

POSITIVE CORRELATION OF CRP, FOLIC ACID, AND D-DIMER WITH PROGRESSION OF CKD STAGING: A CROSS-SECTIONAL STUDY.

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Abstract

Background

Chronic kidney disease (CKD) is characterized by kidney damage or a decline in glomerular filtration rate (GFR) over three months, often leading to various complications and stages of severity. Biomarkers such as C-reactive protein (CRP), D-dimer, and folic acid have been implicated in CKD progression, yet their correlation across different CKD stages remains to be fully elucidated. The study aims to do a comparative study of D-dimer, CRP, and Folic acid in different stages of chronic kidney disease.

Methods

The study employed a cross-sectional design. Serum samples from 150 CKD patients were collected and analyzed for CRP, D-dimer, and folic acid levels using standard laboratory procedures. Statistical analysis was performed using SPSS software and Microsoft Excel to assess the significance of biomarker variations across CKD stages.

Results

Serum CRP, D-dimer, and folic acid levels showed significant variances across CKD stages, displaying lower CRP and higher folic acid in CKD stage I, and elevated D-dimer in later stages. Monitoring these biomarkers is pivotal for gauging CKD progression and severity, notably highlighting the rise in CRP and D-dimer with advancing CKD stages, and the higher folic acid levels seen in earlier CKD stages.

Conclusion

The study underscores the importance of monitoring biomarker levels in CKD progression, with CRP and D-dimer serving as potential indicators of disease severity. Additionally, the observed inverse relationship between folic acid levels and CKD staging warrants further investigation into its potential protective role against CKD progression.

Recommendations

Clinicians should consider incorporating regular assessment of CRP, D-dimer, and folic acid levels into CKD management protocols to better understand disease progression and tailor treatment strategies accordingly.

Keywords: Chronic kidney disease, C-reactive protein, D-dimer, Folic acid.

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INTRODUCTION

According to the KDIGO guidelines, chronic kidney disease (CKD) is defined by kidney damage, either structural or functional, or a decline in glomerular filtration rate (GFR) below 60 mL/min/1.73 m² of body surface area for over 3 months. Initially asymptomatic, later symptoms may include vomiting, fatigue, pedal edema, and confusion. Complications include heart disease, high blood pressure,

bone disease, and anemia. The most common causes are diabetes mellitus, hypertension, and glomerulonephritis [1].

Stage 1: Characterized by slightly diminished kidney function, normal or relatively high GFR (>90 ml/min/1.73 m²), and persistent albuminuria.

Stage 2: Exhibits mild reduction in GFR (60–89 ml/min/1.73 m²) with kidney damage.

Stage 3: British guidelines distinguish between stage 3A (GFR 45-59) and stage 3B (GFR 30-40) for screening and

referral purposes. It involves a moderate reduction in GFR (30-59 ml/min/1.73 m²).

Stage 4: Indicates preparation for kidney replacement therapy with a severe reduction in GFR (15-29 ml/min/1.73 m²).

Stage 5: Represents permanent replacement therapy or end-stage kidney damage with established kidney failure (GFR <15 ml/min/1.73 m²) [2].

D-dimer, a fibrin degradation product (FDP), is a small protein fragment released into the bloodstream during fibrinolysis, the breakdown of blood clots. Its name stems from being composed of two crosslinked D fragments of the fibrin protein. Blood tests measuring D-dimer concentrations help identify thrombosis. However, positive D-dimer results lack clear diagnostic value for end-stage renal disease (ESRD) patients undergoing hemodialysis due to frequent comorbidities like atherosclerosis and malignancy, which also elevate serum D-dimer levels [3, 4]. In these cases, D-dimer levels correlate with the stage of chronic kidney disease (CKD) and disease progression. Moreover, studies suggest that the CKD stage influences D-dimer levels, reducing the specificity of D-dimer testing for pulmonary embolism detection in CKD patients. Hence, this research aims to explore serum D-dimer levels and their correlation with glomerular filtration rate (GFR) in CKD patients [5, 6].

C-reactive protein serves as an inflammation marker, a pentameric protein in plasma with circulating concentrations rising in response to inflammation. Monitoring and charting CRP values can help assess disease progression or treatment efficacy. In kidney failure, CRP levels can elevate even without clinically significant inflammation. Additionally, CRP levels independently mark atherosclerotic disease [7, 8].

Folate, also known as vitamin B9 or folacin, is essential for RNA and DNA synthesis and amino acid metabolism crucial for cell division. Folic acid, a form of folate, is used to treat anemia. Methionine production from homocysteine requires vitamin B12 and folate. Supplementation with folic acid in CKD patients can reduce homocysteine levels, consequently lowering cardiovascular events [9, 10].

Therefore, the study aims to do a comparative study of D-dimer, CRP, and Folic acid in different stages of chronic kidney disease.

MATERIALS AND METHODS

Study Design

A cross-sectional design to assess serum levels of D-dimer, CRP, and folic acid in patients attending the Nephrology OPD at Shri Mahant Indresh Hospital.

Study Setting

The study was conducted at Nephrology OPD of Shri Guru Ram Rai (SGRR) Medical College and Hospital, and Shri Mahant Indresh Hospital, Dehradun, India, between March 2022 to January 2023.

Participants

The participants included 150 patients who visited the Nephrology OPD.

Inclusion Criteria

- Patients diagnosed with chronic kidney disease (CKD) of any stage.
- aged 18 years and above.
- available serum samples for CRP, folic acid, and D-dimer assessment.
- documented CKD staging according to recognized guidelines (e.g., KDIGO staging).

Exclusion Criteria

- Patients with acute kidney injury.
- history of kidney transplantation.
- autoimmune diseases affecting kidney function (e.g., lupus nephritis).
- acute infectious diseases or active inflammation.
- A history of recent major surgery or trauma.
- hematological disorders affecting CRP, folic acid, or D-dimer levels (e.g., leukemia).
- inadequate medical records or incomplete demographic information.

Sample size

To calculate the sample size for this study, the following formula was used for estimating a proportion of a population:

$$n = \frac{Z^2 \times p \times (1-p)}{E^2}$$

Where:

- n = sample size
- Z = Z-score corresponding to the desired level of confidence
- p = estimated proportion in the population
- E = margin of error

Bias

To minimize bias, the selection of participants is randomized, and data collection is performed by trained healthcare professionals following standardized procedures.

Variables

Variables included Serum levels of D-dimer, CRP, and folic acid, participants' demographic information, medical history, and laboratory test results.

Data Collection

Serum samples were taken for serum D-dimer, CRP, and folic acid in patients coming to Nephrology OPD. CRP & Folic acid run on VITROS XT 7600 / VITROS 5600 integrated system using intelli-check technology and for D-dimer Automated latex enhanced immunoassay.

Statistical Analysis

The collected data was analyzed for p-value, student T-test, mean, and standard deviation with the help of SPSS's latest software and Microsoft Excel.

Ethical considerations

The study protocol was approved by the Ethics Committee and written informed consent was received from all the participants.

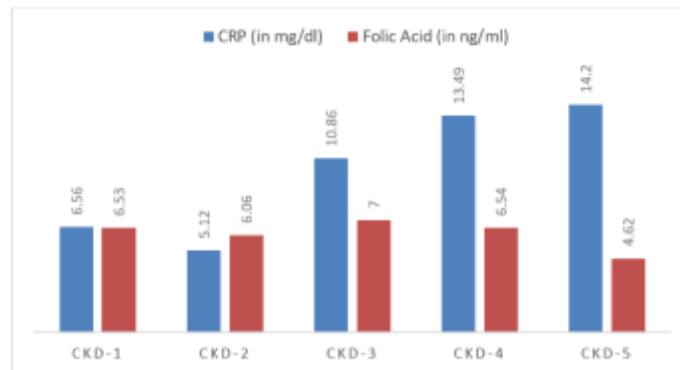
RESULTS

In the study, all 150 samples were analyzed for serum CRP, Folic acid, and D-dimer and subjects were categorized based on chronic kidney diseases. Table 1 shows the comparison and statistical significance of biochemical markers between different stages of chronic kidney diseases.

Table-1: Comparison of serum CRP, Folic acid and D-dimer in different stages of chronic kidney diseases.

CKD Stages	CRP (in mg/dl)	D-dimer (in ng/ml)	Folic acid (in ng/ml)
CKD-1	6.56 ± 4.45	246.30 ± 106.79	6.53 ± 2.62
CKD-2	5.12 ± 2.96	237.13 ± 64.08	6.06 ± 1.87
CKD-3	10.86 ± 9.64	406.23 ± 248.44	7.0 ± 2.78
CKD-4	13.49 ± 9.08	605.50 ± 598.71	6.54 ± 2.83
CKD-5	14.20 ± 12.68	544.54 ± 322.35	4.62 ± 1.87

Graph Chart-1: Comparison of serum CRP and folic acid in different stages of chronic kidney diseases.



Graph Chart-2: Comparison of serum D-dimer in different stages of chronic kidney diseases.

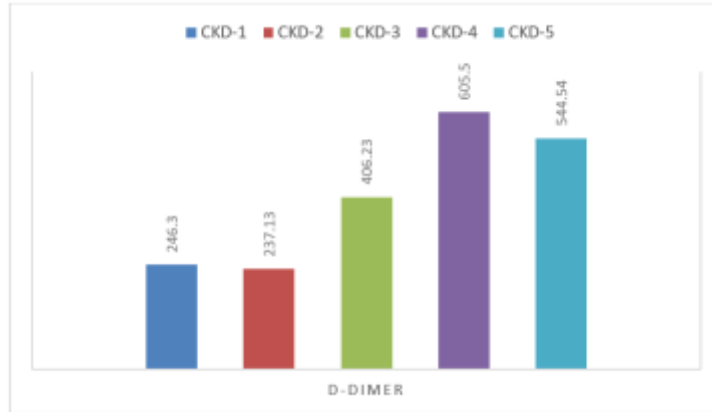


Table 1, graph chart 1 and graph chart 2 show the comparison of serum CRP, folic acid and D-dimer in different stages of chronic kidney diseases. Serum CRP and D-dimer levels were lower in CKD stage 1 than CKD stage 5 and folic acid was higher in CKD stage 3 and lower in CKD stage 5.

Table-2: T-Test and P- Value of serum CRP, D –dimer and folic acid between CKD-1 with different stages of chronic kidney diseases.

STAGES	T-TEST VALUE			P-Value		
	CRP	D-dimer	Folic acid	CRP	D-dimer	Folic acid
CKD-1 V/s CKD-2	4.53	4.44	4.63	0.0001	0.0001	0.000081
CKD-1 v/s CKD-3	4.05	5.83	4.01	0.0003	<0.01	0.00041
CKD-1 v/s CKD-4	2.42	9.13	4.58	0.02	<0.01	<0.01
CKD-1 v/s CKD-5	4.66	7.04	5.39	<0.01	<0.01	<0.01

Graph chart-3: T-Test value of serum CRP, D-dimer and folic acid between CKD-1 with different stages of chronic kidney diseases.

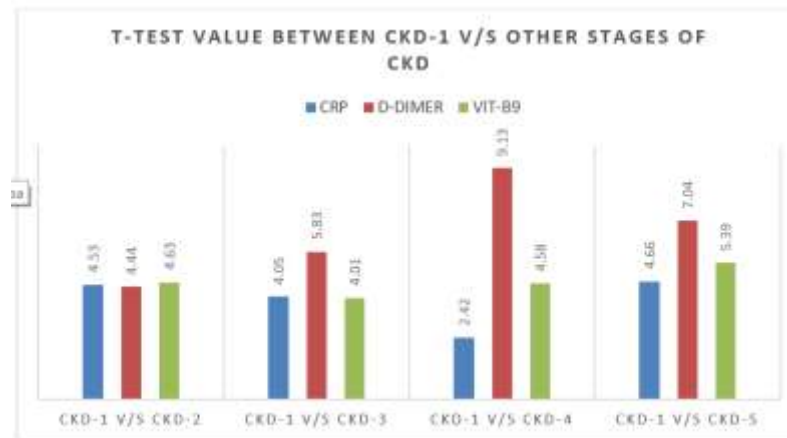


Table-3: T-Test and P- Value of serum CRP, D-Dimer and folic acid between CKD-2 with different stages of chronic kidney diseases.

STAGES	T-TEST VALUE			P-Value		
	CRP	D-dimer	Folic acid	CRP	D-dimer	Folic acid
CKD-2 v/s CKD-3	5.86	9.86	6.40	<0.01	<0.01	<0.01
CKD-2 v/s CKD-4	4.47	14.01	7.7	0.00011	<0.01	<0.01
CKD-2 v/s CKD-5	5.0	9.29	6.53	<0.01	<0.01	<0.01

Graph Chart-4: T-Test Value of serum CRP, D-dimer, and folic acid between CKD-2 with different stages of chronic kidney diseases.

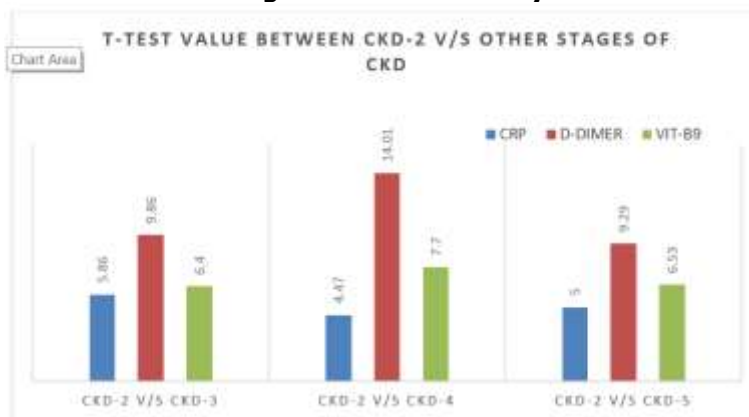


Table 2 and Table 3 show the statistical significance of (the T-test and P-value) of serum CRP, D-dimer, and folic acid between different stages of chronic kidney diseases.

Table 2 shows the T-test value of serum CRP is 4.53 (p-value <0.01) for CKD-1 V/S CKD-2, 4.05 (p-value <0.01) for CKD-1 V/S CKD-3, 2.42 (p-value 0.02) for CKD-1 V/S CKD-4 and 4.66 (p-value <0.01) for CKD-1 V/S CKD-5, which was statistically significant.

The T-test value of serum d-dimer is 4.44 (p-value- <0.01) for CKD-1 V/S CKD-2, 5.83 (p-value-<0.01) for CKD-1 V/S CKD-3, 9.13 (p-value- <0.01) for CKD-1 V/S CKD-4 and 7.04 (<0.01) for CKD-1 V/S CKD-5.

The T-test value of folic acid is 4.63 (p-value-<0.01) for CKD-1 V/S CKD-2, 4.01 (p-value- 0.012) for CKD-1 V/S CKD-3, 4.58 (p-value- 0.01) for CKD-1 V/S CKD-4 and 5.39 (p-value- <0.01) for CKD-1 V/S CKD-5.

Table 3 shows the t-test value of serum CRP is 5.86 (p-value <0.01) for CKD-2 V/S CKD-3, 4.47 (p-value <0.000111) for CKD-2 V/S CKD-4, and 5.0 (p-value 0.01) for CKD-2 V/S CKD-5, which was statistically significant.

The T-test value of serum d-dimer is 9.86 (p-value- <0.01) for CKD-2 V/S CKD-3, 14.01 (p-value-<0.01) for CKD-2 V/S CKD-4, 9.29 (p-value- <0.01) for CKD-2 V/S CKD-5.

The T-test value of folic acid is 6.40 (p-value-<0.01) for CKD-2 V/S CKD-3, 7.7 (p-value- 0.01) for CKD-2 V/S

CKD-4, 6.53 (p-value- 0.01) and 5.39 (p-value <0.01) for CKD-2 V/S CKD-5.

DISCUSSION

The findings from the statistical analysis of serum biomarkers among different CKD stages revealed significant differences. Specifically, lower CRP levels (T-test values ranging from 2.42 to 4.66, $p < 0.02$) and higher folic acid levels (T-test values ranging from 4.01 to 5.39, $p < 0.02$) were observed in CKD stage 1, while D-dimer levels were notably elevated in advanced CKD stages (T-test values ranging from 4.44 to 9.13, $p < 0.01$).

These results suggest a complex interplay between inflammatory, thrombotic, and nutritional markers with CKD progression. Monitoring these biomarkers is crucial for assessing CKD severity, highlighting the heightened inflammatory and thrombotic risks in advanced stages and the potential for nutritional interventions early in CKD to optimize outcomes.

Lalramenga et al. reported increased serum CRP levels in CKD patients [11], while Adejumo et al. found statistically significant CRP levels in chronic renal failure [12]. Annuk et al. also noted elevated serum CRP in CKD patients with a significant p-value of <0.01 [13]. Pravin et al. reported higher CRP levels in chronic kidney disease patients

compared to normal subjects [14]. Abraham et al. concluded that increasing CRP levels were associated with decreased renal function in CKD patients [15]. Similarly, Michael Shlipak et al. reported higher CRP levels in CKD patients compared to normal individuals [16].

Charlotte Mosterd et al. found a positive association between serum D-dimer and CRP in CKD patients [17], which was also supported by Alyaa Abdelmaguid et al. and Sexton et al., who reported higher D-dimer levels in CKD patients [18][19]. Caroline Pereira Domingueti et al. observed elevated D-dimer levels in CKD patients [20], a finding echoed by Hiroyuki Naruse et al. [21]. Mykhaloiko et al. and Meng-Jie Huang et al. also reported significantly higher D-dimer levels in CKD patients [22][23]. Noha Mohammed Siddig Mohammed and Hiba BadrEldin Khalil found increased D-dimer levels in chronic kidney disease patients [24].

On the other hand, Beatriz Baye et al. found lower folic acid levels in CKD patients [25], a finding supported by Christina M. Wyatt and J. David Spence and Alireza Soleimani et al. [26][27]. Anurag A and Yonova D et al. also reported reduced serum vitamin B9 levels in CKD patients [28][29].

Generalizability

The findings regarding serum CRP, D-dimer, and folic acid levels in different stages of chronic kidney disease (CKD) suggest potential generalizability to broader CKD populations. However, the generalizability may be influenced by factors such as the specific demographics of the study sample, variations in CKD etiology, and treatment protocols. Therefore, while the trends observed in this study are valuable indicators, broader generalizability would require validation across diverse CKD cohorts to account for potential confounding variables and ensure applicability across different clinical settings.

CONCLUSION

In the study, highly significant values of D-dimer in different stages of kidney disease mostly in CKD 4 and CKD5 were observed. This study also indicates the presence of a high risk of venous thromboembolism and cardiovascular disease in the later stages of CKD. The later stages of CKD for D-dimer levels should be monitored. Folate levels were decreased in later stages of kidney disease, so supplementation therapy should be done to overcome this.

Limitations

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of a comparison group also poses a limitation for this study's findings.

Recommendation

Clinicians should consider incorporating regular assessment of CRP, D-dimer, and folic acid levels into CKD management protocols to better understand disease progression and tailor treatment strategies accordingly.

Acknowledgment

We are thankful to the patients; without them, the study could not have been done. We are thankful to the supporting staff of our hospital who were involved in the patient care of the study group.

List of abbreviations

CKD - Chronic Kidney Disease
KDIGO - Kidney Disease: Improving Global Outcomes
GFR - Glomerular Filtration Rate
OPD - Outpatient Department
CRP - C-Reactive Protein
ESRD - End-Stage Renal Disease
SD - Standard Deviation

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Conflict of interest

The authors have no competing interests to declare.

REFERENCES

1. National Institute of Diabetes and Digestive and Kidney Diseases. What Is Chronic Kidney Disease? June 2017. Retrieved 19 December 2017.
2. Medline Plus. Kidney Failure. Retrieved 11 November 2017.
3. Adam SS, Key NS, Greenberg CS. D-dimer antigen: current concepts and prospects. *Blood*. 2009 Mar;113(13):2878-87. PMID: 31594601.
4. Tasic N, Paixao T, Goncalves L. Biosensing of D-dimer, making the transition from the central hospital laboratory to bedside determination. *Talanta*. 2020 Jan 15;207:120270. PMID: 19008457.
5. Nelen WL, Blom HJ, Steegers EA, den Heijer M, Thomas CM, Eskes TK. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. *Obstet Gynecol*. 2000 Apr;95(4):519-24. PMID: 10725483.
6. van der Put NJ et al. Folate, Homocysteine, and Neural Tube Defects: An Overview. *Exp Biol Med (Maywood)*. 2001 Apr;226(4):243-270.
7. Dittrich S, Tadesse BT, Moussy F, et al. Target Product Profile for a Diagnostic Assay to Differentiate between Bacterial and Non-Bacterial Infections and Reduce Antimicrobial Overuse in

- Resource-Limited Settings: An Expert Consensus. PLOS ONE. 2016 Aug 25;11(8):e016172.
8. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001 Jul 18;286(3):327-34. PMID: 11466099.
 9. Drugs.com. Folic Acid. American Society of Health-System Pharmacists. January 1, 2010. Retrieved September 1, 2016.
 10. Shen L, Ji HF. Associations between Homocysteine, Folic Acid, Vitamin B12, and Alzheimer's Disease: Insights from Meta-Analyses. J Alzheimers Dis. 2015;46(3):777-90. PMID: 25854931.
 11. Lalramenga PC, Gupta S. Study of C-reactive protein significance in chronic kidney disease.
 12. Adejumo OA, Okaka EI, Okwuonu CG, Iyawe IO, Odujoko OO. Serum C-reactive protein levels in predialysis chronic kidney disease patients in southern Nigeria. Ghana Medical Journal. 2016; 50:31-8.
 13. Garimella PS, Hirsch AT. Peripheral artery disease and chronic kidney disease: clinical synergy to improve outcomes. Advances in chronic kidney disease. 2014; 21:460-71.
 14. Pravin NB, Jayashree SB, Shilpa BA, Suhas SB, Anand PT. Study of serum uric acid and C-reactive protein levels in patients with chronic renal disease. Int J Biol Med Res. 2013; 4:2758-2761.
 15. Abraham G, Sundaram V, Matthew M, Leslie N, Sathish V. C-reactive protein, a valuable predictive marker in chronic kidney disease. Saudi J Kidney Dis Transpl. 2009; 20:811-5.
 16. Shlipak MG, Fried LF, Crump C, Bleyer AJ, Manolio TA, Tracy RP, Furberg CD, Psaty BM. Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. Circulation. 2003 Jan 7;107(1):87-92.
 17. Mosterd CM, Hayfron-Benjamin CF, Van Den Born BJ, Maitland-Van der Zee AH, Agyemang C, Van Raalte DH. Ethnic disparities in the association between low-grade inflammation biomarkers and chronic kidney disease: The HELIUS Cohort Study. Journal of Diabetes and its Complications. 2022 Aug 1;36(8):108238.
 18. Abdelmaguid A, Roberts LN, Tugores L, Joslin JR, Hunt BJ, Parmar K, Nebres D, Naga SS, Khalil ES, Bramham K. Evaluation of novel coagulation and platelet function assays in patients with chronic kidney disease. Journal of Thrombosis and Haemostasis. 2022 Apr 1;20(4):845-56.
 19. Sexton DJ, Clarkson MR, Mazur MJ, Plant WD, Eustace JA. Serum D-dimer concentrations in nephrotic syndrome track with albuminuria, not estimated glomerular filtration rate. American Journal of Nephrology. 2012;36(6):554-60.
 20. Mosterd CM, Hayfron-Benjamin CF, Van Den Born BJ, Maitland-Van der Zee AH, Agyemang C, Van Raalte DH. Ethnic disparities in the association between low-grade inflammation biomarkers and chronic kidney disease: The HELIUS Cohort Study. Journal of Diabetes and its Complications. 2022 Aug 1;36(8):108238.
 21. Naruse H, Ishii J, Takahashi H, et al. Prognostic value of the combination of plasma D-dimer concentration and estimated glomerular filtration rate in predicting long-term mortality of patients with stable coronary artery disease. Circulation Journal. 2017 Sep 25;81(10):1506-13.
 22. Mykhaloiko IS, Dudar IO, Mykhaloiko IJ, Mykhaloiko OJ. D-dimer as a potential predictor of thromboembolic and cardiovascular complications in patients with chronic kidney disease. Ukrainian Biochemical Journal. 2020.
 23. Huang MJ, Wei RB, Wang Y, et al. Blood coagulation system in patients with chronic kidney disease: a prospective observational study. BMJ open. 2017 May 1;7(5):e014294.
 24. Mohammed NM, Khalil HB. D-dimer levels in patients presenting chronic kidney disease in Sudan. Am J Med Med Sci. 2016;6(3):120-2.
 25. Bayés B, Pastor MC, Bonal J, Romero R. "New" cardiovascular risk factors in patients with chronic kidney disease: role of folic acid treatment. Kidney International. 2005 Jan 1;67:S39-43.
 26. Wyatt CM, Spence JD. Folic acid supplementation and chronic kidney disease progression. Kidney International. 2016 Dec 1;90(6):1144-5.
 27. Alireza S, Mojtaba U, Elahe M, et al. Comparison of oral folic acid and folinic acid on blood homocysteine level of patients on hemodialysis.
 28. Anurag A. A Study on the Levels of Folic Acid, Vitamin B12, and Plasma Homocysteine in Patients with Chronic Kidney Disease. The Journal of the Association of Physicians of India. 2022 Apr 1;70(4):11-2.
 29. Yonova D, Dimitrova V, Trendafilov I, et al. Comparative Study of Homocysteine and Vitamin B12 in Patients on Hemodialysis Younger or Older Than 65 Years. Int J Fam Med Prim Care. 2020; 1(3): 1012.

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