Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 2715 - 2727
Received 15 December 2020; Accepted 05 January 2021.

STUDY ON SERUM HEPCIDIN LEVEL IN CHRONIC KIDNEY DISEASE

Jyothi Elizabeth Roy[1], V.S. Kalaiselvi, B. Shanthi[2]
[1,2]Department of Biochemisty, Sree Balaji Medical College & Hospital Chennai -600 044.
* shanthi.b@bharathuniv.ac.in

ABSTRACT (Times New Roman, bold, 10)

AIM
The present study was to estimate and to compare serum hepcidin levels in controls and patients of chronic kidney disease and to check the correlation of hepcidin to anemia and inflammation in chronic kidney disease.
OBJECTIVES
Estimate serum hepcidin levels in controls and patients with chronic kidney disease. Determine and correlated to markers of anemia and inflammations in chronic kidney disease.
MATERIALS AND METHODS
Serum Hepcidin was estimated along with other biochemical parameters such as serum Urea, Creatinine, Iron, Ferritin, Transferrin, Total iron binding capacity (TIBC), hsCRP and Hemoglobin.
RESULTS
Hepcidin could be a prognostic marker in the clinical outcome of CKD especially in the progression of CKD.
CONCLUSION
Serum hepcidin correlated positively with markers of iron status (iron and ferritin) in the CKD group

Keywords
Hepcidin, iron, urea ferritin, transferrin, inflammation and erythropoietin.

Introduction

Chronic kidney disease (CKD) refers to an irreversible progressive deterioration in renal function. CKD has become a worldwide, chronic, non-communicable disease epidemic with adverse outcomes of renal failure, cardiovascular disease and premature death. In developed countries, it affects 10 -15% of adult general population. CKD is a chronic inflammatory state which promotes endothelial dysfunction and vascular remodeling. The deteriorating renal function may result in accumulation of uremic toxins which further stimulate inflammation. Anemia is a complication of chronic kidney disease which starts manifesting in the initial stages and increases its prevalence with the progression of CKD. [1-3] The main causes of anemia in CKD are erythropoietin deficiency, iron deficiency and chronic inflammation. The discovery of hepcidin and its functions has led to a better understanding of iron metabolism disorders in CKD. Hepcidin, a small cysteine rich liver-derived peptide. Hormone is the key regulator of systemic iron homeostasis. Altered homeostasis of hepcidin results in various iron disorders. Hepcidin has evolved as the star mediator of anemia of chronic disease and inflammation. Hepcidin functions by causing degradation of the iron transporter ferroportin. Inflammation increases hepcidin production while erythroid activity and hypoxia decrease hepcidin levels. [4-6] Hepcidin levels reflect various key signals involved in iron regulation and it directly controls iron absorption and its bioavailability in circulation. So its measurement should be useful as a clinical tool for the management of iron disorders.

Previous work has shown that serum hepcidin levels were increased in patients with chronic kidney disease who had co-existent anemia. It is possible that changes in hepcidin may underlie the association between anemia and inflammation associated with chronic kidney disease. Increased serum hepcidin levels result in anemia and resistance to erythropoietin stimulating agents. Treatment with anti-hepcidin drugs may improve anemia of CKD. Hence in the present

International Journal of Research in Medical Sciences
Amitha TA et al. Int J Res Med Sci. 2022 Mar;10(3):660-665
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20220514

Original Research Article

An analysis of vitamin D levels and the factors affecting vitamin D levels among the adult population attending a tertiary care hospital

Amitha T. A.*, Lekshmi G. S., Jyothi Roy

Department of Biochemistry, Sree Gokulam Medical College and Research Centre, Venjaramoodu, Kerala, India

Received: 06 December 2021
Revised: 31 December 2021
Accepted: 27 January 2022

***Correspondence:**
Dr. Amitha TA,
E-mail: amithata162@gmail.com

ABSTRACT

Background: The aim of the study was to determine the vitamin D levels and the factors affecting vitamin D levels among the adult population attending a tertiary care hospital.
Methods: An observational cross-sectional study was conducted among 568 adult individuals attending various outpatient departments at Sree Gokulam Medical College and Research Centre, Venjaramoodu over a period of 3 months from 1st January to 30th March of 2021. 119 males and 449 females were recruited in the study. Only adult patients between the age of 18 to 70 were included in the study. Blood samples were collected randomly from patients attending different OPDs. Serum 25-hydroxyvitamin D (25(OH) D) level was measured using electrochemiluminescence immunoassay (ECLIA) in Roche Cobas e 411 fully automated analyzer.
Results: Among the study population, 52.9% of males had Vitamin D deficiency and 70.8% of females had vitamin D deficiency with a p value 0.001. Based on age, the study population was subdivided into three groups-young, middle age and old age groups. 76.9% of the young age group had vitamin D deficiency. 57.2% of middle age group and 56.8% of old age group were Vitamin D deficient with a p value 0.0001.
Conclusions: Age and gender are significantly related to Vitamin D levels. While individual's occupation, BMI and their religion are less significant factors correlated to vitamin D levels in an individual.

Keywords: Factors affecting vitamin D level, 25-hydroxyvitamin D [25(OH) D], Gender, Age group

INTRODUCTION

Vitamin D is often described as a vitamin, which is indeed a misnomer and is well established as a hormone. Its active form, 1,25-dihydroxy cholecalciferol, also known as Calcitriol, involved in bone metabolism and also in many non-skeletal physiological processes.[1] Calcitriol has an important role in bone metabolism through regulation of calcium and phosphate equilibrium.[2]

Vitamin D is synthesized in the skin through sunlight exposure mostly during summer. It is also found in fish oil, eggs and fortified food products.[3] The production of vitamin D is directly proportional to the skin's exposure to sunlight and inversely proportional to pigmentation of skin. The Solar zenith angle can increase or decrease the wavelength of UV rays. Short wavelength UV rays favour vitamin D synthesis more than long-wavelength UV rays during winter. Calcitriol promotes calcium and phosphorous absorption from the intestinal brush border passively and actively from the intestinal cells to the blood by sodium- calcium exchange mechanism or by pumping calcium-calbindin complex.[4] When calcitriol enters a cell, it binds to VDR (vitamin D receptor), a specific nuclear receptor, forms a heterodimeric complex with RXR (retinoid X receptor), and binds to VDRE (vitamin D response elements) on specific DNA,

resulting in transcriptional activation of a specific gene that codes for calcitriol.[5] In bone, calcitriol coordinates remodelling of bone through the activity of osteoblasts and increases bone mineral density. The kidneys have an important role in activating plasma 25-hydroxycholecalciferol (25-HCC) to 1,25-dihydroxy cholecalciferol (1,25-HCC), the active vitamin D-Calcitriol, by the action of 1-alpha hydroxylase located in the mitochondria of proximal convoluted tubules (PCT).[6] This calcitriol helps the kidney tubules reabsorb calcium and phosphorous, in turn balancing their concentrations in our body. Calcitriol is also involved in the regulation of parathyroid hormone (PTH) action, which raises blood calcium levels through bone resorption.[7] It was also observed that patients with kidney failure are at high risk for developing secondary hyperparathyroidism.[8,9] Most organs and tissues in the body express VDR, so vitamin D modulates the expression of more than 500 genes by regulating the transcriptional activity of vitamin D responsive genes.[10] It has been found that Vitamin D is necessary for signal transduction mechanisms in all organs, especially in the brain and immune cells.

Vitamin D deficiency is common among the general population. Recent studies found an unpredictable high prevalence of vitamin D deficiency in apparently healthy adults in different countries, which could result in major health problems in the future. Vitamin D deficiency is common among pregnant women in certain populations and it is associated with an increased risk of GDM (gestational diabetes mellitus), preterm birth and pre-eclampsia. Vitamin D supplementation and improvement in maternal Vitamin D status reduce the risk of pre-eclampsia, low birth weight and pre-term birth.[11] So, current evidence available infers that vitamin D supplementation in pregnancy improves maternal and infant health outcomes. The health effects of Vitamin D on the musculoskeletal system in children and adults prevent rickets and osteoporosis, respectively. In conditions like respiratory illness, cardiovascular disease, cancers and certain neurological conditions, the potential role of Vitamin D are immense which is proved by recent studies.[12] So, maintenance of adequate Vitamin D status is warranted in reducing the risk of many diseases like cancer, diabetes mellitus, cardiovascular diseases, and autoimmune diseases. Clinical and epidemiological studies support the link between vitamin D deficiency and chronic disease progression such as obesity, diabetes, and hypertension.[13,14] It is also noted that susceptibility to infection has increased in vitamin D deficiency because of impairment of toll-like mediated induction of antimicrobial peptide cathelicidin from macrophages.[11]

25-HCC formed in the liver is used to determine a patient's vitamin D status. Vitamin D status is considered as follows: less than 20 ng/ml indicates a deficiency, levels of 20–29 ng/ml indicate relative insufficiency of vitamin D, and a level of 30 ng/ml or greater can be considered sufficient.[16] Considering this definition, about a billion people around the world have vitamin D deficiency. Even in the sunniest countries like India, vitamin D deficiency is very common due to inadequate exposure to sunlight.[17] Several factors can affect this, including the use of sunscreen, age, skin pigmentation, clothing, and season. This study aims to find the factors affecting the vitamin D levels and estimate the vitamin D deficiency in people visiting our tertiary care hospital.

METHODS

A retrospective observational cross-sectional study was conducted among 568 adult individuals at Sree Gokulam Medical College and Research Centre, Venjaramoodu over a period of 3 months from January to March of 2021. The socio-demographic data, religion, lifestyle, dietary habits and psychosocial factors were noted. Blood samples were collected randomly from patients attending Obstetrics and gynaecology, General Medicine, Orthopaedics and Endocrinology outpatient departments. Only adult patients between the ages of 18 to 70 were included in the study.

Blood samples were obtained by venipuncture and collected in clot-activator tubes. The tubes were centrifuged for 10 minutes at 35000 rpm within 2 hours of sample collection to separate serum. The serum 25-hydroxyvitamin D (25(OH) D) level was determined using a Roche Cobas e 411 electrochemiluminescence immunoassay (ECLIA). Although there is no definite normal level of vitamin D, experts opine that vitamin D deficiency is less than 20 ng/ml, levels of 20-29 ng/ml indicate a relative insufficiency of vitamin D and a level of 30 ng/ml or greater can be considered as sufficient.[14]

Inclusion criteria

Both males and females of the age 18-70 years, visiting our tertiary care hospital.

Exclusion criteria

Subjects under 18 years of age and age more than 70 years of age. Critically ill patients. Liver and kidney pathology. On vitamin D supplements. History of Parathyroid disease.

Data analysis was done in Statistical package for social sciences (SPSS) Software version 22. Quantitative data is presented with the help of mean, median and SD. Correlation among various variables was assessed with the Pearson correlation coefficient. Qualitative data are presented with the help of the frequency and percentage table and the association among study group was assessed with the help of the Chi-Square test. P value less than .05 is taken as a significant level.

RESULTS

568 adults including both genders were included in the study. The mean value of vitamin D from this study is

19.38 ng/ml and the standard deviation is 13.397 (Table 1).

Table 1: Vitamin D level, mean and standard deviation in the sample population.

Total number of samples	568
Mean	19.381
Std. Deviation	13.397

The study found that among 568 adults, 381 individuals had vitamin D deficiency, while 98 individuals had inadequate vitamin D levels and only 89 individuals had adequate vitamin D levels in their bodies.

The comparison of the age among the study group was done and shown in Figure 1. The study population was divided into 3 subgroups based on age as young aged group of 18-35 years (286), middle aged group of 36-59 years (187) and old-aged group with 60-70 years (95). Among the young aged group, 220 (76.9%) individuals were vitamin D deficient, while 33 (11.5%) had inadequate vitamin D levels and 33 (11.5%) had normal vitamin D levels. Among the 187 middle aged individuals, 107 (57.2%) individuals had Vitamin D deficiency, 45 (24.1%) had inadequate vitamin D levels and 35 (18.7%) had normal vitamin D levels. Among the 95 individuals of old aged group, 54 (56.8%) of them were Vitamin D deficient, 21 (22.1%) had inadequate levels and 20 (21.1%) had normal levels of Vitamin D levels. This showed a very significant association with a P value .001 and a chi-square value of 26.29.

Comparing gender with vitamin D levels is shown in Figure 2, among them 63 (52.9%) males were vitamin D deficient and 29 (24.4%) males had inadequate vitamin D levels and 27 (22.7 %) males had normal vitamin D levels. Among the 449 females enrolled in the study, 318 (70.8%) females were vitamin D deficient, 39 (13.4%) females had inadequate vitamin D levels while 71 (15.8%) females had normal vitamin D levels. This was found to be statistically significant with a p value 0.001 and a chi-square value of 14.33.

Table 2: Association of vitamin D levels and body mass index.

BMI	Deficiency (≤20 ng/ml)	Inadequate (21-29 ng/ml)	Normal (≥30 ng/ml)	Total
Underweight	1 (33.3%)	0 (0 %)	2 (66.7 %)	3
Normal	99 (72.8 %)	17 (12.5%)	20 (14.7 %)	136
Overweight	138 (65.4 %)	31 (14.7 %)	42 (19.9 %)	211
Obese	143 (65.6 %)	41 (18.8 %)	34 (15.6 %)	218
Total	381 (67.1%)	89 (15.7%)	98 (17.3%)	568

P value 0.119; Chi square value of 10.136

Table 3: Association of vitamin D with different occupation.

		Deficiency (≤ 20 ng/ml)	Inadequate (21-29 ng/ml)	Normal (≥30 ng/ml)	Total
Occupation	Professional	35 (9.2%)	7 (7.9%)	10 (10.2%)	52 (27.3%)
	Semi-professional	32 (8.4%)	10 (11.2%)	14 (14.3%)	56 (33.9%)
	Clerical work	27 (7.1%)	5 (5.6%)	6 (6.1%)	38 (18.8%)
	Skilled	105 (27.6%)	18 (20.2%)	11 (11.2%)	134 (59%)
	Semiskilled	107 (28.1%)	28 (31.5%)	36 (36.7%)	171 (96.3%)
	Unskilled	30 (7.9%)	10 (11.2%)	14 (14.3%)	54 (33.4%)
	Unemployed	45 (11.8%)	11 (12.4%)	7 (7.1%)	63 (31.3%)

P value of 0.063; Chi square value of 20.22.

While comparing the body mass index (BMI) in the study population (Table 2), 218 (38.5%) individuals, were obese, 211 (37.1%) overweight, 136 (23.9%) normal and 3 (0.5%) underweight subjects. Out of these obese individuals, 143 (37.5%) were Vitamin D deficient, 41 (56.1%) had inadequate vitamin D levels and 34 (34.7%) had normal vitamin D levels. Among the overweight subjects, 138 (36.2%) individuals had a deficiency, 31 (34.8%) had inadequate levels and 42 (42.9%) had adequate vitamin D levels. Among the individuals with normal BMI, 99 (26 %) were Vitamin D deficient, 17 (19.1%) had inadequate vitamin D levels and 20 (20.4%)

had adequate vitamin D levels. This was found to be statistically insignificant with a p value 0.119 and a chi-square value of 10.136.

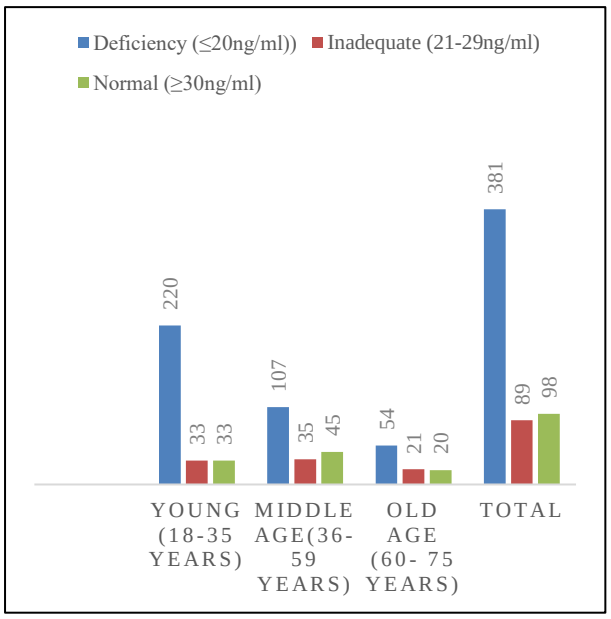
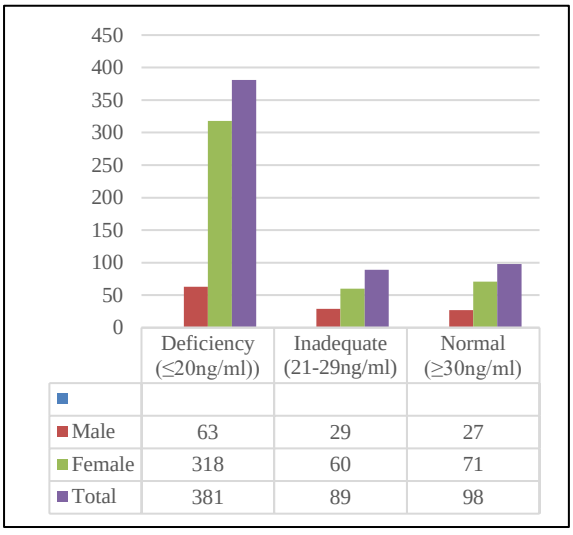


Figure 1: Comparison of vitamin D levels and different age groups.



	Deficiency (≤20ng/ml))	Inadequate (21-29ng/ml)	Normal (≥30ng/ml)
Male	63	29	27
Female	318	60	71
Total	381	89	98

Figure 2: Comparison of vitamin D levels with gender.

The comparison of religion among the study group was found to be statistically insignificant with a p value 0.630 and a chi-square value of 2.58. The study population was categorized into 3 groups based on religion- 343 Hindus, 122 Muslims and 103 Christians (Figure 3). Among Hindus, 223 (65%) individuals were vitamin D deficient, 59 (17.2%) individuals had inadequate vitamin D levels, and 61 (17.8%) individuals had normal vitamin D levels. Out of the Muslim population, 88 (72.1%) individuals were vitamin D deficient, 16 (13.1%) individuals had inadequacy and 18 (14.8%) had normal vitamin D levels. Among Christians,70 (68%) individuals had Vitamin D deficiency, 14 (13.6%) individuals had inadequate levels, and 19 (18.4%) individuals had normal vitamin D levels.

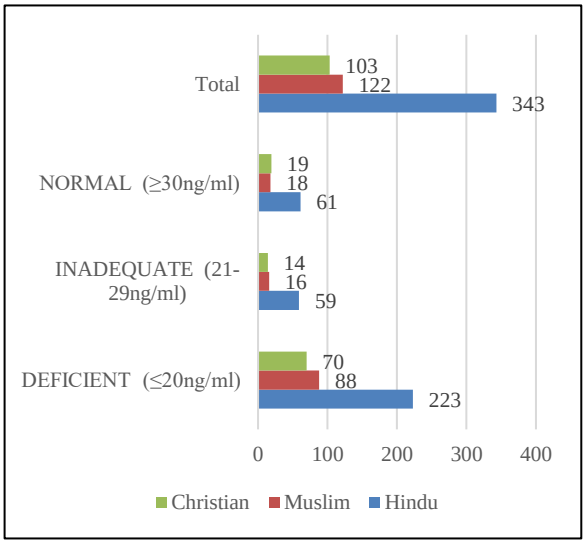


Figure 3: Comparison of vitamin D levels and religion in the study groups.

The comparison of the study population with the occupation (Table 3) also had an insignificant relationship between Vitamin D levels with a p value 0.063 and Chi-square value of 20.22. The study group included 52 (27.3%) professionals, 56 (33.9%) semiprofessionals, 38 (18.8%) clerical workers, 134 (59%) skilled workers, 171 (96.3%) semiskilled workers, 54 (33.4 %) unskilled workers, and 63 (31.3%) unemployed individuals. Among the professionals, 35 (9.2%) had vitamin D deficiency, 7 had inadequate levels and 10 had normal vitamin D values. Of the semiprofessionals, 32 (8.4%) had a deficiency,10 had normal levels and 14 had high vitamin D values. Among the clerical workers, 27 (7.1%) had a deficiency, 5 had inadequate and 6 had normal vitamin D levels. Among the skilled workers,105 (27.6%) were vitamin D deficient, 18 had inadequate and 11 had normal vitamin D levels. Among the semiskilled workers, 107 (28.1%) had a deficiency, 28 had inadequate and 36 had normal vitamin D values. Among the unskilled workers, 30 (7.9%) had Vitamin D deficiency, 10 had inadequate and 14 had normal values for vitamin D and in unemployed subjects, 45 (11.8%) had Vitamin D deficiency, had inadequate and 7 had normal vitamin D values.[11]

DISCUSSION

In this retrospective observational cross-sectional study done among 568 subjects in our tertiary care hospital, it was revealed that there is vitamin D deficiency in the majority of the people 381 (67.1%) and only a small percentage had adequate or normal vitamin D levels 98 (17.3%). This was more evident among females, where 318 (70.8%) had vitamin D deficiency and only 71 (15.8%) had adequate levels of vitamin D. There was a

statistically significant correlation between vitamin D levels and gender (p<0.001). This result was consistent with the study outcomes of Sreekrishnan et al and was contradicting the findings in AlQuaiz et al study.[11,18] Most of the women who had vitamin D deficiency belonged to the reproductive age group. So, the deficiency of vitamin D would affect them seriously. Similar findings were observed in the study done by Hashemipour et al.[19] The reason for vitamin D deficiency among younger females may be due to low dietary intake, less time spent out of doors, insufficient sun exposure, hyperpigmentation, insufficient intake of vitamin D and special dietary habits.[20,21] The extent of clothing due to cultural or religious factors or using topical sunscreen which can block effective dermal synthesis would be the cause. A sunblock of SPF 30 can reduce vitamin D production by 95%.[22] On comparing different age groups in the study population, vitamin D deficiency was more evident among the younger age group, 220 (76.9%), and had a significant correlation between vitamin D levels and age (p value 0.001). But the study of Kader et al had found that vitamin D level decreases in both gender as age progress.[23] Correlation of vitamin D levels with other factors like occupation, religion, and body mass index (BMI) was not significant (p values of 0.063, 0.630 and 0.119 respectively) which was against the study outcomes of Alfawaz et al.[24] Some of the studies mentioned that certain religious practices on the diet, clothing and socioeconomic aspects would affect the vitamin D levels along with other nutrient deficiencies 25 which had not shown any significance in this study.

There were limitations to our study. Firstly, 25-hydroxy vitamin D was checked instead of 1,25-hydroxy vitamin D in the subjects, which would have been a better parameter to detect vitamin D deficiency. Secondly, the correlation of vitamin D and other related parameters like alkaline phosphatase and calcium was not checked. Our observations warrant further studies to define the incidence of vitamin D deficiency in different populations.

CONCLUSION

The study conducted in a tertiary care hospital regarding the vitamin D deficiency in patients coming to the hospital concludes that out of the 568 patients studied, 381 patients were found to be vitamin D deficient irrespective of their BMI, occupation and religion. Of them, 89 were found to have inadequate vitamin D levels, in otherwise healthy adults. Among the 381 vitamin D deficient subjects, the majority of them were females of younger age. It is quite an alarming scenario as vitamin D deficiency can affect the reproductive age group seriously. While considering the occupation of the study subjects, the percentage of individuals who had vitamin D deficiency was found to be less in the professionals (9.2%) and in the semi-professionals (8.4%) compared to the skilled (27.6%) and the semiskilled workers (28.1%). Among the unskilled (7.9%) only a low percentage had vitamin D deficiency and among the unemployed (11.8%) population considerably a high percentage had vitamin D deficiency. This difference may be due to the vitamin D supplement intake in the first group of professionals and semi-professionals. This could be attributed to the high literacy rate of the population in Kerala which could have led to the increased awareness of the importance of supplement intake. In the second population containing the skilled and the semiskilled workers, relatively middle to low socioeconomic status might have affected them, unawareness of supplement intake or difficulties in maintaining a proper diet despite long indoor working hours would be the cause for vitamin D deficiency. Considering the unskilled population, adequate sunlight exposure during their working period would have helped them to maintain adequate vitamin D levels in the majority and among the unemployed group, which mainly consists of homemakers and students who had little sun exposure would have affected them and their poor socioeconomic status, unawareness of importance of vitamin supplements, even diet and clothing habits would have resulted in vitamin D deficiency. Low vitamin D levels in younger and middle age groups in both genders may also derange bone health which ultimately impairs the quality of life of the adults. So, it is better to give Vitamin D supplements to all patients, especially for young adults rather than to advise for a confirmatory test for vitamin D deficiency which costs around 2000 Indian rupees, that cannot be afforded by most of the patients. While Vitamin D supplement of 60000 International units once a week; the only cost is around 25 Indian rupees- initially for 3 months and then monthly once lifelong in order to prevent vitamin D deficiency further. So, in the general population, Vitamin D toxicity should be anticipated, a strict follow-up of regimen should be promoted that would render a good result. It is also imperative to do further studies among the general population to understand the incidence of vitamin D toxicity.

ACKNOWLEDGEMENTS

Dr Jithu SJ nath, Assistant professor, Community Medicine, SGMC & RF, Thiruvananthapuram, Kerala.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Norman AW, Myrtle JF, Midgett RJ, Nowicki HG, Williams V, Popják G. 1,25-dihydroxycholecalciferol: identification of the proposed active form of vitamin D3 in the intestine. Science. 1971;173(3991):51-4.
2. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system

essential for good health. Am J Clin Nutr. 2008;88(2):491S-9S.
3. MacLaughlin JA, Anderson RR, Holick MF. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. Science. 1982;216(4549):1001-3.
4. Pérez AV, Picotto G, Carpentieri AR, Rivoira MA, López MEP, Talamoni NGT de. Minireview on Regulation of Intestinal Calcium Absorption. Digestion. 2008;77(1):22-34.
5. Hui Q, Zhao X, Lu P, Liu S, Nyachoti M, O K. Molecular distribution and localization of extracellular calcium-sensing receptor (CaSR) and vitamin D receptor (VDR) at three different laying stages in laying hens (Gallus gallus domesticus). Poult Sci. 2021;100(5):101060.
6. Warwick T, Schulz MH, Günther S. A hierarchical regulatory network analysis of the vitamin D induced transcriptome reveals novel regulators and complete VDR dependency in monocytes. Sci Rep. 2021;11(65):18-20.
7. Sandra W. Vitamin D and chronic kidney disease. Ethnicity & disease. 2009;19(4):S5-8-11.
8. Melamed, Michal L, Ravi I Thadhani. Vitamin D therapy in chronic kidney disease and end stage renal disease. Clinical journal of the American Society of Nephrology: CJASN. 2012;7(2):358-65.
9. Paul Lips, Vitamin D Deficiency and Secondary Hyperparathyroidism in the Elderly: Consequences for Bone Loss and Fractures and Therapeutic Implications. Endocrine Reviews. 2001;22(4):477-501.
10. Meyer HE, Falch JA, Søgaard AJ, Haug E. Vitamin D deficiency and secondary hyperparathyroidism and the association with bone mineral density in persons with Pakistani and Norwegian background living in Oslo, Norway, The Oslo Health Study. Bone. 2004;35(2):412-7.
11. Sreekrishnan T P, Gireesh Kumar KP. Prevelance of Vitamin D Deficiency In Patients Coming To A Tertiary Care Hospital In India. Int J Recent Sci Res. 2017;8(10):20597-8.
12. Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, et al. 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(10):976-89.
13. Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, et al. Vitamin D and Chronic Diseases. Aging Dis. 2017;8(3):346-53.
14. Vimaleswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med. 2013;10(2):e1001383.
15. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;311(5768):1770-3.
16. Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Berisha A et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr. 2020;74(11):1498-513.
17. Ritu G, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients. 2014;6(2):729-75.
18. Thacher TD, Clarke BL. Vitamin D Insufficiency. Mayo Clin Proc. 2011;86(1):50-60.
19. AlQuaiz AM, Kazi A, Fouda M, Alyousefi N. Age and gender differences in the prevalence and correlates of vitamin D deficiency. Arch Osteoporos. 2018;13(1):49.
20. Hunt SP, O'Riordan JL, Windo J, Truswell AS. Vitamin D status in different subgroups of British Asians. Br Med J. 1976;2(6048):1351-4.
21. Smith R. Asian rickets and osteomalacia. Q J Med. 1990;76(281):899-901.
22. Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis J Clin Endocrinol Metab. 1987;64:1165-8.
23. Kader S, Comaklı H, Tekindal M. A: Evaluation of Serum Vitamin D Levels according to Gender and Age at Karapınar City: A Follow-Up Study from Turkey. Dubai Med J. 2019;2:141-5.
24. Alfawaz H, Tamim H, Alharbi S, Aljaser S, Tamimi W. Vitamin D status among patients visiting a tertiary care center in Riyadh, Saudi Arabia: a retrospective review of 3475 cases. BMC Public Health. 2014;14:159.
25. Chouraqui JP, Turck D, Briend A, Darmaun D, Bocquet A, Feillet F. The Committee on Nutrition of the French Society of Pediatrics, Religious dietary rules and their potential nutritional and health consequences. International Journal of Epidemiology. 2021;50(1):12-26.

Cite this article as: Amitha TA, Lekshmi GS, Roy J. An analysis of vitamin D levels and the factors affecting vitamin D levels among the adult population attending a tertiary care hospital. Int J Res Med Sci 2022;10:660-5.

Journal of Pharmaceutical Research International

32(12): 40-45, 2020; Article no.JPRI.54171
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Serum Hepcidin as an Inflammatory Marker in Chronic Kidney Disease

Jyothi Elizabeth Roy[1*], B. Shanthi[1] and V. S. Kalai Selvi[1]

[1]Department of Biochemistry, Sree Balaji Medical College and Hospital, Chrompet, Chennai – 600044, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i1230558
Editor(s):
(1) Dr. Paola Angelini, University of Perugia, Italy.
Reviewers:
(1) Awofadeju Stephen Olajide, Obafemi Awolowo University Teaching Hospitals Complex, Nigeria.
(2) Sujeet Poudyal, Tribhuvan University Teaching Hospital, Nepal.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54171

Original Research Article

Received 15 May 2020
Accepted 21 July 2020
Published 27 July 2020

ABSTRACT

Background: Hepcidin is known to be the central regulator of iron homeostasis in the body. It is up-regulated by inflammation and downregulated by anemia. CKD is a state of chronic inflammation seen in kidney. Previous work has shown that serum hepcidin levels were increased in patients with CKD. This was surprising as these patients had a chronic inflammatory state and co-existent anemia.
Aim and Objectives: The aim of the study is to estimate the levels of hepcidin in CKD patients and to check the correlation of hepcidin to inflammation in chronic kidney disease.
Methods: This cross-sectional study was conducted at the Department of Biochemistry, Central Laboratory, Sree Balaji Medical College and Hospital, Chromepet, Chennai during January 2017 - June 2018 among 50 patients of chronic kidney disease in the age group of 18-60 years. The blood samples were collected using vacutainer system. Samples for serum hepcidin, ferritin and hsCRP were collected in red topped plain vacuum tube. The samples were centrifuged at 3000 rpm for 15 minutes. The samples were then processed, and values were obtained. The data were analysed using SPSS package.
Results: The mean values of s. Hepcidin, s. ferritin and hsCRP levels were found to be increased

**Corresponding author: E-mail: jothuelza@gmail.com;*

in the study population. The mean value of s. hepcidin was found to have strong positive correlation with the mean values of s. ferritin and hsCRP with r-value > 0.7.
Conclusion: Hepcidin levels are elevated in CKD and hepcidin is a predictor of inflammation since it correlated well with the inflammatory markers hsCRP and ferritin levels.

Keywords: Chronic Kidney Disease (CKD); hepcidin.

1. INTRODUCTION

Chronic kidney disease (CKD) is associated with an irreversible progressive deterioration in renal function. CKD has become a worldwide, chronic, non-communicable disease epidemic with adverse outcomes of renal failure, cardiovascular disease and premature death. In developed countries, it affects 10-15% of adult general population.

The discovery of hepcidin and its functions has led to a better understanding of iron metabolism disorders in CKD. Hepcidin, a small cysteine rich liver-derived peptide hormone, is a key regulator of systemic iron homeostasis. Hepcidin has evolved as a probable mediator of anemia of inflammation.

Increased serum hepcidin levels may contribute to the development and severity of anemia and the resistance to erythropoiesis-stimulating agents. The metabolism of hepcidin is profoundly altered in chronic kidney disease (CKD). What little data are available in this area are inconclusive. Markers of inflammation and iron status were positively associated with serum hepcidin level, regardless of CKD stage. However, glomerular filtration rate was inversely associated with serum hepcidin level, particularly in patients with CKD stages 3b–5 but not in those with CKD stages 1–3a. Hence in the present study, the serum level of hepcidin was estimated in patients with CKD and the relationship between serum hepcidin and inflammation and were analysed.

1.1 Aim

The aim of the present study was to estimate the levels of serum hepcidin in patients with chronic kidney disease and to check the correlation of hepcidin to inflammation in chronic kidney disease.

The objectives of the present study were to:

- Estimate serum hepcidin levels in patients with chronic kidney disease.
- Determine whether hepcidin levels correlated to inflammatory marker in chronic kidney disease.

2. MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Biochemistry, Sree Balaji Medical College and Hospital, Chromepet, Chennai during the period of January 2017 – June 2018 among 50 patients of chronic kidney disease visiting the Nephrology outpatient services in the age group of 18-60 years. Age, gender, duration of chronic kidney disease, general history and medications and blood pressure were recorded. The study was explained to the participants and informed consent was obtained from them before taking the blood sample. After obtaining informed consent, 5 ml of blood was collected from each participant, from a peripheral vein under aseptic precautions in specific vacutainers. Plain tube for hepcidin, ferritin and hsCRP were used. Blood samples collected were used for the estimation of serum levels of hepcidin, ferritin and hsCRP. Transferrin saturation value was obtained by calculation. Blood collected in the plain tube was allowed to clot, then each tube was centrifuged for 10 minutes at 3500 rpm within 2 hours of blood collection to separate serum. Serum sample was used for the estimation of all parameters. Hepcidin was estimated by competitive ELISA kit (Enzyme Linked Immuno Sorbent Assay). Estimation of serum ferritin and hsCRP were done by chemiluminescence assay in Siemens, ADVIA Centaur CP Immunoassay system. Data was categorised based on demographics (Age and Gender).

All the results obtained were statistically analyzed using SPSS software version 16.0. Shapiro-Wilk test was used to test for normality of the data. Mean and standard deviation were used to represent normally distributed data. Median and interquartile ranges were used to represent data which were not normally distributed. Bivariate correlation analyses were done using Pearson correlation to correlate hepcidin with other parameters. The results of

statistical analysis were arranged in tabular form and were plotted in graphs.

2.1 Inclusion Criteria

Known cases of chronic kidney disease on conservative management in the age group 18-60 years.

2.2 Exclusion Criteria

- Age group less than 18 years and greater than 60 years
- Pregnancy
- Any malignancy
- Pre-existing liver disease
- Active inflammatory disease

3. RESULTS

Kruskal-Wallis Test was used to test the statistical significance of hepcidin and other parameters among the study group. The mean values of s. Hepcidin, s. ferritin and hsCRP levels were found to be increased in the study population.

The mean value of s. hepcidin was found to have strong positive correlation with the mean values of s. ferritin and hsCRP with r-value > 0.7.

4. DISCUSSION

Anemia is a significant co-morbidity in patients with CKD. Iron deficiency and inflammation are the most common causes of anemia in CKD. However, despite the reported high and alarming prevalence of anemia in CKD resulting in significant co-morbidity, anemia is often untreated or treated improperly in clinical practice. The reason for this is mainly in the difficulty involved in determining the etiology of anemia in this condition which is vital in providing the right treatment. Hepcidin is the master regulator of systemic iron homeostasis [1] and plays a key role in anemia associated with inflammation. Physiologically, hepcidin inhibition occurs in cases of anemia, iron deficiency or hypoxia. In inflammatory conditions, levels of pro-inflammatory cytokines are increased [2]. It is plausible that increased hepcidin concentrations may cause iron-restricted erythropoiesis in CKD-associated anemia.

In the present study, a total of 50 CKD subjects were studied. Hepcidin levels were significantly higher in patients with CKD than in control subjects. Serum ferritin and hsCRP were found to be elevated in CKD cases.

In the present study, serum hepcidin concentrations were found to be significantly increased in patients with CKD [Mean value: 149.484 ± 47.539 ng/mL]. Serum hepcidin levels were found to be increasing with the progression of CKD. This finding is supported further by the highly significant positive correlation observed between serum hepcidin and creatinine in CKD cases ($r= 0.8437$, $p<0.001$). These findings are in accordance with the study of Tarek et al. [3], which reported an increase in serum hepcidin levels in all stages of CKD among 54 CKD patients under conservative management and 40 CKD patients under hemodialysis.

Hepcidin is cleared from the body by the kidneys. The increase in hepcidin seen in CKD is due to its reduced renal clearance associated with the deteriorating renal function. Depending on the damage of kidney, the elimination of hepcidin is limited and leads to the increase in its serum level. Another reason could be the chronic inflammatory state of CKD which stimulates hepcidin production. Uremia is a state of heightened inflammatory activation. Hepcidin synthesis is induced in the liver as a response to IL-6 stimulation and it expresses its activity by decreasing the absorption of dietary iron and prevents iron release from macrophages. Elevated serum hepcidin levels mediate iron-restricted erythropoiesis and contribute to inducing anemia in CKD patients. Short-term increases in serum hepcidin levels impair the release of storage iron, and long-term increases in serum hepcidin levels result in iron deficiency. High serum hepcidin levels cause iron blockade and anemia in chronic disease. Chronic kidney disease patients with anemia have been found to have elevated serum hepcidin levels, and the high hepcidin levels are likely to contribute to anemia in CKD and to ESA hypo-responsiveness.

Table 1. Descriptive statistics of the study group

Parameters	N	Mean	STD. deviation	Mean rank
S. Hepcidin (ng/ml)	50	149.484400	47.5399263	75.50
S. Ferritin (mg/dl)	50	187.757400	93.1905705	75.22
hsCRP (ng/ml)	50	5.251400	2.5022708	75.22

Table 2. Pearson's correlation between hepcidin and other parameters among CKD cases

Variables		S. hepcidin
S. ferritin	r value	0.907
	p value	0.000
S. hsCRP	r value	0.942
	p value	0.000

***. Correlation is significant at the 0.01 level (2-tailed)*

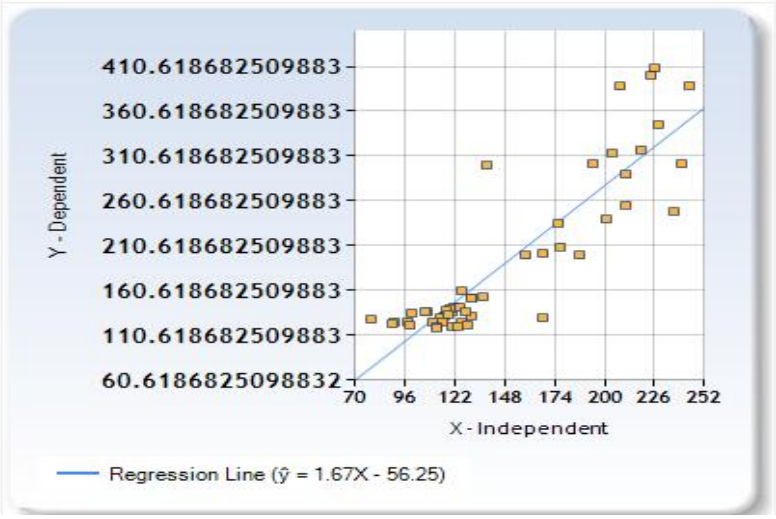


Fig. 1. Scatter plot with linear regression of hepcidin vs ferritin

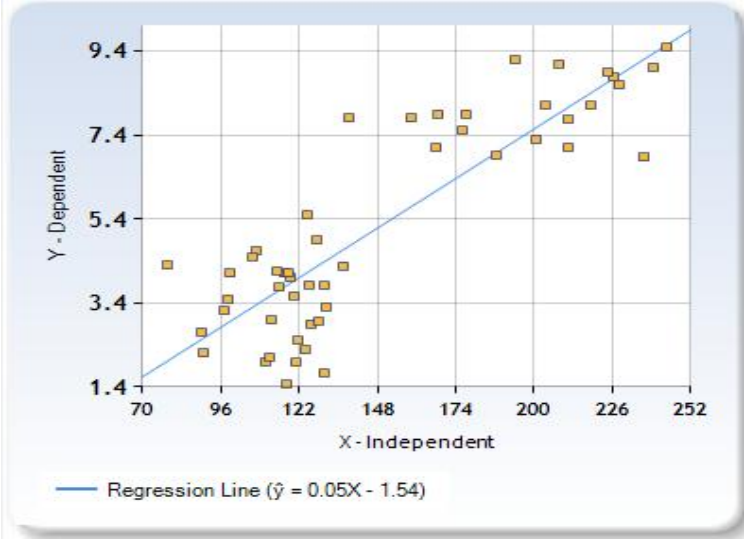


Fig. 2. Scatter plot with linear regression of hepcidin vs hsCRP

Ferritin is considered as marker of iron status. Though ferritin is a marker of body iron stores, it also increases in acute inflammation and therefore becomes less valuable as an indicator of iron status during inflammation seen in CKD. In this study, hepcidin and ferritin were significantly higher in chronic kidney disease patients compared to control subjects. The mean value of ferritin was 187.757 ± 93.19 mg/dl. Also, Malyszko et al. [4] found that in his study on patients with chronic renal failure and hemodialyzed patients, that serum ferritin and

hepcidin were significantly higher than in the healthy volunteers. There was a strong positive correlation between ferritin and hepcidin among the CKD cases ($r = 0.907$, $p< 0.001$) which is in accordance with the study of Mercadal et al. [5]. He observed a positive correlation between hepcidin and ferritin in his study among 199 non-dialysed non-transplanted patients with CKD stages 1-5.

The chronic inflammatory state of CKD is due to the chronic imbalance between prooxidant and antioxidant factors. Oxidative stress induces insulin resistance by decreasing internalization of insulin [6] and increased ferritin synthesis. Functional iron deficiency is present in some patients with chronic renal failure, on conservative treatment and on dialysis, which is characterized by the presence of adequate iron stores with serum ferritin level either normal or elevated. In addition to this, inflammatory iron block occurs among these patients largely due to an underlying inflammatory state. The inflammatory block along with functional iron deficiency cause elevated ferritin level [4]. Hepcidin level of the two study groups in the present study was related to ferritin that agrees with the results of Peters et al. [7], who revealed that serum ferritin concentration was a significant predictor of hepcidin-25 levels in CKD by means of multiple regression analysis. Similar correlation between ferritin and hepcidin was reported by Dallalio et al. [8], in anemic patients undergoing diagnostic bone marrow examination. However, Malyszko et al. [4] could not find a significant correlation between hepcidin and ferritin in his study population of patients with chronic renal failure on conservative treatment and on hemodialysis. Reduced levels of serum ferritin or transferrin saturation (TSAT) are present in most patients with CKD.

CKD is a state of chronic persistent low-grade inflammation with persistent elevation of pro-inflammatory markers. The prototype marker of inflammation in the clinical setting is hsCRP, a positive acute phase reactant and higher level of this inflammatory biomarker is associated with cardiovascular mortality in patients with renal insufficiency. The elevated levels serum hepcidin in patients with CKD indicates an underlying inflammatory state associated with advanced renal failure, with loss of renal function, and development of anemia of chronic disease, the thing that was obvious in the present study in the form of high hsCRP in chronic kidney disease patients when compared to control [Mean level: Cases - 5.25 ± 2.50 ng/mL; $p<0.001$]. This is similar to the finding of Carmen et al. [9]. He observed a positive correlation between hsCRP and hepcidin in anemic CKD patients. However, he could not establish any relation between hepcidin and hsCRP among non-anemic CKD patients. As the renal function declined, there was a progressive increase in the hsCRP levels. Further a strong positive correlation was found between hepcidin and hsCRP levels ($r=0.942$; $p<0.001$) which shows that hepcidin is a positive acute phase reactant. Similar finding of positive correlation between hsCRP and hepcidin was observed by Karthik et al. [10] and Tarek et al. [3] in their studies as well.

5. CONCLUSION

The present study suggests that hepcidin levels are elevated in CKD and hepcidin is a predictor of inflammation since it correlated well with the inflammatory markers hsCRP and ferritin levels. This increase in hepcidin levels reflects both the renal impairment leading to reduced renal clearance of hepcidin and the state of chronic inflammation. These findings highlight the close relationship between inflammation and hepcidin in CKD.

CONSENT

As per international standard or university standard written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Swinkels DW, Wetzels JF. Hepcidin: A new tool in the management of anaemia in patients with chronic kidney disease? Nephrol. Dial. Transplant. 2008;23(8): 2450-2453.
2. McCranor BJ, Langdon JM, Prince OD. Investigation of the role of interleukin-6

and hepcidin antimicrobial peptide in the development of anemia with age. Haematologica. 2013;98(10):1633-1640.

3. Tarek Mohamed Ali, Ashraf Mahmoud Genina, Osama M. Abo-Salem. Corrigendum to "the determinants of hepcidin level in chronic kidney disease and hemodialysis Saudi patients". Beni-Suef University Journal of Basic and Applied Sciences. 2014;3(3):238.
4. Malyszko J, Tesar V, Macdougall IC. Neutrophil gelatin associated lipocalin and hepcidin: What do they have in common and is there a potential interaction? Kidney Blood Press Res. 2010;33:157–165.
5. Lucile Mercadel, Marie Metzger, Jean-Philippe Haymann, Eric Thervet, Jean-Jacques Boa, et al. The relation of hepcidin to iron disorders, inflammation and hemoglobin in chronic kidney disease. PLoS ONE, Public Library of Science. 2014;9(6):e99781. DOI: 10.1371/journal.pone.0099781. hal-01367481
6. Bertelsen M, Anggard EE, Carrier MJ. Oxidative stress impairs insulin internalization in endothelial cells *in vitro*. Diabetologia. 2001;44:605-13.
7. Peters HPE, Laarakkers CMM, Swinkels DW, Wetzels JFM. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. Nephrol Dial Transpl Transpl. 2009;25:848e53.
8. Dallalio G, Fleury T, Means RT. Serum hepcidin in clinical specimens. Br J Haematol. 2003;122(6):996e1000.
9. Carmen Caldararu, Grigore Dogaru, Dorin Ionut Tarta, Adina Hutanu, Maria T Dogaru, Emilian Carasca, Mirela Liana Gliga. Oral iron for anaemia in non-dialysis chronic kidney disease. The relationship between hepcidin-25, iron metabolism and inflammation. Research Article - Biomedical Research. 2017; 28(8).
10. Kalidindi Raja Karthik, Chandru T, Ram Prasad E Varun Kumar B, Soudararajan P, Ramachandra S. Correlation of serum hepcidin levels with inflammation and iron status in patients with Esrd. Paripex - Indian Journal of Research. 2016;5(8): 8–10.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/54171