. The presence of capsular invasion was found to be independent of patient’s age (z=-0.804, p=0.421). A study published in 2013, in a series of 1979 patients in Heraklion hospital in Greece, evaluated the association between patient age and the presence of capsular invasion and found no significant relationship (13).

As for the link between tumor size and the presence of capsular invasion, it was observed that the mean tumor sizes of the group with and without capsular invasion were 7.09±2.23 mm and

<table>
<thead>
<tr>
<th>Variables in the Equation</th>
<th>B</th>
<th>S.E.</th>
<th>Sig.</th>
<th>Exp (B)</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (1)</td>
<td>0.496</td>
<td>1.328</td>
<td>0.709</td>
<td>1.642</td>
<td>0.122</td>
<td>22.167</td>
</tr>
<tr>
<td>Age</td>
<td>0.068</td>
<td>0.036</td>
<td>0.058</td>
<td>1.071</td>
<td>0.998</td>
<td>1.149</td>
</tr>
<tr>
<td>Anti-TPO</td>
<td>0.024</td>
<td>0.039</td>
<td>0.541</td>
<td>1.024</td>
<td>0.949</td>
<td>1.106</td>
</tr>
<tr>
<td>TSH</td>
<td>1.227</td>
<td>1.080</td>
<td>0.256</td>
<td>3.411</td>
<td>0.411</td>
<td>28.340</td>
</tr>
<tr>
<td>TSH Suppressed Tg</td>
<td>-0.019</td>
<td>0.012</td>
<td>0.096</td>
<td>0.981</td>
<td>0.959</td>
<td>1.003</td>
</tr>
<tr>
<td>RAI therapy (1)</td>
<td>19.662</td>
<td>17625.951</td>
<td>0.999</td>
<td>3E+008</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>7.926</td>
<td>8812.976</td>
<td>0.999</td>
<td>2768.654</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Logistic regression analysis of gender, age, anti-TPO, TSH, RAI therapy variables with total neck lymph node metastasis.

a. Variable(s) entered on step 1: Gender, Age, Anti-TPO, TSH, TSH Suppressed Tg, RAI therapy.
The presence of capsular invasion did not seem to affect the tumor size (z=-1.880, p=0.060). The levels of Anti-TPO were analyzed with respect to the presence of total neck lymph node metastasis. The mean levels of Anti-TPO in patients with and without total neck lymph node metastasis were 9.66±5.44 IU/mL and 22.82±42.92 IU/mL, respectively. The Anti-TPO levels were significantly higher (z=-2.53, p=0.011) in the absence of total neck lymph node metastasis. A study conducted in 2016 in China, found the levels of Anti-TPO to be higher in thyroid papillary microcarcinoma patients when total lymph node metastasis was present (14). Once again our study was noted to be incompatible with the literature due to the small number of patients studied. Haymart et al. in 2008, reported the existence of a relationship between thyroid autoimmunity and thyroid malignancy (15), which needs to be investigated in further studies.

No correlation was found between the tumor size and the presence of central lymph node metastasis. The mean tumor size among the patients with central lymph node metastasis was 5.42±2.50 mm and the presence of central lymph node metastasis did not affect the tumor size (z=-0.782, p=0.434). Our results are compatible with those of the study conducted in Spain, in the year 2012, where it was found that the central neck lymph node metastasis does not affect the tumor size (8).

The rate of total neck lymph node metastasis in patients with capsular invasion was 37.5% and the relationship between the presence of capsular invasion and the total neck lymph node metastasis was significant (p=0.020). Page et al. in 2009, reported that 24 out of a group of 41 thyroid papillary microcarcinoma patients, having neck lymph node metastasis, were found to have a capsular invasion. These findings are in accordance with the results of our analysis (16, 17).

When the thyroglobulin values on TSH suppression were evaluated with respect to the presence of capsular invasion, the mean levels of thyroglobulin after TSH suppression in the groups with and without capsular invasion were observed to be 27.10±7.36 ng/mL and 1.80±7.62 ng/mL, respectively. Hence it can be inferred that the levels of thyroglobulin on TSH suppression were higher in patients with capsular invasion and the difference between thyroglobulin values on TSH suppression and capsular invasion was statistically significant (z=-2.379, p=0.017). A published study by Girelli et al. in 429 patients with well-differentiated thyroid carcinoma, reported that the levels of thyroglobulin after TSH suppression was higher in the group with capsular invasion than in the group without capsular invasion (18). Our study was found similar results when compared to this report.

The levels of thyroglobulin after TSH suppression were examined with respect to the presence of total neck node metastasis. The average levels of thyroglobulin after TSH suppression in all the patients with and without total neck lymph node metastasis were 31.07±88.04 ng/mL and 5.18±27.44 ng/mL, respectively. There was no significant relationship between the levels of thyroglobulin after TSH suppression and total neck lymph node metastasis (z=-1.392, p=0.164). Girelli et al. (1985) studied 429 patients with well-differentiated thyroid cancers and found that there were higher levels of thyroglobulin after TSH suppression in the total neck lymph node metastasis group compared to the non-metastasized group (18-20). Our study is not compatible with this study, again due to the smaller number of cases.

Anti-TPO and Anti-Tg levels were compared in patients with and without lateral neck lymph node metastasis. In the presence of lateral neck lymph node metastasis, the mean anti-TPO and the mean Anti-Tg levels were 10.52±8.26 IU/mL and 21.87±15.13 IU/mL, respectively, whereas in its absence the levels were 22.48±42.53 IU/mL and 31.07±52.22 IU/mL, respectively. Thus, higher mean Anti-TPO values were evident in thyroid papillary microcarcinoma patients who did not have lateral lymph node metastasis. The relationship between the presence of lateral neck lymph node metastasis and the Anti-TPO values was statistically significant (z=-2.510, p=0.012) whereas the relationship between the lateral neck lymph node metastasis and Anti-Tg values was non-significant (z=-0.326, p=0.745). A Chinese study conducted in 2016 reported that the mean Anti-TPO and Anti-Tg values were higher in thyroid papillary microcarcinoma patients with lateral neck lymph node metastasis than in the ones without lateral metastasis of the neck lymph node (14).

Logistic regression analysis confirmed that gender, age, Anti TPO, TSH, and the RAI variables did not constitute a risk factor for total neck lymph node metastasis in papillary thyroid mi-
crocarcinoma patients. Furthermore, no statistically significant relationship was found with respect to the other parameters.

There are no data on mortality of thyroid papillary microcarcinoma, either in any previous or in our study. Distant metastases were not detected in any of our patients during their follow-up between the years 2012 and 2014. In conclusion, the values of thyroglobulin after TSH suppression were higher in patients exhibiting lateral neck lymph node metastasis and capsular invasion. The rate of total neck lymph node metastasis was also seen to be higher in patients with capsular invasion. The Anti-TPO values were higher in thyroid papillary microcarcinoma patients who did not have lateral lymph node metastasis.

These findings indicate that the levels of thyroglobulin after TSH suppression and capsular invasion are important risk factors for neck nodal metastasis in papillary thyroid microcarcinoma patients. In a research regarding thyroid papillary carcinoma performed in Colombia between the years 2006 and 2012, 78% of patients with tumor size greater than 20.8 mm exhibited capsular invasion (21). However, the p-value calculated in our study was noted to be at the border. Our results were incompatible with this literature, probably due to the small number of patients that were examined.

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Conflict of Interest: No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Barış Tırman; Design: Barış Tırman; Control/Supervision: Mahmut Başoğlu; Data Collection and/or Processing: Barış Tırman; Analysis and/or Interpretation: Kağan Karabulut; Literature Review: Kağan Karabulut; Writing the Article: Barış Tırman; Critical Review: Barış Tırman; Materials: Barış Tırman.

References


Work Patterns of Mothers Influence the Weights of Their Children

Annelerin Çalışma Şekli Çocuklarının Vücut Ağırlıklarını Etkilemektedir

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Abstract
Objective: Recent studies have reported a positive association between paid work hours of mothers and obesity in their children. Like most of other hospitals, nurses at our center work either on irregular shifts or on a regular basis. The aim of this study was to analyze factors affecting the weights of the children of female nurses.

Material and Methods: We evaluated 100 children of female nurses on duty at our hospital. We prepared a questionnaire including details such as the gender of the child, birth-weight, education level and working status of both parents, duration of breastfeeding, age at which supplementary food was first introduced, exercise habits, snacking habits, and identity of caretakers while the mothers were at work. We calculated the body mass index percentiles using standard growth charts.

Results: According to the standard body mass index percentiles, 13 children were underweight, 53 had normal weight, 14 were overweight, and 20 were obese. The weight patterns of children were positively associated with regular maternal work style (p=0.016), frequency of snacking habits (p=0.003), and cesarian birth (p=0.04). Children of nurses working on shifts were more likely to be underweight compared to those working on fixed schedules. There was no association between the weight patterns of children and education levels of parents, working status of fathers, duration of lactation, birth-weight, gender, order of birth, and supplementary food consumption patterns.

Conclusion: Irregular working hours of mothers can cause irregular and insufficient nutrition for their children. The mother is generally unable to supervise the child during feeding hours. These findings need to be confirmed through more studies on a large scale.

Keywords: Childhood obesity; maternal work style; nurse

Özet

Gereç ve Yöntemler: Hastanemizde çalışan kadın hemşirelerin toplam 100 çocuğu çalışmaya dâhil edildi. Çocukların cinsiyeti, doğum ağırlığı, her iki ebeveyninin çalışma şekilleri, her iki ebeveyninin eğitim durumu, anne sütü alma süresi, ek gıdaya başlama yaşığı, egzersiz alışkanlıklarına, atıştırma alışkanlıklarını, anne çalışma bakanının kim olduğunu ile ilgili bir anket hazırlanıcat ver çek için dolduruldu. Standart büyümeye eğrileri kullanılarak beden kitle indeksi persentilleri hesaplandı.

Bulgular: Standart beden kitle indeksi persentillerine göre çocukların 13’ü düşük kilolu, 53’ü normal kilolu, 14’ü fazla kilolu ve 20’si obez olarak hesaplandı. Çocukların ağırlıkları annenin düzenli mesai çalışması (p=0,016), atıştırma alışkanlıkları (p=0,003) ve sezaryen doğum (p=0,04) ile ilişkilili bulundu. Vardiya usulü çalışan hemşirelerin çocuklarının mesai çalışan hemşire-çocuklarına göre daha çok düşük kilolu olma eğiliminde idi. Çocukların vücut ağırlıkları ile ebeveynlerinin eğitim durumu, babanın çalışma şekli, anne sütü alma süresi, doğum kilosu, cinsiyet, doğum sırası ve ek gıdaya başlama yaşığı arasında ilişki saptanmadı.


Anahtar kelimeler: Çocukluk obezitesi; anne çalışma şekli; hemşire
Introduction

Obesity is a medical condition in which so much adipose tissue has accumulated in the body, that it has an adverse effect on the person’s health (1). Childhood obesity is one of the major public health problems worldwide (2). Childhood obesity is estimated to affect 20-30% of the population in western countries (3). Factors leading to childhood obesity are complex and include genetics, neurology, environment, physiology, sociocultural conditions, and ecology (3, 4).

Parental efficacy, especially maternal efficacy, has been linked to obesity (5). The influence of maternal employment in the development of childhood obesity has garnered the interest of several researchers. Recent studies have established a positive association between maternal paid work hours and pediatric obesity (6, 7). Longer work hours have been suggested to lead to insufficient family interactions. Also, mothers working long hours have insufficient time to prepare nutritious meals and supervise their child’s physical activity (8). In contrast, a recent study reported that childhood obesity was associated with irregular work schedules of fathers (9). Furthermore, this study found no association of obesity with maternal work schedule.

Health personnel in most of health units and hospitals are either on call or need to complete the shift work. The parent’s time at home as well as the time they spend with their children is not fixed. A study performed on nurses and midwives demonstrated that cumulative night-shift work was positively correlated with their own body mass index, waist circumference, hip circumference, and waist/hip ratio (10). However, limited data on their children was available.

The nurses in our hospital comprise a heterogeneous group in terms of education level and working status, along with other factors including age, number of children, and so on. Hence, we aimed to analyze some factors influencing the weight of the children of female nurses.

Subjects and Methods

This study was approved by the Dr. Burhan Nalbantoglu Ethical Committee. We had obtained written informed consent from the children’s parents.

This study was performed on children of nurses working at the Burhan Nalbantoglu state Hospital in Nicosia, Cyprus. Regular working hours at this hospital were from 07:30 am to 03:30 pm. Nurses on a regular schedule worked from Monday to Friday. However, the majority of the nurses worked on either of three shifts: 07:00 am to 02:00 pm, 02:00 pm to 09:00 pm, and 09:00 pm to 07:00 am. The shifts were completely irregular, and a nurse could be assigned any shift on a given day. Nurses working on shifts needed to clock approximately 40 work hours per week. When extra personnel was needed, these nurses would be asked to work overtime. Nurses working on irregular shifts were working in the same format since their first day of employment. Nurses working regularly either had previous experience of shift work or worked regularly since their first day of employment. Nevertheless, they had been working regularly since they had given birth.

The female nurses who worked at our hospital in the same work format since their child was born, who were not divorced, and had at least one child between 2-18 years of age were eligible for the study. A total of 158 female nurses met the criteria. Of these, 120 nurses were working on shifts and 38 were working regularly. Fifty-nine nurses agreed to provide information about their children. Thus, we included 100 children of 59 nurses in this study. We prepared a questionnaire including details such as the gender of the child, birth weight, education level and working status of both parents, the duration of breastfeeding, age at which supplementary food was first introduced, exercise habits, snacking habits, and identity of the caretaker while the mother was at work. The questionnaire form was filled by the participating nurse and was checked by the researcher.

Because of the unavailability and high cost of techniques directly measuring body fat, body mass index (BMI), which is the widely accepted clinical standard identifier of overweight and obese children (2), was used for this study. The child’s body weight was measured to the nearest 0.5 kg and height to the nearest 0.5 cm by the same researcher. The BMI was calculated using the formula:

\[ \text{BMI} = \frac{\text{weight (kg)}}{\text{height}^2 \text{ (m}^2)} \]

BMI percentiles and Z-scores were calculated using standard growth charts established by the Center for Disease Control and Prevention (CDC) (11). Children with BMI percentile <5% were considered underweight; those between 5-84% to be normal weight; 85-94% to be overweight; and 95% to be obese.
Statistical Analysis

We used the statistical program SPSS version 15.0 for Windows. We used chi-square tests and descriptive statistics for analysis of categorical data. We used logistic regression analysis for interpretation of independent variables. A two-sided p-value 0.05 was statistically significant.

Results

We analyzed data from 100 children of female nurses on duty at our hospital. The median age of the children was eight years, and range between 2 and 18 years. The female nurses included in our study were aged between 32 and 46 years, while their median age was 39 years. All nurses were working in the same work format since the birth of their child. The median birth weight was 3000 grams. Only three children weighed less than 2000 grams at birth. The birth weights of many children (69; 69%) ranged between 3000-4000 grams. The remaining 28 (28%) weighed between 2100-2900 grams at birth. The general properties of the study group are summarized in Table 1. According to standard BMI percentiles, 13 (13%) were underweight, 53 (53%) were normal weight, 14 (14%) were overweight, and 20 (20%) were obese. BMI was not associated with birth order (p=0.164), birth weight (p=0.761), duration of breastfeeding (p=0.872), introduction of supplementary food (p=0.166), father’s working status (p=0.457), physical activity (p=0.08), mother’s education level (p=0.558), and father’s education level (p=0.115).

In Table 2, we present the distribution of underweight, normal weight and overweight/obese children according to risk factors (such as snacking habits, method of birth, identity of the caretaker when the mother is at work, and mother’s work format).

Snacking habits were defined as consumption of high-calorie food, including biscuits, crisps, and chocolate. Adiposity was significantly positively associated with snacking habits (p=0.003). Out of 34 children who were obese or overweight, 23 (67.6%) were reported to consume snacks every day. In contrast, only three out of the 13 children (23.07%) in the underweight group were reported to consume snacks every day. The remaining 10 children (76.9%) were reported to either avoid snacks or consume snacks for only 1-2 times a week. This snacking pattern was reported in only six out of 34 children (17.6%) in the overweight/obese group (Table 2).

### Table 1. General properties of the study group.

<table>
<thead>
<tr>
<th>Property</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58 (58%)</td>
</tr>
<tr>
<td>Female</td>
<td>42 (42%)</td>
</tr>
<tr>
<td>Birth Order</td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>57 (57%)</td>
</tr>
<tr>
<td>2nd</td>
<td>38 (38%)</td>
</tr>
<tr>
<td>3rd</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Method of Birth</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>68 (68%)</td>
</tr>
<tr>
<td>C/S</td>
<td>32 (32%)</td>
</tr>
<tr>
<td>Duration of breastfeeding</td>
<td></td>
</tr>
<tr>
<td>0-6 months</td>
<td>54 (54%)</td>
</tr>
<tr>
<td>7-12 months</td>
<td>25 (25%)</td>
</tr>
<tr>
<td>13-24 months</td>
<td>16 (16%)</td>
</tr>
<tr>
<td>&gt;24 months</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Initiation of additional food (months)</td>
<td></td>
</tr>
<tr>
<td>At birth</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>1</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>2</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>3</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>4</td>
<td>24 (24%)</td>
</tr>
<tr>
<td>5</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>6</td>
<td>44 (44%)</td>
</tr>
<tr>
<td>7</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>8</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>10</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Maternal working status</td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>48 (48%)</td>
</tr>
<tr>
<td>Shifts</td>
<td>52 (52%)</td>
</tr>
<tr>
<td>Paternal working status</td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>72 (72%)</td>
</tr>
<tr>
<td>Shifts</td>
<td>28 (28%)</td>
</tr>
<tr>
<td>Maternal education level</td>
<td></td>
</tr>
<tr>
<td>Health School</td>
<td>27 (27%)</td>
</tr>
<tr>
<td>Degree in Nursing</td>
<td>73 (73%)</td>
</tr>
<tr>
<td>Paternal Education Level</td>
<td></td>
</tr>
<tr>
<td>Junior School</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Secondary School</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>High School</td>
<td>40 (40%)</td>
</tr>
<tr>
<td>University</td>
<td>53 (53%)</td>
</tr>
<tr>
<td>Where is the child when mother is at work?</td>
<td></td>
</tr>
<tr>
<td>With Father</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>With Grandparents</td>
<td>37 (37%)</td>
</tr>
<tr>
<td>At School</td>
<td>14 (14%)</td>
</tr>
<tr>
<td>With Babysitter</td>
<td>23 (23%)</td>
</tr>
<tr>
<td>At Home alone</td>
<td>22 (22%)</td>
</tr>
<tr>
<td>Snacking Habits</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>11 (11%)</td>
</tr>
<tr>
<td>Every day</td>
<td>58 (58%)</td>
</tr>
<tr>
<td>1-2/week</td>
<td>23 (23%)</td>
</tr>
<tr>
<td>&gt;3/week</td>
<td>8 (8%)</td>
</tr>
<tr>
<td>Physical Activity</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>30 (30%)</td>
</tr>
<tr>
<td>Once a week</td>
<td>42 (42%)</td>
</tr>
<tr>
<td>2-4/week</td>
<td>20 (20%)</td>
</tr>
<tr>
<td>Everyday</td>
<td>8 (8%)</td>
</tr>
</tbody>
</table>

C/S: Caesarian sections.
Adiposity was significantly positively associated with the method of birth \((p=0.04)\). Out of the 34 overweight or obese children, 28 (82.3%) were born by Caesarian Section (C/S). In contrast, this ratio was 46.1% and 64.1% in the underweight and normal-weight children, respectively (Table 2). Adiposity was also significantly associated with the identity of the caretaker while the mother was at work \((p=0.04)\). Seven out of the 13 underweight children (53.8%) were looked after by a babysitter, whereas only four of 34 children in the obese/overweight group (11.7%) were looked after by a babysitter (Table 2). Adiposity was significantly associated with the mother’s working status \((p=0.016)\). The mothers of 12 out of the 13 underweight children were working on irregular shifts (92.3%), while only one worked on a regular basis (7.7%). On the other hand, the mothers of 20 out of the 34 obese/overweight children were working on a regular basis (58.8%), compared to 14 on irregular shifts (41.1%). The median ages and work experience periods of the nurses in both irregular shifts and regular working groups were similar (37 vs. 38, \(p=0.56\); 12 vs. 13, \(p=0.46\)). There was no association between children’s weight and mothers’ education level, fathers’ education level, fathers working status, duration of lactation, birth weight, children’s gender, birth order, and time of consumption of additional food. We performed a multivariate logistic regression analysis to define the independent variables for childhood obesity and underweight children. The models were adjusted for snacking habits, identity of the caretaker, mothers working status and method of birth. Regular working hours \((p=0.019)\) and C/S birth \((p=0.043)\) were determined to be independent risk factors for childhood obesity. Furthermore, caregiving by a babysitter \((p=0.037)\) and shift patterns of the mother \((p=0.049)\) were determined to be independent risk factors for underweight children.

**Table 2. Distribution of some factors among BMI groups.**

<table>
<thead>
<tr>
<th></th>
<th>Underweight (n/%)</th>
<th>Normal (n/%)</th>
<th>Overweight/obese (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Snacking Habits</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No snacking</td>
<td>4 (30.8%)</td>
<td>3 (5.7%)</td>
<td>4 (11.8%)</td>
</tr>
<tr>
<td>1-2/week</td>
<td>6 (46.2%)</td>
<td>15 (28.3%)</td>
<td>2 (5.8%)</td>
</tr>
<tr>
<td>&gt;3/week</td>
<td>3 (33.0%)</td>
<td>35 (64.0%)</td>
<td>28 (82.4%)</td>
</tr>
<tr>
<td><strong>Method of birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/S</td>
<td>6 (46.2%)</td>
<td>34 (64.2%)</td>
<td>28 (82.4%)</td>
</tr>
<tr>
<td>Normal</td>
<td>7 (53.8%)</td>
<td>19 (35.8%)</td>
<td>6 (17.6%)</td>
</tr>
<tr>
<td><strong>Caretaker</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At School</td>
<td>1 (7.7%)</td>
<td>6 (11.3%)</td>
<td>7 (20.6%)</td>
</tr>
<tr>
<td>Home alone</td>
<td>1 (7.7%)</td>
<td>9 (17.0%)</td>
<td>12 (35.3%)</td>
</tr>
<tr>
<td>Grandparents</td>
<td>4 (30.8%)</td>
<td>22 (41.5%)</td>
<td>11 (32.4%)</td>
</tr>
<tr>
<td>Father</td>
<td>0 (0%)</td>
<td>4 (7.5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Babysitter</td>
<td>7 (53.8%)</td>
<td>12 (22.6%)</td>
<td>4 (11.8%)</td>
</tr>
<tr>
<td><strong>Mothers work type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>1 (7.7%)</td>
<td>21 (39.6%)</td>
<td>20 (58.8%)</td>
</tr>
<tr>
<td>Shifts</td>
<td>12 (92.3%)</td>
<td>32 (41.4%)</td>
<td>14 (41.2%)</td>
</tr>
</tbody>
</table>

BMI: Body mass index; C/S: Caesarian section.
Parental efficacy, especially maternal efficacy, has been linked to the development of childhood obesity (5). Recent studies have established a positive association between maternal paid work hours and child obesity (6, 7). It is argued that longer work hours lead to insufficient family interactions. Also, long-working mothers have insufficient time to prepare nutritious meals and supervise their child’s physical activity (8). Apart from parental working hours, there has been an increase in the proportion of families with either or both parents working at variable intervals (9). A recent study established that childhood obesity was associated with irregular work schedules of fathers. This study found no association of obesity with maternal work schedule (9).

In this study, we observed no association of parental work schedule with the child’s adiposity. The duration of work hours of female nurses working on fixed schedules and those on shift work were identical at our hospital and differed only in the regularity of work. The percentage of underweight children was higher in nurses working irregularly, while the percentage of obese children was higher in nurses working regularly. Furthermore, it was also observed that a high percentage of nurses working on shifts left their children to babysitters while they were at work. More children looked after by babysitters were underweight than those taken care of by someone else (father, grandparents, school, home alone). Babysitters may not be able to handle the nutritional support of the child as well as mothers. In addition, children of regularly working nurses were more likely to be overweight or obese. Despite an increase in the proportion of working mothers over the past decades, the domestic tasks continue to depend on the mother, and fathers’ contributions are minimal (14). Thus, when the mother is not at home, the caretaker does not take as much responsibility of the child in all aspects including nutrition.

Frequent snacking habits were also associated with adiposity, which was expected as it is consistent with several other studies in the literature (15, 16). An interesting finding of our study was that more children born by C/S were obese/overweight than those born normally. This association was independent of the birth weight. A recent meta-analysis demonstrated that children born by C/S had a 1.34 Relative Risk (95% CI 1.18–1.51) compared to children born normally (17). Another study published later reported similar results in pre-school children (18). The authors showed that children born by C/S faced a 24% more chance of being overweight and 29% higher obesity risk. It has been hypothesized that lack of exposure to maternal gut microbiota in children born via C/S could be a possible biological explanation for the development of obesity in early or later childhood (18, 19).

In this study, we also established some results which were like those previously reported, such as positive correlations between birth by C/S and snacking habits with the development of childhood obesity. Interestingly, maternal shift work was associated with more chances of the child being underweight, while regular working was associated with childhood obesity. Although other members of the family aid in feeding the children, their mother still holds a central role in their nutrition. Thus, maternal work patterns may affect their child’s adiposity levels. These findings need to be confirmed by future studies on a larger scale.

Acknowledgements: Mrs. Oya Adsiz Marasuna helped in the preparation and distribution of the questionnaire.

The abstract of this study was presented in the European Congress of Endocrinology in 2016.

Source of Finance: During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest: No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author’s own work and no other author contribution.

References


Expression Level of Circulating miR-93 in Serum of Patients with Diabetic Nephropathy

Diyabetik Nefropati Hastalarının Serumlarında Serbest miR-93 Ekspresyon Düzeyleri

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Abstract

Objective: Diabetic nephropathy is a long-term complication of diabetes and is manifested as reduced glomerular filtration rate, increased urinary albumin excretion, and glomerular lesions. The study aim was to determine the expression level of serum cell-free miR-93 in diabetic patients with or without DN and compare that to healthy controls.

Material and Methods: In this case-control study, 61 type 2 diabetes patients (21 without diabetic nephropathy, 20 with microalbuminuria and 20 with macroalbuminuria), and 22 healthy controls were included. Cell-free microRNA was extracted from the serum of participants and real-time polymerase chain reaction was performed using SYBR Green master mix. The gene of hsa-miR-16 was used as a reference gene.

Results: Our findings revealed a significant downregulation of miR-93 expression in the serum of diabetic patients with or without nephropathy compared to the healthy individuals (p<0.005). However, there was no significant difference between the three groups of diabetic patients presenting different degrees of nephropathy.

Conclusion: Serum miR-93 is a good diagnostic marker for diabetes, but is not useful to distinguish between the diabetics with and without nephropathy.

Keywords: Diabetes; diabetic nephropathy; microRNA; miR-93

Introduction

Diabetes mellitus is a common chronic metabolic disorder with a considerable economic burden worldwide, particularly in low and middle-income countries (1, 2). The prevalence of diabetes in Iranian adults was estimated to be 8.5% by IDF (International Diabetes Federation) in 2015 (3). The number of people with type 2 diabetes mellitus (T2DM) was estimated as 415 million in 2015, and is expected to increase to 642 million by 2040 (3). The high economic burden of diabetes is likely related to its care and manage-
ment, as well as its long-term complications and comorbidities that have been the subject of many studies (4-9). In Iran, about 8.7% of the total healthcare budget is spent on diabetes (10). Diabetic nephropathy (DN) is the most serious microvascular and second most common complication of diabetes and is the leading cause of end-stage renal disease (ESRD) (11, 12). Several mediators and pathways, including hyperglycemia, advanced glycation end products (AGEs), protein kinase C, oxidative stress, inflammation, angiogenesis, renin-angiotensin–aldosterone system (RAAS), and the AGE/RAGE (advanced glycation end-products/receptor AGE) are involved in the pathogenesis and progression of DN (13, 14). In addition, some genetic factors may also predispose some patients to a higher risk of developing diabetes as well as its complications (15). Several studies have so far investigated the association between genetic variants of various genes such as ELMO1 and DN (16).

A growing body of evidence considers circulating miRNAs as potential biomarkers for early detection of various diseases. MicroRNAs are short non-coding RNAs (~21–24 nucleotides in length) that modulate physiological and pathological processes by posttranscriptional regulation of target genes of different pathways (17, 18). Dysregulation of miRNAs is correlated with pathogenesis of diabetes, and mainly influences the function of pancreatic β-cells, insulin resistance or both. In addition, the progression of diabetes is associated with distinct modifications in the profile of serum miRNAs (19). Various microRNAs have been implicated in DN pathogenesis (20), and the signature of circulating miRNAs can be used to predict disease progression (21). MiR-93 regulates microvasculatization in the kidney and reduces angiogenesis by downregulating vascular endothelial growth factor (VEGF) (22, 23). The aim of this study was to determine the expression levels of serum miR-93 in diabetic patients with and without nephropathy and compare that to healthy controls.

**Material and Methods**

**Patient characteristics**

Sixty-one T2DM patients and 22 healthy controls were enrolled at the Diabetes Clinic, affiliated to Diabetes Research Centre (Shariati hospital, Tehran University of Medical Sciences) and Rast Ravesh Clinic (Alorz University of Medical Sciences) from August 2015 to September 2016 for a case-control study. T2DM diagnosis was confirmed by the ADA (American Diabetes Association) criteria (24) or history of using antidiabetic medications. The demographic information and disease history of each participant was recorded; parameters like weight, height, fasting blood sugar (FBS), HbA1c, serum urea and creatinine, and urine albumin/creatinine were measured. Glomerular filtration rate (GFR), body mass index (BMI) and albumin to creatinine ratio (ACR) were calculated based on the above, and persistent proteinuria was confirmed by the urinary albumin to creatinine ratio (ACR).

Patients with at least a 5-year history of diabetes without any evidence of nephropathy were selected as the control diabetes group. The remaining diabetic patients with nephropathy were stratified into the microalbuminuria (ACR: 30–299 µg/mg) and macroalbuminuria (ACR≥ 300 µg/mg) groups according to the KDOQI criteria (25). Healthy individuals older than 20 years of age, without any history or evidence of diabetes and nephropathy, and FBS less than 100 mg/dL were considered as the healthy control group. The exclusion criteria for all groups were pregnancy, lactation, history of smoking and alcohol consumption, obesity (BMI≥30), hypertension (systolic ≥140 or diastolic ≥90 or hypertension medication) and insulin therapy.

Informed consent was obtained from all participants before enrollment. The study protocol was approved by the Ethics Committee at Zanjan and Tehran University of Medical Sciences.

**Sample collection and serum preparing**

Blood samples were collected by Venoject in two tubes, one with and one without EDTA, for measuring HbA1c, and serum isolation and biochemical assays, respectively. To separate the serum, the blood samples were incubated for about an hour at room temperature and then centrifuged at 3000 g for 15 min. The clear supernatant was harvested and divided into two parts: one aliquot was used for biochemical assays and another was stored at -80°C in DNase/RNase free tube till use.

**Biochemical measurements**

Biochemical parameters including FBS and blood urea were measured using enzymatic assays. Serum creatinine was measured by Jaffe method using creatinine kit (Pars Azmun, Iran) and BIOLIS 24i Premium (Tokyo Boeki Machinery Ltd Japan). HbA1c was measured using HPLC with the Tosoh G8 instrument (South San Francisco, CA).
RNA extraction, cDNA synthesis, and real-time PCR

Frozen serum samples were thawed at room temperature and centrifuged at 3000 g for 5 min to pellet the cells, and cell-free microRNA was extracted from 200 µL of serum using miRCURY™ RNA Isolation Kits-Biofluids (Exiqon®, Denmark) according to the manufacturer’s instructions (www.exiqon.com). Any contaminating genomic DNA was eliminated by DNAseI. To detect any inhibitor and also the potential loss of RNA, the serum was spiked with synthetic RNAs (Exiqon, Denmark). The concentration of the extracted RNA was determined by NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). The cDNAs were synthesized by Universal cDNA synthesis kit (Exiqon, Denmark) using 5–10 ng miRNA. Real-time PCR was carried out using LNA™ primers, ExiLENT SYBR Green master mix (Exiqon, Denmark) and 10x diluted cDNA on step one instrument (ABI, USA) according to the manufacturer’s instructions. The hsa-miR-16 and hsa-miR-93 were used as reference and candidate genes respectively. All experiments were performed in duplicate. PCR efficiencies were determined using LinRegPCR 12.x software (AMC, Amsterdam, http://LinRegPCR.nl).

Data Analysis

Real-time PCR data were analyzed by GenEX 5 (MultiD, Sweden) and SPSS (version 21 for Windows, SPSS Inc., USA) programs. All variables (BMI, FBS, HbA1c, serum urea, Cr, urine Alb/Cr ratio and GFR) were compared between the groups using Kruskal-Wallis or one-way ANOVA, as appropriate. For miR-93 gene expression analysis, the data were first adjusted by a spike in RNA, and the fold change in gene expression in the diabetic groups was normalized to the mean of the healthy control group using Pfaffl formula (26). The data were also adjusted for age, HbA1c, and duration of diabetes using the logistic regression model (each time for a covariate). Variables were compared between the groups using Kruskal-Wallis and also Mann-Whitney nonparametric test. A threshold value of 0.05 was considered as statistically significant. Receiver operating characteristics (ROC) curve was plotted to determine the correlation between miR-93 and the diabetic and non-diabetic state. Correlation of GFR (Glomerular Filtration Rate) and miR-93 expression was analyzed using Spearman’s correlation test.

Results

Mean age of participants was 57±10.5 years and 60.25% (50) of them were male. Clinical and biochemical characteristics of all participants in the four study groups (healthy controls, T2DM without nephropathy, T2DM with microalbuminuria and T2DM with macroalbuminuria) are summarized in Table 1. There was a significant difference in BMI, FBS, HbA1c, serum urea, serum Cr, urine Alb/Cr ratio and GFR between the study groups (Table 1).

Table 1. Clinical and biological characteristics of the participants.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal healthy control</th>
<th>Diabetic without nephropathy</th>
<th>Diabetic with microalbuminuria</th>
<th>Diabetic with macroalbuminuria</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>22</td>
<td>21</td>
<td>20</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>9/13</td>
<td>11/10</td>
<td>16/4</td>
<td>14/6</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.2±7.2</td>
<td>61.5±6.5</td>
<td>64.7±6.8</td>
<td>54.8±11.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Duration of diabetes (y)</td>
<td>N/A</td>
<td>12.9±4.8</td>
<td>15.3±4.8</td>
<td>13.1±6.5</td>
<td>0.33</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.88±1.18</td>
<td>23.20±1.88</td>
<td>23.94±1.26</td>
<td>26.25±2.67</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>89.6±6.1</td>
<td>138.3±52.2</td>
<td>187.3±70.5</td>
<td>147±72</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.3±0.2</td>
<td>6.8±0.9</td>
<td>7.7±1</td>
<td>8.5±2.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum urea (mg/dL)</td>
<td>25.9±5.6</td>
<td>37±9.8</td>
<td>45.7±13.8</td>
<td>60.1±30.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum Cr (mg/dL)</td>
<td>0.98±0.2</td>
<td>0.98±0.19</td>
<td>1.9±2.0</td>
<td>1.6±0.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Urine Alb/Cr</td>
<td>23±4.4</td>
<td>26.4±4.7</td>
<td>104±51</td>
<td>819±420</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>83±13.8</td>
<td>72±11.3</td>
<td>67.6±16.6</td>
<td>55.2±28.8</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Values are means ±standard deviation.
GFR: Glomerular filtration rate; FBS: Fasting blood sugar; BMI: Body mass index; HbA1c: Hemoglobin A1c; Cr: creatinine.
*p<0.05 significant.
The extracted RNA was evaluated by NanoDrop, and the OD 260/280 was 2.7-2.2 and RNA concentration was 18±6.2 ng/µl. The mean CT value of miR-93 and miR-16 expression in the entire cohort was 31.72±2.6 and 24±1.5, respectively. Comparison between the diabetic patients (irrespective of kidney function) and the healthy controls showed a significant difference in miR-93 expression (p:0.005), but no significant difference was seen within the diabetic group (Figure 1A). The downregulation of miR-93 expression was observed in 68% of the diabetic patients without nephropathy, 88% of those with microalbuminuria and 72% of the patients with macroalbuminuria (Figure 1B). Furthermore, after adjusting for HbA1c and duration of diabetes, no significant association was seen between GFR (GFR<60 vs GFR≥60) and miR-93 expression among all diabetic patients. However, there was a significant but weak negative correlation between miR-93 expression level and Alb/Cr ratio (r=-0.31, p:0.005). Finally, as per the ROC results, low expression of miR-93 was an independent predictor of diabetes (AUC=0.72, p=0.004, 95% CI: 0.59-0.84) (Figure 2).

Discussion

Our results showed a significant decrease in the expression of miR-93 in T2DM patients compared to the healthy controls, while no significant difference was found among the diabetics with different renal functions. To the best of our knowledge, this is the first study that evaluates circulating cell-free miR-93 in the serum of patients with DN. The AUC (72%) indicated that although miR-93 can accurately discriminate between the diabetics and healthy individuals, it failed to discriminate between patients with DN from those diabetics without nephropathy. MicroRNAs have been considered as therapeutic targets and also potential biomarkers in complex human disorders such as diabetes and cancer (19, 27-29). Circulating miRNAs seem to have the potential of detecting the early development of diabetes several years before the symptoms start manifesting (21, 30). Other studies have reported a decreased expression of miR-93 in some diseases. Salas-Perez et al. showed a downregulation of miR-93 expression in the peripheral blood mononuclear cells (PBMCs) of T1DM patients (0.331±0.05, p<0.02) compared to healthy controls (31).

Figure 1: Comparative expression of miR-93 between different groups of patients (diabetics without nephropathy, diabetics with microalbuminuria, diabetes with macroalbuminuria) versus normal healthy controls. Each sample was normalized to the mean value of gene expression in the normal group. A) Boxplot histograms show the fold change of miR-93 expression in different groups. B) The relative miR-93 gene expression in individual samples.

Long et al. analyzed the expression of miR-93 and VEGF in the glomeruli of diabetic db/db mice, and reported a significant downregulation of miR-93 expression in the diabetic mice compared to the db/m control mice. As VEGF has a critical role in microvascular complications of diabetes, they concluded that mir-93 may act as a key regulator of the VEGF gene (23). In another study, Saito et al. identified a negative correlation between miR-93 expression levels and VEGF-A and concluded that mir-93 probably plays a role in the pathogenesis of acute Kawasaki disease (32). Furthermore, Ulbing et al. reported a decreased expression of miR-93-5p in patients with chronic kidney disease (CKD) at stages 4 and 5 compared to healthy controls, indicating that miR-93 downregulation may drive the progression of DN to CKD (22). A recent study by Shawn et al. showed that in the presence of high glucose lev-
els, miR-93 contributes to chromatin remodeling in podocytes of kidney (33). According to their results, miR-93 had a significant function in angiogenesis and its dysregulation resulted in diabetic complications. The role of miR-93 in VEGF inhibition and angiogenesis has also been reported in other diseases, especially cancer. Some studies have reported an oncogenic role of miR-93, e.g., in gastric (34) and bladder cancers (35), while others have described a tumor suppressor activity of miR-93 in the breast (36), neuroblastoma (37) and colorectal (38) cancers. There are several limitations of our study, including the small number of patients in each study group, lack of mir-93 polymorphism analysis, and the expression of its target genes. Our results, therefore, need to be validated with studies on larger cohorts, along with the evaluation of downstream target gene expression.

Conclusion
The expression of miR-93 is influenced by the diabetic pathology and downregulated in diabetes both with and without accompanying nephropathy. Our results showed that miR-93 can discriminate between diabetes and normal glycemic status with high sensitivity, but not between the diabetic groups with varying degrees of nephropathy. As miRNAs are stable in plasma and serum samples, the detection of circulating miRNAs can help in the early prediction of certain diseases and enable suitable treatment strategies to be developed.

Acknowledgement
This work was supported by a fund from Diabetes Research Center affiliated to Tehran University of Medical Sciences and Zanjan University of Medical Sciences (ZUMS). The authors have no conflict of interests.

Source of Finance: During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest: No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions
Idea/Concept: Alireza Biglari; Masoume Akhbari; Fatemeh Bandarian; Design: Fatemeh Akhbari, Alireza Biglari; Control/Supervision: Fatemeh Bandarian, Alireza Biglari; Data Collection and/or Processing: Masoume Akhbari, Mitra Khalili, Maryam Shahrabi-Farahani; Analysis and/or Interpretation: Mitra Khalili, Fatemeh Bandarian; Literature Review: Mitra Khalili, Fatemeh Bandarian; Writing the Article: Mitra Khalili, Masoume Akhbari; Critical Review: Fatemeh Bandarian, Mitra Khalili; References and Fundings: Diabetes Research Center, Zanjan University Zanjan University of Medical Sciences.

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LEPR Deficiency: Prevalence and Importance of a Novel Mutation and Significant Genetic Variants, Usually Underestimated

Leptin Reseptör Eksikliğinde Tespit Edilen Yeni Bir Mutasyon ile Göz Ardı Edilen Genetik Varyantların Önemi ve Prevalansı

Atil Bişgin
Department of Medical Genetics, Çukurova University Faculty of Medicine, Balcalı Clinics and Hospital, Çukurova University AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Adana, Turkey

Abstract
Objective: Diagnostic testing for leptin receptor deficiency, a rare cause of obesity, should be performed in cases where it may affect the clinical management. Therefore, molecular tests are required to grant a conclusive diagnosis. In this study, the clinical utility of molecular testing and the importance of genetic counselling resulting from all the genetic variants, including both, disease-causing mutations and polymorphisms has been outlined.

Material and Methods: The study consisted of samples of leukocyte-DNA in sixteen clinically deficient patients of leptin receptor. In order to identify the molecular basis, the LEPR gene sequencing was employed using next-generation sequencing platform (MiSeq System, Illumina) for all the exons, introns and exon-intron binding regions. In-silico analyses for novel mutations were carried out using SIFT, Polyphen2 and MutationTaster. Paternal carrier testing was also accomplished.

Results: The causative mutation was identified in three out of sixteen patients with leptin receptor deficiency (18.75%). All these three patients carried the same, novel, homozygous p.P639L (c.1916C>T) mutation. Most interestingly, 62.5% of the patients (n=10) were found to be carrying at least one of the possible disease-risk-polymorphisms related to obesity, increased body mass index, insulin resistance and glucose intolerance.

Conclusion: This study presented with two important outcomes. First, the novel p.P639L mutation could be identified in three different patients and, second, but most important, the fact that polymorphisms of the leptin receptor gene, usually underestimated, is the main genetic predisposition factor for the Turkish population. It is, therefore, critical to identify not only the mutations but all the genetic variants responsible for leptin receptor deficiency, to aid in diagnosis, prevention, prognosis, treatment, and research.

Keywords: LEPR deficiency; morbid obesity; genetic testing; LEPR novel mutation

Özet
Amaç: Leptin reseptör eksikliği, nadir bir obezite sebebi olup, tanı testlerinin yapılmasına klinik sağaltım açısından önemlidir. Dolayısıyla, kesin tanı için moleküler testler gerektirir. Bu çalışmada, sadece hastalık etkeni olan patojenik varyantlar değil, polimorfizm de dahil tüm genetik varyantların saptanmasını genetik danışmanlık ve hastalığın moleküler tanıısı için klinik kullanılabileceği vurgulanmaktadır.


Bulgular: Çalışmaya alınan 16 leptin reseptör eksikliği hastasının 3 (%18,75)’ünde hastalık etkeni bir mutasyon saptanmıştır. Bu hastalarda saptanan varyant aynı olup; yeni, homozigot p.P639L (c.1916C>T) mutasyonudur. İlginç olarak, hastaların %62,5’inde (n=10) hastalığa yatıklikla ilgili en az bir polimorfizm saptanmış olup; bunlar obezite, artmış beden kitle indeksi, insulin rezistansı ve glukoz intoleraşyonu ile ilişkili varyantlardı.


Anahtar kelimeler: LEPR eksikliği; morbid obezite; genetik test; LEPR yeni mutasyon

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Introduction

Leptin receptor (LEPR) deficiency is a rare cause of obesity. LEPR deficiency causes the clinical effects to begin in the first few months of life (1). Though most of the affected patients lie in the normal range of birth weight, yet, they start gaining weight quickly due to the constant hunger. Right from the early childhood, patients have hyperphagia and develop their own abnormal eating behaviors that may include fighting for the food or even eating secretly. Nevertheless, the most important clinical outcome of these patients is the effect on sexual development. LEPR deficient patients have hypogonadotropic hypogonadism which may result in infertility (1-3).

LEPR deficiency is caused by mutations in LEPR gene that encodes the leptin receptor protein on the surface of many cells but is expressed mostly in the hypothalamus (2, 4). It is also one of the rare diseases with an autosomal recessive inheritance pattern, affecting the morbid obesity group (2). Mutations in LEPR gene causes a deficiency in the leptin receptors or an in-effective receptor complex. Consequently, this loss of function prevents the receptor from responding to leptin released from the fat cells. This triggers the activation of a series of signaling pathways, leading to excessive hunger, weight gain and decreased sexual development (4-9). However, the mechanism of these effects is not yet completely understood.

This inherited obesity resulting from LEPR deficiency is difficult to diagnose, due to considerable clinical overlap and since most patients typically do not show signs and symptoms of the condition (10, 11). Hence, molecular tests are required to obtain a conclusive diagnosis as well as to have the benefits of diagnosis including the pre-symptomatic diagnosis and screening, prevention, treatment, prognosis, and research.

Genetic counselling, together with clinical evaluation, plays a critical role in the appropriate use of molecular tests. In this study, the clinical utility of molecular testing and the importance of polymorphisms that are mostly underestimated by many laboratories and clinicians have been outlined by the authors using the experiences they have gained.

Material and Methods

This study was carried out on the biological samples collected from The Cellular and Tissue Biobank of Cukurova University, Balcali Hospital and Clinics that focuses on rare diseases. A total of, sixteen pediatric patients (mean age of 21±1 months) with LEPR deficiency were referred to AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Cukurova University for molecular studies, and genetic counseling.

In order to identify the molecular basis, the genetic analysis including leptin receptor deficiency related LEPR gene was employed as a molecular diagnostics tool by using next-generation sequencing platform (MiSeq System, Illumina). The coverage of the test included all exons for each gene, at least 50 nucleotides upstream and downstream of each exon and 1 kb of both the 5' promoter regions and the 3' UTRs. Sequencing was performed on the leukocyte DNA collected from 16 patients with LEPR deficiency. In-silico analysis for the novel mutations was carried out using SIFT, PolyPhen2 and Mutation Taster. All changes considered to have potential clinical relevance were confirmed by paternal testing to identify the carrier status. ClinCalc (http://clincalc.com) was used to determine the post-hoc power of the study and to apply the Bonferroni correction. All the statistical analyses were performed using the GraphPad Prism software (GraphPad Software, Inc. USA), while the statistical significance was defined at p≤0.05. Hardy-Weinberg equilibrium analysis was performed for each polymorphism identified. A modified version of the human genome (www.varsome.com) was used as the major allele population-specific reference. Confidence interval (CI) as 95% was used to estimate the precision of the odds ratio. The chi-square test was also used to test the frequencies of the alleles and genotypes. All the procedures performed in this study were in accordance with the ethical standards of the institutional ethical and national research committee and the Helsinki declaration. Informed consent of the patients and their families were conducted to keep all the information confidential was taken before the study began.
Results and Discussion

All genetic variants detected in the patients are listed in Table 1 within the column homozygosity status. The causative mutations were identified in three out of the sixteen patients (Patient 1, 2 and 3; sum up 18.75%) affected with LEPR deficiency and there was a novel homozygous p.P639L (c.1916C>T) mutation, in all these three patients, as shown in Figure 1. Neither of these patients had any consanguinity nor did they belong to the same city, but all had a middle-eastern origin. The in-silico analysis revealed that this variant was pathogenic and the mutation was a disease-causing one. Thus, paternal studies were performed and it was found that the mothers and fathers of all these three patients were first degree cousins and were heterozygous for the p.P639L (c.1916C>T) mutation in LEPR gene which confirms the results. This might have occurred because of the probability of carrying this mutation at a high frequency that can be speculated as a founder mutation in LEPR gene. However, further population studies must be carried out to support this conclusion.

The other significant data from this study included the frequency of polymorphisms that are mostly underestimated and usually left unreported in the genetic test reports. Ten out of the sixteen patients (62.5%) had at least one or more polymorphisms, as given in Table 1. Among all the polymorphisms detected (n=10) in patients with LEPR deficiency, p.Q223R (c.668A>G) was the most common. Six out of these ten patients (60%) also showed other polymorphisms along with p.Q223R (c.668A>G). Patients 4, 5 and 6 had two homozygote polymorphisms of p.K109R (c.326A>G) and p.Q223R (c.668A>G). The frequency of these alterations in heterozygous population is high, around 40% (3, 12). However, there are also studies indicating that homozygous p.K109R polymorphism is related to increased birth weight and body-mass index (2, 10, 13). The homozygous p.Q223R variant was found to be significantly associated with obesity in previous studies (14). A study also suggests that when these two variants occur together, they are also associated with glucose intolerance (15, 16).
Patient 7 had three heterozygous polymorphisms of p.K109R (c.326A>G), p.Q223R (c.668A>G) and p.S343S (c.1029T>C). p.S343S (c.1029T>C) is a synonymous variant that does not have any effect on the protein level. Since all these three variants with high population frequency (12) were heterozygous in this patient, no genetic effect was expected in the patient apart from only mild clinical findings, in case of any cumulative effect at all.

Patients 8 and 9 had two heterozygote polymorphisms of p.K109R (c.326A>G) and p.Q223R (c.668A>G). While patients 10 and 11 exhibited only p.Q223R (c.668A>G) heterozygous polymorphism, patients 12 and 13 showed only a single heterozygosity for p.K109R (c.326A>G) polymorphism in LEPR gene. The last three patients (patient numbers 14, 15 and 16) had a normal sequencing data. It is therefore important to carry out MLPA (multiplex ligation-dependent probe amplification) for the deletion and duplication analysis of LEPR gene. Unfortunately, no MLPA probe has been designed for in-vitro diagnosis.

However, the undiagnosed LEPR deficiency patients in whom, genetic testing was not helpful in identifying the disease, might have had yet uncharacterized mutations in other genes.

For each of the 16 patients tested, at least one variant detected in 81.25% of the patients (n=13). In-silico analysis and family studies were performed to help define the pathogenicity of the novel changes. In addition to the polymorphisms, the causative novel mutation was identified in three out of sixteen patients. More interestingly, the identified alterations that are classified as polymorphisms, may have had an effect on the clinical findings, as per the literature review and data from the present study (16). The data in this, therefore, proposes a greater effort in the molecular diagnosis of rare diseases with appropriate genetic reporting in favor of diagnosis and prevention, and not just the control of co-morbidities, with the aim of enhancing the prognosis and a closer follow-up for the patients in order to improve their quality of life.
Conclusion

To summarize, the availability of molecular genetic testing has profound implications for the clinicians, patients and their families. The benefits of genetic testing can be utilized for the diagnoses including the presymptomatic diagnosis and screening, prevention, treatment, prognosis, and research. However, a challenge still exists in the field of genomics that when potential novel genes/mutations/polymorphisms or phenotypes are detected, large group studies, functional studies, and model systems are needed. At the last place, even if a disease is monogenetic as in case of LEPR deficiency, results will still outcome as non-diagnostic or only about susceptibility due to possible defects on other genes or the epigenetic factors such as DNA methylation and histone modification, or epistasis. In this study, a novel mutation, which is a disease-causing mutation, was identified in three different patients. The compound homo/heterozygosity for the polymorphisms was also determined that has helped in a better understanding of its association with obesity, increased body mass index, insulin resistance, and the glucose intolerance. While allelic heterogeneity can result in varying phenotypic severity, phenotypically identical disorders can also have an entirely different genetic basis (genocopy). It is, therefore, critical to identify the mutation/polymorphism-associated risks by molecular analysis. It is also important not to underestimate the disease associated polymorphisms that has to be specified in genetic testing reports.

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Authorship Contributions

This study is entirely author’s own work and no other author contribution.

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Complications of Stump Healing Among Diabetic Population

Diyabetik Populasyonda Güdük Yarasi Komplikasyonları

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Abstract

Lower limb amputation is a major procedure performed in diabetic patients with multiple comorbidities. Almost 10% of the National Health Service budget is taken up by diabetes, with diabetes-related complications accounting for 80% of the costs. The process of wound healing is complex and involves regenerating the cellular organization and the tissue layers. Diabetics are five times more predisposed to wound infection than patients without diabetes mellitus. The amputated stump frequently becomes infected due to inadequate blood circulation, a weak immune system, and poorly controlled diabetes mellitus. Pain, stump edema, and osteomyelitis are significant complications associated with lower limb amputation wounds. A number of factors may substantiate the need for re-amputation, such as stump pain and/or phantom limb pain, delayed stump infection, the formation of symptomatic bone spurs, assessment of the skin flap designed to preserve stump length, and preparation of the stump for the prosthetic device. There are currently no reliable standards that can be referred to prior to leg amputation. The clinicians, therefore, have to rely on their judgment and investigatory parameters. The main purpose of this review is to discuss the difficulties of stump healing in the diabetic population.

Keywords: Diabetes; complication; leg amputation; stump healing

Özet


Anahtar kelimeler: Diyabet; komplikasyon; amputasyon; güdük iyileşmesi

Introduction

Between 2006-2011, the number of people diagnosed with diabetes mellitus (DM) has increased by 25% in the UK alone, from 1.9 million to 2.5 million (1). Furthermore, the number of people with undiagnosed DM is estimated to be around 850,000. Based on the existing trends, the number of diabetics in the UK is expected to reach 5 million by 2025. Type 2 DM is more prevalent (90%) than type 1 DM (10%) (2). Concomitantly, DM-related complications like amputations, stroke, blindness, and end-stage kidney failure are also on the rise and are life threatening with a high mortality rate. Schofield et al. (3) reported
that the median time to death following lower limb amputation was higher in patients with DM than in healthy controls (27.2 months vs. 46.7 months, \( p=0.01 \)). In addition, the study also found that patients with DM had a higher risk of developing congestive heart disease or needing an amputation by factors of 2.26 (95% CI 1.12-4.5) and 1.95 (95% CI 1.14-3.33), respectively. Diabetic foot disease often leads to serious long-term complications, resulting in significant socio-economic and healthcare burdens. The UK National Health Service (NHS) is under enormous financial strain as a result of diabetic foot complications, which is reflected in greater outpatient costs, bed occupancy, and extended hospitalization. The cost of diabetic foot care to the NHS during 2010-2011 was £639-662 million.

The ability and the time required for a patient to walk with a prosthetic limb after a lower limb amputation is determined largely by the process of wound healing (4). In addition, the type of treatment wound characteristics and the condition of the patient also affect the stump healing process. Pino et al. (5) reviewed 19 studies on lower limb amputation in patients with DM and concluded that a complete preoperative workup is desirable before an amputation with emphasis on the probable rate of healing, the functional condition of the limb prior to surgery, control or treatment of any additional diseases, and selection of the level of amputation based on latest techniques. The main purpose of this study was to discuss the problem of stump healing in the diabetic population.

**Types of Healing**

Amputations are considered to have healed primarily if the wound healed without additional debridement or revision. Longer wound healing that prevents prosthetic fitting for at least three months, but eventually leads to closure of the wound is classified as “delayed.” When amputations are complicated by wound necrosis or severe infection, leading to repeated surgery, the wound healing is classified as “failed” (6). The process of wound healing is complex and entails regenerating the cellular organization and the tissue layers. Mercandetti et al. (6) have classified the wound healing process into three categories: 1) category 1 healing, also known as primary wound healing or healing by the first intention, results in minimum damage to cellular structures, 2) category 2 or delayed primary wound healing occurs when the margins of the wound are not reconfigured promptly and may prove beneficial if the wound becomes infected, and 3) category 3 or secondary healing allows a full-thickness wound to close and heal on account of extensive inflammation which stimulates the wound to recede.

**Complications of Stump Healing**

Lower limb amputation surgery is frequently performed in patients who have multiple comorbidities. A large number of patients with DM are hospitalized due to lower limb-related problems (7) such as infection, pain, and osteomyelitis (Figure 1). McIntosh et al. (8) carried out a retrospective study from 2005 to 2007 on 231 patients who underwent major amputations of lower extremities. They observed that 7.3% of the patients experienced wound infection, phantom pain, poor body image, depression and myocardial infarction following the amputations. Diabetics are twice more likely to experience congestive cardiac failure and deep vein thrombosis (DVT; 11% risk) after amputation than patients without DM (3).

**Infection of the Stump**

Patients who have undergone amputation can develop severe problems as a result of infections, especially if they suffer from DM. The amputated stump frequently becomes infected requiring re-amputation (9). The wound infection rates following major lower limb amputation vary between 13% and 40%. Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common cause of post-amputation infections (10, 11). The morbidity and mortality rates usually in-
crease as a result of MRSA infection in vascular patients (12-14). As indicated by Ray (15), patients with DM are five times more predisposed to wound infections than patients without DM, and the presence of peripheral vascular disease further increases the risk of infection. In a study by Aulivola et al. (16), an infection develops in 5.5% of trans-tibial and 6.7% of trans-femoral amputations. A wound infection can produce excessive amounts of discharge that disrupt the suture line (17), and Baxter (18) showed that an extensive infection can even cause wound rupture and tissue death, thus requiring additional surgical interventions. According to the Infection Surveillance Service in England (2006), leg amputations are associated with the highest risk of infection since a large number of patients are subjected to this procedure as a consequence of severely infected ulcerations. Additionally, a number of factors such as inadequate blood circulation, a weak immune system, and poorly controlled DM may increase the likelihood of infection. As noted by Grey (19), cellulitis is another problem which can accompany leg amputation and manifests as inflammation, pain, pus formation, redness, heat, and pyrexia. Serious cases of cellulitis progress to septicemia. The use of prophylactic antibiotics has reduced the incidences of wound infection and cellulitis, resulting in a decline in not only the rate of infection but also in the rate of re-amputation (8). Therefore, it is crucial to ensure effective treatment of wound infections to aid the healing process and to minimize the morbidity and mortality rates.

Pain in the Stump

Pain is a significant and complex problem accompanying lower limb amputation. Incision stump pain and phantom limb pain are the two kinds of pain that amputees experience. Stump pain is limited to the area closely surrounding the stump and amputation site (20). If left untreated, it can adversely influence the wound healing process and consequently, reduce the patient’s quality of life. Opiates and non-steroidal anti-inflammatory agents have been shown to alleviate stump pain. According to Chan et al. (21), 90% of amputees experience phantom limb pain, which has been described as a crushing, tearing pain which the patients feel in the amputated limb (22). Phantom limb pain occurs after amputation and can endure for a few years or, in rare cases, for the rest of a patient’s life (20).

Tissue Necrosis of the Stump

As a large number of amputations are performed due to ischemia, inadequate circulation in the stump area can cause tissue necrosis, which manifests as changes in skin color, dry gangrene, or wet gangrene. Ray (15) pointed out that changes in skin color around the incision line can indicate wound rupture following surgical intervention, or even tissue death a number of weeks after the procedure. Debridement is a frequently used technique to accelerate wound healing (23). Low amounts of dead tissue are usually left to the natural process of autolysis, once it is ascertained that they are harmless. In cases of significant necrosis, however, wound debridement is preferred. The decision as to the optimum method for dead tissue removal (24) depends on a number of factors such as convenience, wound type, location, and costs (25). Larval therapy, which uses sterile maggots, is a frequently used debridement method in the UK. The popularity of this method is attributed to the fact that it is the only viable option in most cases since the presence of co-morbidities often prevents surgical intervention or other methods of stump debridement (26).

Stump Edema

Stump edema is a common problem faced by amputees, especially those who are suitable for prosthetic fitting. According to Ray (15), pre-existing venous deficiency, generalized fluid retention due to congestive heart failure and chronic hyper vascularity are some of the factors causing extensive and protracted edema. Hypervascularity is frequently encountered in DM patients without a severely disrupted circulation. Extensive edema and discharge delay the stump healing process. Scanlon et al. (27) argued that some components of the wound exudates have a negative effect on wound healing, as well as on the surrounding skin. The development and infection of seroma/hematoma are considered to be the causes of exaggerated wound discharge (15). A number of researchers (28, 29) have recommended periodically elevating the amputated leg to limit edema around the stump area. The appropriate wound dressing is also essential to maintain a good moisture level within the wound. Banwell et al. (30) have suggested that topical negative pressure on discharging stump wounds can stimulate fluid elimination and thus decrease the risk of edema (31). In addition, it is vital to maintaining skin hygiene in patients with edema.
as they are more prone to develop infections or even cellulitis due to impaired cellular and lymphatic functions (32). Edema can be reduced by wrapping the stump wound in elastic bandages (33), which protect the healing tissue, keep the dressings in place, limit inflammation and shape the remaining limb, thereby preparing it for the prosthetic device (34).

Limb swelling due to deep vein thrombosis (DVT) often occurs in patients who have undergone leg amputation (35). Apart from DVT, limb swelling can also develop as a result of hypoproteinemia, stump dependency, and infection. It is crucial to determine the exact cause of stump edema in order to prescribe the proper treatment, such as a high protein diet.

**Osteomyelitis**

Osteomyelitis is a dreaded complication post amputation as it can result in life-threatening sepsis (36). The bone in the amputation area can become exposed through the skin as a result of muscle withdrawal from the stump (15). A ruptured wound can facilitate bone exposure, increasing the risk of osteomyelitis. Surgical intervention is needed if the area of exposed bone is extensive, and the granulation tissue cannot cover it through secondary healing. As noted by Lipsky et al. (37), the diagnosis and treatment of osteomyelitis in patients with DM pose considerable difficulties and requires the early involvement of a multidisciplinary team. Diabetes-related osteomyelitis is diagnosed based on clinical, biochemical and radiological evidence as well as findings of some bedside test. It is possible to carry out a probe-to-bone test but its accuracy is doubtful (38, 39). However, the main method used to diagnose osteomyelitis is a microbiological examination of the bone biopsy (40). After initial radiography, magnetic resonance imagining (MRI) is used to assess the development of pedal osteomyelitis and the extent of soft tissue infection, with a sensitivity and specificity of 90% and 83%, respectively (41).

**Stump Hematoma**

Bale et al (42) defined hematoma as a localized accumulation of blood inside an organ, cavity or tissue. A hematoma provides a suitable environment for the development of infection and can generate dead space, undermining the suture line and expanding the level of tension in the wound (18). Hematomas usually drain freely and do not necessitate surgery. Nevertheless, surgical debridement is used to remove considerable quantities of coagulated blood (15). Morrison et al. (43) reported an increased likelihood of hematoma formation under the suture line in the case of wounds without drainage, which can lead to the development of tension, edema, and infection. Furthermore, the blood circulation can also be affected by the increased tension under the suture line, causing wound rupture and tissue death (44). A hematoma should be identified using efficient evaluation methods and the patient must be referred to surgery immediately if needed.

**Wound Dehiscence**

Wound dehiscence refers to the sudden opening of the wound along the suture line and is accompanied by a sharp increase in serosanguineous drainage (45). It usually occurs when the wound is too weak to resist exterior forces such as shear or direct trauma (42), as a result of the premature removal of sutures, or stump edema which creates tension in the wound. Total dehiscence can potentially determine the exposure of muscle and bone (18).

Harker (23) proposed the use of topical negative pressure on the amputated area to prevent wound dehiscence, which has had a higher success rate in previous applications (46). Topical negative pressure therapy, such as the vacuum-assisted closure (VAC) system, can handle extensive quantities of exudates and safeguard the skin against maceration and abrasion, thereby limiting the risk of infection. It also stimulates the generation of granulation tissue inside the cavity wound. The main disadvantage of the topical negative pressure therapy, however, is that many patients cannot tolerate the pain (47). Studies that have discussed the complexity of dehisced amputation wounds and the difficulties in treating them have recommended the collaboration of different specialists to gain positive results (17).

**Non-Healing Requiring a Higher-Level Amputation**

Re-amputation refers to a higher level amputation secondary to non-healing of the stump. There are a number of factors which may substantiate the need for re-amputation, such as stump pain and/or phantom limb pain, delayed stump infection, the formation of symptomatic bone spurs, assessment of the skin flap designed to preserve stump length, and preparation of the stump for the prosthetic device. Dillingham et al. (48) observed that re-amputation is more likely to be performed in the diabetics than in patients
without DM. Reiber (49) estimated that 9% to 20% of patients with DM with an initial leg amputation undergo re-amputation within the first year, and 28% to 51% of patients with DM necessitate re-amputation within five years of the original amputation.

Death Following an Inability to Heal Stump

Criqui et al. (50) revealed that the likelihood of amputees with the peripheral vascular disease to die as a result of cardiovascular complications within ten years of the amputation is six times higher than that of amputees without the peripheral vascular disease. Lee et al. (51) reported that the most common cause of death after a lower limb amputation was DM (37.3%), cardiovascular disease (29.1%), and renal disease (7.3%). According to Mayfield et al. (52), the mortality rate among patients with renal disease, cardiovascular disease or proximal amputation level was high during the first 12 months of the procedure. Tourniakissian et al. (53) reported that the mortality rates increased by 23%, 41%, and 80% in the period immediately following the amputation, after one year, and after five years of the procedure, respectively.

The five-year mortality rates associated with above-knee and below-knee amputation were estimated at 90% and 70%, respectively (54). Ten-tolouris et al. (55) found that 61% of patients with DM aged between 67 and 76 years who had undergone amputation, were likely to die within five years of the operation. Heikkinen et al. (56) reported that the post-amputation mortality rate even among the younger patients with DM was significantly higher than that of non-diabetics. According to Schofield et al. (3), the mortality rate among amputees with DM was 55% higher than among patients without DM. One reason for the high mortality rate may be the greater emphasis on rescuing the limb which delays amputation to only when revascularization is not feasible.

Conclusion

Successful stump healing is a prerequisite for amputation following a lower limb amputation. This review has provided a detailed evaluation of the risks conferred by some of the key complications of lower limb stump healing. There are currently no reliable standards that can be referred to prior to leg amputation surgery. The clinicians, therefore, have to rely on their own judgment and other investigatory parameters including a pre-operative angiogram which has a major role in determining the level of amputation. In order to identify which lower limb stumps would heal following a major lower limb amputation, a pre-surgery prediction rule needs to be formulated and verified.

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Authorship Contributions

Idea/Concept: Suhel Ashraff; Design: Suhel Ashraff, Muhammad A. Siddiqui; Control/Supervision: Suhel Ashraff; Analysis and/or Interpretation: Suhel Ashraff, Muhammad A. Siddiqui; Literature Review: Suhel Ashraff, Muhammad A. Siddiqui, Derek Santos; Writing the Article: Suhel Ashraff, Muhammad A. Siddiqui, Derek Santos, Thomas Carline; Critical Review: Derek Santos, Thomas Carline.

References


Could Retroperitoneal Ganglioneuroma be a Dopamine Secreting Ganglioneuroma?

Retroperitoneal Gangliöroma Dopamin Salgılayan Bir Gangliöroma Olabilir mi?

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Abstract

Ganglioneuromas are rarely occurring benign tumors characterized by hyperplasia of mature ganglia and satellite cells. They are well-differentiated, slow growing, and autonomous nervous system neoplasms, which are usually asymptomatic and do not release any hormones.

A male patient aged 26 years was evaluated for secondary hypertension six months ago. Ultrasonography of the abdomen revealed a mass lesion around the right kidney. An analysis of the 24-hour urine sample of the patient revealed the following parameters: 5-HIAA=3.9 mg/day (2-7), metanephrine=56.3 µg/day (52-341), and normetanephrine=146.1 µg/day (88-444). The computed tomography scan of the abdomen showed a retroperitoneal mass of 10 cm in size, containing minute calcified foci in the right retroperitoneal region. The mass was excised through general surgery and was classified as ganglioneuroma. The blood pressure of the patient returned to normal level after surgery, and he needed no further antihypertensive treatment. Besides, the metanephrine and normetanephrine levels in the 24-hour urine were also observed to be normal as in the preoperative period.

Retroperitoneal masses can actually be ganglioneuromas and an accurate diagnosis can be achieved only through postoperative histopathological evaluation. After the operation, blood pressure of the patient returned to normal. This suggests that retroperitoneal ganglioneuroma could possibly secrete dopamine, epinephrine, or norepinephrine.

Keywords: Retroperitoneal ganglioneuroma; hypertension; catecholamine

Anatarkelimeler: Retroperitoneal gangliöroma; hipertansiyon; katekolamin

This case is presented as a poster in “38th Turkish Endocrinology and Metabolic Diseases Congress” on 11-15 May 2016, Antalya.

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Introduction

Ganglioneuromas are rarely occurring benign tumors characterized by hyperplasia of mature ganglia and satellite cells. They are well-differentiated, slow growing, and autonomous nervous system neoplasms, which are usually asymptomatic and do not release any hormones (1). In the majority of the cases, they are detected incidentally. Histopathological examination is mandatory for differentiation of ganglioneuromas from malignant neuroendocrine tumors such as pheochromocytoma and neuroblastoma. Ganglioneuromas may develop on skull base, neck, posterior mediastinum, retroperitoneum, and adrenal gland through the sympathetic chain (2). They are particularly prevalent in the posterior mediastinum and retroperitoneal region (3). Sixty percent of the cases occur before the age of 20 years. The treatment of these cases is the complete excision of the tumor (2). Our aim in this study was to present a patient with the rarely occurring ganglioneuroma in the retroperitoneal region.

Case Report

A 26-year-old male patient was evaluated for symptoms of secondary hypertension six months ago. Doppler ultrasonography (USG) of the renal artery was done to determine the etiology of early onset of hypertension, and stenosis was not detected. USG revealed a mass lesion around the right kidney. A 24-hour urine sample of the patient was subjected to analysis and the following parameters were recorded: 5-hydroxyindole acetic acid (5-HIAA)= 3.9 mg/day (2-7), metanephrine= 56.3 µg/day (52-341), and normetanephrine= 146.1 µg/day (88-444). The positron emission tomography-computed tomography (PET-CT) revealed a mass lesion in the right adrenal region with high retention of fluorine-18 (F-18) fluorodeoxyglucose (FDG). The 131I/123I-Metaiodobenzylguanidine (MIBG) scintigraphy, carried out to check for pheochromocytoma, showed no retention. In the abdominal CT examination, a retroperitoneal mass of approximately 10 cm in size, containing min calcified foci, was observed in the right retroperitoneal region (Figure 1). The mass was excised through general surgery and diagnosed as ganglioneuroma (Figure 2). The histological examination showed spindle-shaped Schwann cells arranged in small fascicles, clusters of ganglial cells, and fibrous tissue. The ganglial cells had abundant cytoplasm, large eccentric nuclei, and one or more prominent nucleoli. There was no evidence of neuroblastoma. During the period after surgery, the patient’s blood pressure returned to normal levels and he needed no antihypertensive treatment. In addition, the metanephrine and normetanephrine levels in the 24-hour urine were observed to be normal as levels in the preoperative period.

Discussion

Ganglioneuroma originates from neural crest cells comprising sympathetic ganglion and adrenal gland cells. Ganglial cells are composite structures containing fibrous tissue and Schwann cells. Ganglioneuroma is a benign tumor observed three times more frequently in women than in men. Furthermore, it is more prevalent in people under the age of 20. Besides, it is more commonly detected in the mediastinum and retroperitoneal regions. In our case, the patient was a 26-year old male, and
the tumor was located in the retroperitoneum. Ganglioneuromas are usually asymptomatic and release no hormones. Because of a slow growth rate, it is usually diagnosed in the late adolescent period. The diagnosis can be made by monitoring the pressure exerted by the tumor on the surrounding tissues and through the mass effect. If the tumor grows retroperitoneally, abdominal pain and distention could be the main symptoms (4). In our case, abdominal pain was absent although the mass was enormous. The tumors generally do not release any hormones, but when they do, they release vasoactive intestinal peptide, androgen hormone, or catecholamine, which cause hypertension, sweating, diarrhea, virilism, and hypokalemia (3). Although hypertension was historically present in our case, the 24-hour urine levels of metanephrine and normetanephrine were normal. Besides, the potassium levels were normal. Interestingly, the blood pressure of the patient was normal after surgical removal of the ganglioneuroma, which suggested that it could possibly secrete dopamine. However, our efforts at measuring the levels were unsuccessful. Erem et al. (5) had reported a dopamine secreting adrenal ganglioneuroma in a patient. In addition, Yoshida et al. (6) reported a patient diagnosed with retroperitoneal ganglioneuroma who had a history of hypertension. Ganglioneuromas are radiologically well-located solid masses with contoured lobules and may involve partial calcification in some cases (7). Radiological monitoring methods such as USG, CT, and magnetic resonance imaging (MRI) are helpful in evaluating the size and composition of the mass and its relation to adjacent tissues. Previous studies have reported that preoperative diagnosis of ganglioneuroma is usually difficult and the diagnosis is made through histopathological examination (2). In our case, the diagnosis was also made through histopathological examination of the completely excised tumor. Ganglioneuroma is classified as a neurogenic tumor. The histopathological diagnosis for neurogenic tumors relies on the presence of mature ganglion cells. Unlike neuroblastoma, immature cells, atypical mitotic structure, or necrosis are absent in ganglioneuroma (5). Ganglioneuromas can be cured by excision and no relapse has been reported (8). In contrast, metastases of matured neuroblastomas are encountered in the lymph node adjacent to the tumor mass or in the regions away from the tumor. In our case, there was no evidence of metastasis before and after the operation. In conclusion, preoperative diagnosis of retroperitoneal ganglioneuromas is difficult, as it might be radiologically similar to the other tumors.

Conclusion
It is important to remember that retroperitoneal masses can actually be ganglioneuromas, and an accurate diagnosis can be achieved only through postoperative histopathological examination. In case of suspected abnormal clinical parameters such as hypertension, all the catecholamines should be measured before the operation to prevent a possible hypertensive crisis.

An informed consent form was obtained from the patients.

Source of Finance: During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

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Author Contributions
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References


Diagnostic Evaluation and Characterization of Von Hippel-Lindau (VHL) Syndrome by Functional Imaging (\(^{68}\)Ga-DOTANOC, \(^{99}\)mTc-HYNIC-TOC, and \(^{131}\)I-MIBG)

Von Hippel-Lindau (VHL) syndrome is a genetic disorder with high penetrance, leading to various tumors such as hemangioblastomas of retina and cerebellum, renal cell carcinoma, islet cell tumors, endolymphatic sac tumors, etc. In this report, we describe a case of a 22-year-old male with VHL syndrome, where functional \(^{68}\)Ga-DOTANOC PET/CT, \(^{99}\)mTc-HYNIC-TOC SPECT/CT, and \(^{131}\)I-MIBG SPECT/CT were used in the diagnostic evaluation of this disease. This patient presented with bilateral pheochromocytoma, retinal angioma, cerebellar hemangioblastoma, pancreatic neuroendocrine tumor, and pancreatic cysts. This case highlights the emerging role of functional imaging as an adjuvant to conventional tools in the diagnostic evaluations of diseases, an area that has not been addressed to date.

**Keywords:** VHL syndrome; pheochromocytoma; retinal angioma; cerebellar hemangioblastoma; pancreatic neuroendocrine tumor; \(^{68}\)Ga-DOTANOC; \(^{99}\)mTc-HYNIC-TOC; \(^{131}\)I-MIBG, functional imaging

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Introduction

Von Hippel-Lindau (VHL) syndrome is an autosomal dominant genetic disorder with high penetrance (1, 2). A mutation in the tumor suppressor gene present on the short arm of chromosome 3 leads to the development of benign as well as malignant tumors, affecting multiple organ systems (1, 2). Various tumors that are associated with this syndrome are hemangioblastomas of the retina (retinal angioma), cerebellum, and spine, renal cell carcinoma (clear cell type), pheochromocytoma, islet cell tumors of the pancreas, endolymphatic sac tumors, cysts of kidney and pancreas, and cystadenoma in the epididymis and the broad ligament.

Case Report

A 22-year-old non-diabetic male presented with a six-month history of paroxysmal attacks of a headache, sweating, palpitation, weakness, postural dizziness, and about 10 kg weight loss. There was no history suggestive of hypoglycemia, thyrotoxicosis, recurrent fractures, renal calculi, neck swelling, abdominal or body lump, or any drug intake. Family history was unremarkable, except for the history of a certain unknown malignancy of chest in father. The patient also complained of the sudden onset of a painless, permanent visual loss in the left eye, which occurred two months earlier.

Clinical examination of the young male revealed a lean built (BMI = 15.6 kg/m²), with a pulse rate of 77 min⁻¹, supine BP of 186/110 mm of Hg, and standing BP of 64/40 mm of Hg. There was no perception of light in the left eye and its fundus examination revealed a retinal angioma along with total retinal detachment, while the visual acuity and the fundus examination of the right eye were normal. There was no goiter, acanthosis nigricans, mucosal neuromas, café au lait spots, neurofibromas, or any Cushingoid features. Neurological examination, including the cerebellar examination, was unremarkable. Systemic examination, which included the cardiac, pulmonary, and abdominal examinations, was normal.

Investigative workup, including the blood counts, electrolytes, urinalysis, plasma glucose, glycated hemoglobin, renal and liver functions, were unremarkable. Other hormonal investigations included normal fasting plasma insulin (6.52; N 2.6-24.9 mIU/mL), serum T4 (7.66; N 5.1-14.1 mcg/dL), serum TSH (1.73; N 0.27-4.2 mIU/mL), serum morning cortisol (13.86; N 6.2-19.4 µg/dL), and plasma ACTH (9.43; N 7.2-63.3 pg/mL). The 24-hour urinary noradrenaline levels were elevated (275.0; N up to 90.0 mcg/day), with normal urinary adrenaline (4.0; N <30.0 mcg/day) and VMA (11.2; N <15.0 mg/day) levels. The fundus fluorescein angiography revealed a retinal angioma, along with retinal detachment (Figure 1). CECT (Figure 2a) and CEMRI of the abdomen (Figure 2b) revealed bilateral adrenal masses and a pancreatic mass lesion. MRI of the spine and cranium revealed a cerebellar lesion (Figure 2c). Chest roentgenography, electrocardiography, and echocardiogram were all normal (left ventricular ejection fraction = 60%). Audiometry (for endolymphatic tumors), USG of the neck (for medullary thyroid carcinoma), and Ultrasound of the scrotum (for epididymal cysts) were unremarkable.

Figure 1: A fundus fluorescein angiographic image showing retinal angioma with retinal detachment.


131I-MIBG along with SPECT/CT scan revealed bilateral MIBG-concentrating lesions, indicating bilateral pheochromocytoma, with greater uptake on the right side (Figure 3a-c). 68Gallium DOTANOC scan revealed uptake in the cerebellum, suggesting cerebellar hemangioblastoma (Figure 3d). ⁹⁹mTc-HYNIC-TOC (Figure 3e) revealed a somatostatin receptor-expressing tumor in the tail region of the pancreas, which was suggestive of a neuroendocrine tumor, and it also revealed partially-calcified centrally-necrotic right-sided adrenal mass lesions (pheochromocytoma).

In this patient with components such as retinal angioma, bilateral pheochromocytoma, pancreatic neuroendocrine tumor, and cerebellar hemangioblastoma, a diagnosis of VHL syndrome was entertained. The patient was managed with a standard preoperative care procedure for pheochromocytoma and was then subjected to an open laparotomy in a joint approach by the urology and surgical gastroenterology teams. Intraoperative ultrasonography was performed in order to confirm the pancreatic lesions; three lesions were revealed in the ultrasonogram (one in the head region of the pancreas and two in the tail region of the pancreas). The operative procedure included bilateral adrenalectomy, enucleation of the lesion in the head of the pancreas, and distal pancreatectomy for the other two lesions in the tail region of the pancreas. His postoperative period was uneventful. Two months later, he underwent the excision of the cerebellar hemangioblastoma. Postoperatively, the patient remained normotensive and without any neurodeficit, on a glucocorticoid and mineralocorticoid replacement therapy.

Discussion

VHL is usually present as a syndromic complex, characterized by retinal angiomas, hemangioblastoma of central as well as peripheral nervous system, along with cystic lesions in the viscera. The VHL syndrome is a rare genetic cause of pheochromocytoma, which occurs in one among 36,000 people, with >90% penetrance by the age of 65 years (3).

Most of the cases of pheochromocytomas are sporadic, and a familial association is found in up to 25% of the cases. The familial association occurs commonly with neurofibromatosis type 1, multiple endocrine neoplasia type 2, VHL syndrome, and paraganglioma syndrome. The VHL gene is a tumor suppressor gene that regulates the activity
of hypoxia-inducible factor (HIF)–1α, which is involved either in the upregulation of an angiogenic factor or in the downregulation of an inhibitor of angiogenesis. Approximately 80% of the individuals with the VHL disease have a positive family history, and only 20% of the cases develop as a result of de novo mutation. VHL have been classified into two categories based on the absence (type 1) or presence (type 2) of pheochromocytoma, with further sub-classification of the type 2 VHL into 2A (hemangioblastoma and pheochromocytoma), 2B (hemangioblastoma, renal cell carcinoma, pheochromocytoma, and pancreatic cyst or tumor), and 2C (only pheochromocytoma) categories.

The clinical diagnosis of the VHL disease is established in a patient when there is the presence of at least one typical VHL tumor along with a positive family history, or the presence of hemangioblastoma along with one other tumor when the family history is negative. The typical VHL tumors are retinal, spinal, and cerebellar hemangioblastomas; renal cell carcinoma; pheochromocytoma; endolymphatic sac tumors; and multiple pancreatic cysts. Renal and epididymal cysts occur very frequently in the general population as well; therefore, their presence as a sole manifestation is not a reliable indicator of the VHL disease. VHL disease accounts for about 50% of the patients with apparently sporadic familial pheochromocytomas. Pheochromocytomas are the hallmark of VHL type 2. In this study, the presence of bilateral pheochromocytoma, retinal angioma, cerebellar hemangioblastoma, pancreatic cyst, and pancreatic neuroendocrine tumor in the patient indicated the presence of the VHL type 2a syndromic complex. The pheochromocytomas associated with the VHL syndrome exhibit more of a noradrenergic pattern, rather than the adrenergic pattern, of the total catecholamines content. Our patient also exhibited a predominant noradrenergic pattern in the 24-hour urinary catecholamines assay.

The incidental presence of the pancreatic cysts prompted us to suspect VHL syndrome in this patient, and an MRI of brain and spine was performed later in order to search for hemangioblastoma. The most common site for the presence of intracranial hemangioblastoma is cerebellum (37%), followed by spinal cord and brain stem. Our patient had a cerebellar hemangioblastoma (Figure 2c) and a retinal angioma. Retinal angiomias develop in more than one-half of the patients with the VHL syndrome. Most of
these lesions develop in the second or third decade of patient’s life. The patients may also experience a painless loss of vision or certain visual field defects. Exudation or hemorrhage from the hemangioma may lead to macular edema or retinal detachment. Our patient had total retinal detachment, secondary to the left-sided retinal angiomas. Renal cell carcinoma, which is the leading cause of death in the patients with the VHL syndrome, was not present in our patient. In consideration of the raised urinary catecholamine levels and suspected VHL syndrome, functional imaging with $^{68}$Ga-DOTANOC, $^{99m}$Tc-HYNIC-TOC, and $^{131}$I-MIBG was performed. In our patient, the CT scan initially revealed a left-sided adrenal lesion, and the bilateral pheochromocytoma was confirmed later with the $^{131}$I-MIBG imaging. $^{68}$Ga-DOTANOC and $^{99m}$Tc-HYNIC-TOC were a part of our workup for the evaluation of the pancreatic neuroendocrine tumor. Both the scans revealed the presence of a pancreatic neuroendocrine tumor. The use of functional imaging in the diagnostic evaluation of the VHL lesions has not been reported previously. As a result of the multi-organ involvement and the complex nature of the VHL lesions, their treatment requires a multidisciplinary approach.

Our patient required surgical intervention by three surgical specialties: a urologist for the bilateral pheochromocytoma, a neurosurgeon for the hemangioblastoma, and a gastrointestinal surgeon for the pancreatic neuroendocrine tumor. Since the size of the adrenal lesion was small, laparoscopic removal of the bilateral pheochromocytoma was planned on a priority basis. Surgical management of the pancreatic neuroendocrine tumor in the VHL syndrome is controversial. The VHL patients at a lower metastatic risk associated with the pancreatic NET should be spared for the hazards of an operative resection; unlike the patients with sporadic non-functioning NET without the VHL disease, in such as case, surgical resection is generally recommended. Libutti & colleagues (10) recommended the following criteria for the resection of pancreatic tumors: no evidence of metastatic disease; tumor size greater than 3 cm in the body or the tail region, or greater than 2 cm in the head region of the pancreas; or the patient undergoing laparotomy for other lesions. Blansfield et al. (11) proposed the following three criteria to predict the metastatic disease associated with the pancreatic NET in the patients with the VHL disease: tumor size greater than or equal to 3 cm; the presence of a mutation in exon 3; and a tumor doubling time of less than 500 days. If the second or the third criterion is fulfilled, the patient should be considered for surgical management because of a greater likelihood of future malignancy developing from the pancreatic NET.

The blood pressure of our patient was controlled with adequate alpha and beta blockade. We consulted both urologists and surgical gastroenterologists, and planned for an initial bilateral adrenalectomy through laparotomy that would be performed by the urology team; and then if the patient remained stable, we would proceed with the resection of the pancreatic tumors which would be performed by the surgical gastroenterologist within the same setting. The patient underwent laparotomy and the bilateral adrenalectomy was performed for the pheochromocytoma. The intraoperative ultrasonography was performed in order to confirm the pancreatic lesions; the ultrasonogram revealed three lesions (one in the head region of the pancreas and two in the tail region of the pancreas). The patient, therefore, underwent enucleation of the lesion in the head region of the pancreas, and distal pancreatectomy was performed for the removal of the other two lesions in the tail region of the pancreas. His postoperative period was uneventful. He required periodic follow-up with the neurosurgeon and the urologist for the hemangioblastoma and renal cell carcinoma, respectively. Although the renal cell tumor was not observed in this patient, this tumor is the most common cause of death in the patients with the VHL syndrome, and therefore, periodic imaging is recommended, preferably with the MRI of the abdomen.

**Conclusion**

Here, we have described a typical syndromic complex of VHL type 2a in a young male with lesions in the cerebellum, retina, bilateral adrenals, and pancreas. VHL should be suspected in patients with bilateral pheochromocytoma, especially if there is a family history, occurrence at a young age, or if there are associated lesions of pancreas, kidneys or the central nervous system. The treatment of VHL requires a multidisciplinary approach, involving various specialties-endocrinology, urology, surgical gastroenterology, radiology, nuclear medicine, and neurosurgery. The role of functional imaging in the characterization of the lesions associated with the VHL syndrome has been addressed for the first time in
this report. We recommend the addition of functional imaging in the list of comprehensive surveillance of the VHL-related tumors.

**Competing Interests:** None.

**Source of Finance:** During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

**Conflict of Interest:** No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

**Author Contributions**
Concept: Mohd Ashraf Ganie; Design: Alpesh Goyal; Data Collection or Processing: Shyam Kishore; Analysis or Interpretation: Mohd Ashraf Ganie, C.S.Bal, P.N.Dogra, Alpesh Goyal, Shyam Kishore; Literature Search: Alpesh Goyal, Shyam Kishore; Writing: Alpesh Goyal/Shyam Kishore.

**References**
Two Cases of Hypothyroidism with TSH Receptor Gene D727E Polymorphism that Converted to Hyperthyroidism after Many Years

Hipotiroidinin Yıllar Sonra Hipertiroidiye Dönüşüğü İki Vaka ve D727E Polimorfizimi

Murat Dağdeviren, Mustafa Altay, Gülsüm Biten Güven*, Derun Taner Ertuğrul
Keçiören Training and Research Hospital, Clinic of Endocrinology and Metabolism, Ankara, Turkey

Case Report

Hashimoto’s disease and Graves’ disease are common thyroid disorders that are characterized by hypothyroidism and thyrotoxicosis, respectively. An individual may be affected by both the diseases in a sequential manner. However, the occurrence of Graves’ disease with thyrotoxicosis in patients with chronic hypothyroidism is the least common. In the current study, we present the case of two patients with hypothyroidism who developed clinical thyrotoxicosis during chronic levotiroxin treatment. As they developed clinical thyrotoxicosis, levotiroxin treatment was discontinued in both patients. However, thyrotoxicosis persisted and both patients were found be positive for antibodies against thyroid-stimulating hormone receptor. The diagnosis of Graves’ disease was based on clinical signs, laboratory findings, and imaging techniques. Laboratory tests after six weeks of levotiroxin treatment revealed serum thyroid-stimulating hormone 0.004 mIU/L and T4 3.21 ng/dL in the first patient, and thyroid-stimulating hormone 0.004 mIU/L and T4 7.28 ng/dL in the second patient. The patients were treated with methimazole and became euthyroid. Thyroid-stimulating hormone receptor gene sequence analysis revealed D727E homozygous polymorphism in both patients. In patients with chronic Hashimoto’s hypothyroidism, thyrotoxicosis is attributed to D727E polymorphism or other thyroid-stimulating hormone receptor gene mutations-dependent conversion to hyperthyroidism rather than silent thyroiditis. We recommend that patients reporting switching from hypothyroidism to thyrotoxicosis should be tested for D727E polymorphism.

Keywords: D727E polymorphism; hyperthyroidism; hypothyroidism; switching

Özet


Anahtar kelimeler: D727E polimorfizmi; hipertiroidi; hipotiroid; dönüşüm
Introduction
Hashimoto’s thyroiditis and Graves’ disease are two common autoimmune diseases of the thyroid gland that are characterized by different clinical courses. While Hashimoto’s thyroiditis often presents with hypothyroidism, Graves’ disease is usually associated with thyrotoxicosis. The antibodies against TSH receptor (TRAb) play a role in the pathogenesis of both diseases by binding of thyroid stimulating hormone (TSH) to TSH receptor. There are two types of TRAb: thyroid-stimulating blocking autoantibody (TSBAb) and thyroid-stimulating autoantibody (TSAb) (1). Whereas TSBAb causes hypothyroidism in many patients with Hashimoto’s thyroiditis, TSAb causes thyrotoxicosis due to hyperthyroidism in patients with Graves’ disease (1). Graves’ disease and Hashimoto’s hypothyroidism are both relatively common thyroid diseases (2); however, the occurrence of thyrotoxicosis due to Graves’ disease after Hashimoto’s thyroiditis with hypothyroidism is unusual and its etiology is unclear. In the current study, we present two patients who had been treated with levothyroxine (L-T4) for many years and developed thyrotoxicosis due to Graves’ disease.

Case Reports
The first patient was a 46-year-old female who was admitted to the endocrinology clinic with complaints of excessive sweating, weight loss, palpitations, irritability, and redness and pain in her eyes. She also experienced swelling, itching, and erythema of the lower extremities. She had a history of chronic hypothyroidism for at least ten years during which she had received L-thyroxine (L-T4) treatment. Levothyroxine dose for the previous six months was 50 µg/day and L-T4 treatment was stopped by the family physician six weeks before referral to our hospital. On physical examination, her blood pressure was 135/85 mm Hg, pulse rate was 84/min, body mass index was 25.4 kg/m², and her skin was moist. Although she had a brisk gaze, there was no exophthalmos. Her thyroid was not palpable. Thyroid function tests revealed TSH 0.004 mIU/L, fT3 10.11 pg/mL, fT4 2.97 ng/dL, TRAb 348.7 U/L (0-14), anti-TPO antibody 36.09 IU/mL (1-16), and anti-TG antibody 12.33 IU/mL (5-100). The thyroid gland was of normal size; however, ultrasonography revealed bilateral parenchymal heterogeneity. Doppler ultrasonography revealed bilateral increased intraparenchymal perfusion. Thyroid scintigraphy showed bilateral diffused, increased uptake (Figure 1). The uptake with Tc-99m was 15.8% (0.4-4%) (Figure 1). The patient became euthyroid after three months of treatment with methimazole (10 mg/day) and propranolol (2 x 20 mg/day) treatment (Table 1).

The second patient was a 49-year-old woman who was admitted to our out-patient endocrinology clinic with complaints of weight loss, hair loss, palpitations, heat intolerance, sweating, trembling, stinging, and burning sensation in the eyes. She had been on L-T4 treatment for about five years and had received 50 µg/day of L-T4 for the previous six months. On physical examination, her blood pressure was 166/71 mm Hg, pulse rate was 125/min, BMI was 22.8 kg/m², and her skin was moist. Except for a brisk gaze, there were no other eye signs. Her thyroid was not palpable. Thyroid function tests revealed TSH <0.01 mIU/L, fT3 19.01 pg/mL, fT4 6.31 ng/dL, TRAb 118.1 U/L, anti-TPO 190 IU/mL, and anti-TG 12.39 IU/mL. The patient’s levothyroxine treatment was terminated and six weeks later, thyroid function tests showed the following results: TSH 0.04 mIU/L, fT3 16.86 pg/mL, fT4 7.28 ng/dL, TRAb 118.1 U/L, anti-TPO 190 IU/mL, and anti-TG 12.39 IU/mL (Table 2). Four weeks after the administration of methima-

In the current study, we present a case of two patients with chronic hypothyroidism who developed clinically evident thyrotoxicosis during long-term L-T4 treatment. Neither of these patients had undergone laboratory tests for thyroid autoantibodies in the primary care centers where they had been treated for Hashimoto’s thyroiditis. Also, none of the patients had a history of radiation, systemic or local infiltrative disease, or use of drugs with antithyroid properties. Both patients developed thyrotoxicosis while on L-T4 treatment; however, thyrotoxicosis persisted after L-T4 was stopped. Therefore, they received methimazole to achieve euthyroidism. We found that both patients were positive for TRAb and, based on clinical and laboratory findings, they were diagnosed with Graves’ disease. Suggested thyroid stimulator and TSH receptor autoantibodies were less than 14 U/L. Based on clinical and laboratory findings, we concluded that D727E homozygous polymorphism might be related to the transition from hypothyroidism to thyrotoxicosis. TSH: Thyroid stimulating hormone, FT3: Free T3, FT4: Free T4, TRAB: TSH receptor antibodies, Anti-TPO: Thyroid peroxidase antibody, Anti-TG: Thyroglobulin antibody, LT4: Levothyroxine.

**Table 1. The laboratory values of the first patient before admission, on admission, and after the methimazole treatment.**

<table>
<thead>
<tr>
<th>Time</th>
<th>TSH (0.4-4.2 mIU/L)</th>
<th>FT3 (2.2-4.2 pg/mL)</th>
<th>FT4 (0.65-1.7 ng/dL)</th>
<th>TRAB (0-14 U/L)</th>
<th>Anti-TPO (1-16 IU/mL)</th>
<th>Anti-TG (5-100 IU/mL)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six months before admission</td>
<td>5.50</td>
<td>1.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50 mcg LT4</td>
</tr>
<tr>
<td>Four months before admission</td>
<td>3.53</td>
<td>2.7</td>
<td>1.30</td>
<td></td>
<td></td>
<td></td>
<td>50 mcg LT4</td>
</tr>
<tr>
<td>One week before admission</td>
<td>&lt;0.01</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On admission</td>
<td>0.004</td>
<td>3.81</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LT4 terminated</td>
</tr>
<tr>
<td>Four weeks after admission</td>
<td>0.004</td>
<td>10.43</td>
<td>3.21</td>
<td>348.7</td>
<td>36.09</td>
<td>12.33</td>
<td>10 mg methimazole</td>
</tr>
<tr>
<td>Three months after admission</td>
<td>0.070</td>
<td>2.55</td>
<td>0.74</td>
<td>45.97</td>
<td>36.15</td>
<td>5</td>
<td>10 mg methimazole</td>
</tr>
</tbody>
</table>

**Table 2. Laboratory findings of the second patient**

<table>
<thead>
<tr>
<th>Time</th>
<th>TSH (0.4-4.2 mIU/L)</th>
<th>FT3 (2.2-4.2 pg/mL)</th>
<th>FT4 (0.65-1.7 ng/dL)</th>
<th>TRAB (0-14 U/L)</th>
<th>Anti-TPO (1-16 IU/mL)</th>
<th>Anti-TG (5-100 IU/mL)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four weeks before admission</td>
<td>&lt;0.01</td>
<td>19.01</td>
<td>6.31</td>
<td></td>
<td></td>
<td></td>
<td>LT4 terminated</td>
</tr>
<tr>
<td>At admission</td>
<td>0.004</td>
<td>17.03</td>
<td>6.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two weeks later</td>
<td>0.004</td>
<td>16.86</td>
<td>7.28</td>
<td>118.1</td>
<td>190</td>
<td>12.39</td>
<td>20 mg methimazole</td>
</tr>
<tr>
<td>Six weeks later</td>
<td>0.05</td>
<td>3.06</td>
<td>1.16</td>
<td></td>
<td></td>
<td></td>
<td>20 mg methimazole</td>
</tr>
</tbody>
</table>


Discussion

In the current study, we present a case of two patients with chronic hypothyroidism who developed clinically evident thyrotoxicosis during long-term L-T4 treatment. Neither of these patients had undergone laboratory tests for thyroid autoantibodies in the primary care centers where they had been treated for Hashimoto’s thyroiditis. Also, none of the patients had a history of radiation, systemic or local infiltrative disease, or use of drugs with antithyroid properties. Both patients developed thyrotoxicosis while on L-T4 treatment; however, thyrotoxicosis persisted after L-T4 was stopped. Therefore, they received methimazole to achieve euthyroidism. We found that both patients were positive for TRAb and, based on clinical and laboratory findings, they were diagnosed with Graves’ disease. Suggested thyroid stimulator and TSH receptor autoantibodies were less than 14 U/L. Based on clinical and laboratory findings, we concluded that D727E homozygous polymorphism might be related to the transition from hypothyroidism to thyrotoxicosis. TSH: Thyroid stimulating hormone, FT3: Free T3, FT4: Free T4, TRAB: TSH receptor antibodies, Anti-TPO: Thyroid peroxidase antibody, Anti-TG: Thyroglobulin antibody, LT4: Levothyroxine.

Table 1. The laboratory values of the first patient before admission, on admission, and after the methimazole treatment.

Table 2. Laboratory findings of the second patient.
hyperthyroidism. To the best of our knowledge, these two cases are the first in which D727E polymorphism has been associated with such a transition.

The antibody against TR binds to the TSH receptor on thyroid cells and may have inhibitory, stimulatory, or neutral effects on hormone production (5,6). TSAb exerts its effect by increasing the TSH-mediated cAMP synthesis, resulting in hyperthyroidism. TSBAb inhibits cAMP synthesis, thereby leading to hypothyroidism. The analysis of total TSH receptor-binding antibodies detected both blocking and stimulating antibodies, and the balance between the two determines a patient’s thyroid status. Hypothyroidism is found to be consistent with a predominance of TSH receptor-blocking antibodies and thyrotoxicosis being consistent with a predominance of TSH receptor-stimulating antibodies (7). It is now considered that this balance may vary, leading to the concept of a single disease, that is, TRAb disease (5). The factors that determine this balance have not been clarified, but it has been suggested that L-T4 treatment of patients with TRAb-positive hypothyroidism (5,8), administration of anti-thyroid drug administration to patients with TRAb-positive hyperthyroidism, hyperthyroidism in pregnancy, TRAb titer and affinity, and some genetic mutations (1,9) may have a role. However, the present study could not put forward a clear relationship with anti-TPO and anti-TG levels. It is noteworthy that our patients had been treated with L-T4 for many years and that they had D727E homozygote polymorphism of TSHR gene. The relationship between TSH receptor polymorphisms and thyroid dysfunction is complex. Previous studies have identified various germ-line polymorphisms in TSHR, such as P52T, D36T, and D727E, which might affect the thyroid function, especially in Graves’ disease (3). However, only D727E polymorphism is likely to be associated with Graves’ disease or autoimmunity (10,11).

D727E polymorphism occurs by substitution of glutamic acid with aspartic acid, within the intracellular region (carboxy-terminal end) of TSH receptor, at position 2281 in codon 727 of exon 10. Muhlberg et al. reported that 16% of 99 euthyroid healthy volunteers had D727E polymorphism, but only one subject had a homozygous mutation (12). Another study reported the frequency of TSHR D727E polymorphism to be 9.6% in normal individuals, and all polymorphisms were heterozygous (13). The study suggested that germ-line polymorphisms of TSHR did not have a causative effect on hyperthyroidism. Moreover, significant TSHR activation (causing an increase in cAMP levels) was determined in vitro in the sera of patients with D727E polymorphism (13).

Although toxic multi-nodular goiter is not an autoimmune disease, different studies have reported the frequency of mutations in TSH receptor genes in patients with hyperfunctioning thyroid nodules to vary from 3 to 80% (9,10). Additionally, several studies investigated the causative association of D727E polymorphism between toxic adenoma, toxic multinodular goiter, and Graves’ disease (10,11). This polymorphism is considered to be related to toxic multinodular goiter (13). Furthermore, some researchers reported the same polymorphism to be related to Graves’ disease (3,14). A plausible explanation of hyperthyroidism caused by D727E polymorphism could be that it results in activation of the cytoplasmic tail of TSHR, thereby inducing intracellular signal transduction. The resulting increased cAMP responsiveness causes increased synthesis of thyroid hormone synthesis leading to hyperthyroidism. In the light of these data, we hypothesized that TSHR gene D727E homozygous polymorphism found in both cases may sensitize the receptor, leading to thyroid-stimulating effects, as evident in toxic adenoma and Graves’ disease. We may believe that it may be a predisposing factor for switching of thyroid status. To test this hypothesis, further controlled studies need to be conducted to investigate the prevalence of this polymorphism in patients with hormonal switching.

To conclude, in cases with hypothyroidism, the possibility of hormonal switching should be considered, which may occur owing to the TSHR gene D727E polymorphism and the other TSHR gene mutations. We propose that D727E polymorphism should be investigated in patients with interchanging hypothyroid and hyperthyroid status.
Informed Consent: Consent form was filled out by the patient.

Source of Finance: During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

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Successful Preoperative Treatment with Plasmapheresis of Patients with Hyperthyroidism

Hipertiroidili Hastaların Plazmaferez ile Başarılı Preoperatif Tedavisi

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Abstract

Anti-thyroid drugs are used as the first line treatment in the majority of patients with hyperthyroidism, but side effects such as severe hepatotoxicity and agranulocytosis may occur in some rare cases. For patients suffering from such severe side effects and in need of emergent surgical interventions, the removal of circulating thyroid hormones and thyroid antibodies by preoperative plasmapheresis has been reported as an effective adjunct. In this study, we report two patients, one with Graves’ disease who underwent a liver transplantation, and the second with a toxic multinodular goiter and acute cholecystitis due to suspected pancreatic cancer.

Keywords: Hyperthyroidism; plasmapheresis; operation

Introduction

Thyrotoxicosis is characterized by an excess of circulating thyroid hormones that may affect thermoregulatory central nervous and gastrointestinal, hepatic and cardiovascular systems and result in a thyroid crisis along with a high mortality rate (1). There are three treatment options: antithyroid drug therapy, radioactive iodine therapy, and surgery (2). The choice of which depends on the underlying cause. Anti-thyroid drugs are used as the first line treatment in the majority of patients with hyperthyroidism, but side effects such as severe hepatotoxicity and agranulocytosis may occur in some patients (<1%), which can lead to serious consequences (3). Thyroidectomy is the definitive treatment for thyrotoxicosis, especially in patients resistant to other medical treatments or unsuitable for RAI treatment (4). The plasmapheresis an alternative and effective therapeutic option in situations where restoring euthyroidism for the short-term period (5). In this study, we report two patients, one with Graves’ disease who underwent a liver transplantation and the second with a toxic multinodular goiter and acute cholecystitis due to suspected pancreatic cancer.

Anahtar kelimeler: Hipertiroidizm; plazmaferez; operasyon

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Patients

First patient

A 43-year-old male presented with symptoms like weight loss (of 7-8 kg), palpitation, weakness and excessive sweating since three months. His physical examination revealed a heart rate of 100 beats/min, blood pressure of 120/80 mmHg, respiratory rate of 18 breaths per min, 36.5 °C body temperature, and generalized icterus and tremor. Additionally, diffuse palpable thyroid gland was found in the patient. The resulting laboratory test values were as follows; FT4: 45.9 (11.5-22.7 pmol/L), FT3: 12.5 (3.5-6.5 pmol/L), TSH: 0.01 (0.27-4.2 IU/mL), TRAB: 2.43 (<1 IU/L). Ultrasoundography of the thyroid gland indicated mild diffuse enlargement of the thyroid gland, with heterogeneity and the 99m-Tc scan showed diffuse high uptake activity (4.9%) (normal reference range: 0.5-3.75%). These findings were consistent with the diagnosis of Graves’ disease. According to the patient’s history, he had cadaveric liver transplantation due to primary sclerosing cholangitis in the year 2011. After three years, due to persistent ulcerative colitis and high-grade dysplasia from the colon polyps, he had to undergo total colectomy. Antibody profiles including ANA, ASMA, AMA, anti-LKM, and p-ANCA were negative in his earlier report. He had continued treatment with cyclosporin, prednisolone, ursodeoxycholic acid, metoprolol, and cholestyramine as immunosuppressive agents for hepatic transplantation, until the date of admission. During the follow-up, the patient was admitted with symptoms like weakness, weight gain and overt hypothyroidism (TSH: 28 IU/mL, fT4: 8.63 pmol/L) and he had a high level of anti-TPO>1300 with urine iodine in the normal range (121 µg/L). His thyroid ultrasonography was found to be consistent with autoimmune thyroiditis. Levothyroxine therapy was started by gradually increasing the doses up to 100 mcg and once the TSH was suppressed, after three months, the drug was discontinued gradually. Graves’ disease was diagnosed. During this time, the total and direct bilirubin levels were found to be higher (19.69 and 13.48 mg/dL, respectively) and the MELD score was 24. In addition, liver biopsy suggested the recurrence of primary sclerosing cholangitis. Based on these findings, a liver transplantation surgery was planned; however, due to the patient’s high bilirubin levels, anti-thyroid medications and radioactive iodine therapy could not be commenced. Plasmapheresis (4 sessions) was performed using an intermittent flow device (Fresenius-COM.TEC) with the removal of (40 mL/kg) 3100 mL of plasma volume during each plasmapheresis cycle and 5% human albumin/saline solution combined with fresh frozen plasma was employed as the replacement fluid. The levels of free T3 and T4 returned to normal range and a total thyroidectomy was performed. No major complication was observed during surgery but a minor postoperative bleeding at the incision site occurred on the day after the operation that was controlled with a fresh frozen plasma infusion and mechanical tamponade. The pathology results revealed diffuse hyperplasia.

Second patient

A 79-year-old woman was hospitalized for abdominal and back pain and malaise. Physical examination of this patient revealed epigastric tenderness, defense and rebound tenderness and Murphy’s sign for abdominal palpation was found to be positive. The resulting laboratory test values were as follows; ALT: 73 (12-59 u/L), AST: 70 (10-37 u/L), GGT: 307 (5-55 u/L), ALP: 206 (46-116 u/L), T.blb: 0.46 (0.2-1.2 mg/dL), D.blb: 0.3 (0.0-0.2 mg/dL), Amylase: 66 (25-115 u/L), Lipase: 136 (73-393 u/L), CA 19-9: 5273.08 (0-37 U/mL). Abdominal ultrasonography was consistent with acute cholecystitis and the ERCP and EUS investigation showed bile duct strictures with the presence of atypical cells in the biopsy. The patient’s medical history indicated that a toxic multinodular goiter was present for which she had previously used different doses of propylthiouracil during various treatment courses and had later discontinued this therapy on her own. Upon admission we observed that her thyroid test values were as follows TSH: <0.008 (0.25-4.55 uIU/mL), fT4: 46.9 (11.5-22.7 pmol/L), fT3: 7.33 (3.5-6.5 pmol/L), Anti-TPO: <28.0 (0-60 IU/mL), TRAB: 0.27 (<1.0 IU/L). Increased vascularity, with the largest nodule of 39×21 mm dimensions in the right lobe and a second large nodule of 25×19 mm dimensions in the left lobe and multiple hypechoic nodules, were observed during thyroid ultrasonography. We could not use anti-thyroid drugs because of the presence of acute cholecystitis and the probability of pancreatic cancer. There was not enough time to treat hyperthyroidism, because...
the patient had to undergo an emergent surgery. Thus, in her case therapeutic plasmapheresis was employed for three cycles. The plasma volume exchange was performed with 5% concentration of albumin/saline solution and the total volume of plasma that was exchanged at each cycle was 2000 mL. After the third cycle, the free T3 and T4 concentration in her serum were 1.4 pmol/L and 6.07 pmol/L, respectively. Twenty-four hours after the last plasmapheresis the patient underwent a thyroid surgery in which near-total thyroidectomy was performed successfully. Although preoperative screening coagulation tests (prothrombin time, activated partial thromboplastin time and platelet count) were within normal limits, the patient had excessive postoperative bleeding from the operation site leading to a cardiac arrest that necessitated resuscitation and urgent re-operation for bleeding control. After re-operation and supportive treatment with fresh frozen plasma, she recovered completely. The pathology showed multinodular colloidal hyperplasia.

Discussion

In Graves’ disease, TSH-receptor antibodies stimulate the TSH-R to produce excessive amounts of thyroid hormone that lead to hyperthyroidism. The most common treatments for hyperthyroidism are antithyroid drug therapy, radioactive iodine therapy, and thyroidectomy. The most widely used antithyroid drugs cause minor and transient adverse effects like skin rash, itching, and mild leucopenia, in certain cases. The most dangerous effect is agranulocytosis, while other major adverse effects like aplastic anemia, thrombocytopenia, lupus-like syndrome and vasculitis are rare in nature (6). In addition, disturbances of hepatic function is also a complication of antithyroid drug treatment (7). The spectrum of observed changes in such condition ranges from a mild and asymptomatic elevation of cholestatic and cytosolic liver enzymes to fulminant and fatal necrotizing hepatitis (8, 9). Immuno mediated hepatocellular damage may be seen in patients when treated with propylthiouracil, while cholestatics, due to impaired intracellular drug metabolism, may occur in case of treatment with imidazole derivates. Drug-induced intrahepatic cholestasis results from abnormal bile flow arising from disruption of subcellular actin filaments and interruption of proton pumps and mitochondria. Most severe cases of imidazole-induced cholestasis have reported fulminant hepatic failure leading to liver transplantation and other fatal outcomes (10).

Our first patient presented with jaundice and extremely high levels of serum bilirubin due to the rejection of liver transplantation while the second patient presented an acute cholecystitis with suspected pancreatic cancer. We could not use antithyroid drugs in either of these situations and radioactive iodine therapy was not an acceptable choice because these patients could not wait until euthyroid. The first patient was awaiting surgery for an urgent liver re-transplantation and the second patient needed urgent cholecystectomy and probably even the Whipple procedure. In order to perform thyroidectomy, these patients were required to be euthyroid. Therapeutic plasma exchange (TPE) is an alternative treatment that has been proposed since the 1970s for hyperthyroidism and, more precisely, for the thyroid storm, since it leads to rapid clinical response and rapid normalization of circulating thyroid hormone levels, irrespective of the etiology (11). During plasma exchange, the plasma is extracted from the patient’s blood, and a colloid replacement solution (in our cases, FFP and albumin) is infused back to the patient in place of the plasma. TBG along with bound thyroid hormones also gets removed from the plasma during this procedure and the colloid replacement provides new binding sites for the circulating free thyroid hormone. Although albumin binds thyroid hormone with less avidity than TBG, it provides a much larger capacity for low-affinity binding that may contribute to lower levels of free thyroid hormone (12). Even though plasmapheresis has been used mainly for the treatment of thyroid storm, it can be used as a preferred therapy for the prevention of thyroid storm in selected patients in whom thyroid hormone concentrations do not decrease to safer levels in spite of employing medical treatment strategies such as iopanoic acid, lithium, beta blockers or combinations (13).

In thyrotoxic patients with severe complications, TPE should be considered as a valuable pre-operative therapeutic option. However, TPE involves complications like hypotension, hemolysis, anaphylactic or allergic reactions, infection, vascular injury and coagulopathy (14). In addition to this, coagulation proteins and platelets are removed during plasmapheresis and this step is important because when the removed plasma is restored by
colloidal solutions other than fresh frozen plasma, it leads to coagulopathy in the immediate post-plasmapheresis period. After plasmapheresis, factors such as prothrombin time, activated partial thromboplastin time and thrombin time are found to be markedly prolonged (15). In the report by Ezer et al., in 2009, intraoperative bleeding from operation sites occurred in one out of 11 patients who were studied (11). Özbey et al., in 2004, reported in their investigation that two patients had perioperative unusual bleeding despite normal screening coagulation tests (13). Therefore, bleeding complications should be monitored carefully.

In the patients from our study, TPE in combination with standard supportive measures provided safe, rapid and effective treatment for hyperthyroidism.

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An informed consent form was obtained from the patients.

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Conflict of Interest: No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Author Contributions
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References
The Diagnosis of Neuroendocrine Tumours: An Endocrine Perspective

Nöroendokrin Tümörlerin Tanısına Endokrin Bakış

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Abstract

Neuroendocrine tumours are functioning or non-functioning tumours which are derived from neuroendocrine cells scattered throughout the body. The clinical presentation of neuroendocrine tumours depends mainly on the site of the primary tumour and whether it is secretory in nature and thus causing specific symptoms. Neuroendocrine tumour patients may be consulted in endocrinology outpatient clinics with complaints of flushing and sweating, hypoglycaemia, or due to ectopic hormone production-related symptoms, or may be referred from gastroenterology or general surgery units due to incidentally-found gastric neuroendocrine tumours, diabetes, pancreatic lesions, abdominal pain and/or diarrhoea. The current review will focus on presentation, symptomatology and diagnostic markers of tumours arising from the diffuse NE cell system, principally gastroenteropancreatic neuroendocrine tumours. The aim is to present a practical approach for the endocrinologist facing the large numbers of available laboratory tests, and will emphasise the relationship of these tumours with some genetic syndromes such as multiple endocrine neoplasia type 1 (MEN1).

Keywords: Neuroendocrine tumors; gastroenteropancreatic; carcinoid syndrome; ectopic; multiple endocrine neoplasia

Introduction

Neuroendocrine tumours (NETs) are functioning or non-functioning tumours which are derived from neuroendocrine (NE) cells scattered throughout the body. NE cells can form glands (adenohypophysis, parathyroid, adrenal medulla, paraganglia) or may be found in a diffuse/disseminated way throughout the body in the skin, thyroid, lung, thymus, pancreas, gastrointestinal, biliary and urogenital tracts (1). NETs are capable of storing and secreting different peptides and amines, some of which may cause specific clinical syndromes. NET patients may be consulted in endocrinology outpatient clinics with complaints of flushing and sweating, hypoglycaemia, or due to ectopic hormone production-related symptoms, or may be referred from gastroenterology or general surgery units due to incidentally-found
gastric NETs, diarrhoea, abdominal pain and/or pancreatic lesions. From this point of view, the current review will focus on the presentation, symptomatology and diagnostic markers of tumours arising from the diffuse NE cell system, principally gastroenteropancreatic neuroendocrine tumours (GEP-NETs). The aim is to present a practical approach for the endocrinologist facing the large numbers of available laboratory tests, and will emphasise the relationship of these tumours with some genetic syndromes such as multiple endocrine neoplasia type 1 (MEN1).

Epidemiology and Classification

NETs are rare tumours but their incidence and prevalence are increasing owing to increased diagnosis of early stage tumours, and possibly a true increase in incidence. According to a recent retrospective analyses of the SEER database from the USA, the age-adjusted annual incidence of NETs is 60-70 cases per million which indicates an 6.4 fold increase in rate between 1973 and 2012 (2). The prevalence of NETs, which was estimated to be 171,321 cases in 2014 in the USA, is higher than the combined 2013 estimates of other common gastrointestinal malignancies, including oesophageal cancer (36,857 patients), gastric adenocarcinoma (79,843 patients), and pancreatic adenocarcinoma (49,620 patients) (3). About two-thirds of NETs are GEP-NETs, mostly arising from the small intestine: small intestinal NETs are most frequently located in the distal ileum (4). On the other hand, as NE cells are distributed all around the body, there are many reports indicating diverse locations such as the sphenoid sinus (5), middle ear (6,7), renal pelvis (8), mediastinum (9), retroperitoneum (10), medulla spinalis (11), cavernous sinus (12), lymph nodes (13), fallopian tubes (14) and the parotid gland (15). NETs may be benign when small (usually <1 cm), but when malignant (representing less than 2% of gastrointestinal malignancies) they metastasise often before becoming symptomatic, generally when the tumour is larger than 2 cm. They frequently metastasise to regional lymph nodes, liver and less commonly to bone, although the latter site is becoming more obvious with newer imaging techniques. In terms of primary tumour site, unpredictable combinations occur such as thymic carcinoids to the myocardium (16), gastrointestinal carcinoids to the orbit (17), bronchial carcinoids to the spinal cord (18), lung carcinoids to the choroid (19) or breast (20), and visceral carcinoids to the skin (21). However, it does seem that NETs have a general predisposition to metastasise to non-classic sites such as the myocardium, breast and eye. Essentially, histology strongly correlates with specific primary sites; in a registry database from the Netherlands, while grade 1 NETs mostly originate from the gastrointestinal tract, grade 2 NETs often originate from the lung (22), although there is much overlap. According to the SEER data the best prognosis has been reported for rectal and appendiceal carcinoids (often when small and discovered incidentally), and worst for pancreatic and lung NETs (2).

In terms of pathological diagnosis, it is important to ask for an immunohistochemical analysis of biopsy materials in order not to miss the diagnosis of a NET. Tumours may be labelled mistakenly as adenocarcinoma, which may affect the management and treatment protocol and patient prognosis. While there are a number of NE markers, chromogranin A (CgA) and synaptophysin are the principal ones used in diagnostic pathology (Figure 1). CgA is widely used as an immunohistochemical marker in NETs and is recognised as the most useful (23). Importantly, as synaptophysin can be examined in formalin-fixed tissues, it is possible to reevaluate the tumour if it is not considered neuroendocrine initially. The use of Neuron Specific Enolase (NSE) has been discouraged in a recent ENETS consensus guideline due to its low specificity (24).

There are a few proposed classification systems for NETs. Tumour behaviour, secretory patterns, and type of secretory products are similar between NETs of same embryological site. Based on this, NETs have been classified as (25):

- Foregut NETs (bronchus, lung, thymus, oesophagus, stomach, liver, biliary system, pancreas, first portion of duodenum)
- Midgut NETs (second portion of duodenum, jejunum, ileum, appendix, right colon, proximal transverse colon)
- Hindgut NETs (distal transverse colon, left colon, rectum).

On the other hand, although this classification system was introduced both for ease of understanding and gaining a systematic approach to these rare tumours, because of the wide spectrum of their presentations and changing characteristics, neither diagnosis nor designation of a treatment algorithm is valid for all types of NETs. As an example, the molecular profiling of gastrointestinal NETs (GI-NETs) and pancreatic NETs...
(pNETs) have demonstrated different genetic changes, such that these tumours should be regarded as different tumour entities and managed accordingly (26). Thus, the putative embryonic derivation is no longer used, and instead the newer World Health Organization (WHO) classification is used in addition to the standard TNM classification (27). Indeed, neuroendocrine neoplasms (NEN) are heterogeneous tumours now classified into fundamentally two groups: well-differentiated, low-proliferative NENs, called NETs, and poorly-differentiated, highly proliferative NENs, called small- or large-cell neuroendocrine carcinomas (NECs) (28). This dichotomy may be due to an origin from different progenitor cells. The latest WHO classification of gastrointestinal NENs uses the Ki-67 proliferation index to grade NETs as G1 (Ki-67<2%) or G2 (Ki-67 2-20%), and NECs as G3 (Ki-67>20%) (In the 2017 WHO nomenclature, it is suggested that the threshold for pNETs should be changed from 2% to 3%, but other NETs remain unchanged) (Figure 2). In the pancreas, NETs and NECs may overlap in their proliferation index, making the distinction between them difficult and leading to therapeutic uncertainties (28). Because of this, the 2017 WHO Classification of pancreatic NENs introduced a new NET G3 category: well-differentiated G3 tumours with a Ki-67 usually in the range 20-55% are referred to as G3 NETs, while poorly-differentiated G3 tumours with a Ki-67 >55-100% are to be referred to as G3 NECs. At present, this classification officially only applies to pancreatic NETs, but can probably be applied informally to all NETs (27).

Because NE cells can produce a number of hormonal peptides and neuroamines (29,30), another classification system for NETs may be based on secretory products. However, a NET may start as a silent tumour then become secretory, may co-secrete several hormones/amines (31), and on recurrence may secrete another product (32); even its metastases may secrete different peptides from the parent tumour. As a corollary, clinical symptoms and signs may

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**Figure 1:** Chromogranin A (upper) and synaptophysin (lower) staining in a NET.

**Figure 2:** Grading gastrointestinal NETs using Ki-67 according to WHO (27).
change over time due to the secreted peptides (33). Additionally, NETs may secrete substances not related to their cell of origin, such as cytokines and autoantibodies which result in paraneoplastic syndromes (34,35). Although a few of the secreted compounds may be used more generally, there is no ideal NET marker, and the clinician needs to make a choice according to the clinical presentation keeping in mind that the sensitivity and specificity of serum levels differ for each marker.

The complexity and diversity of NETs make the diagnosis of these rare tumours quite difficult. But as mentioned above, although the clinician should expect the “unexpected”, it is of utmost importance to take into consideration the rates and common locations of these tumours before starting laboratory tests and/or imaging studies to prevent useless costs. As an example, for a patient who presents with the carcinoid syndrome (CS), the diagnostic work-up should initially target the small intestine as hindgut NETs are generally silent and non-secretory. On the other hand, secretory pancreatic NETs are most commonly insulinomas, gastrinomas and PPomas, the remaining tumours comprising around 1% of pancreatic NETs. Thus, for a patient with a silent pancreatic mass without diabetes, it is not particular helpful to start laboratory workup with plasma somatostatin and/or glucagon levels.

Clinical Presentation

The clinical presentation of NETs depend mainly on the site of the primary tumour and whether it is secretory in nature and thus causing specific symptoms. To present an overview of clinical presentation, tumour type and location, secreted hormones and diagnostic markers have been listed in detail in Table 1. Corresponding references have also been added not for common but for rare tumour locations and secretory products. NETs are the most common gut endocrine tumours; while they comprise less than 2% of gastrointestinal malignancies, 71% of midgut NETs have metastatic disease at presentation (36). As the most common primary site is the small intestine, around one-third of patients present with many years’ history of intermittent abdominal pain and vague abdominal symptoms (37), and are often misdiagnosed as ‘irritable bowel syndrome’. NETs are slow-growing tumours and the patient may thus be followed as ‘irritable bowel syndrome’ for years (38). Additionally, NETs may present with symptoms due to CS (Table 2), ec-topic hormone production, mechanical complications, or more rarely with paraneoplastic conditions.

Although once considered to occur in less than 10% of patients with NETs, in a recent population-based study from the SEER database excluding patients with pancreatic tumours, small and large cell lung cancers, the frequency of CS at NET diagnosis was reported as 19% (39). In this study, NETs presenting with CS were most commonly duodenal, jejunal or ileal in origin. Additionally, CS was significantly associated with tumour stage, grade and primary tumour site, and led to shorter overall survival.

CS occurs due to secretion of several vasoactive substances such as serotonin, histamine, tachykinins and prostaglandins by the tumour (Table 1). Symptoms of classic CS consist of flushing (occurring in 84%), diarrhoea (70%), abdominal cramping, carcinoid heart disease, telangiectasia, and bronchospasm presenting with wheezing (uncommon) (25,40). The relationship of flushing to diarrhoea is variable. Carcinoid heart disease, which mainly involves the right side of the heart, occurs in more than 50%, and is the initial presentation in 20% of patients with CS (41). Fibrous endocardial thickening occurs mainly in the tricuspid and pulmonary valves which may lead to regurgitation or stenosis (41). Left-sided valvular problems tend to occur when there is a patent foramen ovale. N-terminal pro-brain natriuretic peptide (NT-pro-BNP) is a valid marker in the clinical evaluation of carcinoid heart disease (23,42,43). Additionally, paraneoplastic neuropathy, myopathy, arthropathy, increased skin pigmentation, and peripheral oedema may occur (40). In CS the essential amino-acid tryptophan is mainly used as the precursor for serotonin, leaving inadequate amounts of tryptophan for conversion to niacin (Figure 3). Pellagra may develop due to deficiency of niacin (vitamin B3) with components of dermatitis, diarrhoea, dementia, stomatitis, glossitis and angular cheilitis (40,43). A patient with CS presenting with anaemia, urticaria and angiœdema has also been described (44).

The extent and frequency of symptoms of CS vary and may change according to tumour location due to the difference in secreted products. It is important to note that, in midgut NETs, CS is only possible with hepatic metastases or peritoneal seeding as otherwise the secreted products will be catabolised by the liver (hepatic first-pass effect) (40,45). Additionally, midgut
<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Syndrome</th>
<th>Tumor type</th>
<th>Tumor location</th>
<th>Mediator peptides and hormones</th>
<th>Diagnostic markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flushing</td>
<td>Carcinoid syndrome</td>
<td>NET</td>
<td>Foregut, midgut, rarely hindgut, pancreas, testes (109), presacral (110), pituitary (111)</td>
<td>Serotonin, prostaglandins, kinins, pro-gastrin-releasing peptide (112), VIP, calcitonin gene-related peptide, histamine (45), 5-HTP, substance β, neurotensin, motilin, neurokinin A, kallikrein, neuropeptide K, somatostatin, dopamine (38)</td>
<td>CgA, 24-hour urinary 5-HIAA</td>
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<td>MTC, C-cell hyperplasia</td>
<td>C-cell</td>
<td>Thyroid (38,113)</td>
<td>Calcitonin</td>
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<tr>
<td></td>
<td>Phaeochromocytoma/paraganglioma</td>
<td>Chromaffin cell</td>
<td>Adrenal medulla, sympathetic nervous system</td>
<td>Adrenalin, noradrenalin, rarely dopamine</td>
<td>Plasma or 24-hour urinary metanephrines, normetanephrines, dopamine</td>
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<tr>
<td></td>
<td>Diarrhoea/abdominal pain, dyspepsia</td>
<td>Carcinoid syndrome</td>
<td>Foregut, midgut, appendix (114), rarely hindgut, pancreas, testes (109), presacral (110)</td>
<td>As above</td>
<td>CgA, 24-hour urinary 5-HIAA</td>
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<td>Zollinger-Ellison syndrome (ZE)</td>
<td>Gastrinoma</td>
<td>Duodenum (70%), pancreas (25%), other sites (5%) (40,115)</td>
<td>Gastrin</td>
<td>CgA, gastrin, PP</td>
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<td>Pancreatic polypeptide</td>
<td>PPoma</td>
<td>Pancreas</td>
<td>PP</td>
<td>CgA, PP</td>
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<td>MTC NET</td>
<td>C-cell NET</td>
<td>Thyroid (116), pancreas (116,117), stomach (116), appendix (116)</td>
<td>Calcitonin</td>
<td>Calcitonin</td>
</tr>
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<td>WDHHA (Verner-Morrison syndrome)</td>
<td>VIPoma</td>
<td>Pancreas (90%, adult), other (10%, neural, pheochromocytoma, paraganglioma) (118-121)</td>
<td>VIP, neurotensin</td>
<td>CgA, VIP</td>
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<td>Secretinoma</td>
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<td>Pancreas (122)</td>
<td>Secretin</td>
<td>(tissue immunohistochemistry)</td>
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<td>Carcinoid syndrome</td>
<td>Foregut, midgut, rarely hindgut, pancreas</td>
<td>Substance P, histamine, 5-HT (38)</td>
<td>CgA, 24-hour urinary 5-HIAA</td>
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<td></td>
<td>Ulcer, dyspepsia, epigastric pain</td>
<td>ZE</td>
<td>Duodenum (70%), pancreas (25%), other sites (5%) (40,115)</td>
<td>Gastrin</td>
<td>CgA, gastrin, PP</td>
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<tr>
<td></td>
<td>Hypoglycaemia (123)</td>
<td>Whipple’s triad</td>
<td>Pancreas (~98%), Ectopic insulinoma (~2%), ovarian (125), kidney (126), paraganglioma (127), liver (32), cervical NET (SCC) (128), bronchial carcinoid (129), appendix (114)</td>
<td>Insulin, Glucose, insulin, C-peptide</td>
<td>Insulin</td>
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Table 1. Symptoms, associated syndromes, secretory products and tumor locations of NETs with special emphasis on GEP-NETs (Modified from Vinik AI & Chaya C. Hematol Oncol Clin N Am 2016;30:21-48)(40).
Clinical presentation | Syndrome | Tumor type | Tumor location | Mediator peptides and hormones | Diagnostic markers |
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Non-islet cell tumour hypoglycemia (NICTH)</td>
<td>IGF2-oma</td>
<td>Gastric NET (131), bronchial carcinoid, phaeochromocytoma (132), pancreas (67)</td>
<td>Big IGF2</td>
<td>IGF2 isoforms (thin-layer chromatography) (29) (accompanying low insulin, GH and IGF-1)</td>
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<tr>
<td>IGF1-oma</td>
<td>Large cell Ca of lung (133)</td>
<td>IGF-1</td>
<td>IGF-1</td>
<td></td>
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<tr>
<td>GLP-1 secreting NET</td>
<td>Pancreas (68), ovary (134)</td>
<td>GLP-1</td>
<td>GLP-1</td>
<td></td>
<td></td>
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<tr>
<td>Silent pancreatic mass or diarrhea, liver metastases</td>
<td>Silent</td>
<td>Pancreas</td>
<td>PP</td>
<td>CgA, PP</td>
<td></td>
</tr>
<tr>
<td>Diabetes, diarrhea, steatorrhoea, choledolithiasis, deep vein thrombosis</td>
<td>Somatostatinoma</td>
<td>Pancreas (55%), duodenum/ small intestine (44%) (40), kidney (135), ovary (136)</td>
<td>Somatostatin</td>
<td>CgA, somatostatin</td>
<td></td>
</tr>
<tr>
<td>Phaeochromocytoma</td>
<td>Adrenal medulla (137)</td>
<td>Somatostatin, adrenalin, noradrenalin</td>
<td>Somatostatin, 24-hour urinary or plasma metanephrine, normetanephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-cell</td>
<td>Thyroid (138)</td>
<td>Somatostatin, calcitonin</td>
<td>Somatostatin, calcitonin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes, diarrhea, necrolytic migratory erythema, pellagra (somatitis, glossitis, angular cheilitis)</td>
<td>Glucagonoma</td>
<td>Pancreas</td>
<td>Glucagon</td>
<td>CgA, glucagon</td>
<td></td>
</tr>
<tr>
<td>Glucagonoma NET</td>
<td>Foregut, midgut, rarely hindgut, pancreas</td>
<td>As above</td>
<td>CgA, 24-hour urinary 5-HIAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>With weight loss</td>
<td>All (especially pheochromocytoma)</td>
<td>All</td>
<td>Cytokines (IL-1, IL-6, TNF-α, IFN-γ)</td>
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<tr>
<td>Acromegaly</td>
<td>Acromegaly</td>
<td>Lung (54%), pancreas (30%), jejunum (7%), other (e.g. thymus) (13%) (40,139) Mediastinal parangangioma (61), adrenal medulla (140)</td>
<td>GHRH</td>
<td>GHRH, GH, IGF-1 (pituitary hyperplastic/normal on imaging)</td>
<td></td>
</tr>
<tr>
<td>GHRH-secreting NET</td>
<td>Pancreas (141), bronchial carcinoid (142)</td>
<td>GH</td>
<td>GH, IGF-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaeochromocytoma/paragangioma</td>
<td>Pulmonary carcinoid (52)</td>
<td>GH, IGF-1</td>
<td></td>
<td></td>
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<tr>
<td>Clinical presentation</td>
<td>Syndrome</td>
<td>Tumor type</td>
<td>Tumor location</td>
<td>Mediator peptides and hormones</td>
<td>Diagnostic markers</td>
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<tr>
<td>Cushing’s syndrome</td>
<td>Cushing’s syndrome</td>
<td>ACTH-secreting NET (50% of ectopic Cushing syndrome are due to lung) Phaeochromocytoma C-cell</td>
<td>Foregut (143), midgut, appendix (114), rectum (144), pancreas (59, 60, 145-150), bladder, prostate SCC (151), ovary (152) Adrenal medulla (153) Thyroid (154)</td>
<td>ACTH</td>
<td>ACTH, midnight salivary cortisol, 24-hour urinary free cortisol, dynamic tests</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACTH and/or CRH secreting NET C-cell</td>
<td>Thymus (155), Pancreas (156)</td>
<td>ACTH, CRH</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thyroid (157)</td>
<td></td>
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<tr>
<td>Anorexia, nausea, vomiting, abdominal pain</td>
<td>Hypercalcemia</td>
<td>PTHrP-oma</td>
<td>Pancreas (62, 158,159), thymus (160), liver (161), Phaeochromocytoma (162)</td>
<td>PTHrP</td>
<td>PTHrP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phaeochromocytoma</td>
<td>PTH-oma</td>
<td>Lung (163), MTC (164), ovary (165), pancreas (166), neck NET (167), paraganglioma (168), gastric NET (169)</td>
<td>PTH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,25-dihydroxyvitamin D secreting NET</td>
<td>Pancreas (158, 170)</td>
<td>1,25-dihydroxyvitamin D</td>
</tr>
<tr>
<td>Weakness, lethargy, apathy</td>
<td>Hyponatremia, SIADH</td>
<td>ADH-secreting NET</td>
<td>Larynx (171), Lung (172), rectum (173), cervix (174), pancreas (175,176), prostate (177)</td>
<td>ADH</td>
<td>ADH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADH/ANP-secreting NET</td>
<td>Lung (178), thymus (54)</td>
<td>ADH and ANP</td>
<td>ADH and ANP</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Severe hypertension</td>
<td>Phaeochromocytoma/paraganglioma</td>
<td>Adrenal medulla</td>
<td>Sympathetic nervous system</td>
<td>Adrenaline, noradrenaline, dopamine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NET</td>
<td>Pancreas (66), bronchial carcinoid (179), Phaeochromocytoma (180)</td>
<td>Renin</td>
<td>Plasma renin activity, prorenin</td>
</tr>
<tr>
<td>Hyperandrogenism,virilization</td>
<td>LHoma</td>
<td>pNET</td>
<td>Pancreas (65)</td>
<td>LH</td>
<td>LH</td>
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<tr>
<td>Constipation</td>
<td></td>
<td>NET</td>
<td>Ovary (51)</td>
<td>Peptide YY</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown origin (181), intraabdominal mass (182)</td>
<td>GLP-1, GLP-2, peptide YY</td>
<td></td>
<td></td>
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<tr>
<td>Ovarian hyperstimulation</td>
<td>FSHoma</td>
<td>NET</td>
<td>Mediastinal (183), pancreas (184)</td>
<td>FSH</td>
<td>FSH</td>
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<tr>
<td>Gastroparesis</td>
<td>Ghrelinoma</td>
<td>NET</td>
<td>Stomach (185), presacral region (53)</td>
<td>Ghrelin</td>
<td>Ghrelin</td>
</tr>
<tr>
<td>Diarrhea, peptic ulcer, bile stone attacks</td>
<td>CCKoma</td>
<td>pNET</td>
<td>Pancreas (69)</td>
<td>CCK</td>
<td>CCK</td>
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<tr>
<td>Polycythaemia</td>
<td></td>
<td>pNET</td>
<td>Pancreas (73)</td>
<td>Erythropoietin</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>Syndrome</td>
<td>Tumor type</td>
<td>Tumor location</td>
<td>Mediator peptides and hormones</td>
<td>Diagnostic markers</td>
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<tr>
<td>Neurological / muscular paraneoplastic syndrome</td>
<td>Painful axonal polyradiculoneuropathy, autonomic neuropathy</td>
<td>Mostly SCLC</td>
<td>Lung (187,188)</td>
<td>CRMP5 (collapsin response-mediator protein-5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myopathy</td>
<td>NET</td>
<td>Lung (189)</td>
<td>AChR Ab (anti-acetylcholine receptor Ab)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myasthenia gravis</td>
<td>NET</td>
<td>Lung (190), Small intestine (191), ileum (192), thymus (193)</td>
<td>Anti-Yo Ab (Purkinje cell cytoplasmic Ab type 1-PCA1), Anti-Ri Ab (anti-neuronal nuclear Ab type 2-ANNA-2), anti-GAD Ab (glutamic acid decarboxylase Ab)</td>
<td></td>
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<tr>
<td></td>
<td>Paraneoplastic cerebellar degeneration</td>
<td>Mostly SCLC, also NET</td>
<td>Oropharynx (194), stomach (195), pancreas (196), lung (197), thymus (198), midgut NET (199)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Limbic encephalitis</td>
<td>Mostly SCLC, NET</td>
<td>Pancreas (200), midgut (201), bronchus (199), thymus (202), lung (203), tonsil (204)</td>
<td>Anti-Ma2 Ab, anti-Hu Ab, anti-Ri Ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lambert-Eaton syndrome</td>
<td>Mostly SCLC, NET</td>
<td>Lung (205,206), oropharynx (194), larynx (171)</td>
<td>P/Q type voltage-gated calcium channel Ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autoimmune retinopathy</td>
<td>Mostly SCLC, NET</td>
<td>Lung (206), small bowel (35)</td>
<td>Antirecoverin Ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neuromyelitis optica spectrum disorder</td>
<td>NET</td>
<td>Stomach (34), small bowel (207)</td>
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<td></td>
<td>Axonal Guillain-Barre-like syndrome</td>
<td>Mostly SCLC</td>
<td>Lung (208)</td>
<td></td>
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<tr>
<td></td>
<td>Sensory neuropathy</td>
<td>Mostly SCLC, NET</td>
<td>Lung (206), bronchus (199), duodenum (209)</td>
<td>Anti-Hu Ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Visceral neuropathy (chronic gastrointestinal pseudoobstruction)</td>
<td>Mostly SCLC, NET</td>
<td>Lung (210), bronchial NET (211)</td>
<td>Anti-Hu Ab, Anti-CV2 Ab</td>
<td></td>
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<tr>
<td></td>
<td>Neuromyotonia, amyotrophic lateral sclerosis, multifocal hypertrophic mononeuropathy</td>
<td>SCLC</td>
<td>Lung (212-214)</td>
<td></td>
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<tr>
<td></td>
<td>Brainstem encephalitis</td>
<td>NET</td>
<td>Rectum (215)</td>
<td>Anti-Ri Ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paraneoplastic encephalomyelitis</td>
<td>NET</td>
<td>Lung (216)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paraneoplastic neuropathy</td>
<td>NET</td>
<td>Cecum (217)</td>
<td>HCG (218), CGRP (219), motilin (220,221), dopamine (64), neuropeptide K (222), neurotensin (72), neurokinin A (223)</td>
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</tr>
</tbody>
</table>

Table 1. Symptoms, associated syndromes, secretory products and tumor locations of NETs with special emphasis on GEP-NETs (Modified from Vinik AI & Chaya C. Hematol Oncol Clin N Am 2016;30:21-48)(40) (continued).
NETs have a high 5-hydroxytryptamine (5-HT) content, rarely secrete 5-hydroxytryptophan (5-HTP), and may present with classic CS. However, for some foregut NETs (bronchus, lung, thymus, oesophagus) and gonadal NETs (ovarian and testicular carcinoids) the vasoactive substances can be released into the bloodstream before inactivation, and thus metastases are not essential for CS to occur (40,45,46,47). Foregut NETs have a low content of serotonin and often secrete the serotonin precursor 5-HTP and histamine (40). These differences also reflect in the clinical picture and diagnostic tests of midgut and foregut NETs (see below).

As a common presenting symptom of CS is flushing and these patients are seen in endocrinology outpatient clinics, it is often the endocrinologist who will consider NET as a preliminary diagnosis in these patients. In the work-up of the patient referred with a complaint of flushing, the endocrinologist should obtain a thorough history and perform a physical examination for the differential diagnosis (45,48). Firstly, the absence or presence of sweating should be asked for, as pathophysiology differs between “wet” and “dry” flushing; neurally-mediated flushing is frequently associated with sweating (wet flushing) which can be due to events at both central and peripheral sites, while isolated (dry) flushing is mainly due to circulating vasodilator substances (40,45,48). Additionally, associated symptoms during the attack and triggering events should be sought (emotions, food, alcohol), and importantly a careful drug history is mandatory as flushing can occur as a side effect of several drugs (Table 3). Four types of carcinoid flushing have been described; erythematous, violaceous, prolonged and bright-red (45). The flushing of foregut NETs are described as a bright-red “geographic” flush but bronchopulmonary carcinoids are associated with prolonged flushing lasting several hours to some days which may result in telangiectasia and hypertrophy of the skin of the face and neck (45). The face may take a leonine appearance resembling acromegaly after repeated episodes (40). On the other hand, the flushing of midgut tumours is erythematous, and involves the face and upper trunk down to the nipple line (40). Ileal NETs as part of a midgut syndrome seem to show a patchier and more violaceous flush. Flushing of CS may be spontaneous or triggered by certain foods rich in serotonin (blue cheese, sherry, beer, nuts, avocado, banana, fermented foods, chocolate, red wine, red sausage), alcohol, palpation of the liver, general anaesthesia, and increased adrenergic activity as occurs with pain, anger, embarrassment or exertion (45).

On the other hand, in post-menopausal flushing Tepper and colleagues have described four distinct trajectories which may well explain the spectrum of onset and frequency of symptoms seen in these patients; early onset (onset about eleven years before the final menstrual period with decline after menopause), late onset (onset near the final menstrual period with later decline), high frequency (onset early with persistently high frequency) and low frequency (persistently low frequency) (49). In phaeochromocytoma generally pallor occurs due to the pe-
Carcinoid syndrome
Phaeochromocytoma, paraganglioma
Medullary thyroid cancer
Renal cell carcinoma (due to secretion of gonadotrophin-like hormones)
Systemic mastocytosis
Pancreatic NETs
Cushing’s syndrome
Autonomic neuropathy
Post-menopausal hot flashes (80% of post-menopausal women)
Medical or surgical castration for prostatic cancer (more than 65% of men)
Malignant histiocytoma, neuroblastoma, ganglioneuroma (due to VIP secretion)
Anxiety, panic attacks
Simultaneous ingestion of alcohol and chlorpropamide
Drugs (nitroglycerine, nitro-derivatives, phosphodiesterase-5 inhibitors, calcium channel blockers—mainly dihydropyridine, cholinergic drugs, prostaglandin D2 and E, non-steroidal antiinflammatory drugs, nicotinic acid, vancomycine, rifampicin, cyclosporine, cisplatin, dacarbazine, TRH, bromocriptine, morphine, opioids, triamcinolone, metoclopramide, isoflurane, fentanyl, serotonin reuptake inhibitors—can cause night sweats, radiologic contrast agents)

Table 3. Differential diagnosis of flushing (45,48).

<table>
<thead>
<tr>
<th>Carcinoid syndrome</th>
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<tr>
<td>Phaeochromocytoma, paraganglioma</td>
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<tr>
<td>Medullary thyroid cancer</td>
</tr>
<tr>
<td>Renal cell carcinoma (due to secretion of gonadotrophin-like hormones)</td>
</tr>
<tr>
<td>Systemic mastocytosis</td>
</tr>
<tr>
<td>Pancreatic NETs</td>
</tr>
<tr>
<td>Cushing’s syndrome</td>
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<tr>
<td>Malignant histiocytoma, neuroblastoma, ganglioneuroma (due to VIP secretion)</td>
</tr>
<tr>
<td>Anxiety, panic attacks</td>
</tr>
<tr>
<td>Simultaneous ingestion of alcohol and chlorpropamide</td>
</tr>
<tr>
<td>Drugs (nitroglycerine, nitro-derivatives, phosphodiesterase-5 inhibitors, calcium channel blockers—mainly dihydropyridine, cholinergic drugs, prostaglandin D2 and E, non-steroidal antiinflammatory drugs, nicotinic acid, vancomycine, rifampicin, cyclosporine, cisplatin, dacarbazine, TRH, bromocriptine, morphine, opioids, triamcinolone, metoclopramide, isoflurane, fentanyl, serotonin reuptake inhibitors—can cause night sweats, radiologic contrast agents)</td>
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</table>

Peripheral vasoconstrictive affect of catecholamines but flushing may also rarely occur. In medullary thyroid carcinoma (MTC) the most prominent hormone-mediated symptom is secretory diarrhoea with or without flushing (48). In systemic mastocytosis, in addition to flushing pruritus, nausea, diarrhoea, abdominal pain and even vasodilatory shock may occur due to the release of mast cell mediators (45,50).

Table 1 summarises a number of secretory products and associated symptoms which will not be discussed here in detail. However, it may be important to note that, with the progress in both laboratory and imaging techniques in the last decades, there has been a noticeable increment in the discovery of secretory products and NET locations such as ovarian strumal carcinoid presenting with constipation due to peptide YY secretion (51), a GH and IGF-1 co-secreting pulmonary carcinoid (52), and a presacral ‘ghrelinoma’ causing gastroparesis (53). Patients may also present with acromegaly secondary to secretion of GH-releasing hormone (Table 1).

Mechanical complications in NETs may occur according to the location of the tumour. Obstructive pneumonia, dyspnoea and cough have been reported for bronchial NETs (54). Midgut NETs may cause obstruction in the small bowel but ischaemia may also occur due to mesenteric fibrosis and vessel compression even in the absence of an obvious mass (55). While hindgut NETs are generally silent tumours and rarely cause CS, they may present with bleeding, pain and intestinal obstruction.

In terms of paraneoplastic syndromes, a number of newly-defined neurological conditions and corresponding antibodies have been defined (Table 1), but there also are anecdotal case reports in which the mechanism of the paraneoplastic condition can not be readily explained such as a Budd-Chiari syndrome induced by a stage IV rectal carcinoid (56).

From another point of view, GEP-NETs may also be discovered as incidental masses detected at imaging or endoscopy in gastroenterology outpatient clinics, or with the irritable bowel syndrome-like symptoms. Among these, pNETs and gastric NETs will be mentioned here in detail, particularly because of their relationship with the MEN1 syndrome.

Pancreatic NETs

pNETs are divided into functional (10-30%) and non-functional groups (70-90%)(57). Non-functional pNETs are malignant in 60-90% of cases and CgA and pancreatic polypeptide (PP) levels are elevated (57). Insulinomas are the most common functional pNETs (58), and in decreasing order of frequency gastrinomas, glucagonomas, vasoactive intestinal peptide secreting-tumours
(VIPomas) and somatostatinomas may be seen (40). Rarely, other ectopic hormones are produced such as adrenocorticotropic hormone (ACTH) (59,60), growth hormone-releasing hormone (GHRH) (61), PTH-related peptide (PTHrP) (62) and serotonin (63). More rarely, dopamine (64), luteinising hormone (LH)(65), renin (66), insulin-like growth factor II (IGF-II)(67), glucagon-like peptide-1 (GLP-1)(68), cholecystokinin (CCK)(69), ghrelin (70), calcitonin (71), neurotensin (72) or erythropoietin (73) have all been reported. PNETs may be associated with four autosomal dominant disorders: MEN1 (seen in 30-80% of MEN1 patients in different series) (see below) (74), Von-Hippel Lindau disease (VHL) (seen in 11-17% of these patients), von Recklinghausen disease (neurofibromatosis type 1, seen in 10%) and occasionally tuberous sclerosis (40,75,76). Germline mutations in the VHL gene trigger overexpression of hypoxia-inducible factor (HIF) proteins and cause VHL disease characterised by various tumours and cysts, such as multiple phaeochromocytomas/paragangliomas, haemangioblastomas of the retina and central nervous system, kidney cysts, pancreatic cysts (50%) and NETs, renal cell carcinoma and polycythaemia (77,78).

Patients with insulinoma and non-insulinoma pancreatic islet cell tumours (NIPHS)/nesidioblastosis may present with complaints of hypoglycaemia and are in large part seen in endocrinology outpatient clinics. As the cases of obesity and prediabetes have been on the rise, several patients experiencing postprandial hypoglycaemia quite frequently present nowadays. As opposed to postprandial hypoglycaemia, insulin-related hypoglycaemia classically occurs with fasting either early in the morning or may be exercise-induced (79), but in 5% of patients with insulinomas the hypoglycaemia may be purely post-prandial. Insulin-induced hypoglycaemia generally results in a combination of neurologic (diplopia, blurred vision, confusion, abnormal behaviour and amnesia, seizures, coma) and autonomic (sweating, weakness, hunger, tremor, nausea, feelings of warmth, anxiety, palpitations) symptoms, while post-prandial hypoglycaemia is rarely associated with neurologic symptoms. However, importantly NIPHS patients usually present postprandially but with neuroglycopenic symptoms (within four hours of meal ingestion). NIPHS, which was previously quite rare, is now being seen more frequently as it is most commonly seen after gastric bypass surgery (40).
WDHA (watery diarrhoea, hypokalaemia and achlorhydria) syndrome. VIPomas cause a large amount of stool (>700 mL/day), hypercalcaemia is frequent, and marked metabolic acidosis with bicarbonate and potassium wasting is characteristic of the VIPoma syndrome (40). As noted above, other endocrine causes of diarrhea include CS, MTC, C-cell hyperplasia syndrome, ZES or gastrinoma (38). Diarrhoea in NETs is always secretory, while diarrhoea from other gastrointestinal causes is usually malabsorptive (38). Thus, the key question to ask is whether the diarrhoea persists with fasting, thus clarifying its secretory nature. Importantly, diarrhoea may occur at night (38). Secretory diarrhoea may also be idiopathic or due to a secreting villous adenoma of the rectum or surreptitious laxative abuse (38).

Although irritable bowel syndrome also causes abdominal pain and discomfort, it typically does not disturb sleep, and bleeding, fever, weight loss and persistent severe pain are not features of IBS. It should also be noted that the use of somatostatin analogues changes the character of the diarrhoea from secretory to malabsorptive (38). Although irritable bowel syndrome also causes abdominal pain and discomfort, it typically does not disturb sleep, and bleeding, fever, weight loss and persistent severe pain are not features of IBS. It should also be noted that the use of somatostatin analogues changes the character of the diarrhoea from secretory to malabsorptive (38).

**Gastric NETs**

There are three types of gastric NETs: type I gastric NETs represent around 70-80% and are associated with chronic atrophic gastritis (85). Partial or complete atrophy of parietal cells lead to hypo- or achlorhydria. Through a feedback mechanism loss of gastric acid secretion results in gastrin (G) cell hyperplasia and hypergastrinaemia ensues. Gastrin is trophic to enterochromaffin-like (ECL) cells in the stomach, and especially at levels higher than 1000 pg/mL induces the development of small gastric NETs as polyps or tumours. Premature graying of the hair, anti-parietal cell or intrinsic factor antibodies, pernicious anemia and associated autoimmune conditions may be seen. Type II gastric NETs, comprising around 5-10%, develop from ECL cells in response to constitutively high gastrin levels from a gastrinoma, and are associated with ZES (85). Both type I and II gastric NETs are multifocal and primarily arise in the corpus (85). Type III gastric carcinoids, which are sporadic and solitary, represent approximately 10-15% of the gastrointestine. They are usually detected incidentally, and are associated with ZES (85).
lesions and have the highest risk of metastasis (85). They are not associated with hypergastrinaemia but an atypical CS due to histamine release may occur: extended episodes of flushing, headache, shortness of breath and lacrimation may be seen (rarely). The flushing may be deep purple and last for hours. It may be followed by increased blood flow to the limbs and trunk (40).

**Multiple Endocrine Neoplasia Syndrome Type 1**

MEN1 is an autosomal dominant disorder occurring due to mutations in the tumour suppressor gene \textit{MEN1} which encodes a protein called menin. MEN1 is characterised by the occurrence of parathyroid, pancreatic islet (NE) and anterior pituitary tumours. In a patient having any of these tumours, the other two organ systems should be checked by obtaining a careful medical history and additional laboratory workup. The incidence of MEN1 has been reported to be 1–18% in patients with primary hyperparathyroidism, 16–38% in patients with gastrinoma and less than 3% in patients with pituitary tumours (74). The diagnosis of MEN1 may be established by one of three criteria (74): (1) the development of tumours in two or more primary MEN1-associated endocrine organs \{parathyroid adenoma (90–95%), enteropancreatic tumour (30–70%), pituitary adenoma (30–40%)\}, (2) the occurrence of one of the MEN1-associated tumours in a first-degree relative of a patient with a clinical diagnosis of MEN1 (3), or identification of a germline \textit{MEN1} mutation in an individual who may be asymptomatic (74). It should be kept in mind that, although it is a familial disorder, genetic studies have shown that \textit{de novo} \textit{MEN1} mutations comprise approximately 10% of MEN1 patients (88). In other words, it is possible that the identified patient (index case) may not have a family history regarding MEN1-associated tumours. MEN1-associated pNETs present at an earlier age compared to patients without MEN1 (sporadic insulinomas generally present between 40 and 45 years while the mean age of gastrinomas is 48–55 years) (58), are multiple, and their behaviour is uncertain (74). In terms of enteropancreatic tumour subgroup rates, gastrinoma (40%), insulinoma (10%), nonfunctioning and PPoma (20–55%), glucagonoma (<1%) and VIPoma (<1%) may be seen. Some MEN1 patients may also develop adrenocortical tumours (40%), gastric NETs (10%), bronchopulmonary NETs (2%), thymic NETs (2%), meningiomas (8%), angiofibromas (85%), collagenomas (70%), lipomas (30%), and very rarely phaeochromocytomas (<1%) (74). However, these rates and differences in gender predisposition seem to change according to ethnicity. In association with MEN1, bronchial carcinoids occur predominantly in women, while thymic carcinoids occur predominantly in men (at least in Europe) (74,89). In a recent case series from China, the prevalence of thymic NETs was 7.4% in patients with MEN1, and 55% were in women (90). Compared to sporadic tumours of the same organ, MEN1-associated tumours may be larger, more aggressive and resistant to treatment. As parathyroid tumours are the most common feature of MEN1, patients presenting with primary hyperparathyroidism before the age of 30 years, or multigland hyperparathyroidism, should raise suspicion in terms of genetic predisposition. On the other hand, gastrinomas generally occur in patients who are older than 30 years and many insulinomas occur in patients younger than 20 years (74). Genetic screening is recommended for the index case who meet the clinical criteria of MEN1, who are suspicious for MEN1 (multiple parathyroid adenomas before the age of 40 years, recurrent hyperparathyroidism, gastrinoma or multiple pancreatic NETs at any age) or atypical for MEN1 (development of two nonclassical MEN1-associated tumours, e.g. parathyroid and adrenal tumour) and first-degree relatives of a MEN1 patient even if they are asymptomatic (74). Untreated, patients with MEN1-related endocrine tumours are associated with an earlier mortality. Thus, patients who are found to have MEN1 germline mutations should be screened at least annually for the development of MEN1-associated tumours. An algorithm for tumour screening in patients with known mutation can be found in detail in the guidelines by Thakker et al. which were published in 2012 (74).

**How to Choose Relevant Laboratory Tests?**

Most NE cells have secretory dense core granules. In the diagnosis of NETs both the stored secretory products (bioactive peptides or amines) and the capsular proteins (e.g. CgA) of these granules can be used as diagnostic markers. 5-hydroxy-indole acetic acid (5-HIAA) is the metabolite of serotonin which is formed by monoamine oxidases in the liver, lungs and brain (Figure 3). The biomarkers and/or hormones to be measured should be chosen according to the patient’s presentation, symp-
toms, physical examination and preliminary diagnosis, as noted (Table 1). Currently-used biomarkers are CgA, urinary/plasma 5-HIAA, pancreastatin, PP, NSE, serotonin and neurokinin A (91) (see section below). However, biomarkers are insufficient to identify the primary tumour site so tissue confirmation is necessary for the diagnosis (40,91). For some of the hormones, a cause and effect relationship is clear and the hormone may be used both in diagnosis and follow-up. In others, biomarkers such as CgA need to be identified and used in conjunction with symptoms and imaging during follow-up. It should be noted that CgA is useful for follow-up but is relatively insensitive for diagnosis.

To adopt a problem-oriented approach may be easier if the patient is symptomatic. In terms of endocrinology referrals, the patient with a possible NET may be seen in endocrinology outpatient clinics due to two main symptoms, hypoglycaemia and flushing. Secondly, the patient may be referred on from a gastroenterology clinic due to a gastric NET or a pancreatic mass.

**Hypoglycaemia**

Obtaining a careful history will generally rule out insulin-related fasting hypoglycaemia. In a well patient with documented hypoglycaemia, inappropriate levels of insulin, C-peptide and pro-insulin will suggest either an insulinoma or factitious hypoglycaemia due to drug ingestion. Suppressed levels of insulin, C-peptide and pro-insulin during hypoglycaemia may indicate the presence of an IGF-II-secreting tumour. Post-oesophagectomy or post-bariatric surgery hypoglycaemia should be obvious from the history (40). Whipple’s triad of hypoglycaemia comprises symptoms of hypoglycaemia, a documented plasma glucose level ≤40 mg/dL (2.2mmol/L) and relief of symptoms with administration of glucose. Recently published ENETS Consensus Guidelines recommend concomitant measurements of blood glucose ≤40 mg/dL and an insulin level >6 μU/L (or ≥3 μU/L by ICMA) (if available a β-hydroxybutyrate level ≤2.7 mmol/L will confirm inappropriate insulin release on fasting) during a hypoglycaemic episode to reveal a diagnosis of insulinoma without additional tests (23). According to the 2009 Endocrine Society Guidelines plasma glucose concentrations of glucose less than 55 mg/dL, insulin of at least 3 μU/mL, C-peptide of at least 0.6 ng/mL and proinsulin of at least 5 pmol/L documents endogenous hyperinsulinism (79). However, we consider this glucose threshold is too high and currently prefer the 40 mg/dL. Differential diagnosis includes other possible causes such as counter- regulatory hormone deficiency, autoimmune (insulin antibodies), drug-induced and factitious hypoglycaemia (38).

**Flushing**

It is also vital in a patient with flushing to obtain a good history, as mentioned in the clinical presentation section (Table 3). The laboratory work-up of unexplained flushing should basically include serum fasting CgA and 24-hour urinary 5-HIAA. If CS is excluded, 24-hour urinary metanephrine and normetanephrine, serum calcitonin, and serum total tryptase levels for systemic mastocytosis, should be measured. In general, total tryptase levels are greater than 20 ng/mL in systemic mastocytosis and this is a minor criterion in WHO diagnostic criteria for systemic mastocytosis (50). However, in cutaneous mastocytosis, monoclonal mast cell activation syndrome and systemic mastocytosis limited to bone marrow, tryptase values may be lower (45).

**Patient referred from gastroenterology clinic**

In gastroenterology clinics patients may be seen with symptoms such as dyspepsia, epigastric pain and/or diarrhoea. Furthermore, a gastric NET diagnosed in upper gastrointestinal endoscopy or a pancreatic mass detected on abdominal imaging may be the reason necessitating further laboratory work-up. In these instances the physician needs to choose necessary laboratory tests according to patient’s history and co-existing symptoms. In gastric NETs, measurement of 5-HIAA levels is not recommended as CS is not common. Increased plasma CgA levels are seen in all gastric NETs, and may be used for follow-up and also have prognostic value in patients with metastatic disease. Fasting gastrin levels are elevated in both type I and type II gastric NETs, but there is hypo- or achlorhydria in type I and high acidity in type II gastric NETs. Thus, in a patient having clinical signs and symptoms of ZES, measurement of gastrin and gastric pH levels are necessary (92). As chronic PPI use also leads to high gastrin levels, PPIs must be withheld at least three weeks prior to measuring FSG and gastric pH level as a washout period, if this can be done safely (92). If the gastric pH is above 2 or, regardless of gastric pH, if the fasting gastrin level is normal, than ZES is ef-
fectively excluded. In subjects with low gastric pH and gastrin levels >1000 pg/mL then a gastrinoma is in most situations confirmed in the absence of medication (23), [some authors use gastric pH levels ≤2 with accompanying fasting gastrin levels >10x ULN (upper limit of normal) as a criteria] (92). However, in a recent prospective analysis up to two-thirds of gastrinoma patients were found to have gastrin values below 10-fold normal (93). If fasting gastrin levels are between 1-9.9xULN in a subject with gastric pH ≤2, than the gold standard approach is to perform a secretin test (23,92). Gastrinomas ectopically express secretin receptors and intravenous administration of secretin characteristically causes an exaggerated release of gastrin. Recent ENETS Consensus Guidelines recommend the secretin test for FSG levels between 200-1000 pg/mL, and explains the method of secretin test in detail (23). Gastrin levels also may be elevated in other conditions with hyperchlorhydria such as H. pylori infection, gastric outlet obstruction, renal failure, antral G cell syndromes, short bowel syndrome, and retained gastric antrum (23).

A pancreatic mass work-up needs to be done according to additional disease states and symptomatology (Table 1). For all masses CgA should be measured; with accompanying diarrhoea, duodenal ulcers or ZES, additionally fasting gastrin values, in diabetic patients with accompanying rash plasma glucagon levels, and in patients with severe watery diarrhoea plasma VIP levels (in VIPomas >200 pg/mL), may be added to CgA (25). The diagnosis of a glucagonoma requires demonstration of increased fasting plasma glucagon levels (generally 500-1000 pg/mL) accompanied by a typical clinical presentation (38). Glucagon values may be elevated in other conditions such as diabetes mellitus, burn injury, acute trauma, bacteraemia, cirrhosis, renal failure or Cushing’s syndrome, but it is generally below 500 pg/mL (40). Although rare, it should be remembered that serotonin-secreting pancreatic NETs may also present with CS, and urinary 5-HIAA measurement in pancreatic masses may be helpful in this small group of subjects. In a recent study evaluating the incidence and prognostic value of serotonin secretion in 255 patients with pNETs, 0.8% were diagnosed with CS and 7.8% had a serotonin-secreting pNET without symptoms (63). In the literature somatostatinomas are usually diagnosed incidentally and the events leading to the diagnosis usually occurred in reverse order (38). However, in a patient with a pancreatic or duodenal mass, a combination of diabetes, gallbladder disorder and unexplained steatorrhoea may be a sign of the tumour and plasma somatostatin levels may be measured.

For foregut or hindgut NETs and pNETs that do not secrete serotonin, 5-HIAA is not as useful as a marker compared to CgA in terms of diagnosis, evaluating possible progression and treatment response (38). In patients with foregut NETs the urine contains relatively little amounts of 5-HIAA but large amounts of 5-HTP. It is presumed that these tumours are deficient in dopa-decarboxylase which impairs the conversion of 5-HTP into 5-HT. CgA is positive 80-100% in foregut, midgut and hindgut tumours whereas 5-HIAA detects only 30% of foregut and around 70% of midgut tumours, but fails to recognise the presence of a hindgut tumour.

Biochemical Markers

Thus, the two critical biomarkers are CgA and 5-HIAA. As all laboratory measurements, NET-related biomarkers have their own limitations. CgA, the most important biomarker, is secreted from NETs including foregut, midgut, hindgut gastrointestinal NETs, phaeochromocytomas, neuroblastomas, MTC, some pituitary tumours, functioning and non-functioning pNETs and other NETs (23). However, the sensitivity of CgA is moderate while its specificity is dependent on primary site, grade and status of disease (91,94). CgA is almost universally elevated in patients with gastrinomas, and is often high in midgut NETs and non-functioning pNETs (23). CgA values do not correlate with symptoms but may correlate with tumour type and burden; thus, small tumours may present with normal values while significantly higher levels are found in NET patients with liver metastases, with the highest levels in patients with functioning ileal NET and CS (95,96). There are several available immunoassays for CgA, but with a high level of variability between CgA kits and international standardisation is lacking (91). Individual CgA immunoassays tend to be poorly correlated with each other, making inter-assay assessments difficult (97). In a prospective analysis CgA was underlined as a practical marker in patients with NETs with limited diagnostic power (98). Using ROC curves, a cut-off of 53 ng/mL for IRMA and 16 U/L for ELISA for discriminating between
healthy controls and NET patients yielded moderate sensitivities (71.3% and 83%, respectively) and specificities (71% and 85%, respectively) (98). False-positive CgA due to heterophile antibodies (HAb) which can bind to animal antigens has also been reported, and may be present in up to 40% of the normal population (98). In the CgA immunometric assays HAb interference may be obviated by using HAb-blocking tube (100). CgA needs to be measured under fasting conditions in the morning in plasma or serum and ideally in the same laboratory with the same assay. As CgA is expressed in healthy tissue as well, several neoplastic and non-neoplastic factors may cause elevated CgA levels, but very high levels are rarely found outside the setting of NETs with the exception of gastric acid secretory therapy or those with hypergastrinaemia (Table 4) (4,23,91). PPI therapy may increase CgA concentration just five days after its first intake (45). If the patient is on PPI treatment, measurement needs to be made at least two weeks after stopping the drug and possibly three weeks is the safest (leaving a clearance of at least 3 half-lives, e.g. the half-life of lansoprazole is 12.9 hs and pantoprazole is 45.9 hs) (23,45,92). However, in a patient with a high likelihood of gastrinoma such omission of therapy may be life-threatening and H2-receptor antagonist treatment may be commenced. In this situation measurement can be made after these periods but discontinuing H2-receptor treatment for at least 24 hours (45). It should be kept in mind that somatostatin analogues decrease CgA levels significantly, and thus an increase under treatment may signal loss of control. In patients under somatostatin analogue treatment serial CgA measurements should be made at the the same interval from injection of the drug. In conclusion, CgA may be helpful when there is a known NET and may be a good marker of response to therapy, but it does not have prognostic value, has poor assay reproducibility, moderate sensitivity, and several factors other than NETs may elevate CgA (23,40,101).

As serum serotonin levels changes during the day depending on activity and stress, urinary 5-HIAA as a serotonin degradation product is a useful marker if the tumour is secreting serotonin. It is measured in 24-hour urine specimens collected in hydrochloric acid and usually measured via liquid chromatography tandem mass spectrometry (LC-MS/MS) or high performance liquid chromatography (HPLC) (23). Although not widely available, plasma values have been proven as accurate as urine analysis with suitable care, but they are not routinely available (25,102). In the presence of CS the specificity of 5-HIAA is 90% and sensitivity 70% (23). Urinary 5-HIAA values may found to be normal in non-metastatic tumours, in patients with CS who possibly secrete other biologically-active molecules, and rarely in some CS patients without diarrhoea, while a small number of normal individuals may have elevated urinary 5-HIAA (probably due to diet) (23). It may be used both for diagnosis and follow-up but it has weak cor-

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**Table 4. Factors increasing CgA levels in the absence of GEP-NET (adapted from Verbeek WHM et al. EJE 2017;174:R1-R7) (23).**

<table>
<thead>
<tr>
<th>Organ System or Condition</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal system</td>
<td>Renal insufficiency (CKD 2-3)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Acute coronary syndrome, cardiac insufficiency, giant-cell arteritis, essential hypertension, untreated hypertension and pregnancy</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Chronic atrophic gastritis, pancreatitis, inflammatory bowel disease, irritable bowel syndrome, liver cirrhosis, chronic hepatitis, colon cancer, pancreatic adenocarcinoma, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Pheochromocytoma, hyperparathyroidism, pituitary tumors, medullary thyroid carcinoma, hyperthyroidism, Cushing’s syndrome</td>
</tr>
<tr>
<td>Inflammatory disorders</td>
<td>Rheumatoid arthritis, chronic bronchitis, systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>Non-gastrointestinal cancers</td>
<td>Small-cell lung, prostate, breast, ovary, testis, neuroblastoma</td>
</tr>
<tr>
<td>Drugs</td>
<td>Steroid treatment, proton-pump inhibitors, H2-receptor antagonists</td>
</tr>
<tr>
<td>Other</td>
<td>Parkinson disease, food intake, exercise shortly before measurement</td>
</tr>
</tbody>
</table>
Correct determination of 5-HIAA in urine requires avoidance of certain foods and drugs 72 hours before and during the day of urine sampling (Table 5) (23).

Table 5. The foods and medications that should be avoided 72 hs prior to the test and conditions that affect 5-HIAA collection result are listed below (recommendations differ and the list below may not be definitive) (23,25,38,40,45,224,225). To avoid degradation, preservatives should be used (generally hydrochloric acid) to keep the pH around 3 (224). Although many laboratories recommend keeping urine sample at 4°C in the refrigerator, there is no published data reporting degradation of 5-HIAA in acidified urine at room temperature (224). Drugs and food avoidance has been recommended for two days (224) or five days (45) before urine collection by different authors. Reference values change between laboratories but is approximately 2 to 8 mg/day (23,25,38,40,45,224,225).

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Conditions that decrease 5-HIAA- Renal impairment, being on haemodialysis
Conditions that increase 5-HIAA- Untreated patients with malabsorption (celiac disease, tropical sprue, Whipple disease, intestinal stasis, cystic fibrosis)
they are less reliable than CgA (91). Some authors recommend complementary measurement of CgB with CgA as it does not have false positivity problem (40).

As mentioned above, NT-pro-BNP seems to be an excellent biomarker for carcinoid heart disease (42).

More recent developments have focused on the use of novel technologies to quantify circulating tumor cells. Among these multianalyte technologies based on NE tumour genomics, especially the NETest (a multianalyte qRT-PCR assay based on fifty-one marker genes with algorithmic analysis) has a high sensitivity (>95%) and specificity (>95%) in the detection of GEP-NETs (94). However, further study is required, especially in view of its likely expense, although it does seem to offer a novel and exciting approach to assessing tumour burden.

**Imaging**

Imaging is used in the diagnosis, staging and monitoring treatment in NETs. In patients with hormonal hypersecretion syndromes e.g. CS, positive laboratory tests are not enough to establish the diagnosis and imaging is required to locate the potential tumour (107). On the other hand, when a tumour is identified incidentally by imaging for other purposes, the characteristics of the tumour may give helpful clues to the potential diagnosis (107).

Ultrasonography (US) frequently provides the initial diagnosis of liver metastases and contrast-enhanced US is excellent in characterisation of liver lesions that remain equivocal on CT/MRI (108). US is useful for guiding the biopsy in histopathological NET diagnosis.

Imaging modalities may be divided as anatomic, which includes computed tomography (CT, including CT enterography) and magnetic resonance imaging (MRI), and functional which includes In-111-octreotide scanning and Ga-68 somatostatin analogue PET (107). In terms of anatomic imaging, CT is better for imaging of lung NETs, while MRI is better for the assessment imaging of hepatic NET metastases. For biopsy of thoracic NET lesions CT-guided biopsy is used. CT and MRI enterography are both satisfactory for the diagnosis of small bowel NETs and may detect mucosal NETs as small as 0.5 cm (107). In functional imaging, the In-111-octreotide scan – Octreoscan- has been in clinical use for about thirty years and was the gold standard for NET functional imaging until recently (Figure 5). In-111-labeled octreotide binds to any tissue expressing somatostatin receptor subtype 2 (sst-2) and somewhat to 5 (sst-5). Although the octreotide scan can detect large NETs it can not reliably detect NETs less than 1 cm. PET scans using a positron-emitting radioisotope-labelled somatostatin analogue (SA) have been developed to overcome the limits of the octreotide scan. Additionally, PET is usually combined with CT to provide better signal localisation. Currently, two Ga-68 labelled tracers, DOTATATE (tetraazacyclododecane tetraacetic acid-octreotate) and DOTATOC (DOTA0-D-Phe1-Tyr3-octreotide) are in use. Both are used with approximately equal accuracy, while DOTATATE has around 10-fold higher affinity to sst2. Ga-68 SA PET is certainly superior to octreotide scanning in localising NETs, and should be used whenever available (107). As most NETs do not exhibit high standardised uptake values they are negative on FDG-PET so it is not routinely used for NET imaging. However, positive uptake on an FDG-PET signals a high
proliferative potential and can be useful in choosing the optimal therapeutic strategy, and some would advise complementary FDG- and Ga-68-DOTATATE scanning in most patients, especially those with a high Ki-67 index or more which are rapidly progressive.

In the work-up of GI tract NETs, endoscopy and endoscopic ultrasound (EUS) imaging can directly examine mucosal and mural lesions and can biopsy lesions in the upper (oesophagus, stomach and duodenum) and lower (colon and rectum) GI tract; capsule endoscopy and double balloon enteroscopy are used for the same purpose in the jejunum and ileum (107). Endoscopic EUS is the most sensitive method to diagnose pancreatic NETs and is also suitable for cytology or biopsy (108). Recently, ENETS has released a useful guideline for the imaging of NETs (108).

**Conclusions**

The huge interpatient heterogeneity of NETs and their rarity render their understanding by the clinician complex unless one sees such patients on a regular basis. Endocrinologists need to be alert to the complaints of the patients which may occur over a wide spectrum. In patients with a clinical suspicion but with negative laboratory tests, follow-up may be more appropriate than immediately ruling out a NET, as the biological behaviour of the tumour may be unpredictable and diagnosis may become possible in the long term. While NETs may be seen in several outpatient clinics, a multidisciplinary network may not be available in every centre. In this situation, endocrinologists should still have contact with cardiologists for patients with right-sided cardiac disease, gastroenterologists need to consider NETs in patients presenting with diarrhoea and ‘IBS’, and dermatologists should consider NETs in patients presenting with sweating, flushing and unexplained rashes. All biomarkers may not be available in every laboratory, but a good history, physical examination, basic biomarkers and imaging studies may help the clinician to avoid missing the diagnosis and consequent delay in initiating adequate and effective therapy. It is ideal that when a NET is confirmed, they should be seen at a multidisciplinary centre where all the required diagnostic and therapeutic techniques are available.

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**Authorship Contributions**


**References**


