Original Article

Comparison between Vitamin-D and Urinary Angiotensin Converting Enzyme Levels in Type 2 Diabetic Hypertensive Patients with Non-Diabetic Hypertensive Patients

Mah-e-Jabeen Sear, Sara Naeem, Saima Mukhtar*

[1]Department of Physiology, Rahbar Medical and Dental College, Lahore, Pakistan.

***Correspondence:**

Mah-e-Jabeen Sear
Jabeenraza@live.co.uk

Received: 3 February, 2023

Accepted: 5 April, 2023

DOI 10.51846/jucmd.v2i2.2310

Abstract

Objective: To correlate urinary Angiotensin Converting Enzyme–2 (ACE-2) with serum vitamin D levels in Type 2 Diabetic-Hypertensive and non-Diabetic-hypertensive patients.

Methodology: The Department of Physiology at the University of Health Sciences in Lahore undertook this observational, correlational study from March 2018 to February 2019. The Services Institute of Medical Services (SIMS), Lahore's medical OPD and diabetic centers were used to select 95 hypertension patients between the ages of 30 and 60 divided into 2 groups. Anthropometric parameters as well as Blood Sugar Random (BSR) and serum Vitamin D were recorded. Urine samples for evaluating angiotensin converting enzyme 2 were collected.

Results: Urinary ACE2 showed negative correlation with serum Vitamin D level (r=-0.336, p=0.034) in group A non-diabetic hypertensive patients, but not in group B diabetic hypertensive patients (r=0.07, p=0.677). Despite somewhat greater vitamin D levels in group B, this difference was not statistically significant.

Conclusion: There was no link between ACE2 and blood Vitamin D levels in the diabetic hypertensive patients, despite the fact that we hypothesised that vitamin D would be considered as a viable treatment alternative for treating these individuals.

Keywords: The Renin Angiotensin System (RAS), Serum vitamin D levels, Angiotensin Converting Enzyme 2(ACE-2), Diabetes Mellitus, Hypertension

Introduction

Two of the chronic diseases whose prevalence is increasing globally every day are diabetes and hypertension.[1] Diabetes prevalence among persons over the age of 18 has increased globally, from 4.7% in 1980 to 8.5% in 2014.[2] Recently compiled data shows that approximately 150 million people are afflicted by diabetes mellitus worldwide; the number may double by 2025.[3] Majority of the increase in cases will be in the developing countries due to population growth, aging, unhealthy diets, obesity and sedentary lifestyle.[4] Diabetes is a major cause of stroke, limb amputation, blindness, heart attack and renal failure Diabetes is thought to have directly contributed to 1.6 million deaths in 2015 and by 2030, diabetes is predicted to overtake heart disease as the sixth biggest cause of death.[5] While managing blood pressure and maintaining fluid balance, the Renin Angiotensin System (RAS) is crucial. It maintains a balanced pressure and resistance of blood in the small capillaries regulating hypertension. Many diseases including hypertension are caused by RAS activity that is out of equilibrium. Diabetes alone is not the only health concern in Pakistan. There is also a growing concern among the population about the growing number of vitamin D deficiency cases throughout the country. A steep rise in vitamin D deficiency among South Asians has been documented over the past few decades.[6] It has been observed by health experts that vitamin D deficient subjects are prone to develop diabetes mellitus accompanied with hypertension.[7] Many surveys have been conducted in all provinces of Pakistan over the years to find out the prevalence of Diabetes Mellitus in numerous populations and age groups but less research has been done on the concurrent presence of Type 2 Diabetes Mellitus and hypertension in vitamin D deficient patients. In this study, patients from the local Punjabi population who have Type 2 diabetes and hypertension as well as those who have never developed diabetes are examined to better understand and link urine angiotensin 2 a measure of renal tissue activity with serum vitamin D levels.

Renal failure is one of the leading complications of both diabetes mellitus as well as hypertension increasing the burden of morbidity and mortality linked to them. To be able to detect developing renal derangement very early in its evolvement would be revolutionary to curtailing its dreary fate. Urinary ACE 2 could be a marker which helps to reveal early renal damage in its initial stages. We hoped to prove this in our study thereby decreasing the incidence of chronic renal failure.[8] As vitamin D is involved in down-regulating ACE-2, it may play protective role against development of both diabetes mellitus and hypertension. Keeping this in mind, we hoped to find evidence to support this role of vitamin D suggesting its prophylactic use as well as impeding the progression and severity of hypertension and DM.

Methodology

This observational correlational experimental work was regulated in UHS Physiology depart-

ment, Lahore, within period of approximately 12 months following moral consent by the Institutional Ethical Review Committee. A total of 95 patients were subjected for research work between the age figure of 30-60 years subjected from the life rescue department outdoor patient department and center for diabetic patients of Services Institute of Medicine Sciences (SIMS). There were two groups created from these patients: Group A included non-diabetic hypertensive patients whereas Group B included diabetic hypertensive patients. Group "A" contained 46 patients who were already diagnosed cases hypertensive but not diabetic and Group "B" contained 49 patients who were already diagnosed cases diabetic and hypertension. Each recipient's signed informed consent was obtained before general and systemic analysis was used to eradicate any integral disorders. Sphygmomanometer was used to record the subjects' blood pressure. Asymmetrically blood glucose was observed then and there. Another device with name Enzyme-Linked Immunosorbent Assay (ELISA) kit used to evaluate the urinary ACE margin point. Serum choleciferol levels were evaluated by ELISA kit. (Bio-Rad Laboratories, USA).

Statistical Analysis

The data was examined while using the statistical analytical tool for social sciences (SPSS) version 20. The terms "mean" and "Standard Deviation" refer to normally distributed descriptive variables, whereas median and Inter Quartile ange (IQR) are intended for asymmetrically distributed numerical values. Shapiro-statistical Wilk's tool corrected the data's classification. Student "T" tests and p-value of 0.05 were used to compare group means for quantitative variables with regularly distributed dispersion while Mann Whitney "U" tests were applied to relate group means for quantitative parameters with erratically scattered distributions. The Spearman's "rho" correlation was used to establish the correlation forms among quantitative variables that were thought to be not normally distributed while Pearson correlation (r) was used to find the correlation relationships between variables that were normally distributed. A p-value below 0.05 was typically applied to get statistically meaningful object for all intents.

Results

According to the Shapiro-Wilk Test information gathered from urine ACE 2 levels was not distributed ethically. The median value for each branch must be found. Non-diabetic, hypertension patients (group A) had a median (IQR) of 26.47 (19.5-34.3) mg/dl, while hypertensive patients' with DM (group B) had a median (IQR) of 22.86 (16-28.2) mg/dl (Figure 1).

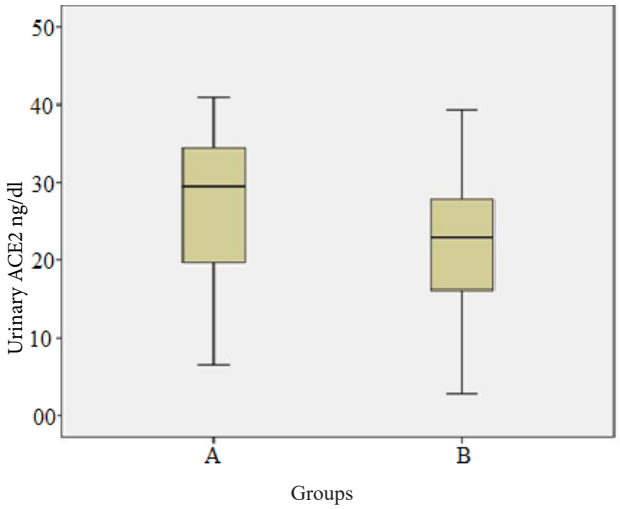

Figure 1. Variation in ACE 2 Levels in the Urine was Substantial. Comparison of Group A's and Group B's Mean Urinary ACE 2

Urinary ACE2 showed negative correlation with serum vitamin D level (rho=-0.336, p=0.034) in non-diabetic, hypertensive patients (Figure 2) but not in diabetic hypertensive patients (Table 2).

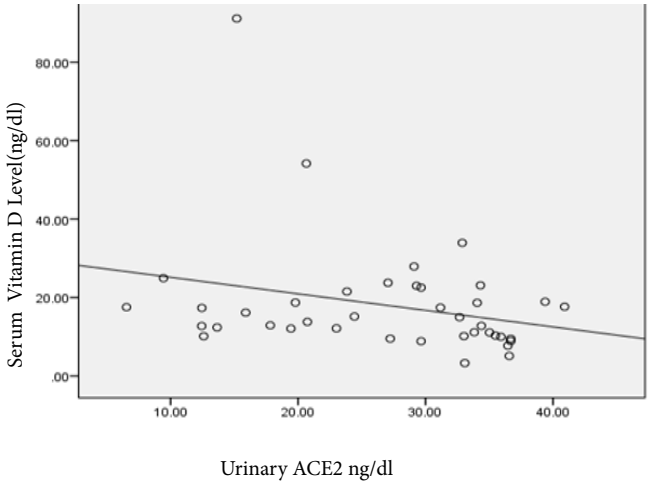

Figure 2. Correlation between uACE2 and Vitamin D in Group A.

Table 1. Comparison of sVitamin D & uACE2 in the two groups

Parameters	Group A	Group B	p-value	Distribution
sVit.D (ng/dl)	14.4(10.2-20.9)	15.8(11.1-23.6)	0.415[b]	Irregular
uACE2 (ng/dl)	26.5(19.5-34.3)	22.9(16.0-28.2)	0.007[b]	Irregular

Where
"s" represents serum
"u" represents urinary.
p less than 0.05 is significant

Table 2. Correlation between urinary ACE2 & serum Vitamin D level in Group A & B

sVit D		Group A	Group B
		sVit D	sVit D
Urinary ACE2	rho	-0.336*	0.07
	p- value	0.034	0.677
	n	40	38

Where rho represents Spearman's correlation coefficient,
"n" represents number of patients.
p less than 0.05 is significant

Discussion

Diabetes causes scarring of the kidneys which in turn leads to salt and water retention, raising blood pressure. Numerous studies are being conducted to better understand the pathology of the onset and development of complications associated with diabetes and hypertension as well as their associated morbidity and mortality. These studies also aim to identify predisposing factors and new biochemical markers of complications. Among the list of predisposing factors vitamin D deficiency seems to be the most relevant and relatable factor as majority of our population is vitamin D deficient. Renin Angiotensin System has two opposing lines of action.[9] One of its actions is secreting Angiotensin II with the help of Angiotensin Converting Enzyme (ACE-I). Antagonistic to this action this system also degrades Angiotensin (ACE-II) via Angiotensin Converting Enzyme II.[9] The first action is responsible for elevating blood pressure causing hypertension whereas the second action is responsible for decreasing blood pressure. As Type II Diabetes Mellitus causes loss of Angiotensin Converting Enzyme (ACE-II) in urine, it predisposes to hypertension. Vitamin D enhances ACE-II and thus may prevent hypertension and diabetes mellitus.[10]

Several studies have shown that diabetes inhibits RAS's protective function which hastens the development of hypertension. Insulin resistance which has been linked to RAS and is thought to be major contributor to type 2 diabetes mellitus, it is also one of the causes of hypertension. on the other hand ACE2 overexpression decreases ROS production and identifies it as a novel antioxidant. Hepatic insulin resistance which is believed to be caused by increased angiotensin II levels has been identified as the primary cause of type 2 diabetes mellitus.

As vitamin D impedes renin creation, with time it reduces raised blood pressure.[11] Thus, it acts as a negative endocrine controller of the renin-angiotensin system (RAS).[11] Evidence to this effect was obtained in a large meta-analysis according to which a 25nmol/L upsurge in 25(OH)D concentrations depresses the threat of incident hypertension by 12%.[12] Deletion of vitamin D receptors in mice demonstrated up-regulation of renin mRNA and protein expression in their kidneys. Consequently, they had hypertension and target-organ damage. The influence of vitamin D on RAS is not a secondary effect via calcium and parathyroid hormone rather a direct suppression of the renin gene transcription through 1,25(OH)2D-liganded VDR.[13] In numerous cross-sectional investigations, it has been discovered that markers for the chance of developing diabetes later in life are inversely associated to vitamin D levels. The hypothesis that vitamin D treatment improves glucose homeostasis has also been upheld by a few animal studies. A study carried out on Korean population showed that reduced 25(OH) D serum levels were coupled with insulin resistance.[14] Similar studies on Asian populations demonstrate a correlation of dwindling 25(OH) D levels with high fasting insulin levels and high HOMA-IR scores. 1,25(OH)D-3 contributes to the pathogenesis of both diabetes mellitus and essential hypertension by inhibiting the RAS.[15]
We anticipated a negative connection between urine ACE2 and vitamin D in patients with either one or both of these illnesses given the mounting evidence for vitamin D's preventive function against both hypertension and diabetes mellitus. Although we obtained a significant negative correlation between the two parameters in hypertensive patients without diabetes mellitus, it was not significant in the patients with both hypertension and diabetes mellitus.

This might have occurred as a result of differences in the standard of care and patient management. Individuals for Group A were drawn from the SIMS medical outdoor patient department and emergency department while group B subjects were chosen from the SIMS diabetes center. Unfortunately, we neglected to account for the variations in medical treatment provided by the two departments at the time of sampling. The diabetes center is a very well-run methodical and organized division with excellent patient counseling and education resources and a sound tracking of patients throughout treatment. Although they also accept incoming poorly controlled diabetes patients, the bulk of their patients are trained enough to keep a good control of their blood glucose levels who come in for follow-up consultations. As a result, they do well and typically experience less difficulties than patients who visit SIMS's other departments. The medical OPD/emergency in contrary interacts with a bigger volume of patients but is less regulated and qualified. The majority of patients are illiterate, misinformed and underprivileged people who know very little about their chronic illnesses let alone the treatments they will receive. In comparison to patients visiting the diabetic clinic and subsequent visits compliance is significantly lower. As a result, those who were recruited from this environment typically had worse health than those who came from the diabetes clinic. We were unaware of this restriction when we conducted the tests and the sampling. The only time we became aware of our unintended mistake in patient selection was during the compiling of the facts and retrograde scrutiny.

Conclusion

We could prove our hypothesis about correlation between ACE2 levels in urine and serum vitamin D levels in patients with hypertension alone, although we could not do so in patients with both hypertension and diabetes mellitus. Therefore, further research is required to undoubtedly declare vitamin D as a negative controller of the RAS.

Limitation of Study

We want to clarify a confounder/limitation of the study at the end of this manuscript. The Services Institute of Medical Sciences (SIMS) located in Lahore, served as the site of the study's sample. Although we made every effort to demographically pair the patients from the two groups, we still found that the patients' quality of care and level of control varied. This was due to the fact that group B subjects were chosen from (SIMS's) diabetic center, having patients trained enough to keep a good control of their blood glucose levels who come in for follow-up consultations, as opposed to group A subjects who were chosen from SIMS's medical OPD and emergency, less organized and less equipped and deal with a higher volume of diabetic patients. Regrettably, we did not account for this difference in medical care at the onset of this study. The only time we noticed the difference in our unintended selection criteria of both groups in patient selection was during the compilation of the findings and retrograde analysis. Pragmatically, we would have chosen patients who were un-medicated diabetics and attending the clinics for the first time.

Authors' contribution: MJS wrote the manuscript and literature review, SN contribute in literature review and statistical analysis; SM helped in data collection and compilation

Conflict of interest: All co-authors have seen and agree with the contents of the manuscript and there is no conflict of interest to report.

Funding: This experiment was funded by University of Health Sciences, Lahore, Pakistan.

Acknowledgement: We are extremely grateful to the untiring and remarkable support and supervision of Late Professor Khalid Pervez Lone. He will always remain an inspiration for many physiologists like us.

References

1. Hossain MB, Khan MN, Oldroyd JC, Rana J, Magliago DJ, Chowdhury EK, et al. Prevalence of, and risk factors for, diabetes and prediabetes in Bangladesh: Evidence from the national survey using a multilevel Poisson regression model with a robust variance. PLOS Global Public Health. 2022 Jun 1;2(6).

2. Arokiasamy P, Salvi S, Selvamani Y. Global burden of diabetes mellitus: prevalence, pattern, and trends. Handbook of Global Health. 2021:495-538.

3. Lopez AD, Mathers CD. Measuring the global burden of disease and epidemiological transitions: 2002–2030. Annals of Tropical Medicine & Parasitology. 2006 Aug 1;100(5-6):481-99.

4. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature reviews endocrinology. 2018 Feb;14(2):88-98.

5. Yuvaraj N, SriPreethaa KR. Diabetes prediction in healthcare systems using machine learning algorithms on Hadoop cluster. Cluster Computing. 2019 Jan 16;22(Suppl 1):1-9.

6. Siddiqee MH, Bhattacharjee B, Siddiqi UR, Rahman MM. High prevalence of vitamin D insufficiency among South Asian pregnant women: a systematic review and meta-analysis. British Journal of Nutrition. 2022 Sep;128(6):1118-29.

7. Jwad SM, AL-Fatlawi HY. Diabetic Nephropathy and Its Effect on the Physiological Factors of The body. Renal Failure. 2022 Jun 14;8:10.

8. Candari CJ, Cylus J, Nolte E. Assessing the economic costs of unhealthy diets and low physical activity: An evidence review and proposed framework. WHO Regional Office for Europe; 2017.

9. Silva AC, Miranda AS, Rocha NP, Teixeira AL. Renin angiotensin system in liver diseases: Friend or foe?. World journal of gastroenterology. 2017 May 5;23(19):3396.

10. Shiota A, Yamamoto K, Ohishi M, Tatara Y, Ohnishi M, Maekawa Y, et al. Loss of ACE2 accelerates time-dependent glomerular and tubulointerstitial damage in streptozotocin-induced diabetic mice. Hypertension Research. 2010 Apr;33(4):298-307.

11. Weng S, Sprague JE, Oh J, Riek AE, Chin K, Garcia M, Bet al. Vitamin D deficiency induces high blood pressure and accelerates atherosclerosis in mice. PloS one. 2013 Jan 22;8(1):e54625.

12. Skaaby T. The relationship of vitamin D status to risk of cardiovascular disease and mortality. Dan Med J. 2015 Feb 1;62(2):1-7.

13. Bouillon R, Carmeliet G, Verlinden L, Van Etten E, Verstuyf A, Luderer HF et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocrine reviews. 2008 Oct 1;29(6):726-76.

14. Webb MJ, Kauer SD, Ozer EM, Haller DM, Sanci LA. Does screening for and intervening with multiple health compromising behaviours and mental health disorders amongst young people attending primary care improve health outcomes? A systematic review. BMC family practice. 2016 Dec;17(1):1-2.

15. Jia Y, Song T, Li Z, Zhou L, Chen S. The Relationship Between Triglyceride Glucose Index and Vitamin D in Type 2 Diabetes Mellitus. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2022 Jan 1:511-25.