Studies on circadian rhythm disturbances and melatonin in delirium

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Citation for published version (APA):

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The tryptophan depletion theory in delirium: not confirmed in elderly hip fracture patients

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Psychosomatics. 2012 May; 53(3):236-43
Abstract

Background: The tryptophan depletion theory assumes that low tryptophan levels are present in delirium and are a contributing factor in the pathophysiology of delirium, possibly because tryptophan is converted in kynurenine.

Aims: To compare plasma tryptophan and kynurenine levels in hospitalised patients with and without delirium.

Methods: Repeated plasma samples were prospectively collected in hip fracture patients, aged 65 years and older. The presence of delirium was assessed daily. The associations of a delirious state and tryptophan, kynurenine, and the kynurenine/tryptophan ratio measured in samples taken ‘before delirium’, ‘during delirium’, and ‘after delirium’ were analysed with linear mixed models.

Results: A total of 469 samples from 140 patients were collected. Adjusted for the days on which they were drawn, there was no difference for all three measured factors in patients with and without delirium.

Conclusions: The results do not confirm the tryptophan depletion theory.
Tryptophan in delirium

Introduction
Delirium is a frequent neuropsychiatric syndrome in acutely hospitalised elderly patients. Due to its associated high morbidity and mortality, delirium has severe consequences for these patients (1). A safe and effective prophylactic or therapeutic treatment that could improve its course and consequences is needed. To reach that goal, a better understanding of the pathophysiology of delirium is necessary. The pathophysiology is multifactorial, and disturbances of one or more neurotransmitter systems is most widely hypothesised as the main cause of delirium (2).

Tryptophan has become a focus in delirium research because low levels of tryptophan were found in patients with delirium (3, 4). Those findings suggested that plasma tryptophan depletion is present in delirious patients and therefore may play a role in the development of delirium. This led to the tryptophan depletion theory, which states that the depletion of tryptophan in the blood leads to a central serotonin deficiency that contributes to the development of a delirious state. Also induction of the enzyme indoleamine 2,3 hydroxylase (IDO) decreases serotonin levels and may contribute to a serotonin deficiency as well (5) (Figure 1).

Figure 1: Tryptophan metabolism

The neurotransmitter serotonin is derived from the large neutral amino acid (LNAA) tryptophan, and the availability of this amino acid is essential for the synthesis of serotonin (6, 7). Tryptophan is able to pass through the blood-brain barrier, and once inside the central nervous system (CNS), it is converted via two main routes (Figure 1). First, it can be hydroxylised by the rate-limiting enzyme tryptophan hydroxylase (TPH) to form serotonin (6). Serotonin can be further metabolised to form melatonin, a hormone that regulates the biological clock (7). Second, tryptophan can be metabolised by the rate-limiting enzyme IDO to form kynurenine (8). Kynurenine can be further metabolised into kynurenic acid (an NMDA receptor antagonist), quinolinic acid (an excitatory neurotoxin and an NMDA receptor agonist), niacin (vitamin B3), and finally in nicotinamide adenine dinucleotide (NAD+), a co-enzyme that is involved in redox reactions. Serotonin has various functions, including the regulation of mood, sleep, and some cognitive functions,
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including memory and learning. Dysregulation of this neurotransmitter pathway could lead to symptoms that are also commonly observed in delirium.

The aim of this study was to test whether an association exists between low plasma tryptophan levels, high plasma kynurenine levels, IDO activity and delirium in our cohort of elderly hip fracture patients. Plasma tryptophan and kynurenine levels were measured in blood samples taken before, during and after delirium and compared to samples taken from non-delirious patients.

Methods

Patients
This was a prospective cohort study conducted from May 2005 through August 2008 that included patients with a hip fracture who were aged 65 years and older (see Figure 2). They were all scheduled for surgery in the Department of Orthopaedic Surgery or Trauma Surgery of the Academic Medical Centre in Amsterdam, The Netherlands. Informed consent was obtained from all patients or from substitute decision-makers in cases of cognitive impairment. Patients were excluded if they were unable to speak or understand Dutch or English. The institutional Medical Ethics Committee approved the study.

Figure 2: Patient and blood sample selection

Excluded patients:
- No informed consent for the study (n=59)
- No informed consent for blood withdrawal (n=75)

Included patients (n=140)

Non-delirious patients
n=69 (46%)

Delirious patients
n=71 (51%)

Total amount of blood samples (n=469)

Excluded blood samples
n=10: failure to determine tryptophan and kynurenine
n=11: CAM missing on day of blood withdrawal
n=10: patient in between delirious episode

Blood samples of non-delirious patients
(n=219)

Blood samples of delirious patients
(n=219)

Before delirium (n=26)
During delirium (n=133)
After delirium (n=58)
**Procedures**

Two geriatricians, a fellow in geriatric medicine, and four research nurses trained in geriatric medicine collected the demographic and clinical data. The presence or absence of delirium was scored during weekdays separately by a physician and a nurse using the Confusion Assessment Method (CAM) (9). We based the diagnosis on our psychiatric examination of the patient, medical and nursing records, including the Delirium Observation Screening (DOS) scale (10), and information given by the patient’s closest relative. When the diagnosis of delirium was doubtful, the patient was discussed with the geriatric consultation team to gain a consensus. For subtyping, we used the Delirium Symptom Interview with the cut-off scores described by Liptzin et al. (11).

Possible confounding factors, including demography, fracture characteristics, type of anaesthesia, type of surgery, and peri- and postoperative complications (including infectious events) were registered for all patients. The severity and number of comorbidities were scored with the Charlson comorbidity index (12). Pre-existent global cognitive functioning was based upon anamnesis, medical history, and the Informant Questionnaire on Cognitive Decline short form (IQCODE-sf) (13). The informant was asked to recall the situation two weeks prior to the hip fracture and compare it with the situation ten years earlier. Patients with a mean score of 3.9 or higher were considered to have global cognitive impairment (14).

To measure pre-existing physical functionality, we asked patients (or their closest relative in cases of cognitive impairment) to complete the 15-item Katz Activities of Daily Living (ADL) scale based on the situation two weeks prior to the hip fracture (15). Patients with a score of 7 or more assisted ADL items of the 15 ADL items assessed were considered functionally impaired.

A maximum of four blood samples were collected during the hospital stay under similar conditions around 11:00 AM using ethylene diamine tetraacetic (EDTA) acid-containing tubes. Samples were taken during weekdays whenever patients were available for venipuncture. Blood was kept on ice after withdrawal. Plasma was obtained by centrifugation for 15 minutes at 1780 g at 4°C, and the aliquots were stored at -80°C. Concentrations of kynurenine and tryptophan were determined via their natural fluorescence using an isocratic, reversed-phase HPLC system (Agilent) and an FP-2020 fluorescence detector (Jasco). The analytical column consisted of a 250 x 2.1 mm i.d. column packed with 5-μm particles of GraceSmart RP-18 (Grace Davison Discovery Sciences), which was protected by a guard cartridge column (4.0 x 2.0 mm i.d.) containing Phenomenex C18 material. Absolute concentrations were calculated using 1-methyltryptophan as an internal standard. Excitation and emission wavelengths for kynurenine (retention time 3.8 min) were 363 and 500 nm and for tryptophan (retention time 6.7 min) and 1-methyltryptophan (retention time 10.3 min) were 285 nm and 365 nm, respectively (16). The kynurenine/tryptophan ratio was calculated to estimate the activity of the enzyme indoleamine 2,3 dioxygenase (IDO) (17).
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Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 15.0 was used for data analysis. We tested for differences in the characteristics of patients with and without delirium using Student’s t-tests, Mann-Whitney tests, and Chi-squared Tests. Variables that were not normally distributed were expressed as median scores and inter-quartile ranges. Differences in tryptophan, kynurenine and the kynurenine/tryptophan ratio in different subtypes were analysed using ANOVA. A two-tailed criterion of $p<0.05$ was considered statistically significant.

To examine the association of tryptophan, kynurenine and the kynurenine/tryptophan ratio with delirium state, we used a linear mixed models approach with these factors as dependent variables in four separate analyses. The state of delirium was classified into four categories based on the CAM scores of the patient at the moment the sample was taken. The samples from patients in the non-delirious group were all categorised as (1) ‘non-delirious’, and the samples from patients in the delirious group were categorised as (2) ‘before delirium’, (3) ‘during delirium’ or (4) ‘after delirium’. Patients who were between delirious episodes (the ones who developed a delirium after recovering from an initial period of delirium) were left out of the analyses. Patient number was taken as a random effect, and the day of sampling and the delirious state were taken as fixed effects. Samples were drawn between the day before surgery and 8 days post surgery. The goodness of fit of the mixed models was inspected by assessing the distribution of the residuals. Pre-operative samples were compared using Student’s t-tests.

Table 1: Characteristics of delirious and non-delirious patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Delirium (n=71)</th>
<th>No delirium (n=69)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>85.1 (6.7)</td>
<td>82.5 (7.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>17 (24)</td>
<td>25 (36)</td>
<td>0.11</td>
</tr>
<tr>
<td>Living at home (%)</td>
<td>34 (48)</td>
<td>60 (87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-admission Katz score (%)</td>
<td>8 (6-11)</td>
<td>3 (1-6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-admission cognitive impairment (%)</td>
<td>47 (66)</td>
<td>11 (16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Charlson comorbidity index</td>
<td>6.3 (1.8)</td>
<td>4.9 (2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fracture characteristics (%)</td>
<td></td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>Femoral neck fracture</td>
<td>30 (42)</td>
<td>33 (48)</td>
<td></td>
</tr>
<tr>
<td>Intertrochanteric fracture</td>
<td>37 (52)</td>
<td>30 (44)</td>
<td></td>
</tr>
<tr>
<td>Spinal anesthesia (%)</td>
<td>22 (31)</td>
<td>24 (35)</td>
<td>0.22</td>
</tr>
<tr>
<td>Type of surgery (%)</td>
<td></td>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>Internal fixation</td>
<td>44 (62)</td>
<td>41 (59)</td>
<td></td>
</tr>
<tr>
<td>Hip replacement</td>
<td>27 (38)</td>
<td>28 (41)</td>
<td></td>
</tr>
<tr>
<td>Complication (%)-total</td>
<td>35 (49)</td>
<td>21 (31)</td>
<td>0.03</td>
</tr>
<tr>
<td>Length of stay – days</td>
<td>14 (7-222)</td>
<td>10 (6-14)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Mean values (SD) are given for continuous variables with a normal distribution,
Median values (inter-quartile ranges) are given for variables that are not normally distributed
Results
During the study period, 304 eligible patients aged 65 years and older were admitted. A total of 89 patients were excluded due to an absence of informed consent and 75 included patients because they did not consent to blood withdrawal. Included patients were older than excluded patients (83.8 vs. 81.5 years, \( p = 0.007 \)). The frequency of male gender was not significantly different than the frequency of female gender (30% vs. 26% \( p = 0.46 \)). A total of 469 samples were drawn from 140 patients. Of these samples, 31 were not used; in 10 samples, the determination of both tryptophan and kynurenine failed; in 11 samples, the CAM score was missing for the day of withdrawal; and in 10 other samples, patients were between a delirious episode.

Figure 3: Tryptophan levels in patients (A) without delirium, and (B) before, (C) during and (D) after delirium in the two days before and the nine days after surgery.
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A total of 219 samples were taken from delirious patients (28 samples before the delirious episode, 133 samples during the delirious episode, and 58 after the delirious episode) and 219 samples were from non-delirious patients (figure 2). The characteristics of the 140 patients, including 71 (51%) with delirium, are presented in Table 1. Delirious patients were older (85 vs 83 years, p=0.03), experienced pre-existing cognitive and functional impairment more often and were more likely to live in either a retirement home or a nursing home than patients without delirium (p<0.001). There was also a difference in the total number of other complications, namely in the frequency of decompensated heart failure, atrial fibrillation, anaemia and infections (49% vs 31%, p=0.03) among the patients with and without delirium. The median length of stay was 14 days for delirious patients and 10 days for patients without delirium (p=0.01).

Figure 4: Tryptophan levels in patients (gray) who never had delirium (n = 219) and in patients (black) who had delirium at any stage (n = 219); mean with interquartile range is shown
Figure 5: Tryptophan levels in patients (black) without delirium ($n=219$ blood samples), (dark gray) before ($n = 28$ blood samples), (light gray) during ($n = 133$ blood samples), and (gray) after delirium($n = 58$ blood samples) in the 2 days before and the 9 days after surgery.

Plasma tryptophan levels in relation to the postoperative day for all samples taken from patients without delirium or before, during or after delirium are shown in figure 3. Figure 4 shows tryptophan levels in patients who never had delirium and in patients who had delirium at any stage. Figure 5 shows tryptophan levels in patients without delirium, before, during, and after delirium. The linear mixed model showed that tryptophan, kynurenine and the kynurenine/tryptophan ratio were not significantly influenced by the delirious state. For all three measured factors, the samples drawn from patients without delirium were not significantly different from the samples drawn from the patients with delirium. No significant association was found between an aberrant tryptophan or kynurenine level or IDO activity in relation to delirium. In preoperative samples, we found borderline higher kynurenine levels in patients with delirium compared to patients without delirium ($2.91$ (SD $1.33$) vs. $2.26$ (SD $0.96$), $p=0.07$). Additionally, in the
preoperative samples, the kynurenine/tryptophan ratio was higher in delirious patients than in non-delirious patients (79.3 (SD 42.8) vs. 57.6 (24.7), p=0.012).

**Discussion**

In this study, we tested whether low levels of plasma tryptophan and/or high levels of kynurenine significantly relate to delirium in acutely admitted elderly hip fracture patients. We found no differences in the concentration or course of plasma tryptophan and plasma kynurenine levels between delirious and non-delirious patients. Thus, we cannot confirm the suggested role of plasma tryptophan depletion in delirium in elderly patients when taking into account all samples. In preoperative samples, the ratio of the kynurenine/tryptophan was significantly higher in patients that experienced delirium. A higher kynurenine/tryptophan ratio indicates an increased activity of IDO. This could lead to a central serotonin deficiency that might contribute to the development of delirium. Furthermore, conversion of tryptophan to kynurenine could lead to an excess of quilonine acid (18). This acid is a selective agonist of the glutamate receptor, and glutamate is one of the neurotransmitters suggested to be involved in the development of delirium (19).

**Table 2:** Studies (incl. the present results) of plasma tryptophan levels in delirious and non-delirious patients

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Type of patients (n) / (% of delirious patients)</th>
<th>Moment of blood withdrawal</th>
<th>Tryptophan levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Jonghe (2010)</td>
<td>Elderly hip fracture patients (n=140) (51%)</td>
<td>Before, during and after delirium</td>
<td>N</td>
</tr>
<tr>
<td>Robinson (2008)</td>
<td>Surgery patients with an anticipated postoperative ICU admission (n=46) (43%)</td>
<td>On postoperative day 2</td>
<td>↓</td>
</tr>
<tr>
<td>van der Cammen (2006)</td>
<td>Hospitalized delirious AD patients (n=17) Community based non delirious AD patients and community based healthy controls (n=46) (27%)</td>
<td>During admission or at home</td>
<td>N</td>
</tr>
<tr>
<td>van der Mast (2000)</td>
<td>Elective cardiac surgery patients (n=269) (13%)</td>
<td>Preoperatively and on postoperative day 1</td>
<td>↓</td>
</tr>
<tr>
<td>van der Mast (1991)</td>
<td>Delirious postcardiotomy patients (n=7) Postoperative controls and healthy controls (n=29) (19%)</td>
<td>Within 5 days of surgery</td>
<td>↓</td>
</tr>
</tbody>
</table>

n=number of inclusions, ICU = Intensive Care Unit, AD=Alzheimers Disease, N= no difference between delirious and non-delirious patients, ↓= levels of Tryptophan were lower in the delirious patients.
Six studies have investigated plasma levels of tryptophan in delirious and non-delirious patients. In two of them, only the ratios of tryptophan to other LNAAs are mentioned (20, 21). The other four studies showed contradictory results (Table 2). In three studies, lower plasma levels of tryptophan were found in delirious patients (3, 22, 23). One of these studies used solely preoperative blood samples. Our results are in accordance with one study, which included medical patients and also found no difference in the plasma levels of tryptophan between delirious and non-delirious patients (22). An explanation for these seemingly contradictory results may be the differences in patient groups and the variety of surgical approaches. All three studies in which lower tryptophan levels were found during delirium included abdominal, cardiac and thoracic surgery. None of the studies measured plasma kynurenine levels. In five studies, the ratios of tryptophan to other LNAAs were determined (3, 20-23). This was based on a theory that LNAAs are transported through the blood-brain barrier by a common LNAA transporter. This transporter has equal affinity for all LNAAs and is usually highly saturated. Thus, when the concentration of one LNAAs increases, the central nervous system entry of other LNAAs declines (24-26). Normal or lower tryptophan levels in the blood with a concomitant rise in the other LNAAs may therefore result in a decreased serotonergic function, and in a normal or increased dopaminergic and noradrenergic function. The results from these five studies are inconclusive. Two studies found no difference in the tryptophan/LNAA ratio between delirious and non-delirious patients (21, 22), two studies found a lower tryptophan/LNAA ratio in delirious patients compared to non-delirious patients (3, 23), and one study found that either very high or very low ratios of tryptophan/LNAA (20) were associated with a delirious state. There are some limitations to the present study. The main limitation is that we measured tryptophan and kynurenine from peripherally obtained blood, whereas delirium takes place in the CNS. Other studies have indicated that blood tryptophan levels correspond to cerebrospinal fluid (26, 27). In addition, 140 of the 304 patients in the original study were included in this study because a number of patients did not consent to multiple blood withdrawals as well as to the collection of demographic information. The strength of our study is the fact that we analysed multiple blood samples per patient. This gave us the opportunity to compare levels before, during and after delirium and, specifically, to compare them preoperatively. Furthermore, the size of our cohort is large compared to the other studies showed in table 2. In general, the patients included in this study represent a real-life elderly hospital population. Patients were included regardless of comorbidities, age, or pre-existing cognitive or functional state. Many of our patients would usually be excluded from clinical research, even though they constitute a substantial part of daily clinical practice. Another strength was that information about age, cognitive status and functional impairment were available.
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**Conclusion**

The results do not confirm the hypothesised association between plasma tryptophan, plasma kynurenine and delirium. These data even contradict the tryptophan depletion theory, which assumes the presence of lower tryptophan levels in delirious patients. Delirium takes place in the CNS, and future measurements in cerebrospinal fluid would add valuable information.
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