Chapter 4

Plasma levels of free metanephrines and 3-methoxytyramine indicate a higher number of biochemically active HNPGL than 24 h urinary excretion rates of catecholamines and metabolites.

N. van Duinen[1], E.P.M. Corssmit[1], W.H.A de Jong[2], D. Brookman[1], I.P. Kema[2], J.A. Romijn[1]

[1]Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, the Netherlands

[2]Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, the Netherlands

Submitted for publication

Abstract

Context: A substantial number of patients with head and neck paragangliomas (HNPGL) have biochemically active tumors, evidenced by increased urinary excretion of catecholamines and metabolites, including 3-methoxytyramine (3MT). It is unclear whether plasma levels of these parameters are more sensitive to detect biochemical activity in HNPGL patients than urinary excretion rates.

Objective: To compare plasma free levels versus urinary excretion rates of deconjugated 3MT and combined metanephrines in patients with HNPGL.

Patients and Methods: We included 124 consecutive patients with HNPGL for screening for catecholamine excess by measurement of 24-hr urinary excretion rates of deconjugated (nor)metanephrine, (nor)epinephrine, dopamine, vanillylmandelic acid, 3MT and plasma free levels of (nor)metanephrine and 3MT.

Results: Plasma free 3MT levels were increased in 35 of the 124 patients (28%), whereas 24-h urinary excretion of deconjugated 3MT was increased in 30 patients (24%) (p=0.13). Plasma free metanephrine (MN) levels were increased in 7 patients (6%), urinary deconjugated metanephrine levels in 6 (5%) (p=1.00). Plasma free normetanephrine (NMN) levels were increased in 7 patients (6%), and 5 patients had increased urinary excretion of deconjugated normetanephrine (4%) (p=0.69). Plasma free combined metanephrine levels (NMN, MN, 3MT) were increased in 41 patients (33%), whereas 24-h urinary excretion rates of deconjugated combined metanephrines was increased in 33 patients (27%, $p<0.05$).

Conclusions: The combined levels of free metanephrines and free 3MT in plasma indicate a higher number of biochemically active HNPGL than the 24 h urinary excretion rates of these markers**.**

Introduction

Head and neck paragangliomas (HNPGL) are rare neuroendocrine tumors derived from parasympathic ganglia (1). Some patients with HNPGL have biochemically active HNPGL, evidenced by increased urinary excretion rates of catecholamines and their metabolites (2-4). The majority of those patients have increased urinary excretion rates of 3-methoxytyramine (3MT), the 3-O-methylated metabolite of dopamine (3).
It is presently unknown whether plasma free concentrations of 3-O-methylated metabolites of catecholamines, including 3MT, are more sensitive parameters of biochemical activity of HNPGL than urinary excretion rates of catecholamines or deconjugated 3-O-methylated metabolites. For the diagnosis of pheochromocytoma, the measurement of plasma free metanephrine concentrations is the optimal biochemical test with the highest sensitivity and specificity (5-8). Therefore, the aim of the present study was to assess whether plasma levels of free metanephrines and 3MT are more sensitive parameters of biochemical activity of HNPGL than urinary excretion rates of free catecholamines and their deconjugated metabolites.

Patients and Methods

We performed a cross sectional study of 130 consecutive patients with HNPGL who were followed at the outpatient clinic of the Leiden University Medical Center, a tertiary referral center for patients with paragangliomas. For this purpose clinical, biochemical and radiological data of all consecutive patients with HNPGL were evaluated. All patients were investigated at the outpatient clinic according to structured standard clinical protocols. These included questions focused at tumor and catecholamine related signs and symptoms, measurement of blood-pressure in the supine position, and after 5 minutes of upright position, in order to screen for orthostatic hypotension. In all patients head-and-neck MRI were performed or had been performed within the previous 2 years.
Urine was collected during 24 hours in duplicate under strict dietary regulations (patients abstained from pineapple, avocado, bananas, kiwi, nuts, plums, coffee, tea and other caffeine containing beverages) and after withdrawal of medication for at least one week or after changing antihypertensive medication to doxazosine for several weeks. In order to ascertain adequacy of urinary collection, 24-hour urinary creatinine excretion rates were measured as well. Blood samples were drawn after the second day of urine collection in the postabsorptive state. Blood samples were drawn from an intravenous catheter inserted into a forearm vein after 30 minutes of rest in the supine position and collected in cold, glutathione containing vacutainers. All blood samples were centrifuged immediately at 3000 rpm for 10 min at 4 ºC. Plasma samples were stored at -80 ºC until analyses.
Biochemical screening included the measurement of urinary excretion rates of deconjugated (nor)metanephrine, free (nor)epinephrine, dopamine and deconjugated 3MT excretion in two 24-hour urinary samples, and the measurement of plasma free (nor)metanephrines and 3MT concentrations.

In case of excessive catecholamine or metabolite excretion (*i.e.*, any value above the upper reference limit in two urine samples or in the plasma sample), radiological assessment by MIBG-scans and MRI and/or CT scans of thorax and abdomen were performed to identify the source of excessive catecholamine production. All patients with documented paragangliomas in the abdomen or thorax were excluded in the present study, because the interpretation of the biochemical results in relation to HNPGL could be confounded by the presence of these other paragangliomas.

We performed screening for succinate dehydrogenase (SDH) mutations in those HNPGL patients, who agreed upon genetic testing. Hereditary disease was diagnosed if mutations in the SDHB, SDHC, or SDHD genes were documented in the HNPGL patient and/or a family member.

We screened 130 consecutive HNPGL patients for catecholamine excess. Four patients with catecholamine excess were excluded from the current analyses, because no additional radiological assessment was performed to exclude the presence of other paragangliomas not related to the head and neck regions. Two patients were excluded because they were diagnosed with a pheochromocytoma. Therefore, the study group comprised 124 patients with HNPGL.

The study was an evaluation of routine patient care. According to the requirements of Dutch law, it was not necessary to obtain permission from the institutional ethical commission. Prior to germ line mutation testing, informed consent was obtained from each patient.

Assays

Free Epinephrine, norepinephrine and dopamine excretion rates in 24 h urine collections were quantified by reversed phase high pressure liquid chromatography (HPLC) with electrochemical detection. Inter- and intra-assay coefficients of variations CVs for epinephrine were 4.3-9.0% ranging from low to high concentrations. For norepinephrine these data were 2.7-3.6% and for dopamine 3.1-4.8%. Vanillylmandelic acid (VMA) excretion in urine was measured using HPLC with fluorometric detection with inter- and intra-assay CVs of 2.4-9.1%. Urinary deconjugated (nor)metanephrine and 3MT were determined by isotope dilution gas chromatography with mass spectrometric detection. The CVs of the 3-O-methylated catecholamine metabolites ((nor)metanephrine and 3MT) ranged from 1.7-4.2% (9). Plasma free metanephrines were determined by automated in-line solid phase extraction and isotope dilution liquid chromatography with mass spectrometric detection (11). Reference ranges were obtained in healthy volunteers. These values were for urinary excretion: free norepinephrine 0.06-0.47 µmol/24h, epinephrine <0.16 µmol/24h, and dopamine 0.46-3.40 µmol/24h, VMA <30 µmol/24h (deconjugated), metanephrine (deconjugated) 33-99 µmol/mol creatinine, normetanephrine (deconjugated) 64-260 µmol/mol creatinine and 3MT (deconjugated) 45-197 µmol/mol creatinine (10). The reference intervals for plasma free metanephrines were determined using blood samples collected in the supine position from 115 volunteers (57 males, 58 females; age range, 36-81 years; median age, 55 years) (11) The reference ranges for plasma free metanephrines

were: metanephrine 0.07- 0.33 nmol/L, normetanephrine 0.23-1.07 nmol/L and 3MT <0.17 nmol/L. SDH mutation analysis was performed by restriction digestion as described by Taschner *et al* (12;13).

Data analysis

SPSS for windows version 16.0 (SPSS inc., Chicago, IL) was used for data analysis. Results are expressed as means ± standard error (SE), unless specified otherwise. Test sensitivity was calculated from the patients with a positive test result divided by the total number of patients tested. Test sensitivities were compared using the McNemar test. The average value of catecholamine and metabolite excretion rates of two urine samples was used for calculation of p values. A p-value <0.05 was considered to represent a significant difference.

Results

Clinical characteristics (Table 1)
The study group comprised 124 patients with HNPGL. Mean age of the patients was 49 years (range 13-77 years). Fifty-five patients (44%) had a single head and neck paraganglioma, whereas 69 patients (56%) had multiple head and neck paragangliomas. Paragangliomas occurred most frequently in the glomus caroticum (70%). Fifty-one patients (41%) had a glomus vagale tumor, 21 (17%) a glomus jugulotympanicum tumor, 13 (11%) a glomus jugulare tumor and 12 (10%) a glomus tympanicum tumor.
Genetic testing for SDHx mutations was performed in 111 of the 124 patients with HNPGL (90%). In 95 patients mutations were found (86 SDHD, 8 SDHB and 1 SDHC), whereas in 16 patients no mutation was found.

Biochemical characteristics (Table 2)
Forty-six patients (37%) had biochemically active HNPGL, evidenced by increased plasma concentrations of free (nor)metanephrine, 3MT and/or increased 24 hour urinary concentrations of free catecholamines, and deconjugated metabolites, including 3MT. There was no difference between the number of subjects with increased plasma free 3MT concentrations compared to the number of subjects with increased urinary excretion rates of deconjugated 3MT (n=35 vs. n=30, 28% vs. 24%, p=0.13). Urinary deconjugated metanephrine excretion rates were increased in 8% (10 of 124 patients), which in combination with the patients with increased urinary deconjugated 3MT concentration, increased the number of patients with biochemically active HNPGL to 27% (33 of 124 patients). Eleven patients (9%) had increased urinary excretion rates of VMA. Increased urinary excretion rates of free catecholamines (adrenaline, noradrenaline, or dopamine) were present in 10% of the patients (12 of 124 patients). Increased urinary excretion rates of combined metanephrines in combination with catecholamines were present in 28% of the patients (35 of 124 patients).

Plasma free (nor)metanephrine concentrations were increased in 11% of the patients (14 of 124 patients). The addition of plasma free 3MT levels to these measurements increased the portion of patients with evidence of biochemically active HNPGL to 33% (41 of 124 patients). The number of HNPGL patients with increased plasma free metanephrine levels was not significantly different from the number of patients with increased 24h urinary excretion rates of deconjugated metanephrines (11% vs. 8%, p=0.34). Increased plasma free metanephrine (MN, NMN) concentrations in combination with increased plasma free 3MT levels were present in significantly more HNPGL subjects than increased urinary excretion rates of combined metanephrines (MN, NMN) including 3MT (33% vs. 27%, p<0.05).
Nine patients tested negative with urinary screening of catecholamine excretion rates but positive with screening of plasma metanephrine levels. Of these patients 6 had increased plasma free 3MT levels, 2 had increased plasma free metanephrine levels and 1 had increased plasma free normetanephrine levels.

Catecholamine and metabolite excretion and tumor load
We identified 55 patients with only a single HNPGL with a mean tumor diameter of 3.3 cm (range 0.3-9.7 cm). In those patients there was a weak positive correlation between tumor diameters and plasma concentrations of free 3MT (r=0.30, p=0.04), and the combined plasma concentrations of free metanephrine, normetanephrine and 3MT (r=0.35, p=0.02). There were also weak correlations between tumor diameters and mean urinary excretion rates of deconjugated normetanephrine (r=0.40, p<0.01), VMA (r=0.32, p=0.03), dopamine (r=0.40, p<0.01), the combined excretion rates of deconjugated metanephrine and normetanephrine (r=0.40, p<0.01), the combined excretion rates of deconjugated (nor)metanephrine and 3MT (r=0.43, p<0.01) and the mean excretion rate of catecholamines (r=0.37, p=0.01). There was no correlation between the number of HNPGL and plasma free metanephrine levels, mean urinary excretion rates of deconjugated metanephrines and catecholamines.

Catecholamine and metabolite excretion, age, BMI and gender
Stepwise linear regression analysis was performed in a model including age, gender and BMI as independent variables and plasma free metanephrine, normetanephrine and 3MT levels as dependant variables. We identified age (β=0.35, p<0.001), BMI (β=-0.29, p<0.001) and gender (β=0.35, p<0.001) as independent predictors of plasma free metanephrine levels. The patients with increased plasma free metanephrine levels were significantly older compared to patients with normal levels (48.0±1.2 vs. 61.5±3.7 yrs, p<0.01). Men had significantly higher plasma free metanephrine levels compared to women (0.21±0.0 vs. 0.15±0.0, p<0.001).
For plasma levels of free normetanephrine there was no relation with age (β=0.09, p=0.35), BMI (β=0.11, p=0.26) and gender (β=0.09, p=0.35). There was a relation between age (β =0.09, p=0.38), BMI (β =0.02, p=0.41) or gender (β =0.08, p=0.41) with plasma free 3MT levels.

Table 1: Clinical characteristics of 124 patients with head and neck paragangliomas (HNPGL).

	All patients with HNPGL N= 124	Hereditary HNPGL N= 95	Sporadic HNPGL N= 16
Patient characteristics			
Age (yr)	49 ± 1	47 ± 1	58 ± 3
Gender N (%)			
Men	64 (52%)	51 (54%)	4 (25%)
Women	60 (48%)	44 (46%)	12 (75%)
Type of glomus tumor N (%)			
Caroticum	87 (70%)	73 (77%)	4 (25%)
Vagale	51 (41%)	42 (44%)	6 (38%)
Jugulare	13 (11%)	11 (12%)	1 (6%)
Jugulotympanicum	21 (17%)	13 (14%)	5 (31%)
Tympanicum	12 (10%)	6 (6%)	5 (31%)
No. of HNPGL N (%)			
1	55 (44%)	37 (39%)	12(75%)
>1	69 (56%)	58 (61%)	4 (25%)
No. of patients with genetic analysis			
SDHD	86 (69%)		
SDHB	8 (7%)		
SDHC	1 (1%)		
Sporadic	16 (13%)		

Data are shown as mean ± SEM, unless mentioned otherwise.

Discussion

The results of this study show that one third of HNPGL patients have biochemically active HNPGL. The combined plasma concentrations of free metanephrines and 3MT indicate a higher proportion of patients with biochemically active HNPGL than 24 h urinary excretion rates of deconjugated metanephrines and 3MT. In addition, the current data indicate that in HNPGL patients, plasma free 3MT concentrations and urinary excretion rates of deconjugated 3MT do not indentify significantly different numbers of subjects with biochemically active HNPGL.

HNPGL have the ability to produce and secrete catecholamines (2;3). Biochemically active paragangliomas are identified by the measurement of plasma (nor)metanephrine and 3MT

concentrations and 24h urinary excretion rates of free catecholamines and their deconjugated metabolites (6;14). The measurements of urinary excretion rates of catecholamine metabolites (*i.e.*, metanephrine and normetanephrine) and especially plasma levels of catecholamines and their metabolites are recommended for the biochemical screening of pheochromocytoma because of their
high diagnostic sensitivity and specificity (7). However, the majority of patients with biochemically active HNPGL secrete 3MT, which is a metabolite of dopamine. Therefore, the measurement of this metabolite should be included if biochemical activity of HNPGL in general is assessed. Three-methoxytyramine can be measured in urine and plasma by HPLC-tandem mass spectrometric detection (XLC-MS/MS) (11). The present study indicates that the assessment of plasma free 3MT levels does not add to the measurement of urinary excretion rates of deconjugated 3MT.

Table 2: Plasma metanephrine levels and mean urinary excretion rates of catecholamines, VMA and metanephrines in 124 HNPGL patients.

		Mean (range)	N tested positive
Plasma			
Normetanephrine	(nmol/L)	0.6 (0.16-6.64)	7 (6%)
Metanephrine	(nmol/L)	0.18 (0.04-0.48)	7 (6%)
3-methoxytyramine	(nmol/L)	0.46 (0.03-12.8)	35 (28%)
MN + NMN	(nmol/L)	0.78 (0.28-6.78)	14 (11%)
MN + NMN + 3MT	(nmol/L)	1.24 (0.32-13.9)	41 (33%)
Urine			
Normetanephrine	(µmol/mol creatinine)	176 (55-1720)	5 (4%)
Metanephrine	(µmol/mol creatinine)	55 (16-162)	6 (5%)
3-Methoxytyramine	(µmol/mol creatinine)	312 (43-6391)	30 (24%)
MN + NMN	(µmol/mol creatinine)	232 (95.5-1783)	10 (8%)
MN + NMN + 3MT	(µmol/mol creatinine)	544 (154-6598)	33 (27%)*
Adrenaline	(µmol/24 h)	0.02 (0.0-0.11)	0
Noradrenaline	(µmol/24 h)	0.40 (0.11-5.2)	6 (5%)
Dopamine	(µmol/24 h)	1.92 (0.77-6.43)	8 (7%)
Catecholamines	(µmol/24 h)	2.34 (0.91-9.78)	12 (10%)
VMA	(µmol/24 h)	22.5 (0-62)	11 (9%)
Catecholamines + combined MNs			35 (28%)

*indicates a significant difference in test sensitivity between urine and plasma.
VMA= Vanillylmandelic acid; combined MNs: MN + NMN + 3MT.

As patients with HNPGL have the ability to (co)secrete noradrenaline (15-17), this catecholamine and its metabolite normetanephrine should be measured as well. Combining the results of free metanephrines and 3MT in plasma resulted in a slightly, but significantly, higher number of patients with biochemically active HNPGL compared to the combined results of urinary excretion rates.

Consumption of catecholamine rich food products can result in substantial increases in urinary excretion rates of deconjugated normetanephrine and 3MT, and to a lesser extend in plasma free 3MT levels. Therefore, dietary restrictions are indicated prior to collection of blood for measurements of plasma free 3MT levels, and urinary excretion rates of deconjugated normetanephrine and 3MT (18). Although patients in our study collected urine during 48 hour under strict dietary regulations, we can not exclude potential confounding effects of the diet. In contrast, the measurements of plasma free metanephrine concentrations are not influenced by the confounding effects of dietary components, this contributes to the highest sensitivity of plasma levels compared to the measurement of urinary excretion rates of metanephrine (18;19). In accordance, plasma free metanephrines (MN, NMN, 3MT) levels were increased in a higher percentage of HNPGL patients compared to urinary excretion rates of metanephrines. Our findings are in agreement with the observations in patients with pheochromocytomas (6-8). Lenders *et al.* reported a test sensitivity of 97% of plasma free metanephrines versus only 60% of urinary combined metanephrines in patients with hereditary pheochromocytomas and 99% of plasma metanephrine levels versus 88% in urinary excretion rates in patients with sporadic pheochromocytomas.

In conclusion, one third of HNPGL patients have biochemically active HNPGL. The combined assessment of plasma concentrations of free metanephrines and 3MT detect a higher number of biochemically active HNPGL than the measurement of 24 h urinary excretion rates of combined metanephrines and 3MT. In addition, the current data indicate that in HNPGL patients urinary excretion rates of deconjugated 3MT and plasma free 3MT levels do not indentify significantly different numbers of subjects with biochemical active HNPGL.

References

1 DeLellis RA, Lloyd RV, Heitz PU, Eng C. World Health Organisation classification of tumors of endocrine organs. 2004. Oxford, UK: Oxford University Press.

2 Erickson D, Kudva YC, Ebersold MJ, Thompson GB, Grant CS, van Heerden JA, Young WF, Jr. 2001 Benign paragangliomas: clinical presentation and treatment outcomes in 236 patients. J Clin Endocrinol Metab 86:5210-5216

3 van Duinen N, Steenvoorden D, Kema IP, Jansen JC, Vriends AH, Bayley JP, Smit JW, Romijn JA, Corssmit EP 2010 Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. J Clin Endocrinol Metab 95:209-214

4 Zak FG, Lawson W. The paraganglionic chemoreceptor system. [1st ed. Springer-Verlag New York Inc.]. 1982.

5 Eisenhofer G, Lenders JW, Linehan WM, Walther MM, Goldstein DS, Keiser HR 1999 Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. N Engl J Med 340:1872-1879

6 Lenders JW, Keiser HR, Goldstein DS, Willemsen JJ, Friberg P, Jacobs MC, Kloppenborg PW, Thien T, Eisenhofer G 1995 Plasma metanephrines in the diagnosis of pheochromocytoma. Ann Intern Med 123:101-109

7 Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, Keiser HR, Goldstein DS, Eisenhofer G 2002 Biochemical diagnosis of pheochromocytoma: which test is best? JAMA 287:1427-1434

8 Sawka AM, Jaeschke R, Singh RJ, Young WF, Jr. 2003 A comparison of biochemical tests for pheochromocytoma: measurement of fractionated plasma metanephrines compared with the combination of 24-hour urinary metanephrines and catecholamines. J Clin Endocrinol Metab 88:553-558

9 Kema IP, Meiborg G, Nagel GT, Stob GJ, Muskiet FA 1993 Isotope dilution ammonia chemical ionization mass fragmentographic analysis of urinary 3-O-methylated catecholamine metabolites. Rapid sample clean-up by derivatization and extraction of lyophilized samples. J Chromatogr 617:181-189

10 Willemsen JJ, Ross HA, Wolthers BG, Sweep CG, Kema IP 2001 Evaluation of specific high-performance liquid-chromatographic determinations of urinary metanephrine and normetanephrine by comparison with isotope dilution mass spectrometry. Ann Clin Biochem 38:722-730

11 de Jong WH, Graham KS, van der Molen JC, Links TP, Morris MR, Ross HA, de Vries EG, Kema IP 2007 Plasma free metanephrine measurement using automated online solid-phase extraction HPLC tandem mass spectrometry. Clin Chem 53:1684-1693

12 Bayley JP, van M, I, Weiss MM, Jansen JC, Oomen PH, Menko FH, Pasini B, Ferrando B, Wong N, Alpert LC, Williams R, Blair E, Devilee P, Taschner PE 2006 Mutation analysis of SDHB and SDHC: novel germline mutations in sporadic head and neck paraganglioma and familial paraganglioma and/or pheochromocytoma. BMC Med Genet 7:1

13 Taschner PE, Jansen JC, Baysal BE, Bosch A, Rosenberg EH, Brocker-Vriends AH, van Der Mey AG, van Ommen GJ, Cornelisse CJ, Devilee P 2001 Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the SDHD gene. Genes Chromosomes Cancer 31:274-281

14 Eisenhofer G, Goldstein DS, Sullivan P, Csako G, Brouwers FM, Lai EW, Adams KT, Pacak K 2005 Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. J Clin Endocrinol Metab 90:2068-2075

15 Hall FT, Perez-Ordonez B, Mackenzie RG, Gilbert RW 2003 Does Catecholamine Secretion from Head and Neck Paragangliomas Respond to Radiotherapy? Case Report and Literature Review. Skull Base 13:229-234

16 Havekes B, van der Klaauw AA, Weiss MM, Jansen JC, van Der Mey AG, Vriends AH, Bonsing BA, Romijn JA, Corssmit EP 2009 Pheochromocytomas and extra-adrenal paragangliomas detected by screening in patients with SDHD-associated head-and-neck paragangliomas. Endocr Relat Cancer 16:527-536

17 van Houtum WH, Corssmit EP, Douwes Dekker PB, Jansen JC, van Der Mey AG, Brocker-Vriends AH, Taschner PE, Losekoot M, Frolich M, Stokkel MP, Cornelisse CJ, Romijn JA 2005 Increased prevalence

of catecholamine excess and phaeochromocytomas in a well-defined Dutch population with SDHD-linked head and neck paragangliomas. Eur J Endocrinol 152:87-94

18 de Jong WH, Eisenhofer G, Post WJ, Muskiet FA, de Vries EG, Kema IP 2009 Dietary influences on plasma and urinary metanephrines: implications for diagnosis of catecholamine-producing tumors. J Clin Endocrinol Metab 94:2841-2849

19 Eisenhofer G, Coughtrie MW, Goldstein DS 1999 Dopamine sulphate: an enigma resolved. Clin Exp Pharmacol Physiol Suppl 26:S41-S53