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Association of hsCRP and vitamin D levels with mild cognitive impairment in elderly type 2 diabetic patients

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Abstract

Aims
The aim of the study was to determine the serum levels of 25-hydroxyvitamin D and high-sensitivity C-reactive protein (hsCRP) in elderly diabetic patients with and without mild cognitive impairment (MCI) and to examine factors (including 25-hydroxyvitamin D and hsCRP) associated with MCI in elderly patients with type 2 diabetes (T2DM).

Methods
A total of 194 T2DM elders were evaluated: 62 subjects with MCI and 132 controls. Data was collected concerning biochemical parameters and biomarkers.

Results
HsCRP concentration was elevated and 25-hydroxyvitamin D level was decreased in MCI patients to controls. HsCRP level was negatively correlated with 25-hydroxyvitamin D level and with MoCA score, and highly correlated with HbA1c level. The multivariable analysis indicated that less years of formal education, previous CVD and hypertension, increased number of co-morbidities, higher level of hsCRP and lower level of 25-hydroxyvitamin D, are the predisposing factors for MCI.

Conclusions
Higher hsCRP level and lower 25-hydroxyvitamin D may be regarded as a state of cognitive impairment in elderly patients with T2DM. Further prospective larger studies should be conducted to check the association between decreased vitamin D and risk of cognitive decline and to clarify whether this association may be mediated by systemic inflammation.

Key words: diabetes mellitus; elderly; mild cognitive impairment; 25-hydroxyvitamin D; hsCRP
1. Introduction

Both type 2 diabetes (T2DM) and dementia are the most common disorders in late life, and patients with type 2 diabetes show an increased risk to develop the cognitive impairment. Many studies reported these subjects have problems with verbal memory, immediate recall, delayed recall, psychomotor speed and verbal fluency. Cognitive impairment may lead to difficulties in self-management and an increase risk of diabetes-related complications. Mild cognitive impairment (MCI) represents the transitional stage from the cognitive changes of normal ageing to very early dementia and 10-15% of patients with MCI have progress to dementia per year compared to 1-2% of healthy controls. Due to the growth of the elderly population both conditions: MCI and T2DM have recently become a focus of research. Several mechanisms contributing to the etiology of cognitive impairment in diabetes has been proposed. One of the theories underlies the causative role for vascular pathology in diabetic brain. Some studies showed that small vessel diseases in the brain (white matter lesions and lacunae) affect cognitive function in older diabetics without overt dementia or symptomatic stroke. Other hypotheses suggest that hyperglycemia lead to higher formation of advanced glycation end products (AGEs), increased glucose shunting in the hexosamine pathway, diacylglycerol activation of protein kinase C, and polyol pathway activation.

Vitamin D (25-hydroxvitamin D; 25[OH]D) deficiency has been associated with various neuropsychiatric symptoms, and it has also been reported to have an important role in the development of dementia. Some studies showed that serum 25-hydroxvitamin D levels are lower in subjects with impaired cognitive function and dementia than in healthy controls. The precise mechanism associating dementia risk and vitamin D deficiency is not fully explained. Some studies have shown that low level of vitamin D has been related to increase white matter hyperintensities and larger ventricular volumes. Another study has shown that reduced serum vitamin D concentration is associated with low level of β-amyloid in cerebrospinal fluid. Recent evidence suggests that low vitamin D concentrations are associated with increased levels of inflammatory markers. In one large study performed in older adults have reported that those subjects who have low serum levels of 25(OH)D have increased inflammatory biomarker profiles, including increased CRP, plasma fibrinogen and WBC. The authors suggested a potential role of vitamin D in chronic inflammation. Vitamin D receptors are also present in the cells of the immune system and potentially exert profound immunemodulating effects. Interestingly, it has been reported that low grade inflammation may be a contributing factor to cognitive dysfunction. Inflammatory mediators may
influence on the development of cognitive decline in patients with diabetes either by a direct effect on the brain or through the enhancement of atherosclerosis, vascular disease and endothelial dysfunction.\textsuperscript{15} Although there are some studies that described separately relationship of inflammation or vitamin D levels with MCI in diabetes, data about common pathogenesis included low-grade inflammation measured by hs-CRP concentrations and serum vitamin D levels in elderly subjects with coexisting mild cognitive impairment and type 2 diabetes are lacking. Therefore, the aims of the study were twofold: Firstly, evaluate levels of serum 25-hydroxvitamin D and high-sensitivity C-reactive protein (hs-CRP) in elderly patients with T2DM with and without MCI and secondly, identify the factors (including vitamin D levels and hs-CRP levels) associated with MCI in elderly patients with T2DM.

2. Material and Methods

2.1. Population

194 participants were obtained from the study previously described elsewhere.\textsuperscript{16} We conducted a cross-sectional study among T2DM subjects patients recruited from outpatient diabetology clinic affiliated with the university hospital no 1 in Lodz, Poland from November 2013 to February 2014. As vitamin D status depends in part on sunlight exposure all participants were required in the same season (winter). All subjects were Caucasian race. Inclusion criteria were: age $\geq$ 65 years old; diagnosed as type 2 diabetic patients for at least one year, and capable of understanding and cooperating with study procedures. Individuals with diagnosed dementia or depression, who were being used of possible or known cognition-impairing drugs in the previous 3 month, taking vitamin D supplements, with presence of neoplasm, constant alcohol or substance abuse, severe visual, mobility, or motor coordination impairment, history of head trauma, inflammatory or infectious brain disease, severe neurological or psychiatric illness were excluded.

2.2. The study design

The study comprised a single visit divided into two parts. All patients were required to sign the written informed consent before inclusion, once they had read and perfectly understood the patient information paper. The first part of visit included complete physical examination height and weight assessment and blood pressure measurements. Venous blood samples were drawn from the antecubital vein on the morning after an overnight fast. Then patients had
eaten a snack followed by capillary glucose level measuring to ensure that participants were not hypoglycemic at the time of cognitive testing. The second part of visit took place in a private area in the clinic. After collecting demographic data from a questionnaire all subjects underwent cognitive testing.

2.3. Measurements

2.3.1. Serum 25-hydroxyvitamin D level
Serum 25-hydroxyvitamin D level was determined by ELISA kit (Demeditec Diagnostics GmbH, Kiel, Germany), according to the instructions of the manufacturer. Limit of detection was 2.81 ng/ml.

2.3.2. high-sensitivity C-reactive protein
Serum high-sensitivity CRP (hsCRP) was measured using commercially available ELISA kits (Demeditec Diagnostics GmbH, Kiel, Germany), according to the instructions of the manufacturer. Minimum detectable concentration was 0.02 µg/ml.

2.3.3. The Montreal Cognitive Assessment (MoCA)
The following tests were administrated to evaluate the participants' the cognitive functions: the Montreal Cognitive Assessment (MoCA), 17 Katz Basic Activities of Daily living (BADL) and Lawton Instrumental Activities of Daily Living (IADL) questionnaires to collect information on daily activities. 18,19 The MoCA assesses multiple cognitive domains, including attention and concentration, executive functions, immediate and delayed memory, language ability, visuoconstructual skills, conceptual thinking, calculation performance, and orientation. The normal MoCA score is ≥26, with one point added if the subject has fewer than 12 years of formal education. The MoCA is recommended to detect MCI in the elderly patients with type 2 diabetes. 20 Diagnosis of MCI was performed according criteria proposed by the MCI Working Group of the European Alzheimer’s Disease Consortium which are currently available standard test. 21,22 These criteria included absence of dementia. Therefore we excluded from the study subjects with MOCA score 19 and below and sent them to psychiatrist for further care. The cut-off points for MoCA scores (19/30) are recommended for the diagnosis of ‘dementia’ in epidemiological studies. These criteria included also absence of major limitations in daily life (measured by Katz BADL and Lawton IADL).
We evaluated 2 groups of subjects: group 1 - patients with MCI and group 2 - patients without MCI (controls).

2.3.4. Covariates
The outcome variable was mild cognitive impairment. The explanatory variables are sociodemographic, clinical, and biochemical factors that were collected by patient interview, medical record review and blood samples analyses. Sociodemographic factors included: age, gender, years of education and smoking status. Clinical factors included: duration of diabetes, the treatment for diabetes, number of comorbidities, presence of diabetic complications: nephropathy, retinopathy, neuropathy, cardiovascular disease (CVD), stroke, hyperlipidemia and hypertension. BMI was calculated as weight (kg)/height (m^2). Biochemical factors, including glycosylated hemoglobin (HbA1c), total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were measured in a centralized laboratory.

2.4. Ethics
The study was operated in accordance with the World Medical Association’s Declaration of Helsinki. Each participant was assigned a number by which he/she was identified to keep his or her privacy. The approval was obtained from the independent local ethics committee of Medical University of Lodz. The purpose, nature, and potential risks of the experiments were fully explained to the subjects, and all subjects gave written, informed consent at the beginning of the study. We included only patients who had been fully able to understand and cooperate with study procedures.

2.5. Statistical Analysis
All data are presented as means ± SD. This study was designed to detect significant changes between the diabetic patients with MCI and control (diabetic patients without MCI). The mean prevalence of MCI in diabetic patients is 14-31 % thus with prediction of MCI in this study about 25%, using a two tails test with power of 90% and α = 0.05 a calculated minimal sample size of 60 for diabetic MCI positive patients was required to yield a statistically significant result [http://www.gpower.hhu.de/]. Normality of distributions was assessed using the Shapiro-Wilk tests. The descriptive statistics for the categorical variables were tested using the χ^2, and the continuous variables using the Student’s t or the Mann Whitney-U tests whenever applicable. Pearson correlation analysis for normally distributed
variables and Spearman rank correlation for nonnormally distributed variables were used to assess relationships. Simple logistic regression model was done in order to select so-called independent factors which increase the selection risk of MCI in elderly patients with T2DM. Then multivariable regression model in order to select the “strongest” factor from independent risk factors. To “optimize” the multivariable model, a stepwise approach was used (backward elimination with Wald criteria). Odds ratios (OR) were computed and presented with the 95% interval of confidence (CI). A P value of less than 0.05 was considered statistically significant. Statistica 13.1 (StatSoft, Poland) was used for analysis.

3. Results

3.1. General description of patients with MCI and controls

The demographic and clinical characteristics of the study group are presented in Table 1.

Table 1. Demographic and clinical characteristics of type 2 diabetic elderly patients.

|                                      | All subjects | MCI     | Controls | χ^2 /Z | P value |
|--------------------------------------|--------------|---------|----------|--------|---------|
| Number of patients                   | 194          | 62      | 132      |        |         |
| Age (years) *                        | 73.25 ± 4.74 | 74.7±3.9| 72.5±4.9 | -3.39  | P<0.001 |
| Gender, female/male                  | 82/112       | 32 (51.6%) | 50 (37.8%) | 3.26   | 0.07    |
| Education-years*                     | 11.37 ± 2.4  | 9.8±1.9 | 12.1±2.2 | 6.72   | P<0.001 |
| Smoked tobacco regularly             | 46 (23.7%)   | 12 (19.4%) | 34 (25.7%) | 0.96   | 0.32    |
| Duration of T2DM (years) *           | 7.79 ± 5.8   | 10.63±6.2| 6.45±5.07| -5.59  | P<0.001 |
| Microvascular complications          | 86 (44.3%)   | 43 (69.4%) | 43 (32.6%) | 23.12  | P<0.001 |
| Retinopathy (%)*                     | 70 (36.1%)   | 32 (51.6%) | 38 (28.8%) | 9.53   | 0.002   |
| Neuropathy (%)                       | 23 (11.8%)   | 10 (16.1%) | 13 (9.8%)  | 1.59   | 0.21    |
| Macrovascular complications Previous CVD (%)* | 76 (39.2%) | 48 (77.4%) | 28 (21.2%) | 55.93  | P<0.001 |
| Stroke (%)                           | 7 (3.6%)     | 2 (3.2%)  | 5 (3.78%) | 0.04   | 0.84    |
| Previous HA/ use of HA drugs (%)*    | 153 (78.8%)  | 60 (96.7%) | 93 (70.4%) | 17.53  | P<0.001 |
|                      | Patients with MCI | Controls | p-value  |
|----------------------|-------------------|----------|----------|
| **Hiperlipidemia (%)** | 142 (73.2%)       | 56 (90.3%) | 86 (65.15%) | 13.62 | P<0.001 |
| **Co-morbidity (n)** | 3.98 ± 2.8        | 6.3±3.06  | 2.8±1.8  | -7.26 | P<0.001 |
| **Treatment Insulin (%)** | 70 (36.1%)       | 23 (37.1%) | 47 (35.6%) | 0.04 | 0.84 |
| **OAD (%)**          | 187 (96.4%)       | 61 (98.4%) | 126 (95.4%) | 1.04 | 0.31 |
| **MoCA score**       | 25.43 ± 2.98      | 21.5±1.5  | 27.3±1.2 | 11.21 | P<0.001 |
| **BMI (kg/m^2)**     | 29.02 ± 3.32      | 29.8±3.5  | 28.6±3.1 | -2.48 | 0.01 |
| **HbA1c (%)**        | 7.2 ± 0.64        | 7.62±0.69 | 7±0.5   | -5.83 | P<0.001 |
| **Serum cholesterol (mmol/l)** | 4.54 ± 0.9     | 4.72±1.03 | 4.46±0.82 | -1.86 | 0.06 |
| **Serum LDL-C (mmol/l)** | 2.65 ± 0.7      | 2.7±0.7   | 2.63±0.7 | -1.15 | 0.24 |
| **Serum triglycerides (mmol/l)** | 1.95 ± 0.44     | 2.09±0.54 | 1.88±0.36 | -5.04 | P<0.001 |
| **Serum HDL-C (mmol/l)** | 1.19 ± 0.25     | 1.1±0.3   | 1.24±0.21 | 4.61 | P<0.001 |
| **hsCRP (ng/ml)**    | 1.35 ± 1.21       | 2.38 ± 1.41 | 0.85 ± 0.67 | -6.82 | <0.001 |
| **Vit D (ng/ml)**    | 18.76 ± 4.72      | 16.06 ± 4.26 | 20.02 ± 4.40 | 5.53 | <0.001 |

*significance, p<0.05; comparing patients with MCI and those without MCI (controls)
T2DM – diabetes type 2, OAD- oral anti-diabetic drug, CVD - cardiovascular disease, HA- hypertension, BMI – body mass index, CHOL - total cholesterol; hsCRP - high-sensitivity C-reactive protein; Vit D - 25-hydroxyvitamin D; HbA1c - glycosylated hemoglobin; HDL-C - high-density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol; MoCA - Montreal Cognitive Assessment, Data are mean – SD values. Mann-Whitney U test (Z), or χ² test was used to test for significant differences

The results of the χ² test indicated that patients with MCI were significantly more likely to be diagnosed with CVD, hypertension, hyperlipidemia, retinopathy and nephropathy compared to controls. There were no significant differences between the groups with regard to gender, smoking habit, the presence of stroke, neuropathy, and treatment. Furthermore, the Mann–Whitney U test and T-test showed that patients with MCI were older, less educated, had a longer duration of diabetes, higher number of co-morbidities, higher BMI and the level of HbA1c, triglycerides and lower concentrations of HDL cholesterol (table 1). MoCA score was significantly lower in subjects with MCI compared with controls. Lastly, no significant differences were found between the groups in levels of total cholesterol and LDL cholesterol (p>0.05).

### 3.2. 25-hydroxyvitamin D level and high-sensitivity C-reactive protein in MCI subjects and controls
Serum 25-hydroxyvitamin D level was significantly decreased in patients with MCI compared to controls (p<0.001) (Table 1). As expected, in the group of patients with MCI serum 25-hydroxyvitamin D levels was negatively correlated with BMI (r=-0.78, p<0.001) and with hsCRP level (r=-0.28, p=0.028). Furthermore, serum 25-hydroxyvitamin D concentration was positively correlated with MoCA score (r=0.31, p=0.016).

High-sensitivity C-reactive protein level was significantly increased in patients with MCI compared to controls (p<0.001) (Table 1). In the group of patients with MCI hsCRP concentration was highly correlated with HbA1c level (r=0.55, p<0.001). The results indicated that hsCRP level was inversely correlated with MoCA score (r=-0.59, p<0.001). A positive but weak correlation was found between this parameter and total cholesterol, or BMI. The results are presented in Table 2.

**Table 2.** Relationship of serum levels of hs-CRP and 25-hydroxyvitamin D with other clinical indicators in group of diabetic elderly patients with MCI

|                      | hsCRP    | Vit D     |
|----------------------|----------|-----------|
|                      | r        | p         | r        | p         |
| MoCA score           | -0.59*   | P<0.001  | 0.31*    | 0.016     |
| HbA1c (%)            | 0.55*    | P<0.001  | -0.18    | 0.145     |
| Serum cholesterol (mmol/l) | 0.27*   | 0.036     | -0.06    | 0.63      |
| Serum LDL-C (mmol/l) | 0.21     | 0.09      | -0.11    | 0.39      |
| Serum triglycerides (mmol/l) | 0.08    | 0.54      | -0.23    | 0.07      |
| Serum HDL-C (mmol/l)  | -0.01    | 0.92      | 0.24     | 0.06      |
| hsCRP (ng/ml)        | 1        |           | -0.28*   | 0.028     |
| Vit D (ng/ml)        | -0.28*   | 0.028     | 1        |
| BMI                  | 0.27*    | 0.03      | -0.78*   | P<0.001   |

*significance, p<0.05; r-correlation coefficient
BMI – body mass index; CHOL - total cholesterol; hsCRP - high-sensitivity C-reactive protein; Vit D - 25-hydroxyvitamin D; HbA1c - glycosylated hemoglobin; HDL-C - high-density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol; MoCA - Montreal Cognitive Assessment

3.3. Logistic regression models
The univariate logistic regression models revealed that variables which increased the likelihood of having been diagnosed with MCI in elderly patients with type 2 diabetes were higher levels of HbA1c, hsCRP, triglycerides and lower levels of 25-hydroxyvitamin D and HDL cholesterol, presence of CVD, hypertension, hyperlipidemia, retinopathy and nephropathy, longer duration of diabetes, higher BMI, increased number of co-morbidities, older age and less years of formal education (Table 3).

**Table 3.** Assessment results of the risk of having MCI in a simple logistic regression model in elderly patients with type 2 diabetes

| Variables analyzed                  | β     | SE of β | p value | OR     | 95% CI    |
|-------------------------------------|-------|---------|---------|--------|-----------|
| Age (years)*                        | 0.1   | 0.03    | 0.003   | 1.1    | 1.03-1.18 |
| Gender: female                      | 0.55  | 0.31    | 0.07    | 1.7    | 0.95-3.21 |
| Education (years)*                  | -0.62 | 0.11    | P<0.001 | 0.54   | 0.44-0.66 |
| Smoked tobacco regularly            | 0.18  | 0.08    | 0.33    | 1.2    | 0.83-1.74 |
| Duration of DM2 (years)*            | 0.13  | 0.03    | P<0.001 | 1.14   | 1.07-1.21 |
| Previous stroke                     | 0.2   | 0.08    | 0.84    | 0.84   | 0.16-4.4  |
| Previous CVD*                       | 2.54  | 0.37    | P<0.001 | 12.7   | 6.15-26.3 |
| Previous HA or use of HA drugs*     | 2.53  | 0.74    | P<0.001 | 12.5   | 2.92-54.0 |
| Hyperlipidemia*                     | 1.61  | 0.46    | P<0.001 | 4.99   | 2.0-12.46 |
| Retinopathy*                        | 1.54  | 0.33    | P<0.001 | 4.68   | 2.44-8.98 |
| Nephropathy*                        | 0.97  | 0.31    | 0.002   | 2.64   | 1.14-4.92 |
| Neuropathy                          | 0.56  | 0.45    | 0.21    | 1.76   | 0.75-4.27 |
| Co-morbidity (n)*                   | 0.55  | 0.08    | P<0.001 | 1.74   | 1.48-2.04 |
| BMI (kg/m2)*                        | 0.11  | 0.04    | 0.015   | 1.12   | 1.02-1.23 |
| HbA1c (%)*                          | 1.65  | 0.28    | P<0.001 | 5.2    | 2.95-9.14 |
| CHOL mmol/l                         | 0.01  | 0.004   | 0.06    | 1.01   | 0.99-1.02 |
| LDL (mmol/l)                        | 0.01  | 0.006   | 0.51    | 1.004  | 0.99-1.02 |
| TG (mmol/l)*                        | 0.01  | 0.005   | 0.002   | 1.014  | 1.005-1.02 |
| HDL (mmol/l)*                       | -0.07 | 0.01    | P<0.001 | 0.93   | 0.89-0.96 |
Abbreviations: ß: regression coefficient; CI: confidence interval for odds ratio; OR: odds ratio; SE: standard error; *significance, p<0.05
T2DM – diabetes type 2, CVD - cardiovascular disease, HA- hypertension, BMI – body mass index, CHOL - total cholesterol; hsCRP - high-sensitivity C-reactive protein; Vit D - 25-hydroxyvitamin D; HbA1c - glycosylated hemoglobin; HDL-C - high-density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol;

Table 4 shows the risk of MCI occurring based on multivariable regression. Less years of formal education, previous CVD and hypertension, increased number of co-morbidities, higher level of hsCRP and lower level of 25-hydroxyvitamin D, are the factors increasing the likelihood of having MCI in elderly patients with type 2 diabetes in multivariable model.

Table 4. Assessment results of the risk of having MCI in a multivariable logistic regression model in elderly patients with type 2 diabetes

| Variables analyzed                  | ß    | SE of ß | p value   | OR    | 95% CI     |
|-------------------------------------|------|---------|-----------|-------|------------|
| hsCRP (ng/ml)*                      | 1.27 | 0.19    | P<0.001   | 3.56  | 2.46-5.15  |
| Vit D (ng/ml)*                      | -0.21| 0.04    | P<0.001   | 0.81  | 0.75-0.87  |
| Education (years)*                  | -0.55| 0.15    | P<0.001   | 0.57  | 0.43-0.77  |
| Previous CVD*                       | 1.69 | 0.58    | 0.004     | 5.47  | 1.73-17.27 |
| Previous HA or use of HA drugs*     | 2.3  | 0.94    | 0.014     | 9.98  | 1.58-62.9  |
| Co-morbidity (n)*                   | 0.21 | 0.10    | 0.048     | 1.24  | 1.0-1.53   |

4. Discussion

The results of this study showed that serum vitamin D level was significantly decreased in patients with cognitive impairment. Furthermore, serum 25-hydroxyvitamin D concentration was positively correlated with MoCA score. Several previous studies had reported an association between low levels of serum vitamin D and cognitive impairment or dementia in elderly population. 23,24 In a comprehensive systematic review and meta-analysis including twenty-six cross-sectional and longitudinal cohort studies and three intervention studies comparing vitamin D supplementation with a control group, Goodwill et al. found that
low vitamin D concentrations were related to poorer cognition. However, interventional studies didn't show a clear benefit from vitamin D supplementation in midlife and older adults. Some other studies had reported opposing results as well. In one longitudinal investigation performed in 3369 middle-aged and elderly European men, the authors didn't show any association between vitamin D levels and visuocinstructural abilities, visual memory, or processing speed over on average 4.4 years. Studies reported vitamin D levels in cognitive impairment in diabetes are very rare in the literature. Chen et al. examined 95 diabetic patients with MCI and 70 subjects with no MCI and they found that cognitive impairment is associated with lower level of vitamin D. These data are consistent with our results, however their patients were younger with mean age around 55 years and they excluded subjects older than 70 years where cognitive impairment has greater impact on health in general. Similar to our results the authors also showed that vitamin D levels were independently associated with MoCA score and they concluded that vitamin D might have a potential protective effect against MCI in patients with diabetes. Several biological mechanisms had been proposed that might explain the association between low serum vitamin D levels and cognitive impairment. In the recent study the authors showed that vitamin D deficiency is related to reduced hippocampal volume and disrupted structural connectivity. The researchers had analyzed an existing structural and diffusion MRI dataset of elderly patients with MCI and found that low vitamin D is associated with reduced volumes of hippocampal subfields and connection deficits in elderly people with MCI, which may exacerbate neurocognitive outcomes. Other studies showed that low vitamin D concentration was associated with reduced β-amyloid levels in cerebrospinal fluid, which is a well-known risk factor for dementia. Vitamin D receptors are widely expressed in the human brain areas including hippocampus, hypothalamus, prefrontal cortex and may be potentially involved in many neuropsychiatric processes.

In our study we have also found that in the group of patients with MCI serum 25-hydroxyvitamin D levels was negatively correlated with BMI and with hsCRP level. This observation is consistent with previous investigations. In systematic review and meta-analysis of fifty-five observational studies, the authors showed the inverse relationship between serum vitamin D status and body mass index (BMI) in studies of both diabetic and non-diabetic subjects. The researchers suggest that many pathologic conditions like obesity, metabolic syndrome, insulin resistance and diabetes mellitus share common background and may develop as a result of hypovitaminosis D. The association between deficiency of vitamin D and adiposity may be because of mechanisms such as adipose sequestration. The adipose
tissue of obese subjects has increased uptake and storage of vitamin D which is fat-soluble. On the other hand obesity could be due to low level of vitamin D by effects on lipogenesis or adipogenesis that can occur in adipose tissue. A lot of studies have suggested that vitamin D deficiency may cause diabetes via different pathways including insulin resistance, impaired pancreatic ß cell function, or systemic inflammation. It has been proposed for potential role of vitamin D in inflammation because vitamin D receptors are expressed in most immune system cells including T-lymphocytes, dendritic cells, macrophages and neutrophils. There are limited studies investigating the associations between 25(OH)D serum levels and inflammatory markers in elderly population. In one large, nationally representative older adults from the general population the authors had found that those who have low serum levels of 25(OH)D have increased inflammatory biomarker profiles, including increased CRP, plasma fibrinogen and WBC. They concluded a potential anti-inflammatory role for vitamin D in elderly subjects. Other study showed that decreased vitamin D concentration in male was associated with higher risk of T2DM and hemoglobin A1c was mediated by serum CRP. Thus provide new evidence that the effect of low vitamin D level on T2DM may proceed through increased systemic inflammation.

In our study we found that hsCRP level was significantly increased in patients with MCI compared to controls. Hs-CR - an acute-phase protein is a sensitive and dynamic marker for low grade inflammation in diabetes. Other researchers had also confirmed higher concentration of hs-CRP in diabetic elderly patients with MCI compared to those without cognitive impairment. We also notice that hsCRP concentration was highly correlated with HbA1c level. Patients with MCI were more likely be diagnosed with retinopathy and nephropathy compared to controls. Persistent hyperglycemia and development of diabetes micro complications could enhance cognitive dysfunction by aggravating inflammation. Other authors showed that chronic hyperglycemia and increased HbA1c levels are risk factor for the cognitive impairment. CRP is also associated with increased risk of development and prognosis of cardiovascular diseases and for cardiovascular events in T2DM. We showed that higher level of hsCRP, lower level of 25-hydroxyvitamin D, presence of cardiovascular disease or hypertension are the independent factors increasing the likelihood of having MCI in elderly patients with type 2 diabetes in multivariable logistic regression model. Thus the mechanism of the involvement of low-grade inflammation reflected by serum hs-CRP levels in cognitive impairment is not explained entirely by the presence of cardiovascular disease or deficiency of vitamin D in diabetic patients. CRP is present around small-vessel damages and in amyloid plaques and could lead to neurodegeneration via activating the complement
system. We known that that stepwise analysis may greatly bias the final results for lots of reasons therefore we compared the results of our regression analysis with evidence found from literature finding. In consistent to our data some authors found that poor education and hypertension are the risk factors in MCI of T2DM patients. Another researchers in multivariate logistic regression analysis showed that vascular diseases were significantly related to increase the odds of MCI and its specific subtype. Chen et al revealed that low level of vitamin D, and history of hypertension are independent factors predicted MoCA score in multivariate regression analysis. They concluded that vitamin D may be a potential protective factor for cognitive impairment in patients with type 2 diabetes.

This study provides important insights into common pathogenesis included low-grade inflammation measured by hs-CRP concentrations and serum vitamin D levels underlying cognitive impairment in elderly diabetic subjects however, it is not without limitations. First, it was a single-center study, most subjects lived in urban areas in central Poland, therefore the study should be extended to the inhabitants of other regions and the results may be obtained from entire Polish population. Our participants were recruited from one race/ethnicity, which may limit the generalizability of findings to other populations. Second, the study wasn’t designed as longitudinal prospective investigation. It could be interesting to check the contribution of low-grade inflammation and hypovitaminosis D to cognitive decline and late-life dementia risk, although the exact mechanism is uncertain. Thirdly because the stepwise analysis may greatly bias the final results for lots of reason some sensitivity analyses in modifying the pre-determined list of variables may be performed.

5. Conclusions

In summary, the current study demonstrated that T2DM elderly individuals with mild cognitive impairment presented decreased serum vitamin D levels and increased hsCRP concentrations. Furthermore, high hsCRP level, low 25-hydroxyvitamin D level, previous CVD and hypertension, increased number of co-morbidities, and less years of formal education are the potential predictors of cognitive dysfunction among elderly patients with type 2 diabetes. Further prospective larger studies should be conducted to check the association between decreased vitamin D and risk of cognitive decline and to clarify whether this association may be mediated by systemic inflammation.

Declarations of interest: none

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Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Association of hsCRP and vitamin D levels with mild cognitive impairment in elderly type 2 diabetic patients

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Highlights

1. Diabetes, aging and cognitive impairment are associated with pro-inflammatory state.
2. Low vitamin D levels are associated with increased levels of inflammatory markers.
3. Pathogenesis of mild cognitive impairment in diabetic patients remains unclear.
4. Higher hsCRP and lower vitamin D levels could be regarded as a state of cognitive impairment.