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ORIGINAL ARTICLE

Screening for phaeochromocytoma in patients with acute cerebrovascular disease: Is it necessary?

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Abstract

Introduction: Phaeochromocytoma may present with uncontrolled hypertension leading to haemorrhagic stroke (HS), ischaemic stroke (IS) and transient ischaemic attack (TIA). False elevation in the levels of CATS/ METS has been reported in acute cerebrovascular disease. Our aim was to analyse the frequency and pattern of elevations of CATS/METS in patients with acute cerebrovascular disease and to determine associated factors. *Materials and Methods:* This is a retrospective study of 112 samples of CATS/ METS received by the laboratory over a two-year period, from patients with acute cerebrovascular disease. CATS/METS were measured using LC/MS/MS method. Clinical details and CATS/METS level were obtained from the database. Mann-Whitney U test and Kruskal Wallis test were used for statistical analysis. These statistical analyses were performed using SPSS v.20.0 (IBM Corp., Armonk, NY, USA). *Results:* Of the 112 patients, 39% had HS, 54% had IS and 7% had TIA. A total of 29% of patients had elevated CATS/ METS. Elevated levels of CATS/METS were noted in 41% and 25% of HS and IS patients, respectively (p=0.53). Median norepinephrine, epinephrine and metanephrine levels in HS were significantly higher than IS ($p< 0.05$). Systolic blood pressure was higher in those who had elevated CATS/ METS (p=0.04). Only for two patients with elevated CATS/METS repeat testing was performed. Age, diastolic blood pressure and the time of sample collection in relation to the presentation, for CATS/METS were not significantly different between groups that had elevated levels of CATS/ METS versus those who did not. *Conclusion:* We noted that CATS/METS were elevated in one-third of patients, especially in patients with high systolic blood pressure. Increase in CATS/METS should be appropriately followed up with repeat testing. Since false elevation in CATS/METS has been reported in cerebrovascular disease, screening for phaeochromocytoma is best deferred for a month.

Keywords: phaeochromocytoma, acute cerebrovascular disease, catecholamines, metanephrines, screening

INTRODUCTION

Patients with phaeochromocytoma often present with uncontrolled hypertension and its complications including stroke.[1] The diagnosis of phaeochromocytoma is based on the biochemical evidence of elevated catecholamines (CATS) and its metabolites in plasma or urine.[1–4] The diagnosis of phaeochromocytoma is straightforward when the catecholamines/metanephrines (CATS/ METS) levels are elevated more than three times the upper reference limit (3X URL).[4,5] A high diagnostic sensitivity of plasma free or urine fractionated METS indicates that most of the cases of symptomatic phaeochromocytoma are likely to be detected by significant (>3X) elevations in CATS/METS level.[4] Nevertheless, it does not imply that all positive results indicate the presence of phaeochromocytoma. The interpretation of the results for the diagnosis of phaeochromocytoma can be challenging in patients with borderline elevated CATS/ METS, comprising 20 - 25% of patients with phaeochromocytoma.[5-7] In these patients, repeat biochemical testing is required after eliminating factors responsible for false-positive results.[5] A number of conditions may falsely increase the

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levels of CATS/METS, including catecholamine rich diet, stress, medications such as beta-blockers or tricyclic antidepressants and acute cerebrovascular disease.[2]

Phaeochromocytoma is a rare cause of stroke but the prevalence of acute stroke in patients with phaeochromocytoma has not been formally reported.[8] Excessive catecholamines may lead to stroke as a consequence of uncontrolled hypertension.[8] Although it is rare, phaeochromocytoma should be excluded in younger hypertensive patients with stroke. It has been reported that false-positive results for urinary and plasma CATS/ METS is possible in acute stroke when these investigations are carried out within a month of the presentation.[10] The aim of this study was to determine the frequency and pattern of elevations of urinary CATS/METS in patients screened for phaeochromocytoma, presenting with acute haemorrhagic stroke (HS), ischaemic stroke (IS) or transient ischaemic attack (TIA).

MATERIALS AND METHODS

Study Subjects

A total number of 115 patients diagnosed with acute stroke were investigated for phaeochromocytoma between 1st January 2015 and 31st May 2017. Clinical information and biochemical investigations of each subject were extracted from the medical records and laboratory information system, respectively. The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Research Ethics Committee of our institution.

Patients with a known history of phaeochromocytoma, head trauma, seizures or who were on sympathomimetic drugs were excluded. Three patients were excluded from the analyses as they presented with seizures. Of the 112 patients included, 75% were male with the median age of 47 years (range: 32 - 64). The diagnosis of stroke was based on clinical evaluation and confirmed with computed tomography (CT) brain findings on admission. All urinary CATS/METS samples sent within 30 days of presentation of acute stroke were included.

Measurement of urinary catecholamines/ metanephrines

A 24-hour urine collection is required for measurement of urinary fractionated CATS/ METS. The urine samples were collected in containers with an acid preservative containing 20 ml of 6 mol/L hydrochloric acid (HCl). All aliquots of these samples were stored at -20 °C until the biochemical analysis. CATS/METS were measured using liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. For catecholamines, urine was processed directly to isolate only free norepinephrine, epinephrine and dopamine. While for urine metanephrines, HCl was added to release the conjugated forms. Prior to chromatography, sample pre-treatment (solid-phase extraction) was done to eliminate interferences such as lipid and protein. The sample pre-treatment involved the addition of diphenylboronic acid ethanolamines to the urine sample in alkaline condition with optimum pH between 7.5 to 9.5. The chemical forms a stable negatively charged complex with CATS/METS, which will be retained in Bond Elute Plexa Cartridge. Subsequently, the analytes were eluted with formic acid.

The extracts were injected directly onto a pentaflourophenylpropyl (PFP) column and separated isocratically in 0.2% formic acid in water and methanol. Detection was accomplished using an Agilent 6460A triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA), which operates in positive Agilent Jet Stream (AJS) electrospray mode. A borderline increase in CATS/METS results was defined as an elevation of more than the URL but less than 3X URL.

Statistical Analysis

Kolmogorov-Smirnov test was performed to determine the distribution of data. Since the data were not normally distributed, they were presented as median and range. Differences in age, Glasgow coma score, systolic and diastolic blood pressure between the HS, IS and TIA groups were evaluated using Kruskal Wallis test. While the Mann-Whitney U test was used for the comparison between HS and IS groups as well as between patients with elevated and non-elevated CATS/METS level. p-value <0.05 was considered significant. All statistical analysis was performed using SPSS v.20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Of the 112 patients who were investigated for phaeochromocytoma, 39% had HS, 54% had IS and 7% had TIA. In 83% of patients, urinary CATS/METS were requested less than

TABLE 1: Baseline characteristics of the patients with HS, IS and TIA

Variable	HS (n = 44)		IS (n = 60)		TIA (n=8)		*Kruskal-wallis test p-value
	Median	Range	Median	Range	Median	Range	
Age (years)	47	32-64	47	31-72	43	33-49	0.12
Systolic blood pressure (mmHg)	195	150-254	187	113-278	155	132-177	0.002
Diastolic blood pressure (mmHg)	113	73-162	102	67-172	89	78-100	0.001
Glasgow coma score (GCS)	15	5-15	15	7-15	15	14-15	0.09

*Statistically significant ($p < 0.05$); Kruskal-Wallis test
Haemorrhagic stroke (HS), Ischaemic stroke (IS), Transient ischaemic attack (TIA)

10 days from presentation and for the remaining 17% between 10 days to a month. Baseline characteristics of the patients with HS, IS and TIA is summarised in Table 1. Blood pressure was significantly higher in HS versus IS and TIA.

A total of 29% (33/112) of patients had elevated CATS/ METS. Out of the 33 patients, only one patient had CATS/METS elevations of more than 3X URL but did not have any repeat testing. This patient had HS and passed away one year later due to intraabdominal sepsis. The CT of the abdomen done did not show any evidence of phaeochromocytoma. In patients with HS, CATS/METS were elevated in 41% of patients (18/44) versus 25% (15/60) of patients with IS (p=0.09). None of the patients with TIA had elevated CATS/ METS. Median norepinephrine, epinephrine and metanephrine levels in HS were significantly higher than IS as shown in Table 2.

When we compared characteristics of patients with elevated CATS/METS versus those with normal values, systolic blood pressure was significantly higher in the elevated group. Age, diastolic blood pressure and time of sample collection in relation to the presentation were not different between the two groups. This is shown in Table 3.

Of the 33 patients who had increased CATS/ METS, 23 patients had been followed up at neurology/ neurosurgical clinics. One patient has passed away, five had defaulted follow up and four had been transferred to another centre and we could not trace if repeat testing was performed in those centres. Only two of the 23 patients with follow up had repeat investigations

TABLE 2: Catecholamine and its metabolites in HS and IS patients

Variable	HS (n = 44)		IS (n = 60)		z-value	*p-value
	Median	Range	Median	Range		
Norepinephrine	388.5	44-2302	284	0-867	-2.174	0.03
Epinephrine	33.5	15-231	25	6-142	-3.226	0.001
Dopamine	1467	203-4036	1099	530-4126	-1.119	0.26
Normetanephrine	2.42	0.73-11.39	2.04	0.46-9.18	-0.794	0.42
Metanephrine	0.87	0.11-1.67	0.6	0.23-1.3	-2.116	0.03
Methoxythramine	0.78	0.25-2.57	0.86	0.27-1.92	0.008	0.99

* Statistically significant ($p < 0.05$); Mann - Whitney test (z-value)
Haemorrhagic stroke (HS), Ischaemic stroke (IS), Transient ischaemic attack (TIA)

TABLE 3: Comparison between patients with elevated and non-elevated CATS/METS level

Variable	Patients with elevated CATS/METS (n =33)		Patients with non-elevated CATS/METS (n = 71)		z-value	*p-Value
	Median	Range	Median	Range		
Age	49	33-72	46	31-68	0.266	0.86
Systolic blood pressure (mmHg)	200	152-260	185	113-278	2.040	0.04
Diastolic blood pressure (mmHg)	116	73-152	108	67-172	1.624	0.10
Time of sample collection (days)	5	2-26	5	2-26	0.317	0.75

* Statistically significant ($p < 0.05$), Mann Whitney test (z-value)
CATS: catecholamines; METS: metanephrines

of urinary CATS/METS within one year and the values had normalised in both of these patients (data not shown).

DISCUSSION

Although phaeochromocytoma is a rare disorder, it is important to exclude this cause as it carries fatal consequences if untreated. Diagnosis can be easily made by measurements of catecholamines or its metabolites in urine or plasma. However, managing clinicians should be aware of the medical disorders and medications that can interfere with the interpretation of CATS and METS.[5] As more than 20% of patients may have false-positive results, judicious interpretation of test results is critical to select high-risk patients for further studies in order to avoid unnecessary imaging and patient anxiety.[9]

Patients with phaeochromocytoma may present with stroke.[8] It is standard practice to exclude phaeochromocytoma as a cause of acute stroke in young patients with refractory or labile hypertension. In our study, we noted that CATS/METS were increased in 1/3rd of our patients, with almost all being borderline elevations and it was measured within 30 days of presentation with acute stroke. Out of 29% of patients with elevated CATS/METS, all had elevation less than 3X URL except for one patient who had elevations of more than 3X in CATS/METS level. False elevations of more than 2X URL had been reported by Leow *et al*. in haemorrhagic stroke patients.[10] Studies also have shown that CATS/METS excess occurs temporarily in acute stroke and it is postulated to be due to autonomic storm corresponding to sympathetic dysautonomia.[10,11,15] This is likely one of the reasons for elevated levels of CATS/METS in our patients with acute stroke.

We noted that urinary CATS/METS were significantly elevated in HS compared to IS. Our findings differ from Akil *et al*. who noted no significant difference in norepinephrine and epinephrine between HS and IS.[11] Our study measured urinary CATS/ METS as opposed to the study by Akil *et al*. that measured plasma CATS/METS. Plasma norepinephrine and epinephrine can vary widely in a day due to pulsatile release from the sympathetic nervous system. As such, plasma norepinephrine and epinephrine may not reflect the true picture of sympathetic activity as compared to urinary CATS/METS. Moreover, the sympathetic activity cannot be solely determined from plasma catecholamine due to its short half-life and rapid degradation.[12]

Myers *et al*. have shown increased levels of plasma norepinephrine in IS compared to TIA and attributed this to increased peripheral sympathetic activity.[13] Our findings are similar as we found no patients with TIA to have any increase in urinary CATS/ METS compared to IS in which 25% had elevations.

We noted in our study there was no significant difference between the groups that had elevated levels of CATS/METS versus those who did not in terms of age, diastolic blood pressure and time of sample collection except systolic blood pressure. Significantly higher systolic blood pressure was observed in HS patients. In patients with essential hypertension, Jacques *et al*. observed elevated levels of CATS who had higher systolic blood pressure compared

to those with normal levels.[14] They suggested that hyperactive sympathetic activity may be responsible for the severity of hypertension. This could be the probable reason for higher systolic blood pressure in the group that had elevated levels of CATS/METS versus those who did not in our study. This might probably explain the higher levels of CATS/METS in our HS patients.

In view of the falsely elevated levels of CATS/METS, Leow *et al*. have suggested that screening patients with an intracranial haemorrhage for phaeochromocytoma is best avoided in the first week and deferred for at least a month.[10] In our study, we noted that the majority of the patients had borderline elevations in CATS/METS level and hence to confidently rule out phaeochromocytoma repeat investigations should have been carried out. Of the 33 patients with a borderline increase in CATS/METS, one patient has passed away, four had been transferred to other hospitals while another five patients had defaulted their follow up. The remaining 23 patients had been followed up in our hospital. However, only two received repeat testing and CATS/METS levels were within the reference interval. Our findings are similar to Anas *et al*.[5] that reported only 28% of patients with positive results received appropriate follow-up. They raised the concern that the diagnosis of phaeochromocytoma could have been missed in these patients. Similar to their audit, we also noted that there was no repeat testing of urinary CATS/METS in patients with elevated levels.

From this audit, we conclude that borderline elevations of CATS/METS occur in patients with cerebrovascular disease especially in patients with high systolic blood pressure. Elevated levels are seen more commonly in HS patients compared to IS patients. Increase in CATS/METS should be appropriately followed up with repeat testing or further investigations so that the diagnosis of phaeochromocytoma is not missed. Since false elevation in CATS/METS has been reported in cerebrovascular disease, screening for phaeochromocytoma is best deferred for a month.

Conflict of interest: The authors declare they have no conflict of interest.

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