Is mercury in Tibetan Medicine toxic? Clinical, neurocognitive and biochemical results of an initial cross-sectional study

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Abstract

Mercury an important therapeutic substance in Tibetan Medicine undergoes complex “detoxification” prior to inclusion in multi-ingredient formulas. In an initial cross-sectional study, patients taking Tibetan Medicine for various conditions were evaluated for mercury toxicity. Two groups were identified: Group 1, patients taking “*Tsothel*” the most important detoxified mercury preparation and Group 2, patients taking other mercury preparations or mercury free Tibetan Medicine. Atomic fluorescence spectrometry of Tibetan Medicine showed mercury consumption 130 µg/kg/day (Group 1) and 30 µg/kg/day (Group 2) ($P \leq 0.001$), levels above EPA (RfDs) suggested threshold (0.3 µg/kg /day) for oral chronic exposure. Mean duration of Tibetan Medicine treatment was 9 ± 17 months (range 3–116) (Group 1) and 5 ± 1.96 months (range 1–114) (Group 2) (NS) with cumulative days of mercury containing Tibetan Medicine, 764 days $\pm$ 1214 (range 135–7330) vs. 103 days $\pm$ 111 (range 0–426), respectively ($P \leq 0.001$). Comparison of treatment groups with healthy referents (Group 3) not taking Tibetan Medicine showed no significant differences in prevalence of 23 non-specific symptoms of mercury toxicity, abnormal neurological, cardiovascular and dental findings and no correlation with mercury exposure variables; consumption, cumulative treatment days, blood/ urine Hg. Liver and renal function tests in treatment groups were not significantly increased compared to referents, with mean urine Beta₂ Microglobulin within the normal range and not significantly associated with Hg exposure variables after correcting for confounding variables. Neurocognitive testing showed no significant intergroup differences for Wechsler Memory Scale, Grooved Pegboard, Visual Retention, but Group1 scores were better for Mini-Mental, Brief Word Learning, Verbal Fluency after correcting for confounding variables. These results suggest mercury containing Tibetan Medicine does not have appreciable adverse effects and may exert a possible beneficial effect on neurocognitive function. Since evidence of mercury as a toxic heavy metal, however, is well known, further analysis of literature on mercury use in other Asian traditional systems is highly suggested prior to further studies.

Keywords: Tibetan Medicine, mercury, toxicity, detoxification, neurocognitive testing

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Introduction

Mercury and other heavy metals added intentionally as therapeutic ingredients or present due to unintended contaminated sources are found in many Asian medicines including Ayurveda, Traditional Chinese Medicine (TCM) and Traditional Tibetan Medicine (TTM).^{1–3}

Individual cases of lead and to a lesser extent mercury and arsenic poisonings have been reported from several countries following ingestion of Asian Medicines,^{3–6} while

random analysis of samples of Chinese, Tibetan, and Ayurvedic medicines available for sale to the public have demonstrated levels of these metals significantly above international safety limits.^{1,2} These findings are of concern and have resulted in the call for improved regulations governing dietary supplements imported from Asia into the US and Europe.^{1,2} Historically, mercury has been considered an important therapeutic ingredient in TTM for over a

1000 years. Never used in its natural or raw form, it undergoes varying degrees of processing before it is added to complex formulas containing many other ingredients.⁷⁻¹¹ Two types of Hg preparation *Tsothel* and *Karthul* are prepared from elemental Hg (quicksilver) following a complex “detoxification” process involving addition of sulfur, various salts, medicinal plants and pigments and subject to repeated cycles of heating, cooling, washing, mixing, and separation. *Tsothel*, a coarse black powder is the most important and widely used “detoxified Hg” preparation, added to some 30 different types of Tibetan Medicines (TMs) including the highly prized “Precious Pills”; *Karthul* based on “cold processing” involves different ingredients, less cycles of heating and cooling and is used in some 10 medications. Both are considered “detoxified”, safe for oral use and are used particularly in chronic problems including cancer, blood disorders, arthritis, wounds, neurological, and cerebral disorders and as tonics and antidotes to chemicals and poisons.⁹⁻¹¹; *Chokla* a third type of Hg preparation is derived from *Cinnabar*, a red, naturally occurring mineral ore of mercuric sulfide. Subject to a short period of processing including heating and cooling, it is used in 10-12 TMs (and formally as a coating for several others^a) and has a narrower therapeutic range.⁹⁻¹¹

Clinically and scientifically little is known about the toxicity or therapeutic value of these Hg containing preparations. While high levels of Hg have been found on screening TMs sold for oral use,² no studies have conclusively documented whether they are in fact associated with toxic effects in patients.

Hg toxicity is determined by many factors including its chemical form, dose, duration, and rate of exposure.¹²⁻¹⁴ As with toxic agents in general, acute symptoms produced within hours or days by high doses of mercury can develop particularly after inhalation of Hg vapor. Chronic symptoms caused by lower doses repeated over time and appearing within weeks or months of exposure are dominated by neurological abnormalities affecting the motor system, intellectual capacity, and emotional state, a triad known as *erethism*, and by dysfunction in other systems including renal, liver, respiratory, and cardiovascular.¹⁴ As the dose drops into the sub-clinical range, more subtle sub-chronic symptoms are also recognized including weakness, fatigue, anorexia, weight loss, and fibromyalgia.¹²

In a small case series that we conducted in 2005, Tibetan patients taking TM containing Hg primarily as *Tsothel* and many times above the EPA established reference doses (RfDs) for chronic oral exposure (although well below levels associated with minimal toxicity) did not appear to show any major pathological consequences.¹⁵ Analysis of *Tsothel*, showed its main constituents were Hg (44.7%), primarily mercuric sulfide (HgS) (with smaller amounts of mercuric sulfite and mercuric sulfate), and sulfur (42.5%). Lesser amounts ($\leq 1.5\%$) of calcium, silver, iron, and copper were also detected but no lead, arsenic, or other heavy metals.¹⁵

The current, cross-sectional study expands on the previous one by evaluating in greater depth the effects of chronic and sub-chronic Hg exposure in Tibetan patients who had been taking TM for variable periods of time. Clinical,

neurologic, neurocognitive, renal, hepatic, and biochemical indicators of Hg toxicity are evaluated and correlated with indicators of Hg exposure including daily dose, length of exposure time, and blood and urine levels. Comparisons are made to referents, Tibetans not taking TM but exposed to the background of Hg in the living environment of the target population with results compared to established safety limits for inorganic Hg compounds, since studies have consistently indicated Hg in Asian Medicines including TTM is primarily in this form.^{2,5,15,16}

Materials and methods

Setting

The study was carried out in Dharamsala, Himachal Pradesh, India, home to the largest community of refugee Tibetans outside the Autonomous Region of Tibet (China). Patients who had been previously been prescribed TM for various complaints by a Traditional Tibetan doctor were recruited from the outpatient clinic of Men-Tsee-Khang Tibetan Medical & Astro Institute of H.H the Dalai Lama (TMAI). Referents were local Tibetans not taking any form of TM and recruited from administrative workers at TMAI, local governmental institutions, and/or their families.

Materials

TM taken by the study participants was made locally at TMAI pharmacy according to classical Tibetan pharmaceutical texts with ingredients made from herbal and/or raw materials. Production of the three Hg containing preparations follows technical guidelines developed by TMAI pharmacy, under the supervision of the Dept. of Health, Central Tibetan Administration and The Central Council of TM, Dharamsala, India.¹⁰

Study design

This was a non-randomized, cross-sectional, controlled, observational study with volunteers who agreed to participate in the study entered into one of three groups. Our original plan was to compare patients who were taking TM containing any of the three types of Hg preparation with those taking TM free of mercury. In practice, this was difficult to implement due to the wide number of medicines prescribed for each patient and the presence of even small amounts of mercury in many of these medications. We therefore revised our original plan and instead compared the effects of taking high dose of Hg in TM with a lower dose as follows: **Group 1:** Patients taking TM containing *Tsothel*, (the most widely used Hg preparation) mainly in the form of “Precious Pills” and/or other TMs containing *Karthul* and/or *Chokla*. This group was anticipated to have the **highest level** of ingested mercury. **Group 2:** Patients taking either **Hg free TM** or only containing *Karthul* and/or *Chokla*. This group was anticipated to have **lower levels** of added mercury. **Group 3:** Referents composed of healthy Tibetans living locally in Dharamsala not taking any form of TM.

Sample size

Estimation was based on our earlier pilot study¹⁵ using differences in urinary Hg levels between patients taking Hg as *Tsothel* in Precious Pills (Group 1) and patients taking other types of TM (Group 2). Assuming mean urinary levels in Group 1 will be 67 µg/L (SD 91.36) and the mean level in Group 2 will be 1.7 µg/L (SD 91.36), based on a significance level of 5% (two-tailed) and a power of 80%, a sample of 32 patients in each of Groups 1 and 2 would be sufficient to prove a statistically significant difference. In the current study, 50 patients were recruited to each of Groups 1 and 2 giving a power of almost 95%. The third population (Group 3) ($n=20$) was not included in the calculations as they served only to provide an indication of normative parameters for this population based on background Hg exposure.

Inclusion criteria

For Groups 1 and 2: patients attending TMAI outpatient clinic who had been taking TM for at least one month prior to the onset of the current study. For Group 3 (referents): healthy local participants not suffering from any known disease, with no significant medical history and no history of taking TM for at least three months prior to the study. All study participants were volunteers aged between 18 and 65 years who were Tibetan speakers and willing to sign an informed consent.

Exclusion criteria

For all participants: documented evidence based on medical records of a pre-existing condition associated with abnormal renal, liver, and/or hematological function; severe medical conditions including cancer or HIV; serious alcoholic, drug and/or psychiatric problems preventing the signing an informed consent; mental retardation and/or psychiatric history preventing neurocognitive testing. To reduce the confounding effects of background/environmental Hg exposure, both patients and referents were excluded if they had worked in a known Hg containing environment in the six months prior to the study including TMAI pharmacy, agriculture (with exposure to pesticides and fertilizers) and industry (e.g. battery production).

Study tools

All patients and referents recruited into the study were interviewed by research staff at TMAI and the following information recorded in case report forms (CRF):

1. *Background data.* Including: demographic details, conventional past medical history and diagnosis (when known), concurrent treatment (if any) with conventional medication, dental history.
2. *TTM diagnosis and medication.* For Groups 1 and 2, information on TM usage was obtained from a retrospective review of their "Personal Health Record" (PHR), a booklet provided to all patients attending TMAI outpatient clinics detailing TTM diagnosis and treatment by the treating physician. Patients

routinely bring their PHR to all OPD appointments with a new booklet provided when space is filled. In the current study, details of each TM in the PHR including: name, dosage (i.e. no of pills), date of commencement, and date of completion were recorded in the CRF.

3. *Symptoms of Hg toxicity.* Patients and referents were questioned by the interviewers on the current presence of 23 non-specific symptoms of mercury toxicity associated principally with chronic and sub-chronic exposure particularly to mercuric salts.^{14,17} These included: nausea, vomiting, diarrhea, weakness, (may also be present also in acute Hg toxicity), metallic taste, burning sensation in the mouth, pain in mouth or throat, hematemesis, abdominal pain, excess saliva, fatigue, insomnia, anorexia, weight loss, headaches, loss of concentration, dizziness, delirium. Symptoms suggestive of impaired emotional state included: easy blushing, extreme shyness, excessively emotional, anxiety, depression. (All symptoms were translated into Tibetan beforehand using the method described below.) In treatment Groups 1 and 2, only participants who currently suffered from any of the above symptoms were retrospectively questioned as to whether these symptoms had been present before taking TM or had developed subsequently and if so whether they were currently better, worse, or unchanged since taking TM.
4. *Physical examination.* Performed by authors YD and YB^b on all participants with particular focus on problems associated with chronic/sub-chronic Hg toxicity. This included cardiovascular examination (*hypertension, arrhythmias*), dermatological (*acrodynia*) and neurological (*visual, muscle weakness, motor/ sensory neuropathy, sensorineural deafness, increased reflexes, cerebellar signs; tremor, nystagmus, etc*).^{17–20} Basic dental status was assessed for signs suggestive of longer term chronic Hg toxicity (*gum problems, loose teeth*). In addition, the presence and number of dental fillings containing mercury amalgam were recorded in all participants as potentially additional sources of Hg exposure. Height and weight were measured and body mass index (BMI) calculated. Blood pressure was recorded manually by trained staff using a cuff and sphygmomanometer and consisted of an initial reading after the patient had sat for 5 min followed by two further readings 5 min apart with the mean value for systolic and diastolic pressure based on second and third examinations.
5. *Neurocognitive evaluation.* A neurobehavioral test battery was administered to all participants using tests sensitive in detecting subtle cognitive deficits associated with lower levels of chronic neurotoxicant exposure particularly Hg.²¹ The test battery was administered by four trained interviewers: authors YD and YB and two TMAI staff (bilingual in English and Tibetan) trained prior to staff the study by YD and

YB. Results of testing were compared between exposed groups and referents and related to normative values for each test validated by age, sex, and education.

Translation

As the neurobehavioral test battery administered was unavailable in Tibetan, and English was limited amongst participants, the tests selected for the study (and also non-specific symptoms of mercury toxicity) were translated beforehand using the back translation method.²² The source language (English) was initially translated into the Target language (Tibetan) by designated local Tibetan physicians with a high command of both languages. This version was then translated back into the source language by independent translators recruited from the Translation Dept. of TMAI who were blinded to the original questionnaire. The two source language questionnaires were compared by senior Tibetan physicians who approved the linguistic and medical translations as far as possible in keeping with the rigorous requirements of cross-cultural research. Finally, before implementing the study, a validation process of translated questionnaires was undertaken using a focus group of potential research subjects (referents).

The following neurobehavioral tests were included:

(i) *Standardized Mini-Mental State Examination (MMSE)*²³. A sensitive, validated, reliable 30-point questionnaire used extensively for *cognitive screening* and examining functions of *registration, attention, calculation, recall, language, ability to follow simple commands, and orientation*. Test scores range from 0 to 30 with higher scores denoting better cognitive functioning, and values available for cognitive impairment,²⁴ age, and educational level.²⁵

To assess relatively motor independent measures of *memory, attention and concentration*, the following tests were selected: (ii) Wechsler Memory Scale (WMS): Mental Control Tests, subset III.^{26,23} Two of the original three tests of subset III were used; simple conceptual tracking (counting by threes from 1 to 40) and counting backwards from 20 to 1. Normative data (for all three tests) are available²⁷ based on number of errors and time to completion (iii) Brief Word Learning (BWL) tests²⁸: Task A: no. of repeats before subject can say seven unrelated familiar words (normative recall scores (=5) for lists beyond five words with no significant differences for 4–7 words in various age groups)²⁹; Task B: no. of words remembered at 1 min; Task C: no. of words remembered at 5 min. (iv) Verbal Fluency (VF) test: Adaption of the word naming test³⁰ with subjects asked to name as many animals as they can within 60s (normal subjects without cognitive impairment naming 16 to 18 animals)³¹ (v) Visual Retention (VR) test: Adapted version of the visual recognition task³² evaluating *visuospatial perceptual processing* shown to be particularly sensitive to sub-clinical neurotoxicity.³³ (The current study used five cards containing a 5 × 5 grid with four cells colored red positioned so no two stimuli are alike.) Patients reconstruct the pattern after 10s

exposure and 10s delay (maximum possible error score = 20), normative data available.³⁴ To assess *higher executive function and complex motor function* two additional tests were implemented. (vi) Clock Drawing (CD) test³⁵: Frequently used screening tool for cognitive assessment including *verbal understanding, memory, spatial knowledge, abstract thinking, concentration, visual-constructive skills, and executive functions* shown not to be influenced by language or cultural factors and available in several forms. In the current study, the 10-point version³⁶ was used with higher scores (max = 10) indicating better functioning. (vii) Grooved Peg board (GP) test:³⁷ Testing complex *psychomotor function, manual dexterity, and motor performance*, uses a board containing 5 × 5 set of slotted holes angled in different directions (Lafayette Instrument Co. Lafayette, IN 47903 Model 32025). Each peg has a ridge along one side requiring it to be rotated into position for correct insertion. Time to completion is compared for dominant and non-dominant hands with normative data based on age and sex).^{38,39}

Laboratory analysis. Blood was taken from all participants for renal, hepatic, and hematological function. Analysis at Delek Hospital laboratory, a local western style institution serving the population of Dharamsala, was performed for the following tests; serum chemistry (*sodium, potassium, urea, creatinine, bilirubin, Aspartate aminotransferase (SGOT/AST), Alanine aminotransferase (SGPT/ALT), Alkaline phosphatase (ALK P) Gamma-glutamyl transferase (GGT)* using the fully automated Biochemical Analyzer (BioSystems, Reg: 831051880, Model A15, Barcelona, Spain) Hematology: (CBC, WBC platelet count) using Sysmex 100 Hematology Analyzer A 2019 (Sysmex Asia Pacific Pte Ltd & Sysmex Corporation of Japan). A 24-h urine sample was collected from all participants in the study and volume recorded. An aliquot tested at Delek hospital laboratory used paper strip (dipstix) for qualitative estimations of pH, SG, protein, RBC, WBC, glucose, ketones, bilirubin.

Measurement of urinary Beta₂ Microglobulin (B₂M). This small-molecular-weight protein filtered through the glomerular membrane and reabsorbed in the proximal renal tubules is normally excreted in the urine in small amounts (<0.3 µg/mL) when tubular function is normal. In conditions of impaired absorption, e.g. due to Hg or other heavy metal accumulation in the kidney, B₂M may be excreted in significantly greater amounts acting as a sensitive, early indicator of renal tubular damage. B₂M analysis was performed on an aliquot (3–4 ml) of the 24 urine collection, added to a sterile container. Quantitative determination was carried out at the Oncology Laboratory, Hadassah Medical Organization, Israel by ELISA (LIAISON[®] β₂ kit) according to manufacturers' instructions for B₂M – U using the fully automated LIAISON Analyzer (DiaSorin Ltd, Vercelli, Italy) with levels directly proportional to color intensity measured spectrophotometrically at 450 nm.

Measurement of urine and blood inorganic mercury levels. Urine samples (3–4 ml) from the 24 h collection were added to a sterile container. Venous blood (5 ml)

taken from all participants was added to EDTA tubes placed on ice. Urine and blood Hg analysis was performed at the Institute of Toxicology, Tel Hashomer Hospital, Israel using Flow Injection Mercury Hydride Analysis on a Perkin Elmer FIMS (Flow Injection Mercury System). Prior to analysis, samples were incubated with an acidic solution containing potassium permanganate to eliminate all organic material. After centrifugation, clear supernatant was transferred to the instrument where mercury was reduced into its elemental state with sodium borohydride, and measured. A calibration curve prepared in the same matrix as the measured samples was used for quantitation. Calibrators were prepared by spiking the matrix with different amounts of a mercury standard solution (Merck, traceable to National Institute of Standards, USA). The limit of quantitation (LOQ) (lowest concentration of mercury reliably measurable within method uncertainty of measurement ($\pm 20\%$)) was for urine: $0.5 \mu\text{g/L}$ and blood $0.01 \mu\text{g/dL}$. The limit of detection (LOD) (lowest concentration of mercury reliably discernible from blank) was for urine $0.25 \mu\text{g/L}$ and blood $0.005 \mu\text{g/dL}$. Blood and urine samples for analysis in Israel were stored at TMAI during the course of the study at -5°C . To prevent contamination and blood clotting during transfer to Israel, all samples were placed on dry ice and on arrival at Hadassah and Tel Hashomer Hospitals stored at -80°C until analysis was performed.

Analysis of mercury concentration in TM. Samples of TM pills were transferred to the Division of Geochemistry and Environmental Geology, Geological Survey of Israel. For each 0.5 g of ground pill sample, 8 ml of Aqua regia (10 mL HNO_3 (69% INSTRA), (Baker INSTRA analyzed) and 30 mL HCl (38% INSTRA) was added, the mixture placed on a hot plate for 20 min at 130°C , followed by addition of 5 mL of tap water to each sample which was then left on the hot plate for a further 10 min. After cooling the mixture was transferred to a 50 mL tube and diluted to 50 mL producing a total dilution of 1:100. Samples were further diluted to 10^6 and subjected to Tin (II) chloride reduction, which converts Hg (II) into Hg (0) vapor. After separation, the resulting atoms of Hg were detected by atomic fluorescence spectrometry using PS analytical atomic fluorescence (PSA Merlin).

Definition of Hg exposure variables. In order to explore potential dose-response and time-dose response relationships in patients taking TM containing Hg with outcome variables associated with toxicity, the following variables of Hg exposure were defined: (i) Daily Hg dose/kg BW based on analysis of Hg content of TM and calculation of the previous three months of treatment as recorded in patient's PHR (in the 3% of patients taking TM for less than three months this was calculated from the equivalent period), (ii) blood Hg level, (iii) urine Hg level, (iv) total duration (months) of taking TM (Hg and non Hg containing) based on a continuous period of therapy derived from patients PHR, (v) cumulative number of days of taking TM containing Hg (this variable was included as TM treatment varied

considerably with multiple medications prescribed on a daily/ alternate day and /or weekly basis often with recurring patterns of re-prescription over several months and even years).

Ethical approval. The study was approved by the joint local IRB/Ethics committee of TMAI and Delek Hospital according to the Declaration of Helsinki. All participants considered eligible for the study were requested to sign an informed consent form, following a full explanation of the study and assurance that data would be analyzed anonymously by the projects research staff.

Data analysis. All data were entered into a digital database (Microsoft Office Excel) and statistical analyses performed using Statistical Package for the Social Sciences (SPSS) version 19. Quantitative (continuous) variables between two groups were compared with the two sample t-test and Mann-Whitney non-parametric test. The ANOVA with Scheffe *post hoc* adjustments of *P*-value for multiple pair wise comparisons and Kruskal-Wallis non-parametric tests were applied for comparing more than two groups. Association between two categorical variables was assessed using Chi-square or Fisher's exact tests. To evaluate relationships between Hg exposure variables and outcomes, Pearson correlation coefficient and Spearman non-parametric correlation coefficient were calculated for assessing the strength of an association between two quantitative variables. Non-parametric tests were applied when sample size was small and data not normally distributed. ANCOVA multivariate linear model was used to test simultaneous effect of several variables on a quantitative outcome variable. All statistical tests applied were two-tailed and *P*-value of 5% or less considered statistically significant.

Results

Demographic characteristics of participants (Table 1)

The three groups were all ethnic Tibetans but differed in several demographic findings: Group 1 (40 ± 10.7 years) was older than Group 3 (32.3 ± 6.8 years) ($P = 0.016$) and males were more prevalent in Group 3 (60%) vs. Groups 1 (34%) and 2 (19%), ($P = 0.005$). The most significant difference between groups was in occupation with most participants in Group 1 being monks or nuns (72%) compared to Groups 2 (44%) or Group 3 (0%), ($P < 0.0001$); Group 3 had a higher percentage (75%) of skilled/semi-skilled workers ($P = 0.0001$). The three groups did not differ significantly in mean years of education (approx. 10 years including elementary/high school/further education and monastic studies), those with less than four years of education (16%, 18%, and 20%, respectively) and/or those illiterate (12%, 6%, and 5% respectively).

Western diagnosis and treatment with conventional medicine

In each of the study Groups 1 & 2, one patient was receiving conventional medicine for hypertension. Other medications included non-steroidal anti-inflammatories, anti-reflux,

Table 1 Demographic characteristics of participants

	Group 1 (n = 50)	Group 2 (n = 50)	Group 3 (n = 20)	P
Age (year)				
mean $\pm$ SD	40.04 $\pm$ 10.74	37.21 $\pm$ 10.32	32.3 $\pm$ 6.76	
Range	22–65.81	21.05–62.77	21.90–46.72	.016
Sex: n (%)				
female	33 (66%)	40 (81%)	8 (40%)	
male	17 (34%)	9 (19%)	12 (60%)	.005
Marital status: n(%)				
single	38 (76%)	30 (61%)	11 (55%)	.16
married	12 (24%)	19 (39%)	9 (45%)	
Place of Birth n (%)				
Tibet	44 (88%)	29 (59%)	17 (85%)	.03
India	6 (12%)	20 (41%)	3 (15%)	
Education ^a (yr)				
mean $\pm$ SD	10.2 $\pm$ 6.51	9.9 $\pm$ 6.29	10 $\pm$ 6.26	.97
median (range)	10 (0–29)	10 (0–26)	12 (0–17)	
<4 years education: n(%)	8 (16)	9 (18)	4 (20)	.92
Illiterate ^b : n(%)	6 (12)	3 (6)	1 (5)	.47
Occupation: n(%)				
Unskilled ^c	4 (8)	8 (16)	5 (25)	.17
Skilled/semi-skilled ^d	10 (20)	20 (40)	15 (75)	.0001
Monk/nun	25 (72)	22 (44)	0 (0)	<.0001

^aIncludes: basic studies (elementary), high school, university and further education, monastic studies.

^bNo reading and/or writing skills in any language.

^cHousekeeper /laborer/tradesman.

^dAdministrative staff/student/ technician.

analgesics, iron, and Vitamin B12 taken by five patients in Group 1 and 2 patients in Group 2. In Group 1, one patient took antibiotics and prednisolone for treatment of an abscess and two patients had a history of Hepatitis B infection with normal Liver Function tests documented on previous blood tests.

Non-specific mercury associated symptoms

No significant differences were found in the mean number of current non-specific symptoms/patient between Group 1 (mean 2.69 ± 2.33 median 2.0, range 0–10), Group 2 (2.92 ± 2.42 , median 3.0, range 0–9) and Group 3 (2.30 ± 2.20 , median 2.0 range 0–6) ($P = .61$). Overall prevalence per group (i.e. total number of symptoms $\div$ total number of possible symptoms²³ $\times$ number of patients) was Group 1 (11.7%), Group 2 (12.6%), and Group 3 (10%). ($P = 0.40$). Only “headache” was more prevalent in Groups 1 and 2 (57% and 63%, respectively) vs. Group 3 (20%), ($P = 0.004$). “Easy blushing” was more prevalent in referents (20%) vs. Groups 1 or 2 (2% and 8%, respectively) ($P = 0.035$). Mean no. of symptoms reported only after taking TM did not differ significantly between Group 1 (0.28 ± 0.78) and Group 2 (0.45 ± 1.02), ($P = 0.39$) with an overall prevalence of 1.6% (Group 1) and 2% (Group 2), ($P = 0.43$). Of these dizziness was the commonest symptom reported in Group 1 and headache and nausea in Group 2, with no symptom reported as worsening during TM treatment.

Physical examination (Table 2)

Group 1 was heavier ($P = .03$), with a higher BMI ($P = .02$) vs. Group 2. Group 3 was taller than Group 2. These differences disappeared when stratified by sex (not shown).

Cardiovascular. Systolic and diastolic blood pressure and pulse rate did not differ significantly between groups with means within the normal range for age according to criteria of the American Health Association.⁴⁰ Heart murmurs were detected in Group 1 (6%) and Group 2 (2%) (NS).

Dermatological. Skin lesions were present in eight (16%) of Group 1 and 3 (6.1%) of Group 2 and absent in Group 3 ($P = 0.08$), none resembling acrodynia or pink disease associated with Hg toxicity.

Neurological. Mean overall number of abnormal neurological findings/patient did not differ significantly between groups ($P = 0.35$) although *Cranial nerve abnormalities* (II–XII) were more prevalent in Group 1 (50%) than Group 2 (28.4%) and Group 3 (25%) ($P = 0.043$). Evaluating cranial nerves separately, however, including those associated with visual problems, showed no significant differences between groups. Increased *reflexes* and abnormal *motor, sensory, and cerebellar function* were low in all groups without significant intergroup differences except nystagmus which was higher in Group 3 (10%) ($P = 0.074$). Tremor was present in Group 1 (10%), Group 2 (8%) and absent in Group 3 although differences

Table 2 Physical examination and principal abnormal findings

	Group 1 (n = 50)	Group 2 (n = 49)	Group 3 (n = 20)	P
Weight (kg) mean $\pm$ SD	67.3 $\pm$ 12.3	61.55 $\pm$ 9.35	66.53 $\pm$ 11.52	.03
median (range)	65.5 (47–96)	59 (46–85)	65.5 (47–90)	
Height (cm) mean $\pm$ SD	160.89 $\pm$ 9.21	159.64 $\pm$ 7.6	165.5 $\pm$ 8.83	
median (range)	160.5 (142–196)	159 (142–182)	164.5 (152–184)	.04
BMI mean $\pm$ SD	25.89 $\pm$ 3.51	24.04 $\pm$ 3.28	24.16 $\pm$ 2.77	
median (range)	25.13 (21.01–34.85)	23.55 (19.61–34.93)	24.51 (19.27–34.93)	.02
Systolic BP: mean $\pm$ SD	107.34 $\pm$ 14.99	105.12 $\pm$ 14.85	111.2 $\pm$ 8.98	.27
median (range)	105 (90–182)	100 (82–160)	110 (99–129)	
Diastolic BP: mean $\pm$ SD	73.06 $\pm$ 9.61	72.16 $\pm$ 12.09	74.95 $\pm$ 6.78,	.53
median (range)	70(60–120)	70(50–100)	74.5 (64–86)	
Pulse rate/min mean $\pm$ SD	70.48 $\pm$ 5.21	72.26 $\pm$ 6.49	73.4 $\pm$ 7.57	0.15
median (range)	70 (57–83)	73 (54–85)	72.5 (59–86)	
Abnormal findings: n(%)				
Cranial nerves (II–XII)	25 (50)	14 (28.4)	5 (25)	0.04
Impaired vision	7 (14)	5 (10.2)	1 (5)	0.58
Decreased visual fields	1 (2)	1 (2)	0	
Reduced ocular movements	3(6%)	2(4)	0	0.84
Impaired swallowing	0	0	0	–
Impaired hearing	9 (18)	4 (8.2)	4 (20)	0.23
Muscle tone – increased	0	2 (4.1)	0	0.78
– decreased	1 (2)	0	0	
Muscle strength – decreased	1	1	0	0.83
Motor function Fine – decreased	2 (4)	2 (4)	0	0.51
Gross – decreased	2 (4)	0	0	
Motor neuropathy	1 (2)	0	0	1.0
Reflexes – Increased ^a	5 (10)	3 (6.1)	1 (5)	0.69
Sensory neuropathy	3 (6)	1(2)	0	0.46
Cerebellar signs: DDH ^b	1 (2)	0	0	1.0
Dysmetria ^c	1 (2)	0	0	1.0
Ataxia	0	0	0	–
Nystagmus	0	3 (6.1)	2 (10)	0.07
Intention tremor	5 (10)	4 (8.2)	0	0.53
Mean no. neuro. signs /patient ($\pm$ SD)	1.02 $\pm$ 1.87	0.6 $\pm$ 1.11	0.45 $\pm$ 0.69	0.35
median (range)	0(0–11)	0(0–5)	0(0–2)	
Gingivitis	16 (32)	15 (30)	1 (5)	0.04
Loose teeth	8 (16)	8 (16.3)	0	0.15
Missing teeth	27 (57.4)	26 (53.1)	11 (55)	1.0
Dental fillings	29 (59.2) ^d	27 (54)	5 (25)	.04
Blue line gum margins	1 (2)	1 (2)	0	.83
Mean no Dental/patient ($\pm$ SD)	1.41 $\pm$ 1.73	1.5 $\pm$ 1.9	0.5 $\pm$ 1.9	.03
median (range)	1(0–9)	1 (0–6)	0 (0–5)	

^a $\geq$ Three reflexes (out of max four).^bDDH = Dysdiadochokinesis (inability to perform rapid alternating movements).^cDysmetria (finger-nose and heel-shin test).^dGroup 1 = 49

were not high enough to show significance ($P=0.53$). *Hearing loss* was found in Group 1, (18%), Group 2 (8.2%), and Group 3 (20%) ($P=0.23$) but was reported to have developed after TM in only 3(6%) of Group 1 and 2 (4%) Group 2 with one patient in each group appearing to have unilateral *sensorineural hearing loss*. These findings were based on preliminary Rinne-Weber screening test and could not be confirmed by audiometric testing unavailable in the current study.

Dental. *Gingivitis* (which can lead to loose and missing teeth) was more prevalent in Group 1 (32%) and Group 2 (30%) than Group 3 (5%) ($P=0.04$); *Loose and missing teeth* showed no significant intergroup differences. A *blue line* along gum margins was found in one patient in each of Groups 1 and 2. *Dental fillings* were more common in Groups 1 and 2 (59% and 54%, respectively) vs. Group 3 (25%) ($P=0.04$). The mean number of fillings/patient was higher in Groups 1 and 2 vs. Group 3

($P=.03$). Overall poor dental care was reflected in all groups by the number of participants who had never visited a dentist (26%, 18%, and 15%, respectively) (NS), a last dental visit more than a year ago (54%, 62%, 75%, respectively) and high prevalence of tooth extraction before TM in Groups 1 and 2 (54%, 48%, respectively) and referents (50%).

Neurocognitive testing (Table 3)

Several neurocognitive tests required basic reading and writing skills. For the purpose of analysis, we therefore identified two groups; those with less than four years of education and/or who were also illiterate whom we speculated would have problems performing these tests and those with more than four years of education and/or who were also literate. In Table 3 inter- and intra- group differences are shown; however, findings are discussed only for those with ≥ 4 years of education, etc. using *post hoc* analysis to identify the pattern of a given dataset.

MMSE. Mean scores in all groups were above those associated with cognitive impairment (<24).²⁵ Significant intergroup differences were found ($P=0.01$) arising from higher scores in Group 1 vs. Group 2. Normative values (28 ± 1.9 according to age and years of education) were most similar to Group 1 (28.03 ± 2.09) and least so for Group 3 (26.29 ± 2.27).²⁵

WMS: Subset III, Mental Control Tests. Total mean scores for two parts of WMS subset III (max=6) showed no significant differences between Group1 ($4.35 \pm .86$), Group 2 (4.0 ± 1.36), and Group 3 (4.07 ± 1.44). (Normative data available only for the combined three parts of WMS (max=9) ranges from 6.75 ± 1.92 to 6.61 ± 1.9 based on similar years of and age).²⁷

BWL tests. Significant intergroup differences were seen ($P=0.05$) for Task A, with Group 1 needing less repeats than Group 3. There was no significant intergroup differences for Task B (1 min recall) and significant differences for Task C (5 min recall) ($P=0.04$), Group 3 recalling more words than Group 2. All groups scored above normative recall scores of five words at 1 and 5 min.²⁹

Verbal Fluency test (VF). Significant inter-group differences ($P=0.003$) with Group 1 scoring higher than Group 2 and all groups above normative values (17–18 words).³¹

VR test. There were no significant intergroup differences ($P=.11$) (max error score=20 based on five cards). Normative data (max. error score=40 based on 10 cards with mean error score=5.5)³⁴ suggest that error scores for all groups were relatively high.

CD test (max. score = 10). All groups scored highly (>9.0) with significant intergroup differences ($P=.02$) arising mainly from Group1 ($9.8 \pm .46$) scoring higher than Group 2 (9.05 ± 1.7).

GP test. No significant intergroup differences were found for either dominant ($P=.95$) or non-dominant hand ($P=.68$). Times compared well with normative values according to age, sex,^{38,39} and years of education.⁴¹ When Group 1 who were significantly older ($40 \text{ years} \pm 7.1$) than the other groups were compared with normative values for this age group, results of performance were further enhanced.^{38,39}

Laboratory examination (Table 4)

Clinical biochemistry and hematology results were within the normal adult range⁴² except *AlkP* that was high in all groups. Group 3 had significantly higher levels of *Alk P*, *urea*, *K*, *SGOT*, and *SGPT* than other groups. No significant intergroup differences in pH or SG were detected but RBC were found in 12% of Groups 1 and 2 and absent in Group 3. WBC were present in 16%, 10%, and 5%, respectively. Glucose ($\leq 2+$) was detected in Group 2 (4%) and undetected in Groups 1 and 3. Protein was undetectable in all the three groups. Urinary Beta₂ - Microglobulin level: Mean levels for Groups 1 and 2 were within the normal range ($<0.3 \mu\text{g/mL}$) with no significant intergroup differences ($P=0.3$). One patient in Group 1 had high B₂M ($2.26 \mu\text{g/mL}$) (but negative urinary protein on dipstick, no medical history of renal problems or hypertension and normal biochemistry). Urine results on referents were unfortunately lost in transit and unavailable for analysis.

Hg exposure in TM (Table 5)

Number and duration of TM. A total of 108 different TMs were used by patients. Group 1 consumed significantly more types of TM than Group 2 (11.5 ± 5.7 vs. 8.4 ± 3.4) ($P=.002$), more TMs containing Hg (5.1 ± 1.73 vs. 1.74 ± 1.2 , $P \leq .001$), for an overall longer duration (Hg and non Hg containing) (9.2 ± 17 month vs. 5.1 ± 1.96 month) ($P=.093$) and had a greater cumulative number of days of taking TM containing Hg (764 ± 1214 days vs. 103 ± 111 days, $P \leq 0.001$). In both groups, 97% of patients had been taking TM for at least three months prior to the study.

Analysis of TM Hg content/pill (Table 6). In 24 out of 27 TMs (88%) to which Hg is added therapeutically, Hg content per pill ranged from $3.7 \times 10^{-1} \text{ mg}$ to 6.12 mg in TM containing *Tsothel* (principally “Precious Pills”) and $1 \times 10^{-3} \text{ mg}$ to 15.3 mg in TM containing *Karthul* and/or *Chokla*. Analysis of five medicines to which Hg is not added therapeutically and considered Hg free showed Hg levels ranging from $2 \times 10^{-2} \text{ mg}$ to $4 \times 10^{-4} \text{ mg}$.

Daily Hg consumption (Table 5). Mean daily Hg consumption showed Group 1 consumed significantly more Hg ($8.4 \pm 10.05 \text{ mg}$, median 4.83, range 0.2–51.3 mg) than Group 2 ($2.2 \pm 3.82 \text{ mg}$, median 0.24, range 0–15 mg) ($P \leq .001$). Mean daily Hg consumption (μg) /kg body weight was Group 1 ($130 \pm 160 \mu\text{g}$, median 70, range 3–860 μg) and Group 2 ($40 \pm 70 \mu\text{g}$, median 3, range 0–240 μg) ($P \leq 0.001$). Values for both groups were well

Table 3 Neurocognitive testing adjusted for years of education and literacy

	Group 1		Group 2		Group 3		Intergroup	
	<4 years	≥4 years	≥4 years	≥4 years	≥4 years	≥4 years	≥4 years	≥4 years
<i>MMSE (max 30)</i>	(n = 9)	(n = 40)	(n = 9)	(n = 38)	(n = 5)	(n = 14)		
mean ± SD	23.78 ± 2.86	28.03 ± 2.09	22.22 ± 3.99	26.82 ± 2.24	23.6 ± 2.7	26.29 ± 2.27	.82	.012 ^a
median (range)	24 (20–29)	28 (21–30)	22 (13–27)	27 (22–30)	24 (20–27)	26 (23–30)		
<i>P</i> ^e	<.001		<.001		.087			
<i>WMS (max 6)</i>	(n = 8)	(n = 40)	(n = 9)	(n = 38)	(n = 5)	(n = 14)		
mean ± SD	3.13 ± 1.13	4.35 ± .86	3 ± 1.66	4.0 ± 1.36	3 ± 0.7	4.07 ± 1.44	1.0	.41
median (range)	3 (2–5)	4 (2–6)	3 (0–5)	4.0 (0–6)	3 (2–4)	4.0 (2–6)		
<i>P</i>	.008		.092		.19			
<i>BWL test: Task A</i>	(n = 8)	(n = 40)	(n = 9)	(n = 37)	(n = 9)	(n = 14)		
mean ± SD	4.63 ± 1.6	3.63 ± .81	4.89 ± 1.16	4.24 ± 1.36	5.21 ± 1.79	4.43 ± 1.4	.66	.05
median (range)	4 (3–8)	3 (3–6)	5 (3–6)	4 (2–8)	4 (4–8)	4.5 (2–8)		
<i>P</i>	.05		.13		.56			
<i>Task B mean ± SD</i>	6.38 ± .74	6.53 ± .6	6.373 ± .70	6.32 ± .71	6.4 ± .55	6.5 ± .65	.99	.38
median (range)	6.5 (5–7)	7 (5–7)	6 (5–7)	6 (5–7)	6 (6–7)	7 (5–7)		
<i>P</i>	.65		1.0		.69			
<i>Task C mean ± SD</i>	5.5 ± 2.5	6.1 ± .67	5.44 ± 1.01	5.81 ± .37	6.0 ± .71	6.43 ± .64	.47	.043 ^b
median (range)	7 (0–7)	6 (5–7)	6 (4–7)	6 (4–7)	6 (5–7)	6.5 (5–7)		
<i>p -value</i>	.67		.37		.30			
<i>VF</i>	(n = 9)	(n = 39)	(n = 9)	(n = 38)	(n = 5)	(n = 14)		
mean ± SD	20.11 ± 5.09	27.33 ± 4.73	17.44 ± 4.1	23.6 ± 4.91	20.2 ± 3.03	23.21 ± 7.07	.44	.003 ^c
median (range)	20 (12–29)	28 (14–39)	17 (11–23)	5 (14–34)	20 (16–24)	21 (15–39)		
<i>P</i>	.001		.002		.62			
<i>VR</i>	(n = 9)	(n = 40)	(n = 9)	(n = 38)	(n = 5)	(n = 14)	.62	.11
mean ± SD	8.22 ± 2.28	4.58 ± 2.62	9 ± 3.04	6.16 ± 3.53	7.8 ± 4.27	4.93 ± 4.38		
median (range)	8 (4–11)	5 (0–8)	8 (5–15)	7 (1–12)	6 (3–14)	5 (0–15)		
<i>P</i>	.001		.067		.16			
<i>CD (max 10)</i>	(n = 8)	(n = 40)	(n = 9)	(n = 37)	(n = 5)	(n = 14)	.48	.02 ^d
mean ± SD	3.5 ± 4.84	9.8 ± .46	5.44 ± 4.33	9.05 ± 1.7	7.4 ± 2.07	9.71 ± .61		
median (range)	0 (0–10)	10 (8–10)	8 (0–10)	10 (1–10)	8 (4–9)	10 (8–10)		
<i>P</i>	<.001		.005		.002			
<i>GP (Dominant)</i>	(n = 7)	(n = 39)	(n = 7)	(n = 36)	(n = 5)	(n = 14)	.25	.95
mean ± SD	83.57 ± 13.37	65.92 ± 12.23	76.14 ± 13.81	66.14 ± 13.36	72.2 ± 12.22	67.14 ± 7.72		
median (range)	85 (65–90)	64 (40–110)	73 (60–90)	64 (41–115)	75 (55–85)	65 (55–80)		
<i>P</i>	<.001		.08		.34			
<i>Non-dominant</i>	(n = 7)	(n = 37)					.16	.68
mean ± SD	92 ± 13.89	74.89 ± 12.79	84 ± 15.45	76.08 ± 17.84	74.4 ± 11.59	72 ± 9.24		
median (range)	90 (75–120)	75 (50–105)	90 (65–105)	70 (51–135)	70 (65–92)	71 (55–90)		
<i>P</i>	.003		.17		.96			

^{a–d}Group pairs contributing most to overall significance (Scheffe post hoc): ^aGroup 1 vs. 2, ^bGroup 3 vs. 2, ^cGroup 1 vs. 2, ^dGroup 1 vs. 2, ^eintra-group *P*-value: comparing <4 years education and /or illiterate vs. ≥4 years education and literate.

Table 4 Clinical chemistry and haematology

Mean values $\pm$ SD median (range)	Range ⁴²	Group 1 (n = 50)	Group 2 (n = 50)	Group 3 (n = 20)	Intergroup P value
Na (mmol/L)	135–145	136.36 $\pm$ 2.94 137 (123–141)	136.66 $\pm$ 1.78 136 (133–142)	131.33 $\pm$ 27.76 138 (136–141)	.18
K (mmol/L)	3.5–5	4.08 $\pm$ 0.36 4 (3.5–4.9)	3.92 $\pm$ 0.34 3.9 (3.3–5)	4.1 $\pm$ 0.3 4.1 (3.8–4.8)	.02 ^a
Urea (dL/mg)	8–25	24.04 $\pm$ 7.52 23 (11–47)	22.76 $\pm$ 6.31 21 (11–44)	29 $\pm$ 6.6 28 (16–40)	.004 ^b
Creatinine (dL/mg)	0.6–1.5	.86 $\pm$ 0.1 .83 (.5–1.3)	.83 $\pm$.15 .8 (.6–1.2)	.91 $\pm$ 0.16 .9 (.7–1.3)	.2
Bilirubin (dL/mg)	0–1	.61 $\pm$ 0.26 .54 (.31–1.4)	.62 $\pm$.29 .59 (.18–1.57)	.69 $\pm$ 0.23 .66 (.26–1.2)	.52
SGOT (U/L)	10–40	26.16 $\pm$ 13.7 23 (14–100)	22.96 $\pm$ 8.13 22 (11–56)	33.65 $\pm$ 11.76 28 (20–63)	.002 ^c
SGPT (U/L)	7–55	32.86 $\pm$ 24.36 27 (10–139)	25.16 $\pm$ 12 23 (8–61)	42.1 $\pm$ 31 29 (12–104)	.04 ^d
ALK PHOS (U/L)	30–115	150.68 $\pm$ 52.01 138 (79–324)	145.7 $\pm$ 37.84 146.5 (82–224)	184.25 $\pm$ 61.87 167.5 (105–380)	.01 ^e
GGT (U/L)	(f) 5–25 (m) 7–47	24.24 $\pm$ 24.89 17 (5–143) 33.59 $\pm$ 17.7 31 (13–81)	21.15 $\pm$ 14.53 16 (7–69) 28.7 $\pm$ 13.64 20 (18–57)	20.38 $\pm$ 6.97 19.5 (11–33) 39 $\pm$ 32.8 26.5 (10–114)	.75 .57
WBC ($\times 10^9$ L)	4.5–11	6.13 $\pm$ 1.59 6.15 (3.3–11.9)	6.02 $\pm$ 1.43 6 (2.9–9.5)	5.6 $\pm$ 1.02 5.5 (3.9–7.7)	0.38
Platelets ($\times 10^3$ L)	150–350	273.6 $\pm$ 66.5 269.5 (145–448)	256.28 $\pm$ 68.7 253 (90–488)	238.7 $\pm$ 79.4 212.5 (121–405)	0.15
Haemoglobin (g/dL)	13–18	13.07 $\pm$ 2.1 12.8 (7.7–17.8)	12.34 $\pm$ 1.99 12.45 (5–17)	13.39 $\pm$ 2.18 (7.9–16.3)	0.09
Urine analysis		(n = 47)	(n = 41)		
B ₂ M (μ g/mL)	0–0.3	.07 $\pm$ 0.32 .01 (.01–2.26)	.02 $\pm$.006 .012 (.012–.04)	Missing data	.3
pH		5.7 $\pm$ 0.65 6 (5–7)	5.82 $\pm$ 0.79 6 (5–8)	5.63 $\pm$ 0.5 6 (5–6)	.51
SG		1.01 $\pm$ 0.01 1.02 (1–1.03)	1.01 $\pm$ 0.004 1.02 (1–1.02)	1.02 $\pm$ 0.004 1.02 (1.01–1.02)	.20

^{a–e}Group pairs contributing most to overall significance (Scheffe *post hoc* adjustment): ^a Group 2 vs. 3 ^b Group 3 vs. 2 ^c Group 3 vs. 2 ^d Group 3 vs. 2 ^e Group 3 vs. 1.

above EPA (RfDs) for oral chronic exposure (0.3 μ g/kg per day mercuric chloride),⁴³ but below levels for chronic oral intake causing minimal toxic effects (estimated at 28,000 μ g/day for inorganic mercuric salts).⁴⁴

Blood Hg levels. Hg was detected in blood samples at the LOQ in 76%, 74%, and 10% of Groups 1, 2, and 3, respectively ($P \geq 0.0001$). Mean blood Hg levels did not differ significantly between Group 1 (0.22 $\pm$.19 μ g/dL), Group 2 (0.18 $\pm$.011 μ g/dL), and Group 3 (0.35 $\pm$ 0.35 μ g/dL) and were below the EPA (RfD) suggested threshold for oral chronic exposure (0.58 μ g/dL).⁴⁵ In 6% of Group 1 and 5% of referents (Group 3), blood Hg levels were above EPA (RfD) but below the biological exposure index (BEI) (1.5 μ g/dL).⁴⁶ Compared to adult blood Hg levels reported by NHANES (US National Health and Nutrition Survey)⁴⁷ 8%, 4%, and 5% of Groups 1, 2, and 3, respectively, had

blood Hg levels above the 95th percentile (0.46 μ g /dL) but below FAO/WHO recommended levels for Hg²⁺⁺ (2.8 μ g/dL).⁴⁸

Urine Hg levels. In the majority of patients Hg was undetectable. Hg was detected in urine at the LOD in Groups 1(4%) and 2 (8.3%) and at the LOQ in Groups 1(14%) and 2 (6%) ($P=0.2$). A major limitation of the current study was the missing data on Group 3 urine samples which were lost in transit. Mean urine Hg levels $\geq$ LOQ did not differ significantly between Group 1(1.5 $\pm$ 1.24 μ g/L) and Group 2 (1.57 $\pm$ 0.55 μ g/L) ($P=0.9$) with only one participant in Group 1 having a urine Hg above NHANES 95th percentile reference level (3.33 μ g/L).⁴⁹ Mean Hg urinary excretion/24 h (μ g) calculated from 24 h urine volume in participants in whom Hg was detected (LOQ and LOD) did not differ

Table 5 Mercury levels in urine and blood and mercury consumption in TTM

	Group 1 (n = 49)	Group 2 (n = 48)	Group 3 (n = 20)	P
Urinary Hg: undetected, no (%)	40 (81.6)	41 (85)	NA	.6
Urinary Hg $\geq$ LOQ $\mu\text{g/L}$: no (%)	7 (14.2)	3 (6.25)		.2
Mean	1.5 $\pm$ 1.24	1.57 $\pm$ 0.55	NA	.9
Median (range)	1.0 (0.5–4.0)	1.3 (1.2–2.2)		
Urinary Hg $\geq$ LOD $\mu\text{g/L}$: no (%)	2 (4)	4 (8.33)		.44
Mean	0.3 $\pm$.14	0.3 $\pm$.08	NA	1.0
Median (range)	0.3 (.2–.4)	0.3 (0.2–0.4)		
Mean urinary Hg excretion/24 h (μg)	3.0 $\pm$ 3.08	1.43 $\pm$ 1.19	NA	.41
Median (range)	2.69 (.13–10.4)	.98 (0.36–3.30)		
Blood Hg: undetected no (%)	12 (24%)	13 (26%)	18 (90%)	.007
Blood Hg $\geq$ LOQ $\mu\text{g/dL}$: no (%)	38 (76%)	37 (74%)	2 (10%)	>.0001
Mean	0.22 $\pm$.19	0.18 ($\pm$ 0.11)	0.35 ($\pm$ 0.35)	.83
Median (range)	0.1 (0.1–0.8)	0.10(0.1–0.5)	0.35 (0.1–0.60)	
Mean no. of different TMs consumed	11.5 $\pm$ 5.76	8.46 $\pm$ 3.35	–	.002
Median (range)	10.5 (3–27)	8.0 (3–17)		
Mean duration of different TMs (mth)	9.21 $\pm$ 17	5.08 $\pm$ 1.96	–	.093
Median (range)	5.5 (3–116)	5 (1–114)		
Mean no. Hg containing TMs consumed	5.18 $\pm$ 1.73	1.74 $\pm$ 1.18	–	<.001
Median (range)	5 (1–10)	2 (0–5)		
Mean cumulative exposure Hg TMs (days)	764.04 $\pm$ 1214	103 $\pm$ 111.21	–	<.001
Median (range)	421 (135–7330)	62 (0–426)		
Mean daily Hg consumption (mg) ^a	8.44 $\pm$ 10.05	2.2 $\pm$ 3.82	–	<.001
Median (range)	4.83 (0.2–51.3)	0.24 (0–15.1)		
Mean daily Hg consumption/kg BW (mg)	.13 $\pm$.16 .07	.04 $\pm$.07	–	
Median (range)	(.003–.86)	.003(0.–.24)		<.001

BW: body weight; NA: not available.

^aCalculated over a three-month period.

significantly between Groups and were within the normative range (0–9 μg).⁵⁰

Dose–time–response relationships. Hg exposure variables were entered into a correlation analysis to ascertain if they were associated or independent of each other. No significant correlations were found (not shown) except between overall duration of TM and cumulative days of TM containing Hg ($r = .87$, $P = < .001$). Hg exposure variables were then individually analyzed to see if they were related to quantitative outcome measures associated with Hg toxicity (Table 7) (Urine Hg was not included because of its low detection (<20%) in Groups 1 and 2 and missing values in Group 3.). No significant correlations were found between Hg exposure variables and number of non-specific symptoms of mercury toxicity, abnormal neurological signs, abnormal dental findings, and Beta₂M (Table 7). Hg consumption (increasing dosage) showed a significant positive correlation with the Mini-Mental ($P < .05$) and Verbal Fluency Tests ($P < 0.01$) (where higher scores indicate better performance) and a significant negative correlation with SGOT levels ($P < .05$); Cumulative days of treatment were negatively correlated with Learning Task A (lower scores indicating better performance) ($P < 0.05$) and positively correlated with SGOT ($P < 0.05$), SGPT ($P < 0.01$), and Gamma GT ($P < 0.01$); Blood Hg levels were negatively correlated

with diastolic blood pressure ($P \leq .05$), Learning Task A, ($P < 0.05$) and urea, creatinine, SGOT, SGPT, and gamma GT ($P < 0.01$). Overall duration of TM treatment showed no significant correlation with any outcome variables.

Where significant correlations were found, Hg exposure variables were entered into a multiple linear (Ancova) model for the entire population to test the simultaneous effect of several variables including potential confounders (age, sex, occupation, BMI, years of education, and dental fillings) on quantitative outcome measures (Table 8). After correcting for confounding variables, Hg consumption was positively correlated with Mini-Mental ($P = .02$) and Verbal Fluency Tests ($P = .007$); Cumulative days of treatment was negatively correlated with Learning Task A ($P = 0.2$) although these relationship were not sufficient to show a strong linear association. No significant association was found between Hg exposure and diastolic blood pressure, liver function tests, and hemoglobin.

Discussion

Mercury is an important therapeutic ingredient in certain traditional TMs. Never used in its raw or natural form, it undergoes varying degrees of processing to produce three distinct types of preparation (Tsothel, Karthul, and Chokla) that vary in their use and therapeutic range.^{7–11} While all

Table 6 Analysis of mercury content in Tibetan Medicines (TM)

TM Name (dry weight of pill) (g)	Mercury preparation			Mean Hg content (mg/pill)	TM Name (dry weight of pill) (g)	Mercury preparation			Mean Hg content (mg/pill)
	TS	K/C				TS	K/C		
Precious pills ^a									
<i>Rinchen Tsajor Chemo</i> (1.2)	+	—		1.8	<i>Samnor</i> (1.01)	—	+		15.3
<i>Rinchen Rana Samphen</i> (1.09)	+	—		6.1	<i>Mutik 25</i> (1.03)	—	+		11.62
<i>Rinchen Chakril Chenmo</i> (1.13)	—	+		3.71	<i>Gurkhyung Chakdor</i> (.49)	—	+		10.62
<i>Rinchen Dangjor Chemo</i> (1.06)	+	+		1.2	<i>Yukhyung Kamen</i> (.46)	—	+		1.2
<i>Rinchen Mangjor Chemo</i> (1.11)	+	+		1.8	<i>Gawa 16</i> (.41)	—	+		5.45
<i>Rinchen Tso-Tru Dhasel</i> (1.18)	+	—		2.0	<i>Dashel 37</i> (1.19)	—	+		1.16
<i>Rinchen Jumar 25</i> (1.26)	—	+		5.2	<i>Jangchoe 37</i> (.97)	—	+		1.0
Other Hg containing TM					<i>Khyung nga Nila</i> (.037)	—	+		0.1
<i>Yukar</i> (0.38)	—	+		.71	<i>Samkhyung^b</i> (0.41)	—	+		0.001
<i>Sangdak Daryaken</i> (0.26)	+	—		.37	TM not containing Hg				
<i>Sangdeng 25</i> (0.45)	—	+		2.29	<i>Yuril 13</i> (0.45)				0.001
<i>Lama 25</i> (1.12)	—	+		3.83	<i>Serdok 5</i> (0.64)	—	—		0.02
<i>Nyulchu 18</i> (0.44)	—	+		0.93	<i>Phakmo drupar</i> (0.52)	—	—		0.0004
<i>Nyenpo18</i> (0.43)	—	+		3.80	<i>Tsanglha</i> (0.77)	—	—		0.002
<i>Sarkyung</i> (0.36)	—	+		12.86					
<i>Goyu Daepak</i> (1.18)	—	+		1.29					

T: Tsothel; KC: Karthul and/or Chokla.

^aPrecious pills in which Hg is principally in the form of Tsothel although Karthul and/or Chokla may also be present. ^bHg as Chokla (Cinnebar) used as coating for pill (removed since 2010).

Table 7 Significant associations* between Hg exposure variables and clinical, neurocognitive and biochemical/hematological outcomes

	Hg exposure variables			
	Mercury consumption /kg/day BW	Overall duration of all TM	Cumulative days of TM + Hg	Blood Hg
Clinical Symptoms ^a	NS	NS	NS	NS
Neurological signs ^b	NS	NS	NS	NS
Dental findings ^c	NS	NS	NS	NS
BP: systolic	NS	NS	NS	NS
diastolic	NS	NS	NS	−0.201 ^d
Neurocognitive: MMSE	0.241 ^e	NS	NS	NS
WMS	NS	NS	NS	NS
LTA	NS	NS	−0.249 ^d	−0.211 ^d
LTB	NS	NS	NS	NS
LTC	NS	NS	NS	NS
VR	NS	NS	NS	NS
VF	0.262 ^e	NS	NS	NS
CD	NS	NS	NS	NS
PB – Dominant	NS	NS	NS	NS
Non-dominant	NS	NS	NS	NS
B2M	NS	NS	NS	NS
Urea	NS	NS	NS	−0.284 ^e
Creatinine	NS	NS	NS	−0.354 ^e
SGOT	−0.220 ^d	NS	0.209 ^d	−0.276 ^e
SGPT	NS	NS	0.292 ^e	−0.259 ^e
GGT	NS	NS	0.279 ^e	−0.259 ^e
Alk Phos	−.225 ^d	NS	NS	−.314 ^f
Haemoglobin	NS	NS	NS	−.194 ^d

*Spearman correlation coefficient. ^{a-c}Number/patient associated with Hg toxicity ^d $P < .05$, ^e $P < .01$, ^f $P < 0.001$

three are historically considered safe for oral consumption, little is known clinically and scientifically about their potentially toxic effects in patients. In this cross-sectional observational study, we attempted to document for the first time whether patients exposed to Hg in TM had clinical, neurocognitive, biochemical, and hematological findings suggestive of chronic and/or sub-chronic Hg toxicity. In the two groups of patients taking TM those taking what we estimated would be higher doses of Hg (Group 1) had a daily Hg consumption of 130 µg/kg/day compared to Group 2 taking what we estimated would be a lower Hg dose (30 µg/kg/day). Both were $\times 400$ and $\times 100$ respectively above the established EPA safety level (0.3 µg/kg per day)⁴³ with Group 2 consuming less than what is considered a low chronic dose (<40 µg/kg BW).¹⁹ Duration of TM was more than three months in 97% of patients in both groups, with a median of five months and maximum of nine years, a time period we believe is sufficient to justify our initial observations on clinic-pathological effects associated with chronic/sub-chronic Hg exposure in TM.

In contrast to acute symptoms appearing within hours or days, symptoms of chronic Hg exposure become apparent within weeks or months, can be difficult to document especially as dose drops into the sub-clinical range and in the most toxic forms, e.g. methyl mercury only after a latency of

many years.^{5,12–14} In TTM as in other Asian traditional systems including Ayurveda and TCM, studies have consistently indicated that Hg used therapeutically is present only as inorganic Hg compounds^{2,5,15,16} and not as the more toxic elemental or organic forms associated with industrial exposure and fish consumption. Nevertheless, inorganic mercuric salts can cause a variety of clinic-pathological effects including a number of non-specific symptoms, CNS disturbances, renal tubular dysfunction, tooth loss (long-term exposure) cardiovascular problems, and progressive anemia.^{12,14,19}

In this study, few significant intergroup differences were found between patients exposed either to higher or lower Hg doses in TM and referents, healthy Tibetans not taking any TM and providing an indication of normative parameters for this population based on background Hg exposure. Non-specific symptoms of chronic and sub-chronic exposure showed that only headache was more prevalent in treatment groups, although no patients reported this symptom had worsened since taking TM; tremor a neurological problem highly suggestive of chronic toxicity^{14,19} was found in several patients taking TM but in such low numbers that statistically it was a non-significant finding; sensorineural deafness, which can be a consequence of long-term Hg exposure^{14,19} was found in one patient in each treatment

Table 8 Multiple linear (Ancova) model evaluating association between Hg exposure and outcome variables, controlling for potential confounders

Outcome variables	MMSE	LTA	VF	Creat.	Urea	SGOT	SGPT	GGT	ALK P	Hb	Diastolic BP
ρ (β) ^a Age	.001 (-.09)	.02 (.037)	.52	.28	.15	.51	.88	.58	.59	.88	.36
Sex	.135	.04 (.63)	.04 (2.57)	<0.001 (.199)	.007 (4.1)	.96	.02 (10.06)	.02 (11.09)	.03 (24.39)	<.0001 (2.9)	<.001 (9.12)
BMI	.003 (.24)	.01 (-.105)	.72	.93	.26	.05 (.782)	<.001 (2.48)	.001 (2.13)	.53	.06	.02 (.70)
Education ^b	.001 (-2.72)	.02 (1.003)	.02 (-4.46)	.56	.13	.005 (11.79)	.003 (19.17)	.03 (13.89)	.27	.69	.97
Occupation	.14	.71	.53	.43	.17	.09	.08	.23	.17	.63	.38
Hg fillings	.38	.43	.45	.71	.89	.87	.16	.65	.32	.62	.03 (-1.11)
TTM consumption /kg/day BW	.02 (4.05)	.007 (10.38)		-	.22	.43	-	-	.08	-	-
Cumulative days Hg containing TTM	-	.02 (-.00036)	-	-	-	.72	.60	.47	-	-	-
Blood Hg	-	.43	-	.23	-	.62	-	.23	.95	.24	.61

^a β value shown where p is significant.^b < 4 years education and/or illiterate and > 4 years education and literate
MMSE (Mini-Mental Test), LTA: learning task A test; VF: Verbal Fluency test; BP: diastolic blood pressure.

group and needs further confirmation by audiometry. Dental findings such as a blue line along gum margins and gingivitis leading to tooth loss associated with long-term chronic Hg exposure were more prevalent in the treatment groups; however, these results should be interpreted with caution in view of the poor dental care in all groups and that examinations were not carried out by dental professionals. Hg accumulation in the kidneys and liver, an important feature of chronic toxicity associated with inorganic mercuric salts¹⁹ causes elevated liver enzymes and renal problems including tubular dysfunction, immunologic glomerulonephritis, and nephrotic syndrome.^{18,19} In both treatment groups, mean values for liver and renal functions tests including urinary Beta₂ - macroglobulin, (B2M) a sensitive indicator of renal tubular damage, were within the normative range. One patient in Group1 had a high B2M level, a finding that in view of other normal renal function tests and medical history suggests (but does not confirm) that this result may be due to laboratory error. Nevertheless, red blood cells in the urine of several participants receiving TM require further investigation.

Neurocognitive testing in the current study is particularly interesting due to the well-documented neurotoxic effects of chronic exposure to mercurials in humans.^{18,19} Treatment and referent groups were not homogenous; although, when results were compared to normative values for these tests stratified and validated by age, sex, and education, they were largely within the normal range. Group1 (highest Hg consumption), however, scored significantly better than Group 2 (lower Hg consumption) and referents in the *Mini-Mental*, *Brief Word Learning (Task A)*, *Verbal Fluency* and *Clock Drawing tests* examining functions of attention, calculation, recall, language orientation, and relatively motor independent measures of concentration and memory.

To further explore this relationship and also any association between Hg exposure variables with evidence of chronic/sub-chronic toxicity, dose-response and dose-time relationships were examined. After controlling for potential confounders (age, sex, BMI, occupation, years of education, dental fillings), no significant correlations were found except for a positive outcome correlating better scores on certain neurocognitive tests with higher doses of Hg and cumulative days of treatment.

This finding is particularly surprising since Hg is not known to have any beneficial effect in humans. In TTM however "detoxified Hg" especially *Tsothel* is credited with important therapeutic activity including treatment of chronic neurological problems.^{7,9,10} Whether our results indicate a potentially **hormetic** response (characterized by low dose stimulation but high dose toxicity or inhibition) can only be conjectured. Such an effect for Hg is unknown in humans although it has been documented for other heavy metals in several species.⁵¹ Our results suggest that hormesis should be examined for in future studies on TM taking into account the life expectancy of the target group. The relative lack of toxic effects found in the current study may be related to the presence of mercuric sulfide (HgS) in the three types of Hg preparations used in TM. In our

previous study¹⁵ *Tsothel* contained Hg principally as HgS a finding also demonstrated in other analysis of TMs⁵²; *Chokla* is derived directly from Cinnabar (HgS)⁵³ and while formal analysis of *Karhul* has not been performed, its similar preparation to *Tsothel*⁹ suggests it also contains primarily HgS. While the therapeutic effect of HgS is unknown in humans, its reportedly low toxicological profile⁵³ has probably contributed to its historic inclusion in Asian Medicines including TCM and Ayurveda. In-vivo studies in rodents have shown that HgS water solubility is far lower than other forms of Hg,⁵⁴ with absorption from the gastric tract only 0.2% compared with mercuric chloride (7–15%) and methyl mercury (>95%).⁵³ Oral bioavailability is also very low, 30–60 and 1000 fold less than mercuric chloride and methyl mercury, respectively,^{55,56} with excretion mainly in faeces and to a lesser extent urine.⁵⁷ Taken orally HgS can be toxic; high doses in rodents (1.0 g/kg) have been associated with hearing loss, memory, and behavioral problems with long-term administration causing gastrointestinal symptoms, renal dysfunction, and blurred vision.^{58–60} While not considered to cross the blood-brain barrier or placenta, a small portion of HgS can be reduced in the tissues and exhaled as Hg vapor with accumulation in the cerebral cortex and cerebellum.^{56,61} In the current study, Hg in TM was taken at doses far below those associated with HgS toxicity, while Hg in blood and urine (the latter detected in only 20% of patients) were well below international safety levels. These results tend to confirm the relatively poor gut absorption of Hg in TM and indicate a pattern of low grade chronic Hg exposure. The finding that 10% of referents also had detectable blood Hg levels, half of whom were above the EPA (RfD) also suggests that Tibetans in this study were exposed to other sources of Hg. Dental fillings containing Hg amalgam may be one such source since they were prevalent in all groups and are associated with long term, sub-chronic Hg toxicity.¹³ Nevertheless when treated as a confounding variable, the effect of dental fillings on outcome measures was not significant. It is possible that other factors may also contribute to the relatively low toxicity of TM. TMs are complex formulas containing many other ingredients including medicinal plants, minerals and metals, which in addition to their therapeutic role could act as natural chelators helping the body rid itself of excess Hg. Various plants and foods have been identified in this role including dietary fiber, sulfur-rich foods, e.g. garlic, brassicas (e.g. broccoli) and *Coriandrum sativum*.^{62–64} The role of natural chelation in TM is speculative but it should be investigated further.

Strengths and weakness

This is the first study to systematically examine Hg toxicity in a cohort of Tibetan patients taking TM using a wide range of outcome measures including symptomatic, clinical, neurocognitive, renal, biochemical, and hematological assessment. Evaluation of blood and urine Hg levels and analysis of TM Hg content allowed us to make quantitative estimates of mercury exposure in patients, while laboratory examination including sensitive ELISA B2M test enabled us to test the possibility of renal tubular

damage. Nevertheless, the study design had a number of limitations. As a cross-sectional observational rather than prospective study, we were not able to accurately document whether symptoms and signs of Hg toxicity were secondary to TM or features of disease for which treatment had been sort. Patients were enrolled only after having taken TM for at least one month and therefore acute toxicity could not be evaluated. Comparison with referents based on our inclusion criteria may also have been problematic. Hg exposure in TM could theoretically have occurred before three months and affected results particularly of neurocognitive testing. Hg however accumulates in the brain and nervous tissue slowly following chronic exposure (particularly to Hg vapor or organic Hg).⁶⁵ As referents were healthy without any prior medical history justifying TM treatment, we believe this process was unlikely to have occurred. The Tibetan translation of certain neurocognitive tests (e.g. Mini-Mental) also presented certain limitations. These tests were not previously available in the Tibetan language and were translated from English by staff at TMAI using accepted back translation methods and local validations. Standard validation processes if available would have provided greater authenticity. A significant problem in the current study was the loss of urine samples for referents and subsequent missing data on urine mercury levels and B₂M that would have provided further important information on the background Hg exposure of this group. Background exposure to Hg other than dental fillings and potential effects of a “lifetime” exposure to TM could also not be differentiated for their effect on outcome variables. The real long-term consequences of TM also remain unknown, since although some patients in the current study had a maximum range of treatment extending over several years, the mean duration in the current study was only nine months.

Conclusion

Results of an initial cross-sectional study suggest that patients taking TM containing Hg at doses well above those considered safe show little if any evidence of chronic or sub-chronic toxicity. An unexpected finding is the weak but significant association between higher Hg ingestion and improved neurocognitive testing even after correcting for confounding variables. These results should be interpreted with caution, however, due to the small sample size and retrospective study design which do not provide sufficient power to give a definitive answer. Nevertheless, the putative neurocognitive effect has not previously described in scientific research and warrant’s further investigation. Since toxicological effects of mercury are well known, in-depth analysis of literature on Hg use in Tibetan and other Asian traditional systems is highly recommended prior to implementing larger, prospective studies to produce more conclusive evidence of the current study’s findings. Such studies should include relevance to other ethnic groups, as genetic susceptibility to Hg toxicity is increasingly recognized¹³ and also attempt to quantify effects of individual TMs particularly those such as *Sammor* containing very high

Hg content although this will be challenging due to the significant numbers of TMs collectively used for each patient.

Future research should also examine the therapeutic activity of “detoxified” Hg preparations used in TM particularly *Tsothel* in degenerative brain disorders such as Alzheimer’s and Parkinson’s Disease. As our results are based on Hg processing at TMAI Dharamsala, research is needed to evaluate Hg preparation at other centers of TTM as well as in other Asian traditional systems. For maximum safety, effectiveness, and reproducibility, we also suggest the complex Hg processing at TMAI be fully standardized and subject to GMP.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

NOTES

- a. Personal communication TMAI pharmacy (Note: The practice of using Chokla as a coating in some TTMs was discontinued in 2010 as it was considered to interfere with the overall therapeutic activity of the medicine).
- b. Authors YD & YB were final year medical students at Hadassah School of Medicine, Israel at the time of the study)

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