Study of the possible relations between vitamin D, telomere length, and high-sensitivity C-reactive protein in older people

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Background
Ageing is a multifactorial process, and telomere shortening is one of the possible mechanisms of cellular ageing. Many factors can affect telomere length (TL). Our study investigated the effect of vitamin D and high-sensitivity C-reactive protein (hs-CRP), as one of the inflammatory markers, on TL in older people.
Patients and methods
This was a cross-sectional study on 100 older people who did not have malignancy, autoimmune diseases, or renal disease and did not take vitamin D supplementation. Measurement of TL was done using real-time PCR, and for each sample, calculation of the T/S ratio was done. Telomere (T) signals in experimental DNA samples and single-copy gene (S) signals were measured in separate wells, in comparison with a reference DNA, to yield relative T/S ratios that are proportional to average TL.
Results
The results showed that vitamin D was significantly lower in females than males, and there was a significant positive correlation between vitamin D and the level of education of our participants. We found a highly significant negative correlation between vitamin D and hs-CRP. However, there was no significant correlation between T/S ratio and either vitamin D or hs-CRP.
Conclusion
In conclusion, vitamin D has an anti-inflammatory effect that decreased the hs-CRP level in our study. However, factors other than vitamin D and hs-CRP may be responsible for shortening telomere in elderly patients.

Keywords:
elderly, high-sensitivity C-reactive protein, telomere length, vitamin D

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Introduction

Ageing is a multifactorial biological process characterized by a progressive decline of organ functions, leading to an increased risk of age-associated diseases and death [1].

Several molecular and biochemical pathways contribute to ageing, and one of the most important of these is cellular senescence [2]. Cellular senescence is an irreversible arrest of cell proliferation induced in different ways including genomic damage, toxins, irradiation, oxidative stress, oncogene expression, tumor suppressor gene activation, and epigenomic alterations [3]. Deterioration of genomic integrity and genomic instability are critical aspects of ageing at a cellular level.

Telomeres are the end caps of chromosomes. Telomeres appear to be of critical importance for genomic stability and cellular ageing [1]. Telomeres participate in the maintenance of genomic and cellular stability and replication; in fact, they protect the genome from degradation, unwanted recombination, and chromosomal fusion [4–6]. With increasing age, most human somatic tissues and adult stem cells undergo telomere attrition, as they do not express sufficient amounts of telomerase to maintain telomere length (TL) indefinitely [7]. Telomere shortening and dysfunction have been proposed as indicators of cellular ageing and are associated with age-related diseases, including cardiovascular disease, type 2 diabetes mellitus (T2DM), neurodegenerative diseases, cancer, or chronic obstructive pulmonary disease [8].

Many methods are available for TL measurement. These include Southern blot technique, quantitative fluorescence in situ hybridization, and quantitative PCR [9].

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Micronutrients, such as vitamins, and trace elements play an essential role in cell metabolism, and some studies suggest a direct effect of these micronutrients on telomere biology and cellular ageing [10]. Vitamin D, for example, is a steroid hormone with genomic and nongenomic activities that are involved in the regulation of cell proliferation, differentiation, and apoptosis [11].

1,25(OH)2D3 primarily exerts its effects on target tissues through genomic actions via the vitamin D receptor. The vitamin D receptor has been located in many cell types, including enterocytes, myocytes, immune cells (including activated T and B lymphocytes, macrophages and dendritic cells), as well as neurons and glial cells of the central nervous system, among many others [9].

Epidemiological data support an association between vitamin D deficiency and numerous conditions. These include various muscle function parameters, multiple autoimmune diseases, upper respiratory tract infection, tuberculosis, insulin resistance, T2DM, coronary heart disease, heart failure, peripheral vascular disease, and all-cause mortality [12–18]. Observational data suggest an association between hypovitaminosis D and cognitive function, depression, bipolar disorder, and schizophrenia [19]. Furthermore, there is an association between vitamin D and a number of cancers, including colorectal and prostate cancers [20].

The role of vitamin D in cellular ageing and senescence is the consequence of its numerous functions in the regulation of cellular proliferation, differentiation, and apoptosis. One pathway through which vitamin D can delay cellular ageing is the preservation of telomere biology [21].

Previous studies demonstrated a positive correlation between serum 25-hydroxy vitamin D[25-(OH)D] and leukocyte TL in humans, which remained significant after adjustment for age [22,23]. Another study observed longer telomeres in hemodialysis patients treated with calcitriol or analogs for at least 6 months compared with hemodialysis patients without such treatment [24].

However, all studies published so far harbor significant limitations, and results are sometimes conflicting. Further systematic studies are needed to understand the role of vitamin D in telomere biology and cellular ageing [9].

The aim of this work is to study the possible relationships between vitamin D level, TL, and high-sensitivity C-reactive protein (hs-CRP) in a random sample of older people.

Patients and methods

The study was a cross-sectional study carried out on 100 older people aged 65 years or more.

Exclusion criteria were as follows:

(1) Patients having malignancy.
(2) Patients having an autoimmune disease.
(3) Patients with renal diseases.
(4) Patients taking vitamin D supplementation.

The following was done for each patient:

(1) Informed consent was taken from each participant.
(2) Thorough history taking.
    (a) Complete clinical examination.
    (b) Basic laboratory tests in the form of the following:
    (c) Complete blood count [25].
    (d) Fasting and postprandial plasma glucose levels [26].
    (e) Liver function tests: SGOT, SGPT, and serum albumin [26].
    (f) Renal function tests: estimated glomerular filtration rate [27].
    (g) hs-CRP [26].
(3) Measurement of TL using real-time PCR, and for each sample, T/S was calculated: telomere (T) signals in experimental DNA samples, and single-copy gene (S) signals in separate wells, in comparison with a reference DNA, to yield relative T/S ratios that are proportional to average TL [28].
(4) Assessment of 25-hydroxyvitamin D level [29].
(5) A correlation study was done between TL, 25-hydroxyvitamin D levels, and hs-CRP to detect if there are any significant correlations between them.

The study was approved by the ethical committee of Faculty of Medicine, Alexandria University.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package, version 20.0. (IBM Corp., Armonk, New York, USA). Qualitative data were described using number and percent. The Kolmogorov–Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, SD, median, and interquartile range. Significance of the obtained results was judged at the 5% level.

Results

Demographic data

Sex

According to sex of our studied group, we have 50 (50%) males and 50 (50%) females.

Age

All participants are elderly (≥65 years). All participants were in the range of 65–87 years with a mean of 71.06 years, a standard deviation of 5.03 years, and a median of 70.0 years.

Level of education

According to their level of education, our participants were divided into illiterate, primary level, secondary level, and university level. A total of 20 (20%) participants were illiterate, 16 (16%) participants were at the primary level of education, 26 (26%) participants were at the secondary level of education, and 38 (38%) participants were at the university level of education (Tables 1 and 2, and Figs 1 and 2).

Table 1 Distribution of the studied cases according to demographic data (*N*=100)

	n (%)
Sex	
Male	50 (50.0)
Female	50 (50.0)
Minimum–maximum	65.0–87.0
Mean±SD	71.06±5.03
Median (IQR)	70.0 (67.0–75.0)
Level of education	
Illiterate	20 (20.0)
Primary level	16 (16.0)
Secondary level	26 (26.0)
University level	38 (38.0)

IQR, interquartile range.

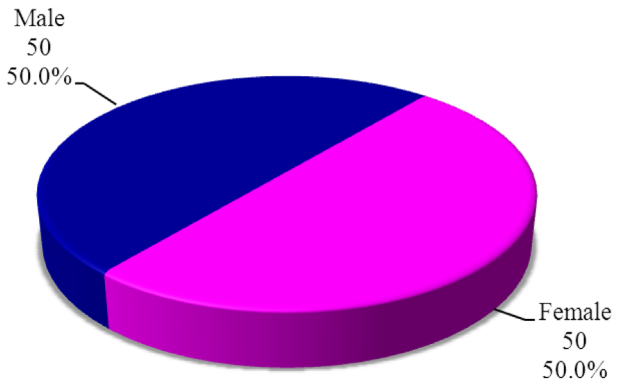
The basic laboratory tests

Relations

There was no significant relation between T/S ratio and either sex or level of education (Table 3).

There was a significant relationship between vitamin D and sex. Female participants have significantly lower vitamin D level compared with male ones. Moreover,

Figure 1



Distribution of the studied cases according to sex (*n*=100).

Table 2 Descriptive analysis of the studied cases according to different laboratory parameters (*N*=100)

Laboratory parameters	Minimum–maximum	Mean±SD	Median (IQR)
Hemoglobin	6.40–16.70	13.46±1.74	13.70 (12.5–14.4)
MCV	67.60–98.10	87.38±5.81	87.90 (48.8–91.4)
MCH	20.0–33.10	28.54±2.72	28.95 (27.6–30.1)
MCHC	28.70–36.20	32.52±1.59	32.65 (31.7–33.4)
WBCs	2.09–16.27	6.60±2.21	6.29 (5.6–7.5)
Platelets	33.0–472.0	226.9±72.50	220.0 (186.0–262.0)
Neutrophils	0.58–12.46	3.67±1.80	3.37 (2.63–4.2)
Lymphocytes	0.93–3.36	2.10±0.63	2.10 (1.6–2.5)
Fasting plasma glucose	66.0–197.0	109.0±31.72	99.50 (90.0–116.0)
2-h postprandial plasma glucose	105.0–273.0	153.9±39.04	140.0 (130.0–157.0)
ALT	13.0–69.0	29.70±10.73	27.0 (23.0–34.0)
AST	13.0–78.0	28.72±14.35	23.50 (19.0–36.0)
Albumin	3.10–4.30	3.77±0.26	3.80 (3.6–4.0)
Urea	18.0–68.0	34.80±10.54	33.0 (26.0–40.0)
Cr	0.30–1.65	0.87±0.28	0.80 (0.70–1.0)
eGFR (ml/min)	43.32–319.8	91.99±42.28	79.97 (70.5–103.2)
T/S ratio	0.21–3.71	1.17±0.73	1.02 (0.60–1.5)
Vitamin D	7.0–54.0	24.74±10.73	23.50 (17.0–30.0)
hs-CRP	0.50–16.0	4.96±2.87	5.0 (3.0–7.0)

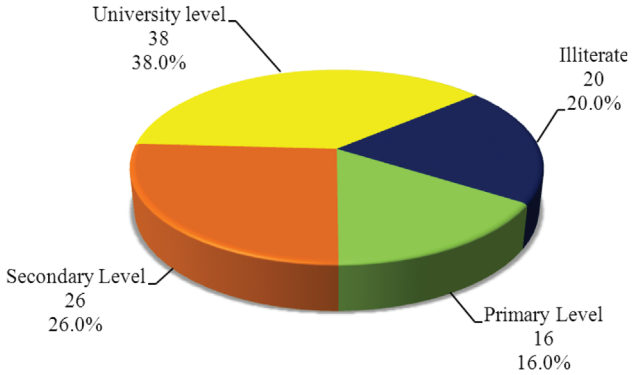
ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; WBC, white blood cell.

there was a significant positive relation between vitamin D level and level of education (Tables 4 and 5, and Figs 3 and 4).

There was a statistically significant negative correlation between T/S ratio and white blood cells (WBCs) (Fig. 5).

There was a statistically significant negative correlation between T/S ratio and neutrophil count (Fig. 6).

Figure 2



Distribution of the studied cases according to level of education (*n*=100).

There was a statistically significant negative correlation between T/S ratio and 2-h postprandial plasma glucose (Fig. 7).

There was a statistically significant positive correlation between hemoglobin and vitamin D levels (Fig. 8).

There was a statistically significant negative correlation between vitamin D and fasting plasma glucose levels (Fig. 9).

There was a statistically significant negative correlation between vitamin D and 2-h postprandial plasma glucose (Fig. 10).

There was a statistically significant positive correlation between vitamin D and albumin (Fig. 11).

There was a statistically significant positive correlation between vitamin D and estimated glomerular filtration rate (Fig. 12).

There was a statistically significant negative correlation between vitamin D and hs-CRP (Fig. 13).

There was no significant correlation between T/S ratio and either vitamin D or hs-CRP.

Table 3 Relation between T/S ratio and either sex or level of education (*N*=100)

	N	T/S ratio			Test of significance	*P**
		Minimum–maximum	Mean±SD	Median		
Sex						
Male	50	0.21–2.91	1.08±0.68	0.97	*U*=1040.0	0.149
Female	50	0.39–3.71	1.25±0.77	1.10		
Level of education						
Illiterate	20	0.39–3.71	1.14±0.94	0.93	*H*=2.218	0.528
Primary level	16	0.47–2.06	1.25±0.48	1.20		
Secondary level	26	0.21–2.91	1.11±0.65	0.94		
University level	38	0.29–3.25	1.18±0.76	0.80		

U, Mann–Whitney test; *H*, *H* Kruskal–Wallis test. *P*, *P* value for comparing between the studied categories. *Statistically significant at *P* value less than or equal to 0.05.

Table 4 Relation between vitamin D and either sex or level of education (*N*=100)

	N	Vitamin D			*U*	*P*
		Minimum–maximum	Mean±SD	Median		
Sex						
Male	50	10.0–54.0	29.60±10.79	27.0	552.0*	<0.001*
Female	50	7.0–42.0	19.88±8.25	19.0		
Level of education						
Illiterate	20	7.0–26.0	15.0±5.75	15.0	*H*=29.897*	<0.001*
Primary level	16	9.0–48.0	22.75±11.33	21.50		
Secondary level	26	10.0–54.0	27.92±11.86	24.0		
University level	38	16.0–46.0	28.53±8.32	27.0		

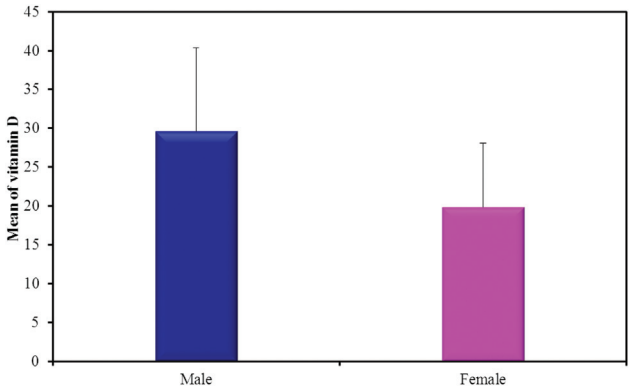
U, Mann–Whitney test. *P*, *P* value for comparing between the studied categories. *Statistically significant at *P* value less than or equal to 0.05.

Table 5 Correlation between T/S ratio and vitamin D with different parameters (*N*=100)

	T/S ratio		Vitamin D	
	r_s	*P*	r_s	*P*
Age (years)	−0.041	0.686	−0.069	0.495
Hemoglobin	−0.031	0.762	0.439*	<0.001*
WBCs	−0.244*	0.014*	0.004	0.972
Neutrophils	−0.285*	0.004*	−0.004	0.967
Lymphocytes	−0.036	0.721	−0.114	0.258
FPG	−0.161	0.109	−0.246*	0.014*
2hPPPG	−0.217*	0.030*	−0.280*	0.005*
ALT	−0.092	0.363	0.112	0.268
AST	−0.160	0.111	0.013	0.898
Albumin	−0.058	0.565	0.264*	0.008*
Urea	−0.130	0.196	0.051	0.614
Cr	−0.013	0.896	−0.023	0.823
eGFR (mL/min)	0.072	0.478	0.291*	0.003*
hs-CRP	−0.099	0.326	−0.551*	<0.001*
T/S ratio			0.065	0.521
Vitamin D	0.065	0.521		

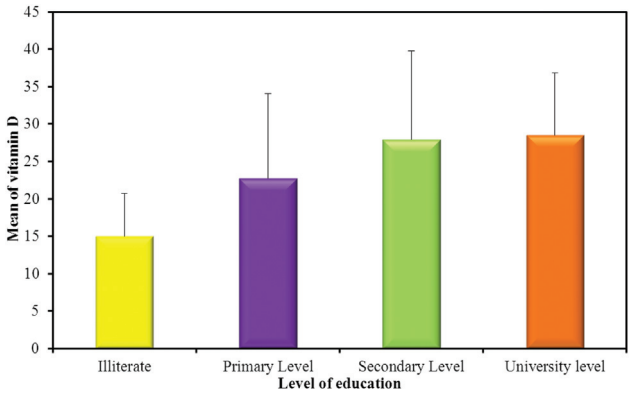
2hPPPG, 2-h postprandial plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; hs-CRP, high-sensitivity C-reactive protein; r_s, Spearman coefficient; WBC, white blood cell. *Statistically significant at *P* value less than or equal to 0.05.

Figure 3



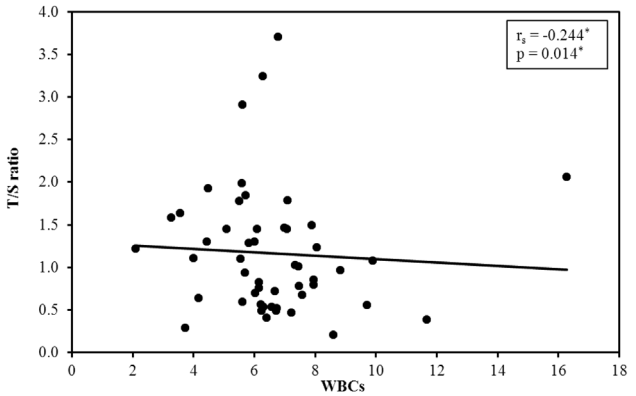
Relation between vitamin D and ex (*n*=100).

Figure 4



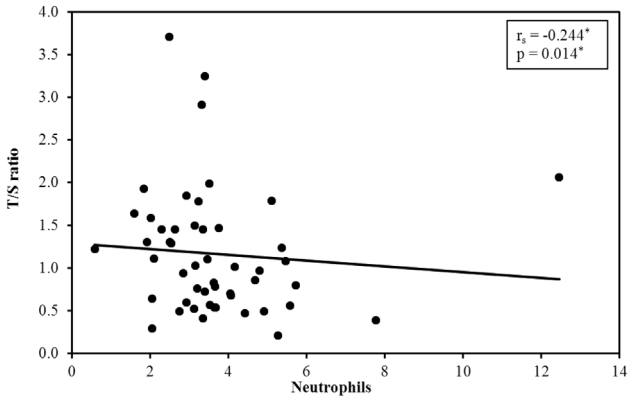
Relation between vitamin D and level of education (*n*=100).

Figure 5



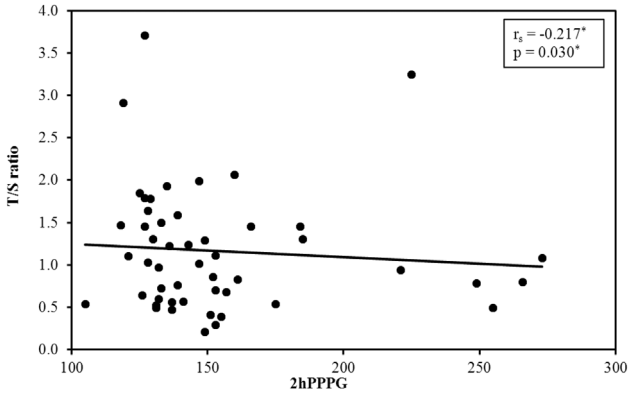
Correlation between T/S ratio with WBCs (*n*=100). WBC, white blood cells.

Figure 6



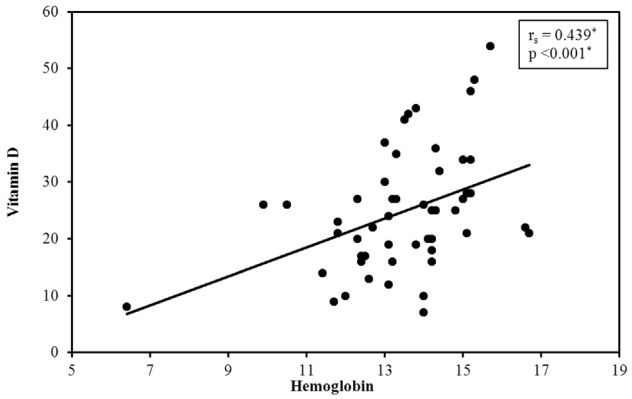
Correlation between T/S ratio with neutrophils (*n*=100).

Figure 7



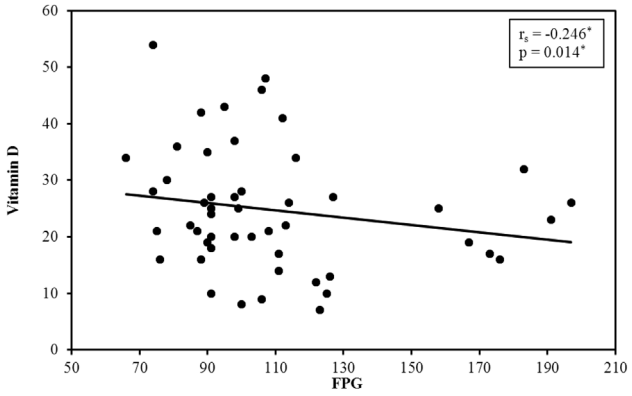
Correlation between T/S ratio with 2hPPPG (*n*=100). 2hPPPG, 2-h postprandial plasma glucose.

Figure 8



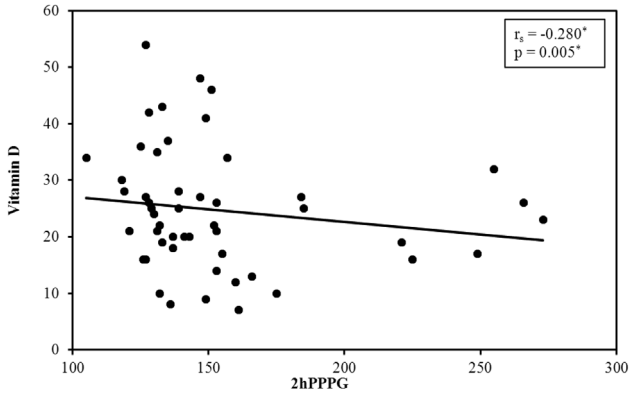
Correlation between vitamin D with hemoglobin (*n*=100).

Figure 9



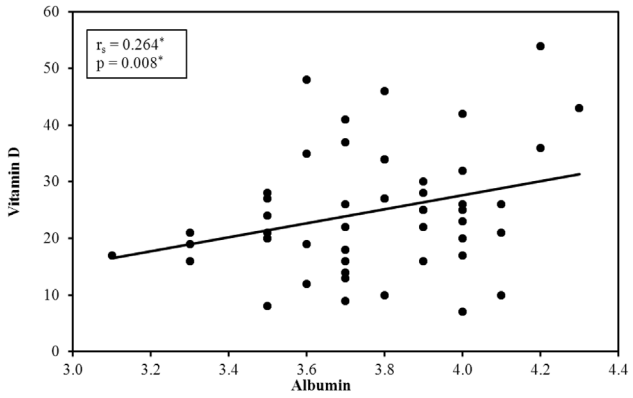
Correlation between vitamin D with FPG (*n*=100). FPG, fasting plasma glucose.

Figure 10



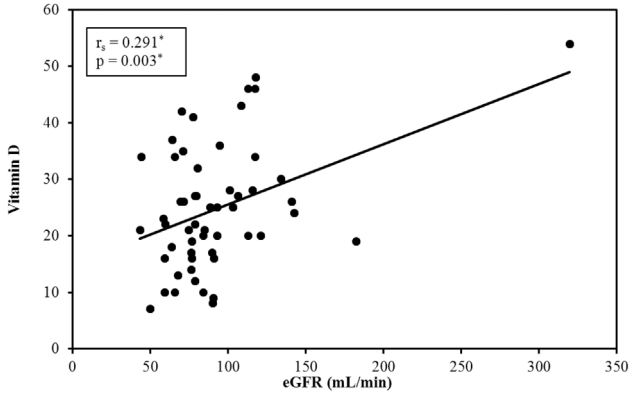
Correlation between vitamin D with 2hPPPG (*n*=100). 2hPPPG, 2-h postprandial plasma glucose.

Figure 11



Correlation between vitamin D with albumin (*n*=100).

Figure 12

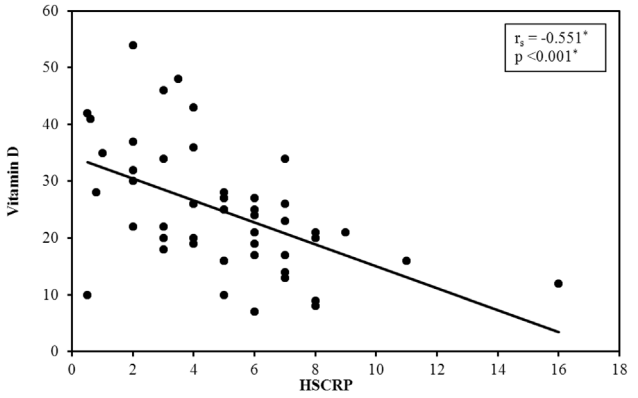


Correlation between vitamin D with eGFR (ml/min) (*n*=100). eGFR, estimated glomerular filtration rate.

Discussion

In this study, we aimed to study the possible relations between vitamin D level, TL, and hs-CRP as an inflammatory marker in a random sample of elderly people aged 65 years or more. We found a highly significant negative correlation between vitamin D and hs-CRP. However, no significant correlation between T/S ratio and either vitamin D or hs-CRP could be detected.

Figure 13



Correlation between vitamin D with hs-CRP (*n*=100). hs-CRP, high-sensitivity C-reactive protein.

Our study found a highly significant negative correlation between vitamin D and hs-CRP. Faivre *et al.* [30] found 25(OH) vitamin D to be inversely correlated with CRP in psychiatric patients, and this correlation remained significant in multivariate analysis, including factors such as age and BMI. Vitamin D supplementation decreased CRP in a nonpsychiatric population according to the meta-analysis of Chen *et al.* [31], and in the recent study of Tabatabaeizadeh *et al.* [32]. Mirzavandi *et al.* [33] found that two intramuscular injections of 200 000 IU of vitamin D at weeks 0 and 4 in patients with T2DM and vitamin D deficiency resulted in significant reductions in CRP. A systematic review and meta-analysis, which included 20 trials with a total of 1270 individuals with T2DM, has shown that vitamin D intake had significant reducing effects on the hs-CRP level [34]. In another meta-analysis which included 20 randomized controlled trials, it was shown that vitamin D supplementation could exert beneficial effects on hs-CRP in patients with diabetic nephropathy [35]. In another trial that was performed in patients with coronary artery disease, 12-week intervention of oral 50 000-IU vitamin D supplements had no significant differences in the measures of E-selectin and hs-CRP [36]. Hejazi *et al.* [37] found that treatment of vitamin D deficiency had no significant effect on hs-CRP after 3 months among patients with DVT/PE.

Activated vitamin D decreases the mediators of systemic inflammation, such as interleukin-2 and tumor necrosis factor-α. Vitamin D receptors are ubiquitously expressed on T and B lymphocytes, natural killer cells, and monocytes, and through the downregulation of cytokines and other pro-inflammatory factors, activated vitamin D exerts anti-inflammatory actions [38]. This may explain the negative correlation between vitamin D and hs-CRP. The absence of hs-CRP response to vitamin D supplementation in the latter studies may be explained by the smaller dose of vitamin D supplementation compared with dose used in patients with T2DM or the short duration of supplementation (3 months).

In our study, we found a positive, though an insignificant, correlation between vitamin D and TL. Our findings are in line with several studies. In a recent study by Liu *et al.* [39] among 1154 US radiologic technologists aged 48–93 years old, little evidence was found in support of the association of vitamin D and TL. Moreover, there were no associations of 25(OH)D and leukocyte TL in a sample of male health professionals [40] or in another study of young adults (at age 31) from Finland [41].

In contrast to our study, some studies have found a positive correlation between vitamin D and TL. In a study of 2160 adult twin women in the United Kingdom, 25(OH)D levels were positively associated with TL [22]. Moreover, in a study of 1337 registered female nurses in the United States, Liu *et al.* [23] also reported a positive association of 25(OH)D with TL. Mazidi *et al.* [38] did not find any significant trend in mean 25(OH)D across quartiles of the TL. However, in linear regression models adjusted for age, race, marital status, education, and CRP, overall and stratified by sex, they found a statistically significant association between vitamin D and TL in the overall sample with no evidence of statistical interaction by sex. However, after they further adjusted for the previous covariates (age, race, marital status, education, and CRP) plus smoking, BMI, and physical activity, no significant association was found in the overall sample, in men, or in women [38].

Many factors can affect the vitamin D and TL relationship. For instance, cigarette smoking, obesity, and a sedentary lifestyle are associated with shortened TL [42–44]. In our study, many factors may have led to an insignificant correlation between vitamin D and TL. First, the cross-sectional nature does not allow inference about causality. Moreover, 25(OH)D was measured at one time point, which may not reflect the lifetime vitamin D status. Moreover, a larger sample may have given a better result. Furthermore, the inclusion of a comparison group of adult people may have made the effect of vitamin D on TL on older people more evident.

Our study also showed a negative correlation between T/S ratio and both the WBCs and the neutrophils.

This may be owing to that the increased WBCs or neutrophils are associated with more cellular multiplication which ultimately results in telomere shortening.

There was a statistically significant negative correlation between vitamin D and the glycemic profile as well as the renal function of our participants. Vitamin D is activated in the kidney to the active form by hydroxylation to yield 1,25 dihydroxycholecalciferol. In people with impaired renal function, vitamin D activation is impaired as well, resulting in vitamin D deficiency. This explains the negative correlation between vitamin D and renal functions of our participants. Diabetes is the most common cause of impaired renal function. However, the negative correlation of vitamin D level and the participants' glycemic profile may be owing to its effect on the renal function or something else, and this needs to be investigated.

Conclusion

From the previous discussion, we can conclude that vitamin D decreases the release of mediators of inflammation, as shown in different studies discussed previously. The relation of vitamin D and TL is controversial; some studies found a positive correlation, whereas others did not find, because TL is multifactorial.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Varela E, Blasco MA. 2009 nobel prize in physiology or medicine: telomeres and telomerase. Oncogene 2010; 29:1561–1565.

2 Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 2013; 123:966–972.

3 Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol 2013; 75:685–705.

4 Blasco MA. Mice with bad ends: mouse models for the study of telomeres and telomerase in cancer and aging. EMBO J 2005; 24:1095–1103.

5 Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet 2005; 6:611–622.

6 Blasco MA. Telomere length, stem cells and aging. Nat Chem Biol 2007; 3:640–649.

7 Mathon NF, Lloyd AC. Cell senescence and cancer. Nat Rev Cancer 2001; 1:203–213.

8 Armanios M. Telomeres and age-related disease: how telomere biology informs clinical paradigms. J Clin Invest 2013; 123:996–1002.

9 Pusceddu I, Farrell CJ, Di Pierro AM, Jani E, Herrmann W, Herrmann M. The role of telomeres and vitamin D in cellular aging and age-related diseases. Clin Chem Lab Med 2015; 53:1661–1678.

10 Paul L. Diet, nutrition and telomere length. J Nutr Biochem 2011; 22:895–901.

11 Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357:266–281.

12 Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ. Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). Am J Cardiol 2008; 102:1540–1544.

13 Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karlson EW, *et al.* Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. Am J Clin Nutr 2004; 80:752–758.

14 Ginde AA, Mansbach JM, Camargo CA. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009; 169:384–390.

15 Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int J Epidemiol 2008; 37:113–119.

16 Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab 2007; 92:2017–2029.

17 Schöttker B, Haug U, Schomburg L, Köhrle J, Perna L, Müller H, *et al.* Strong associations of 25-hydroxyvitamin D concentrations with all-cause, cardiovascular, cancer, and respiratory disease mortality in a large cohort study. Am J Clin Nutr 2013; 97:782–793.

18 Herrmann M, Sullivan DR, Veillard AS, McCorquodale T, Straub IR, Scott R, *et al.* Serum 25-hydroxyvitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes. Diabetes Care 2015; 38:521–528.

19 Cherniack EP, Troen BR, Florez HJ, Roos BA, Levis S. Some new food for thought: the role of vitamin D in the mental health of older adults. Curr Psychiatry Rep 2009; 11:12–19.

20 Gandini S, Boniol M, Haukka J, Byrnes G, Cox B, Sneyd MJ, *et al.* Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. Int J Cancer 2011; 128:1414–1424.

21 Gloth FM, Gundberg CM, Hollis BW, Haddad JG, Tobin JD. Vitamin D deficiency in homebound elderly persons. JAMA 1995; 274:1683–1686.

22 Richards JB, Valdes AM, Gardner JP, Paximadas D, Kimura M, Nessa A, *et al.* Higher serum vitamin D concentrations are associated with longer leukocyte telomere length in women. Am J Clin Nutr 2007; 86:1420–1425.

23 Liu JJ, Prescott J, Giovannucci E, Hankinson SE, Rosner B, Han J, *et al.* Plasma vitamin D biomarkers and leukocyte telomere length. Am J Epidemiol 2013; 177:1411–1417.

24 Borras M, Panizo S, Sarró F, Valdivielso JM, Fernandez E. Assessment of the potential role of active vitamin D treatment in telomere length: a case-control study in hemodialysis patients. Clin Ther 2012; 34:849–856.

25 Lewis SM, Bain BJ, Bates I, Dacie JVS, Dacie JVSPh. Dacie and Lewis practical haematology. 10th ed. Philadelphia: Churchill Livingstone/ Elsevier 2006.

26 Burtis CA, Ashwood ER, Bruns DE, Tietz NW. Tietz textbook of clinical chemistry and molecular diagnostics. 5th ed. St Louis, MO: Saunders 2013.

27 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16:31–41.

28 Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002; 30:e47.

29 Miller M, Chen TC, Holick MF, Mikota S, Dierenfeld E. Serum concentrations of calcium, phosphorus, and 25-hydroxyvitamin d in captive African elephants (*Loxodonta africana*). J Zoo Wildl Med 2009; 40:302–305.

30 Faivre S, Roche N, Lacerre F, Dealberto MJ. Vitamin D deficiency in a psychiatric population and correlation between vitamin D and CRP. Encephale 2019; 45:376–383.

31 Chen N, Wan Z, Han SF, Li BY, Zhang ZL, Qin LQ. Effect of vitamin D supplementation on the level of circulating high-sensitivity C-reactive protein: a meta-analysis of randomized controlled trials. Nutrients 2014; 6:2206–2216.

32 Tabatabaeizadeh SA, Avan A, Bahrami A, Khodashenas E, Esmaeili H, Ferns GA, *et al.* High dose supplementation of vitamin D affects measures of systemic inflammation: reductions in high sensitivity C-reactive protein level and neutrophil to lymphocyte ratio (NLR) distribution. J Cell Biochem 2017; 118:4317–4322.

33 Mirzavandi F, Talenezhad N, Razmpoosh E, Nadjarzadeh A, Mozaffari-Khosravi H. The effect of intramuscular megadose of vitamin D injections on E-selectin, CRP and biochemical parameters in vitamin D-deficient patients with type-2 diabetes mellitus: a randomized controlled trial. Complement Ther Med 2020; 49:102346.

34 Mousa A, Naderpoor N, Teede H, Scragg R, de Courten B. Vitamin D supplementation for improvement of chronic low-grade inflammation in patients with type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. Nutr Rev 2018; 76:380–394.

35 Wang Y, Yang S, Zhou Q, Zhang H, Yi B. Effects of vitamin D supplementation on renal function, inflammation and glycemic control in patients with diabetic nephropathy: a systematic review and meta-analysis. Kidney Blood Press Res 2019; 44:72–87.

36 Sokol SI, Srinivas V, Crandall JP, Kim M, Tellides G, Lebastchi AH, *et al.* The effects of vitamin D repletion on endothelial function and inflammation in patients with coronary artery disease. Vasc Med 2012; 17:394–404.

37 Hejazi ME, Modarresi-Ghazani F, Hamishehkar H, Mesgari-Abbasi M, Dousti S, Entezari-Maleki T. The effect of treatment of vitamin D deficiency on the level of P-selectin and hs-CRP in patients with thromboembolism: a pilot randomized clinical trial. J Clin Pharmacol 2017; 57:40–47.

38 Mazidi M, Michos ED, Banach M. The association of telomere length and serum 25-hydroxyvitamin D levels in US adults: the National Health and Nutrition Examination Survey. Arch Med Sci 2017; 13:61–65.

39 Liu JJ, Cahoon EK, Linet MS, Little MP, Dagnall CL, Higson H, *et al.* Relationship between plasma 25-hydroxyvitamin D and leucocyte telomere length by sex and race in a US study. Br J Nutr 2016; 116:953–960.

40 Julin B, Shui IM, Prescott J, Giovannucci EL, De Vivo I. Plasma vitamin D biomarkers and leukocyte telomere length in men. Eur J Nutr 2017; 56:501–508.

41 Williams DM, Palaniswamy S, Sebert S, Buxton JL, Blakemore AI, Hyppönen E, *et al.* 25-hydroxyvitamin D concentration and leukocyte telomere length in young adults: findings from the Northern Finland Birth Cohort 1966. Am J Epidemiol 2016; 183:191–198.

42 Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, *et al.* Obesity, cigarette smoking, and telomere length in women. Lancet 2005; 366:662–664.

43 Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, *et al.* The association between physical activity in leisure time and leukocyte telomere length. Arch Intern Med 2008; 168:154–158.

44 Nawrot TS, Staessen JA, Gardner JP, Aviv A. Telomere length and possible link to X chromosome. Lancet 2004; 363:507–510.