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# Cancer and Blood Concentrations of the Co-mutagen Harmane in Essential Tremor

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## Abstract

**Background—**Blood concentrations of harmane, a tremor-producing neurotoxin, are elevated in essential tremor (ET). Harmane is also a co-mutagen.

**Objective—**To compare the prevalence of cancer in ET cases vs. controls, and determine whether blood harmane concentrations are elevated among ET cases with cancer.

**Methods—**Case-control design.

**Results—**66/267 (24.7%) ET cases vs. 55/331 (16.6%) controls had cancer (adjusted OR 1.52, 95% CI 1.01 – 2.30, p = 0.04). Among specific cancer types, colon cancer was more prevalent in ET cases than controls (2.6% vs. 0.6%, p = 0.04). Log blood harmane concentration was higher in ET cases vs. controls (p = 0.02) and in participants with vs. without cancer (p = 0.02). Log blood harmane concentration was highest in ET cases with cancer when compared with other groups (p = 0.009).

**Discussion—**These links between cancer and ET and between high blood harmane and cancer in ET deserve further study.

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**Keywords**

essential tremor; epidemiology; cancer; toxin; harmane

## Introduction

Harmane (1-methyl-9H-pyrido[3,4-*b*]indole), a potent neurotoxin,[1] is present in many foods in the human diet.[2] Laboratory animals exposed to harmane and other heterocyclic amines develop an acute essential tremor (ET)-like action tremor.[3] Interestingly, blood harmane concentration has been found to be elevated in ET cases compared with controls.[4]

Many heterocyclic amines are also mutagens and are linked with several types of cancer (esp. colon and prostate).[1,5,6] While harmane itself is not mutagenic, it exerts co-mutagenic activity in bacteria and mammalian cells.[7,8]

Medical co-morbidity has not been studied extensively in ET; indeed, there are no data on the prevalence of cancer in ET cases. By contrast, this topic has been examined extensively in Parkinson's disease (PD), which is associated with increased risk of melanoma and reduced risks of other cancer types.[9]

Harmane is a co-mutagen and a high blood concentration could conceivably predispose an individual to cancer. Blood harmane concentrations also seem to be elevated in ET.[4] We therefore tested the hypothesis that we would find particularly high blood harmane concentrations among ET cases with cancer.

The aims of this study were to: (1) compare the prevalence of cancer in ET cases vs. controls, and (2) determine whether blood harmane concentrations are elevated among ET cases with cancer.

## Methods

All participants were enrolled (2000 – present) in an ongoing study of the environmental epidemiology of ET.[4] ET cases were patients at the Neurological Institute of New York, Columbia University Medical Center (CUMC) or the Weill Medical College of Cornell University (WMC). Their ascertainment, including identification from a computerized database, has been described previously.[4] Controls were identified from the New York Tri-state area using random digit telephone dialing and, as described in detail,[4] came from the same source population as the cases. Controls were frequency-matched to cases based on gender, race, and age and were screened so as to be free of tremor. The CUMC and WMC Internal Review Boards approved of all study procedures and written informed consent was obtained upon enrollment. To date, 352 cases and 331 controls have enrolled, although 85 cases were excluded because their ET diagnosis could not be confirmed (see criteria below); their diagnoses were: PD (11), dystonia (13), psychogenic tremor (1), myoclonus (1), enhanced physiological, drug-induced, and other tremors (59). Sixteen (18.8%) of 85 had cancer. Data on the remaining 267 cases and 331 controls were used to estimate cancer prevalence. Blood harmane data were available on 147 cases and 187 controls (data were unavailable in the remainder for several reasons: patient refused, laboratory moved locations, failed phlebotomy, quantity not sufficient). Participants with vs. without blood harmane data were similar (Table 1).

Participants were evaluated in person by a trained tester using structured questionnaires to collect demographic and medical data. Cancer was reported by the participants and was confirmed by a review of medical records and pathology reports. In a sub-study of 75 ET cases

and 75 controls who reported no malignancy, we determined based on medical record review that the proportion of false negative reports was low (1/75 or 1.3% [cases] and 1/75 or 1.3% [controls]). Severity of illness in 14 disease systems was rated using the Cumulative Illness Rating Scale.[10]

The tester videotaped a tremor examination[11] and each of 12 videotaped action tremor items was rated by Dr. Louis (0 to 3), who was blinded to cancer status, and who confirmed the ET diagnosis using published diagnostic criteria (moderate or greater amplitude action tremor during ≥3 activities or head tremor in the absence of PD or dystonia).[11]

Phlebotomy was performed and blood harmane concentration was quantified using a high performance liquid chromatography method described previously.[12]

Analyses were performed in SPSS Version 15.0. Chi-square ($\chi 2$), t tests, analysis of variance (ANOVA) with Tukey's post test comparisons, and Pearson's correlation coefficients were used to test for associations. Blood harmane concentrations were log transformed ($\log_{10}$) because they were not normally distributed. In regression analyses, covariates were included in adjusted models when they were associated with the dependent or independent variables in univariate analyses or when prior analyses[4] indicated such an association.

## Results

### Cancer Odds in ET

The 267 ET cases and 331 controls were similar in terms of demographic characteristics, years since last hospitalization and Cumulative Illness Rating Scale scores (Table 1). 66/267 (24.7%) ET cases vs. 55/331(16.6%) controls had cancer (Table 1). In a logistic regression analysis in which cancer was the dependent variable and ET vs. control was the independent variable, unadjusted odds ratio (OR) = 1.65, 95% Confidence interval (CI) = 1.10 – 2.46, p = 0.01; OR adjusted for age in years, gender, ever cigarette smoker = 1.52, 95% CI = 1.01 – 2.30, p = 0.04. Further inclusion of cigarette pack-years as a covariate in the same adjusted regression model did not change the results (OR = 1.52, 95% CI = 1.01 – 2.29, p = 0.047). The prevalence of colon cancer, in particular, was higher in ET cases than controls (2.6% vs. 1.6%, p = 0.04) and the prevalence of skin cancer was marginally elevated (8.2% vs. 5.1%, p = 0.13). The prevalence of melanoma was similar in cases and controls (Table 1). After stratifying by gender, 33/122 (27.0%) male cases vs. 18/137 (13.1%) male controls had cancer (unadjusted $OR_{men}$ = 2.45, 95% CI = 1.30 – 4.63, p = 0.006; adjusted [age, ever cigarette smoker] $OR_{men}$ = 2.23, 95% CI = 1.16 – 4.29, p = 0.016), and 33/145 (22.8%) female cases vs. 37/194 (19.1%) female controls had cancer (unadjusted $OR_{women}$ = 1.25, 95% CI = 0.74 – 2.12, p = 0.41; adjusted $OR_{women}$ = 1.17, 95% CI = 0.68 – 2.01, p = 0.57). In 34 ET cases, there were precise data both on age of tremor onset and age at cancer diagnosis; in 30 (88.2%), tremor preceded the cancer diagnosis (mean latency = 15.6 ± 2.7 years, median = 10.0).

### Blood Harmane, Cancer, and ET

147 ET cases and 187 controls with blood harmane results were similar, except for a 3.7 year age difference (Table 1). Among controls, log blood harmane concentration was not associated with age, gender, education, cigarette smoking (current, ever, or pack-years), years since last hospitalization, or Cumulative Illness Rating Scale score (all p values > 0.3).

Log blood harmane concentration was higher in ET cases vs. controls (0.61 ± 0.63 $g^{-10}$/ml vs. 0.44 ± 0.68 $g^{-10}$/ml, p = 0.02) and in participants with vs. without cancer (0.70 ± 0.68 $g^{-10}$/ml vs. 0.48 ± 0.65 $g^{-10}$/ml, p = 0.02). Log blood harmane concentration was highest in ET cases with cancer (0.87 ± 0.68 $g^{-10}$/ml, Table 2, p = 0.009). In a linear regression analysis, ET cases with cancer had higher log blood harmane concentrations than controls without cancer

(b = 0.15, p = 0.002), even after adjusting for age in years, gender, ever cigarette smoker, and cigarette pack-years (b = 0.14, p = 0.003). Stratifying by gender did not change the results (unadjusted b = 0.16 and p = 0.01 [men], and unadjusted b = 0.13 and p = 0.048 [women]).

Log blood harmane concentration was stratified into quartiles. ET cases with cancer were nearly twice as likely as controls without cancer to be in the highest vs. lowest log blood harmane concentration quartile (unadjusted OR = 1.72, 95% CI = 1.10 – 2.68, p = 0.017, and adjusted [age, gender, ever cigarettes smoker and cigarette pack-years] OR = 1.62, 95% CI = 1.02 – 2.60, p = 0.04).

## Discussion

Odds of cancer have been studied extensively in PD[9] but they have never been studied in ET. In general, there are very few data on medical co-morbidities in ET. With the current analyses, we begin to examine the epidemiologic evidence that patients with ET have increased odds of cancer. In this sample, the odds of cancer were increased by approximately 50% in ET cases compared with matched controls from the same source population. Colon cancer, in particular, was more prevalent in ET cases than controls. This is of interest because of established links between dietary heterocyclic amines and colon cancer.[6] Melanoma, which is more prevalent in PD,[9] was present in a similar proportion of ET cases and controls. In one other study,[13] cancer was marginally more common in ET families (23.3%) than in control families (17.2%), but this area has not otherwise been studied.

As previously demonstrated,[4] blood harmane concentrations were elevated in ET cases. Furthermore, they were most elevated in ET cases with cancer, among whom levels were double those of controls without cancer. Harmane is both tremorogenic and co-mutagenic, so that it is possible that elevated blood harmane concentration is a common determinant for both diseases (i.e., ET and cancer). One possibility is that differences in dietary harmane intake predispose to these diseases. Another possibility is that genetic differences lead to differences in the ability to metabolize dietary harmane, leading the accumulation of blood concentrations in individuals who then develop both ET and cancer. Each of these models requires further exploration. An alternative model is that treatment for cancer could precipitate ET and lead to increased blood harmane concentrations. However, this is less biologically-plausible and, furthermore, in the large majority of our cases, tremor preceded the cancer diagnosis by many years.

The molecular mechanisms that underlie the tremorogenic toxicity of harmane are unknown. One possibility is that harmane has acidifying properties that change neural membrane potentials.[14]

One issue is whether our controls were systematically selected for their overall-health, thereby leading us to underestimate the prevalence of cancer in our comparison group. Controls were selected to be tremor-free but were not selected with regards to overall health. Indeed, their overall morbidity, as assessed through Cumulative Illness Rating Scale scores and years since last hospitalization, was similar to that of our ET cases. In addition, using published estimates of cancer rates in New York State,[15] we calculated that the cumulative incidence of all cancers among persons living up to age 70 years is approximately 8.0% and, among persons living up to age 85 years, approximately 16.0%. These expected proportions are lower than or similar to those we observed in our controls (mean age = 66.6 ± 13.1 years), indicating that our comparison group was not selected to be cancer-free.

This study was cross sectional rather than longitudinal; we were not able to assess whether high harmane concentrations preceded either ET or cancer. Yet the study also has considerable strengths. It is the only study to examine the association between cancer and ET and the only

study to look at the relationships between ET, cancer and blood harmane concentrations. We used a large sample of ET cases and matched controls from the same source population.

In summary, the links between cancer in ET and especially between high blood concentrations of the tremorogenic co-mutagen harmane and cancer in ET deserve additional investigation.

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**Table 1**

Demographic and clinical characteristics of ET cases and controls

| | All ET Cases (N = 267) | All Controls (N = 331) | ET Cases with Harmane Data (N = 147) | Controls with Harmane Data (N = 187) |
|---|---|---|---|---|
| **Demographic and Clinical Characteristics** | | | | |
| Age in years | 68.5 ± 14.4 | 66.6 ± 13.1 | 67.7 ± 14.6* | 64.0 ± 13.8 |
| Female gender | 145 (54.3%) | 194 (58.6%) | 81 (55.1%) | 107 (57.2%) |
| Years of education | 14.8 ± 4.1 | 15.1 ± 3.5 | 15.0 ± 4.0 | 15.2 ± 3.6 |
| Current cigarette smoker | 16 (6.0%) | 30 (9.1%) | 10 (6.8%) | 18 (9.6%) |
| Ever cigarette smoker | 131 (49.1%) | 169 (51.1%) | 75 (51.0%) | 98 (52.4%) |
| Cigarette pack-years | 9.4 ± 19.6 | 11.0 ± 21.8 | 8.0 ± 17.9 | 9.6 ± 20.3 |
| Years since last hospitalization | 13.9 ± 20.5 | 14.6 ± 19.9 | 13.8 ± 18.9 | 14.5 ± 18.9 |
| Cumulative Illness Rating Scale Score | 5.6 ± 3.7 | 5.3 ± 3.7 | 5.4 ± 3.7 | 5.0 ± 3.8 |
| **Cancer** | | | | |
| Cancer | 66 (24.7%) | 55 (16.6%) | | |
| Colon* | 7 (2.6%) | 2 (0.6%) | | |
| Skin | 22 (8.2%) | 17 (5.1%) | | |
| Melanoma | 8 (3.0%) | 9 (2.7%) | | |
| Basal Cell Carcinoma | 9 (3.4%) | 4 (1.2%) | | |
| Squamous Cell Carcinoma | 4 (1.5%) | 3 (0.9%) | | |
| Basal and Squamous | 1 (0.4%) | 1 (0.3%) | | |
| Prostate | 8 (3.0%) | 7 (2.1%) | | |
| Breast | 18 (6.7%) | 22 (6.6%) | | |
| Uterine/ovarian | 4 (1.5%) | 4 (1.2%) | | |
| Lung | 1 (1.4%) | 0 (0.0%) | | |
| Other** | 6 (2.2%) | 3 (0.9%) | | |

* $p < 0.05$ compared with controls ($\chi^2$ and t tests).

** including bladder, brain, kidney, thyroid, and lymphoma/leukemia.

**Table 2**

Log blood harmane concentrations ($g^{-10}$/ml) by ET diagnosis and cancer

| | ET Cases (N = 147) | Controls (N = 187) |
|---|---|---|
| Participant with cancer (N = 58) | 0.87 ± 0.68 (N = 30) | 0.51 ± 0.64 (N = 28) |
| Participant without cancer (N = 276) | 0.54 ± 0.60 (N = 117) | 0.43 ± 0.69 (N = 159) |

For comparison of all four groups, ANOVA F = 3.90, p = 0.009.

In Tukey post hoc comparisons:
ET cases with cancer vs. controls without cancer, p = 0.005.
ET cases with cancer vs. controls with cancer, p = 0.16.
ET cases with cancer vs. ET cases without cancer, p = 0.07.
Controls with cancer vs. controls without cancer, p = 0.56.

Log blood harmane concentration was higher in ET cases vs. controls (0.61 ± 0.63 $g^{-10}$/ml vs. 0.44 ± 0.68 $g^{-10}$/ml, p = 0.02) and in participants with vs. without cancer (0.70 ± 0.68 $g^{-10}$/ml vs. 0.48 ± 0.65 $g^{-10}$/ml, p = 0.02).