Follow-up evaluation of outliers with elevated SSAT-1 activity

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ABSTRACT

Background: In the evolving role of biomarkers, proteinomic signatures related to up-regulation of polyamine synthesis including spermine/spermidine acetyltransferase-1 (SSAT-1) appear to be promising markers of malignancy. Acetylamantadine (AA) excretion, a measure of SSAT-1 up-regulation, has been shown to be a marker for malignant proliferation. In preliminary analyses of ostensibly normal individuals from Canada (Winnipeg) and Bangladesh (Dhaka), a proportion were identified to have evidence of SSAT-1 up-regulation above the expected non-malignant range (outliers). These outliers were assessed and followed for development of identifiable medical conditions. **Methods:** SSAT-1 up regulation was assessed in 60 ostensibly normal individuals by analysis of urinary excretion of AA after ingestion of amantadine 200 mg x 1 dose. AA was assayed using HPLC-mass spec techniques as previously described. Outliers who consented were followed-up by clinical examinations, routine biochemical and hematological tests and radiographs, where indicated. **Results:** Total AA average (median) excretion at 6 hrs in the Winnipeg cohort was 579+/-252 vs 1699+/-633 ng in the Dhaka cohort. Average urinary concentration at 6 hrs was 3.75+/-0.75 vs 21+/-20 ng/ml. In outliers consenting to follow-up, 1 case of invasive malignancy was identified, 6 cases of pre-malignant neoplastic change, chronic liver disease in 7 cases and chronic inflammation in 2 cases. In others, no malignant or inflammatory conditions have yet been identified. The range of SSAT-1 activity in individuals living in Bangladesh was significantly higher than in the Canadian cohort. Bangladesh volunteers live in an area of known to have high natural contamination with arsenic, a recognized carcinogen and they are exposed to high levels of air pollution. **Conclusions:** By this assay, high SSAT-1 activity has been confirmed in patients harboring malignancy; however, pre-malignant and inflammatory conditions may result in a positive test. Follow-up of individuals with elevated SSAT-1 activity may reveal unrecognized malignancies, pre-malignant neoplasms, liver disease, inflammatory conditions and possibly false-positivity in individuals exposed to carcinogens such as arsenic.

METHODS

Experimental subjects

Healthy controls (n=40) were recruited by the National Institute of Cancer Research & Hospital, Department of Medical Oncology, Mohakhali, Dhaka, Bangladesh within the local area. Twenty healthy adult controls were also recruited locally at the Asper Clinical Research Institute, St. Boniface Hospital, Winnipeg, Canada. All participants provided a signed informed consent for participation. Volunteers aged between 18 and 80 years were included in the study. Exclusion criteria were declared as follows: alcohol consumption within 5 days of amantadine ingestion, previous adverse reaction to amantadine, currently pregnant or lactating females, and history of liver or kidney disease. After overnight-fasting, participants were requested to provide a complete urine collection on the day of the study prior to ingesting amantadine. They then ingested orally amantadine capsules, 200 mg (2 x 100 mg) (Mylan-Amantadine, amantadine hydrochloride, USP). Urine was then collected at 2, 4 and 6 hr post-amantadine ingestion for analysis of acetylamantadine (AA).

Blood analyses and other clinical assessments

Hematological tests, mammogram and ultrasound were conducted using standard procedures at the National Institute of Cancer Research & Hospital, Dhaka, Bangladesh (Bangladesh outlier cohort). A questionnaire and review of electronic medical records was conducted for the outliers in the Winnipeg cohort. The urine levels of AA were measured by LC/MS/MS (Biopharmaceutical Research Inc (Vancouver, BC, Canada).

CONCLUSION

By this assay, high SSAT-1 activity has been confirmed in patients harboring malignancy; however, pre-malignant and inflammatory conditions may result in a positive test. Follow-up of individuals with elevated SSAT-1 activity may reveal unrecognized malignancies, pre-malignant neoplasms, liver disease, inflammatory conditions and possibly false-positivity in individuals exposed to carcinogens such as arsenic.

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RESULTS

In some of the cases, higher than expected urinary AA concentration was linked to increased serum carcinoembryonic antigen. Clinical and radiographic assessments revealed underlying abnormalities in some cases that could represent pre-malignant conditions. Hematology tests revealed elevations in white blood cells and platelets which are markers of inflammation.

Subject I.D.	2hr [AA] (ng/ml)	Urine (ml)	Total AA (ng)	4hr [AA] (ng/ml)	Urine (ml)	Total AA (ng)	6hr [AA] (ng/ml)	Urine (ml)	Total AA (ng)
BM0001	2.0	220	429	2.2	205	445	3.5	130	452
BM0002*	10.5	20	210	10.6	40	424	10.7	75	803
BM0003*	1.7	180	297	3.4	238	809	1.4	228	309
BM0004*	1.1	410	442	2.8	320	880	4.5	130	589
BM0005*	3.8	40	152	3.2	190	614	1.4	180	252
BM0006*	1.2	85	99	8.7	145	1260	2.0	165	333
BM0007*	3.0	165	501	14.9	120	1788	4.0	175	707
BM0008	1.6	190	296	1.7	205	344	5.0	170	841
BM0009*	3.9	170	660	9.2	130	1191	5.8	210	1210
BM0010	1.1	7	8	0.8	120	99	0.4	100	42
BM0011*	0.8	410	314	3.2	400	1288	1.7	390	655
BM0021*	2.7	220	603	9.3	460	4264	5.3	190	1005
BM0022	2.3	90	207	2.0	95	194	2.1	100	211
BM0023	0.6	315	201	3.3	85	282	2.4	155	369
BM0024	4.4	55	239	4.6	90	413	4.4	130	569
BM0025	1.4	50	69	4.0	50	198	2.3	100	229
BM0026*	0.6	245	142	2.6	305	790	3.5	75	264
BM0027	5.2	160	837	4.4	80	353	4.6	130	593
BM0028*	3.8	220	834	10.7	100	1070	14.8	115	1702
BM0029	4.6	120	550	5.8	100	575	4.5	140	631

Table 1. Urinary AA concentration in healthy adult volunteers recruited in Winnipeg

Subject I.D.	2hr [AA] (ng/ml)	Urine (ml)	Total AA (ng)	4hr [AA] (ng/ml)	Urine (ml)	Total AA (ng)	6hr [AA] (ng/ml)	Urine (ml)	Total AA (ng)
H21	9.0	75	677	10.2	80	816	4.7	80	378
H22	6.8	60	408	30.9	95	2934	5.2	90	466
H23	4.3	60	256	7.1	60	425	3.6	80	284
H24	7.5	65	488	14.3	100	1430	9.6	75	722
H25	12.8	45	576	11.6	40	464	2.8	110	306
H26*	63.3	90	5697	35.8	75	2685	10.5	200	2100
H27	21.5	90	1935	30.7	50	1535	22.6	55	1243
H28	3.2	80	254	80.6	60	484	12.6	115	1449
H29	1.5	110	164	6.1	110	673	13.7	105	1439
H30*	2.9	70	204	13.8	55	759	96	30	2880
H31*	3.5	120	414	14.7	70	1029	34.6	70	2422
H32*	18.0	100	1800	14.5	90	1305	42.5	70	2975
H33*	6.3	100	626	34.6	70	2422	52.6	80	4208
H34	47.2	100	4720	2.8	80	227	7.2	75	542
H35	11.3	110	1243	13.9	110	1529	13.5	100	1350
H36	23.1	100	2310	26.7	105	2804	11.5	100	1150
H37	7.7	80	617	13.7	100	1370	15.2	90	1368
H38	1.6	80	127	8.1	75	609	5.6	95	535
H39*	22.0	80	1760	24.2	100	2420	24.0	95	2280
H40	7.4	105	781	19.2	85	1632	11.20	85	286

Table 2. Urinary AA concentration in healthy adult volunteers recruited in Bangladesh

Subject I.D.	Sex (M/F)	Age (Yrs)	Follow up questionnaire/EMR
BM0021	M	50	No health issue
BM0026	M	43	No health issue
BM0028	M	18	No health issue
BM0002	F	24	No health issue
BM0003	F	57	Thyroid nodules (2013)
BM0004	F	34	Ovarian cancer stage 1C (2014)
BM0005	F	49	Lung nodules NYD (2016)
BM0006	F	46	Registered with CCMB, but no diagnosis or information currently available
BM0007	F	28	No health issue
BM0009	F	22	No health issue
BM0011	F	25	No health issue

Table 3. Clinical features of the healthy adult volunteers recruited in Winnipeg

Subject I.D.	Sex (M/F)	Age (Yrs)	Mammogram/Ultrasound	Hematology (cells/µL)
H03	F	44	Enlarged axillary LN, mild fibrotic change in both breasts; mild hepatomegaly with fatty change; cholelithiasis	Normal
H07	M	42	Mildly enlarged prostate (PSA normal); mild fatty change in liver	Elev ated WBC (15,000)
H11	F	56	Mild fibrotic change in both breasts; mild hepatomegaly with fatty change	Elevated platelets (450,000)
H12	F	34	Underlying mass lesion, dense breasts; mild fatty change in liver	Elevated WBC (12,600)
H17	F	38	Mild fibrotic c hange in both breasts; mild fatty change in liver	High platelets (420,000), eosinophilia
H18	F	52	Mild fibrotic change in both breasts; mild fatty change in liver	Normal
H26	M	57	Upper limit of normal prostate (PSA normal); mild hepatomegaly with fatty change	Normal

Table 4. Clinical features of the healthy adult volunteers recruited in Bangladesh