Research Article

Vitamin K Concentration and Cognitive Status in Elderly Patients on Anticoagulant Therapy: A Pilot Study

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Received 29 May 2019; Revised 20 November 2019; Accepted 26 December 2019; Published 19 February 2020

Objectives. Recent studies have suggested that vitamin K may exert significant effects on the central nervous system. The present study investigates the relationship between vitamin K plasmatic levels and cognitive functions in elderly patients on oral anticoagulant therapy (OAT).

Design. At the Thrombosis Centre of Haematology, "Sapienza" University of Rome, 85 patients on OAT, aged between 75 and 92, were randomly enrolled in the study. Patients were on OAT with vitamin K antagonists (VKAs). Vitamin K1 concentrations were determined using standardized High-Performance Liquid Chromatography (HPLC). Cognitive functions were assessed using the Milan Overall Dementia Assessment (MODA).

Results. MODA scores are positively correlated to vitamin K1 concentration. Patients with vitamin K1 below 0.100 μg/L and between 0.100 and 0.400 μg/L showed a mean MODA score of 79 ± 5 and 82 ± 3, respectively. Patients with vitamin K1 above 0.400 μg/L had a significantly greater MODA score (89 ± 1). After binning the data into bicentiles, MODA scores are shown to be linearly dependent on vitamin K1 concentrations (p < 0.001). Even long-term OAT (>10 years) does not affect MODA scores. Education seems to exert a greater role on the cognitive status in comparison with aging.

Conclusions. The study shows a positive association between vitamin K1 concentration and cognitive status in elderly patients (≥75 years) on OAT. The relationship between vitamin K1 concentration and MODA scores is described by a linear model. Cognitive status is not influenced by the duration of OAT but by the years of education.

1. Introduction

Vitamin K includes a group of lipid-soluble molecules based on a common 2-methyl-1,4-naphtoquinone ring but with a different side chain at the 3-position [1–3]. Phylloquinone (2-methyl-3-phytyl-1,4-naphtoquinone), or vitamin K1, is mainly found in vegetables [1, 2]. A second class of vitamers is the menaquinones (MK). MKs are produced by bacteria as provitamins and present unsaturated 5-carbon (prenyl) side chains at the 3-position [1–3]. One of the menaquinones, MK-4, is not commonly produced by bacteria, but synthesised from vitamin K1 [2, 3]. Vitamin K has a key role in the carboxylation of glutamate residues in proteins leading to gamma-carboxyglutamate (Gla) residues. Gla residues bind calcium and are essential for the activity of the so-called Gla proteins. Gla-proteins are involved in blood coagulation (coagulation factors II, VII, IX, and X and protein C, S, and Z), as well as in bone and vascular metabolism (osteocalcin and growth arrest-specific protein 6, Gas-6, respectively) [1, 2].

Vitamin K has been found in rat and human brain. Here, the most common vitamer is MK-4 [3, 4]. These findings have contributed to an increasing interest in the role of vitamin K in cognitive impairment and neurodegenerative diseases. Recent studies seem to suggest that vitamin K may exert some significant effects on the central nervous system.
Different studies, however, show discrepancies on whether vitamin K would act as a protection factor against cognitive impairment or would contribute to neurodegeneration and loss of cognitive functions. On the one hand, Gas-6 helps the development and survival of many central nervous system’s cellular lines [5]. On the other hand, Gas-6 has been shown to inhibit, in a concentration-dependent manner, the overexpression of Tyro3. Tyro-3 is a tyrosine kinase which reduces β-amyloid production [6].

Vitamin K may influence cognitive functions through other vitamin K-dependent proteins. Protein S, for example, seems to act as a protective factor during ischemic damage [7] and as a preserving factor for the integrity of the blood-brain barrier [8].

Vitamin K seems to regulate multiple enzymes involved in the sphingolipid biosynthesis [3, 9, 10]. Sphingolipids are present in high concentrations in brain tissues where they are important membrane constituents and major signalling molecules [10]. Their altered metabolism is involved in the pathological mechanisms leading to β-amyloid accumulation [11] as well as in the development of cognitive impairment in murine models [12, 13].

In the last five years, clinical observational studies have started investigating the impact of vitamin K on cognitive functions [14–16]. An increase in the dietary vitamin K intake has been shown to improve cognitive performance in geriatric patients [14, 15]. This is confirmed by the fact that a decrease in serum concentrations of vitamin K is associated with deterioration in verbal episodic memory [16]. A case control study conducted by our group on the relationship between vitamin K and the percentage of time in therapeutic range (TTR%) confirmed that subjects with low vitamin K1 plasmatic concentrations (below 0.060 μg/L) were more likely to show signs of neurodegenerative diseases [17].

The impact of vitamin K on cognitive functions is indirectly confirmed by the effects of vitamin K antagonists (VKAs) which interfering with vitamin K metabolism seem to worsen neurodegenerative diseases. Rats treated with VKAs (either warfarin or acenocoumarin). Table 1 shows the distribution of patients by therapeutic indication. OAT quality was assessed by TTR%. TTR% was calculated according to the Rosendaal method [19], considering PT/INR values over the last 9 months. The PT/INR target was 2.5 (range: 2.0–3.0) in patients with atrial fibrillation, deep venous thrombosis, pulmonary embolism, and arterial disease [17]. The PT/INR target was 3.0 (range: 2.5–3.5) in those with mechanical heart prosthesis [17].

2. Materials and Methods

2.1. Patients. At the Thrombosis Centre of Haematology, “Sapienza” University of Rome, 625 OAT patients older than 75 years are regularly followed as outpatients. Of these, 456 patients are on OAT with VKAs or with direct oral anticoagulants (DOACs).

From 1 November 2016 to 15 December 2017, a random sample of patients has been selected for the study. Of 456 possible patients, 105 were chosen and gave their informed consent to participate in the study. The selection method was that the first patient who underwent a prothrombin time/international normalized ratio (PT/INR) test or other coagulative tests for each hour between 8 a.m. and 11 a.m., from Monday to Wednesday, was enrolled. Patients were excluded if their alcohol assumption was higher than a single serving/day, or if they showed evidences of an active hepatopathy. We excluded 12 subjects who were on OAT with DOACs. This is due to the fact that the limited number of patients treated with DOACs would not allow any statistically significant analysis. The final cohort was made up of 85 patients.

Education background significantly varied ranging from less than 4 years of studies (lack of primary school diploma) to more than 17 years (equivalent to university degree).

These 85 patients (52 males and 33 females) were aged between 75 and 92 (mean = 83.4 ± 0.4) and were on OAT with VKAs (either warfarin or acenocoumarin). Table 1 shows the distribution of patients by therapeutic indication. OAT quality was assessed by TTR%. TTR% was calculated according to the Rosendaal method [19], considering PT/INR values over the last 9 months. The PT/INR target was 2.5 (range: 2.0–3.0) in patients with atrial fibrillation, deep venous thrombosis, pulmonary embolism, and arterial disease [17]. The PT/INR target was 3.0 (range: 2.5–3.5) in those with mechanical heart prosthesis [17].
evaluated through participation in the Vitamin K External Quality Assessment Scheme (KEQAS) [20].

2.3. Cognitive Assessment. Different tests are currently available to investigate cognitive functions and abilities. The most widely used test is the Mini Mental State Examination (MMSE) [21]. This is a rapid 30-point questionnaire which does not require any training and provides reliable results in the diagnosis of cognitive impairment. MMSE, however, has a significantly low sensitivity in identifying Mild Cognitive Impairment (MCI) [22]. Therefore, we decided to use the Milan Overall Dementia Assessment (MODA) [22–25]. MODA is a test with a significantly higher sensitivity in the detection of signs of MCI [22]. Cognitive evaluation of the patients was performed after the venous puncture.

The MODA test is based on a three stages examination:

1. **Autonomy Scale.** This is found altered only in the most deteriorated patients

2. **Orientation Enquiry.** This part of the test investigates 4 different orientation areas: temporal, spatial and personal orientation, and family relationships

3. **Neuropsychological Tests.** This investigation evaluates the levels of attention, memory, intelligence, space cognition, visual perception, and language

We used age and years of school correction tables to obtain the final score for each patient. According to the protocol’s instructions, cognition values were considered normal if the score was ≥85/100 and pathological below 80/100. Values between 80 and 85 are considered borderline. Even if some authors quote MODA score up to one decimal place [22, 24, 25], we took the error on the MODA to be ±1. This was performed so as to avoid any potential overestimation in the precision of the method.

2.4. Data Analysis. Data was retrieved and statistically analysed using a specific program, which was developed by one of the authors using Python programming language. The program evaluated the correlation between MODA scores and vitamin K1. Data were analysed considering a threshold value for the vitamin K1 of 0.060 μg/L. This value is the LLOQ for the HPLC method [20]. Advanced analysis was conducted binning the data according to the vitamin K1 centiles (0.100–0.199 μg/L, 0.200–0.299 μg/L, and so on) and bicentiles (0.100–0.299 μg/L, 0.300–0.499 μg/L, and so on). For each bin, the MODA score and the corresponding error were taken to be the mean and the standard error, respectively. When binning the data, vitamin K concentrations below 0.100 μg/L were excluded. This was performed so as to remove any potential bias among centiles. The centile from 0 to 0.100 μg/L would be significantly different from the others. The lower detection limit for vitamin K1 being 0.060 μg/L, this centile would not include any values coming from the first half of the centile itself. Furthermore, the HPLC method is potentially affected by lower precision for concentrations below 0.100 μg/L [20]. A further analysis was conducted according to whether the patients had vitamin K1 plasmatic levels below or above 0.400 μg/L.

Data analysis was performed using Pearson’s chi-squared test. The χ² test was implemented into our custom-made Python program. The relationship between MODA scores and vitamin K1 levels was evaluated considering the reduced χ² (χ²r). For a system with ν degrees of freedom, the goodness of the fit is proved for χ²r = 1. The other condition to test is whether χ² is within ±2 standard deviations of the mean, that is, within the range ν ± 2√2ν. The null hypothesis of a relationship between the two quantities is further tested by the cumulative probability function (P). The significance threshold being set to less than 0.001 (p < 0.001), P needs to be greater than p so as to not reject the null hypothesis [26, 27]. Further data analysis on the goodness of the linear fits is performed assessing the correlation with the Pearson correlation coefficient (r). A value for r of 1 shows that the linear regression predictions perfectly match the observed data [27, 28]. We evaluated also the correlations between, on the one hand, MODA scores and vitamin K1 levels, and, on the other hand, education, age, OAT length, and comorbidities. Also here, we set a threshold value for the vitamin K1 of 0.060 μg/L, corresponding to the LLOQ for the HPLC method [20].

3. Results

The characteristics of all the patients are reported in Table 1. Out of the 85 patients on OAT, 52 were males and 33
Table 2: MODA scores and percentage of time in therapeutic range (TTR%) according to the plasmatic levels of vitamin K1. For both the MODA score and the TTR%, the value and the associated error are the mean and the standard error, respectively.

<table>
<thead>
<tr>
<th>Vitamin K1 (μg/L)</th>
<th>&lt;0.060</th>
<th>&lt;0.100</th>
<th>0.100–0.400</th>
<th>&gt;0.400</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTR (%)</td>
<td>59 ± 4</td>
<td>57 ± 6</td>
<td>66 ± 4</td>
<td>61 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>MODA score</td>
<td>81 ± 4</td>
<td>79 ± 5</td>
<td>82 ± 3</td>
<td>89 ± 1</td>
<td>—</td>
</tr>
<tr>
<td>Pts (number)</td>
<td>19</td>
<td>8</td>
<td>32</td>
<td>26</td>
<td>85</td>
</tr>
</tbody>
</table>

Table 3: MODA scores according to patients’ characteristics.

<table>
<thead>
<tr>
<th>MODA score</th>
<th>&gt;90</th>
<th>90–85</th>
<th>84–80</th>
<th>79–70</th>
<th>&lt;70</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>15</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>52</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75–80</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>80–84</td>
<td>15</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>85–89</td>
<td>10</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>&gt;90</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Education (years of school)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>4–7</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>8–12</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>13–16</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>&gt;16</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

females. Out of the entire cohort, 71 (84%) patients were aged 80 years or above. Atrial fibrillation was the most common diagnosis among patients (49%) followed by mechanical heart prosthesis (34%) and by deep venous thrombosis or pulmonary embolism (8%). Patients enrolled in the present study had been receiving OAT for at least 18 months and 65 (76%) patients for more than 10 years.

As shown in Table 2, vitamin K1 concentration was below 0.100 μg/L in 58 patients. Considering the patients with vitamin K1 concentration above 0.100 μg/L, 32 of them had vitamin levels between 0.100 and 0.400 μg/L; the remaining 26 had vitamin concentrations above 0.400 μg/L.

TTR% tends to increase with vitamin K concentration (Table 2). Patients with vitamin K levels below 0.100 μg/L had a mean TTR% of 57 (±6) %. For vitamin K levels between 0.100 and 0.400 μg/L, patients showed a slight increase in the mean TTR% (82 ± 3). Patients with vitamin K above 0.400 μg/L had a significantly greater mean MODA value (89 ± 1). Cognitive functions seem to be related to vitamin K1 levels by a logarithmic-like function with MODA scores dispersion decreasing for increasing levels of vitamin K1 (Figure 1). This is confirmed by further analysis dividing the patients according to vitamin K1 concentration centiles (Table S1 in the Supplementary Material). For the low concentration centiles, MODA scores show a large variability, that is, standard deviation (SD) > 10. An increase in vitamin K1 above 0.400 μg/L corresponds to a decrease in SD below 10 (see also Figure 1). Therefore, 0.400 μg/L was taken to be our threshold value. Given that some centiles have a very small number of patients, further analysis was performed binning the data into bicentiles. This is pictorially represented in Figure 2 showing a linear relationship between vitamin K1 concentrations and cognitive capacities. The goodness of the fit is confirmed by χ² and χ², being 1.21 and 0.3, respectively. Here, χ² is within 2 standard deviations of the mean, and χ² is close to the ideal value of 1. The hypothesis of a linear relationship between vitamin K1 levels and cognitive functions is statistically significant (p < 0.001) as shown by the calculated value for the cumulative probability function P = 0.85 being close to the ideal value of 0.5 [26, 27]. The r value of 0.94 shows a high positive correlation between vitamin K levels and MODA scores. This further
The results are particularly remarkable considering that there do not seem to be any clear relations between, on the one hand, comorbidities and, on the other hand, vitamin K plasmatic concentration and cognitive functions (see Tables S2 and S3 and in Figures S5–S8 in the Supplementary Material).

4. Discussion

The role of vitamin K in coagulation processes has been extensively studied and characterised [29]. Recent studies have suggested that the vitamin may also play a key role in cognitive performance [13–16]. Studies on animal models have suggested that vitamin K may be involved in memory consolidation. Rats fed with a low vitamin K diet presented an altered sphingolipid profile in their hippocampus, which is the key cerebral region for memory [13]. Clinical studies on human subjects confirmed a positive association between vitamin K levels and MODA scores. This is supported by the normalised residuals being randomly distributed around the zero and not showing any clear structures. The value for the Pearson correlation coefficient of 0.94 shows a very high positive correlation between MODA scores and vitamin K levels, thus further confirming that the linear regression is a good model for the data.
OAT with VKAs. Previous studies mainly focused on animal models or human tissues analysed post mortem [30]. Some investigations had evaluated the relationship between vitamin K and cognitive functions in human subjects [30]. These studies, however, relied on simple cognitive tests, such as the MMSE. Here, the cognitive functions of the patients have been assessed using a more accurate test, that is, the MODA score. The MODA is a complex tool which allows evaluating the levels of attention, memory, intelligence, space cognition, visual perception, and language. The MODA has shown significantly high sensitivity in the detection of MCI signs [22].

This study shows a positive association between plasmatic levels of vitamin K1 and cognitive status in patients older than 75 years on OAT. There is a linear relationship between vitamin K1 concentration per bicentile and cognitive functions as measured by the MODA score. The MODA values tend to be borderline or pathological for vitamin K1 concentrations smaller than 0.400 μg/L. This would support the role of vitamin K in ensuring and preserving cognitive functions. Vitamin K1 concentration of 0.400 μg/L may be a threshold value below which important signs of cognitive impairment appear. The reasons behind a positive impact of vitamin K on cognitive status may be explained with the fundamental role of this vitamin on the synthesis of protein S [7, 8] and sphingolipids [3, 9, 10]. These molecules have been shown to have a key role in ensuring brain signalling and metabolism [7–10, 30].

Interestingly, the cognitive capacities do not correlate with the anticoagulant therapy and with its duration. These findings would suggest that long-term OAT can be prescribed to elderly patients without any major impacts on their cognitive status.

Education seems to have a greater influence on cognitive functions in comparison with aging. Despite applying correction factors for both education and aging, the former still seems to influence MODA scores. Only 9% of subjects with high education had a MODA score below 80, whereas low education levels are associated to a pathological MODA value in 41% of the patients.

Further studies on larger cohorts of patients are needed so as to confirm the findings of this investigation.

5. Conclusions

This pilot study has shown a positive correlation between vitamin K1 concentration and cognitive status. This is in particular true for vitamin K1 concentrations above 0.400 μg/L. A multicentre research is strongly recommended so as to confirm the data of the present investigation.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

This work is a pilot study which was performed as part of the authors’ habitual work at the University of Rome, Sapienza.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

L. Alisi interviewed the patients and collected their data. C. Cafolla conducted the data analysis and wrote the paper. A. Gentili performed the HPLC experiments. S. Tartaglione collected the blood samples. R. Curini contributed to the design of the study. A. Cafolla designed and supervised the study. Ludovico Alisi and Clodomiro Cafolla contributed equally to this work.

Supplementary Materials

Supplementary materials include Figure S1 (MODA score vs. age of the patients), Figure S2 (vitamin K levels vs. therapy length), Figure S3 (MODA score vs. therapy length), Figure S4 (MODA score vs. education), Table S1 (mean values and standard deviations of MODA scores for vitamin K1 centiles), and Table S2-3 and Figures S5-8 (MODA scores and vitamin K levels vs. comorbidity). (Supplementary Materials)

References


