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Original Research Article

A Case Control Study to Explore the Relationship Between Vit-D and Cellular Senescence Measured Using the Enzyme Telomerase in pre-HTN

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Abstract

Aim: The aim of the study was to explore the relationship between Vit-D and cellular senescence measured using the enzyme telomerase in pre-HTN.

Materials and Methods: This investigation was carried out in 50 pre-hypertensives and equal number of age- and gender-matched controls. Cellular senescence was measured by increased levels of telomerase and Vit-D was assessed with using commercially available ELISA assay kits.

Results: Weight and BMI, Systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial pressure (MAP), rate pressure product (RPP) and telomerase levels were high and vi-D levels were low in pre-HTN group. Low levels of Vit-D were negatively correlated with telomerase, HR, SBP, and PP.

Conclusion: The lower Vit-D levels in pre-HTN could lead to derangements in cardiovascular homeostatic mechanism and enhance the speed of cellular senescence measured by telomerase.

Keywords: Cellular Senescence; Pre-hypertension; Telomerase; Vitamin D

Introduction:

Vitamin D deficiency has recently emerged as a public health problem, affecting almost 50% of the population worldwide [1]. In addition to the reduced exposition to sunlight[2], also genetic and environmental factors have been suggested as a cause of this pandemic, such as pollution, diet, sedentary life style and stress[3]. Moreover, vitamin D is no longer considered as only a pivotal mediator of calcium metabolism and skeletal health, but it also regulates several cell functions, including differentiation and metabolism. This aspect may explain the reason why hypovitaminosis D has been proved to be an independent risk factor for overall mortality in various cohort

analyses[4], whereas vitamin D supplementation significantly reduced mortality[5]. Moreover, similar data were collected from different clusters of inflammatory and chronic diseases, such as infections[6], autoimmunity[7], and neurodegenerative pathologies[8], as well for cancer[9]. However, a special interest was conferred to the potential relationship between vitamin D and cardiovascular (CV) disorders.

Although in human cohorts low vitamin D levels were associated with impaired CV outcomes[10], a causal relationship remains unknown, and the general enthusiasm about the benefits of vitamin D supplementation have been recently replaced by words of caution.

Another report from Lucknow with 1746 population in this study, they reported 32.3% pre-HTN,[11]A Puducherry based study with 300 medical college staff had reported prevalence of 22%[12]another report from Uttar Pradesh showed 27.2% prevalence.[13]In military adults, the highest prevalence of 80% was reported.[14]With 500 medical students in Karnataka reported a higher prevalence of 55%.[15]

The telomerase is an enzyme to inhibit the telomere shortening process. Telomeres become short with each cell division and this process reaches a crucial extent leads to replicative cellular senescence.[16, 17]

Materials and methods:

After obtaining Patna Medical College & Hospital, Patna volunteers were recruited from students.

Inclusion criteria: The pre-hypertensive group (pre-HTN) (n = 50) were both genders between 18 and 25 years of age with SBP between 120 and 139 mmHg and DBP between 80 and 89 mmHg in apparently healthy individuals were included in the study. The controls (n = 50) population were healthy individuals with 18–25 years of age with SBP between 100 and 119 mmHg and DBP between 60 and 79 mmHg.

Exclusion Criteria: Individuals suffering from diabetes, hypertension, endocrine disorders, kidney diseases, and hypertensive patients already receiving medication were not considered to take part in this research.

Sample Collection: The volunteers were asked to not participate in heavy exercises, not drink alcohol and coffee 1 day before the data collection. Baseline, anthropometric parameters were recorded before recording of the BP by sphygmomanometer as per standard protocol. [18] Then, 5 ml of blood was collected, allowed to clot, and subjected to centrifugation to separate the serum. Serum was stored at –80°C for processing of Vit-D and telomerase levels as per the instructions provided in the commercially available kits.

Statistical Analysis: To study the between-group differences, independent *t*-test, to assess the correlation of Vit-D with telomerase and other parameters, Pearson's correlation coefficient analysis was applied.

Results:

The study population included 100 apparently healthy individuals. Forty four of 100 were pre-hypertensives with the age of 18.22 ± 0.79 and the age of controls was 18.52 ± 0.81. Among 50 in each group, 29 males, 21 females in pre-HTN group and 27 males, 23 females in the control group.

Table 1, a significant difference, was not found between-group differences in height and waist-hip ratio. However, pre-HTN group subject's BMI ($P < 0.000$) and weight ($P < 0.000$) was more compared to controls.

Table 2 indicates that in pre-HTN group, significantly higher HR ($P < 0.000$), SBP ($P < 0.000$), DBP ($P < 0.000$), MAP ($P < 0.000$), and RPP ($P < 0.000$) were seen when

compared to controls. No significant difference was seen in PP but it was slightly high in pre-HTN group and negatively associated with Vit-D (r: –0.327).

Table 3 depicts the values of Vit-D, telomerase in both groups. Significantly low levels of Vit-D ($P < 0.000$) and high telomerase ($P < 0.000$) were seen in pre-HTN group when compared to controls.

Table 4 shows the correlation of Vit-D and other parameters in pre-HTN group. Low levels of Vit-D have no correlation with BMI, waist-hip ratio, DBP, and MAP. However, significant correlation was seen with HR.

Further, as shown in Table 5, high telomerase levels have correlation with waist-hip ratio (r: 0.309), SBP (r: 0.322), DBP (r: 0.445), MAP (r: 0.691), and RPP (r: 0.390) but no significant correlation was seen with BMI, HR, and PP.

Table 1: Comparison of anthropometric characteristics between pre-HTN and controls

Parameter	**Pre-HTN (*n*=41)**	Controls (*n*=41)	***P*- value**
Age	19.11±1.25	17.69±0.91	0.339
Gender (male/female)	29/21	27/23	NA
Height (cm)	171.26±9.21	167.1±7.81	0.421
Weight (kg)	77.21±10.28	57.81±8.22	0.000
BMI (k/m2)	27.17±4.61	23.71±5.83	0.000
Waist to hip ratio	0.91±0.06	0.86±0.07	0.589

Table 2: Comparison of cardiovascular parameters between pre-HTN and controls

Parameter	**Pre-HTN (*n*=41)**	**Controls (*n*=41)**	***P*- value**
HR (BPM)	90.21±4.81	81.71±4.73	0.000
SBP (mmHg)	132.71±5.91	120.63±5.83	0.000
DBP (mmHg)	88.99±3.54	79.64±4.82	0.000
PP (mmHg)	41.85±7.82	35.02±5.75	0.272
MAP (mmHg)	94.87±2.51	82.90±2.81	0.000
RPP	12781.27±892.02	8721.72±581.62	0.000

Significant difference was seen in PP but it was slightly high in pre-HTN group and negatively associated with Vit-D (r: –0.327).

Table 3: Comparison of Vitamin D and telomerase levels between pre-HTN and controls

Parameter	**Pre-HTN (*n*=41)**	**Controls (*n*=41)**	***P*-value**
Vitamin D (ng/ml)	18.22±5.01	22.71±8.03	0.041
Telomerase (IU/ml)	41.82±20.66	9.51±5.90	0.000

DBP (r: 0.462), MAP (r: 0.724), and RPP (r: 0.309) but no significant correlation was seen with BMI, HR, and PP.

Table 4: Correlation between Vitamin D and other parameters in pre-HTN

Parameter	Vitamin D (ng/ml)	
	R-value	***P*-value**
BMI (kg/m2)	0.152	0.571
Waist hip ratio	0.227	0.191
HR (BPM)	–0.402	0.056
SBP (mmHg)	–0.672	0.000
DBP (mmHg)	0.372	0.261
PP (mmHg)	–0. 491	0.017
MAP (mmHg)	–0.181	0.621
RPP	0.572	0.001
Telomerase (IU/ml)	–0.482	0.011

Table 5: Correlation between telomerase and other parameters in pre-HTN

Parameter	Telomerase	
	R-value	***P*-value**
BMI (kg/m2)	0.251	0.121
Waist-hip ratio	0.521	0.028
HR (BPM)	0.111	0.732
SBP (mmHg)	0.523	0.001
DBP (mmHg)	0.472	0.001
PP (mmHg)	0.082	0.681
MAP (mmHg)	0.720	0.000
RPP	0.472	0.021
Vitamin D (ng/ml)	–0.301	0.022

Discussion:

Vitamin D has been implicated in the regulation of several pathways, besides its well-known role as regulator of the calcium-phosphate metabolism, it has been suggested that it may be implicated in immune system modulation [19],in the regulation of muscle strength and metabolism and in the cognitive decline [20].

In humans, more than 80% of vitamin D requirements is produced through the ultraviolet-B (UVB)-induced conversion of 7-dehydrocholesterol to vitamin D in the skin, whereas only 10%-20% is absorbed with the diet[1].

The photosynthesis of vitamin D evolved over 750 million years ago, first in the phytoplankton and then in early plants and animals[21]. From an evolutionary stand point it is interesting to note that the first living beings synthetizing vitamin D were missing calcific skeleton. This suggests that a new recognized non-metabolic role (called “non-classical effects”) of vitamin D might actually be the oldest. Regardless of the source, vitamin D require sliver

hydroxylation [through 25-hydroxylase (CYP2R1 or

CYP27A1)] to form 25-hydroxyvitamin D [25(OH) vitamin D or calcidiol], inactive form but used as reference for vitamin D status, because abundant, stable and easier to quantify[1]. In the kidney 25(OH) vitamin D is then hydroxylated to 1, 25-dihydroxyvitamin D [1, 25(OH) 2 vitamin D or calcitriol] the active form of vitamin D [through 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1)]. This latter step is a pivotal effector of calcium homeostasis and thus highly controlled by the up-regulation of parathyroid hormone (PTH) and the suppression of FGF23/ klotho axis [22]. Although the exact contribution of extra renal hydroxylation in determining the circulating levels of 1, 25(OH) 2 vitamin D is still unknown, it has been recognized also an extra-renal activity of CYP27B1. Finally, the recent identification of a role of vitamin D binding proteins on vitamin D catabolism has further increased complexity of the system[23].

Earlier reports have shown that higher Vit-D is related to longer telomere length, which underscores the conceivably advantageous impacts of this hormone on cell senescence and age-related conditions.[24]In this study, cellular senescence was assessed using telomerase. This enzyme attempts to inhibit the process of telomere shortening.[16, 17]Since the cell telomere loss appears to result from cell division just to a fractional degree, different components, particularly oxidative stress, were attested to assume a job in the expanded rate for shortening of telomeres.[26,27]The exact mechanism by which lower Vit-D levels are associated with this cellular senesce is hypothesized dependent on the perceptions recommends that the degrees of the telomerase may really be related to oxidative stress, with higher oxidative stress prompting higher telomerase levels. Cells of nearly complex organism may not have an ability to divide. This marvel was depicted by Hayflick in 1961.[16, 25, 26]

Low Vit-D advances insulin resistance,[27]endothelial dysfunction,[28]production of pro-inflammatory cytokines,[29]hyperparathyroidism, and hypocalcaemia influencing vascular smooth muscles.[30]

Conclusion:

The reduced Vit-D levels in pre-HTN may cause derangements of cardiovascular homeostatic mechanism, enhance the speed of cellular senescence measured by telomerase.

Vitamin D deficiency is highly prevalent, particularly amongst older person and hypovitaminosis D may accelerate senescence. Aging and, in particular, senescence is associated with an increased risk of MetS.

Gender differences in the biological mechanisms leading to senescence have been described and these differences may influence different prevalence of MetS according to gender.

References:

1. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357: 266-281 [PMID: 17634462 DOI: 10.1056/NEJMra070553]
2. Lucas RM, Ponsonby AL, Dear K, Valery PC, Taylor B, van der Mei I, McMichael AJ, Pender MP, Chapman C, Coulthard A, Kilpatrick TJ, Stankovich J, Williams D, Dwyer T. Vitamin D status: multifactorial contribution of environment, genes and other factors in healthy Australian adults across a latitude gradient. J Steroid Biochem Mol Biol 2013; 136: 300-308
3. Holick MF. Environmental factors that influence the cutaneous production of

vitamin D. Am J Clin Nutr 1995; 61: 638S-645S [PMID: 7879731]
4. Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, Shoenfeld Y, Lerchbaum E, Llewellyn DJ, Kienreich K, Soni M. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence. Autoimmun Rev 2013; 12: 976-989
5. Amer M, Qayyum R. Relationship between 25-hydroxyvitamin D and all-cause and cardiovascular disease mortality. Am J Med 2013; 126: 509-514
6. Gunville CF, Mourani PM, Ginde AA. The role of vitamin D in prevention and treatment of infection. Inflamm Allergy Drug Targets 2013; 12: 239-245
7. Pelajo CF, Lopez-Benitez JM, Miller LC. Vitamin D and autoimmune rheumatologic disorders. Autoimmun Rev 2010; 9: 507-510
8. Annweiler C, Rolland Y, Schott AM, Blain H, Vellas B, Beauchet O. Serum vitamin D deficiency as a predictor of incident non-Alzheimer dementias: a 7-year longitudinal study. Dement Geriatr Cogn Disord 2011; 32: 273-278
9. Freedman DM, Looker AC, Abnet CC, Linet MS, Graubard BI. Serum 25-hydroxyvitamin D and cancer mortality in the NHANES III study (1988-2006). Cancer Res 2010; 70: 8587-8597
10. Liu L, Chen M, Hankins SR, Nùñez AE, Watson RA, Weinstock PJ, Newschaffer CJ, Eisen HJ. Serum 25-hydroxyvitamin D concentration and mortality from heart failure and cardiovascular disease, and premature mortality from allcause in United States adults. Am J Cardiol 2012; 110: 834-839
11. Yadav S, Boddula R, Genitta G, Bhatia V, Bansal B, Kongara S, et al. Prevalence and risk factors of pre-hypertension and hypertension in an affluent North Indian population. Indian J Med Res 2008;128:712-20.
12. Asmathulla S, Rajagovindan D, Sathyapriya V, Pai B. Prevalence of prehypertension and its relationship to cardiovascular disease risk factors in Puducherry. Indian J Physiol Pharmacol 2011;55:343-50.
13. Mahmood SE, Ansari SH. Prevalence of prehypertension and hypertension in rural Bareilly. Natl J Med Res 2012;2:291-4.
14. Ray S, Kulkarni B, Sreenivas A. Prevalence of prehypertension in young military adults and its association with overweight and dyslipidaemia. Indian J Med Res 2011;134:162-7.
15. Seĵy SS, Naik A. Prevalence of prehypertension amongst medical students in Coastal Karnataka. J Evol Med Dent Sci 2012;1:975-80.
16. von Zglinicki T. Telomeres and replicative senescence: Is it only length that counts? Cancer Lett 2001;168:111-6.
17. Joosten SA, van Ham V, Nolan CE, Borrias MC, Jardine AG, Shiels PG, et al. Telomere shortening and cellular senescence in a model of chronic renal allograft rejection. Am J Pathol 2003;162:1305-12.
18. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension 2003;42:1206-52.
19. Sassi F, Tamone C, D'Amelio P. Vitamin D: nutrient, hormone,and immunomodulator. Nutrients 2018;10:1656.
20. D'Amelio P, Quacquarelli L. Hypovitaminosis D and aging: is there a role in muscle and brain health? Nutrients 2020;12:628.
21. Reschly EJ, Bainy AC, Mattos JJ, Hagey LR, Bahary N, Mada SR, Ou J, Venkataramanan R, Krasowski MD.

Functional evolution of the vitamin D and pregnane X receptors. BMC Evol Biol 2007; 7: 222
22. Bergwitz C, Jüppner H. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. Annu Rev Med 2010; 61: 91-104
23. Malik S, Fu L, Juras DJ, Karmali M, Wong BY, Gozdzik A, Cole DE. Common variants of the vitamin D binding protein gene and adverse health outcomes. Crit Rev Clin Lab Sci 2013;50: 1-22
24. 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;86:1420-5.
25. Greider CW, Blackburn EH. The telomere terminal transferase of tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 1987;51:887-98.
26. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 1965;37:614-36.
27. 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;92:2017-29.
28. Talmor Y, Golan E, Benchetrit S, Bernheim J, Klein O, Green J, et al. Calcitriol blunts the deleterious impact of advanced glycation end products on endothelial cells. Am J Physiol Renal Physiol 2008;294:F1059-64.
29. Talmor Y, Bernheim J, Klein O, Green J, Rashid G. Calcitriol blunts pro-atherosclerotic parameters through NFkappaB and p38 in vitro. Eur J Clin Invest 2008;38:548-54.
30. Freundlich M, Quiroz Y, Zhang Z, Zhang Y, Bravo Y, Weisinger JR, et al. Suppression of renin-angiotensin gene expression in the kidney by paricalcitol. Kidney Int 2008;74:1394-402.