EFFECT OF VITAMIN D3 SUPPLEMENTATION ON HEPSIDIN AND FERRITIN SERUM LEVELS IN CHILDREN WITH CHRONIC KIDNEY DISEASE

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Protocol

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Summary:

This study aim to assess the effect of Vitamin D administration on Hepcidin and Ferritin serum level in children with chronic kidney diseases. It is a clinical trial involving 43 chronic kidney disease patients at Dr. Wahidin Sudirohusodo Hospital in Makassar, Indonesia. Subjects are enrolled into two different groups, one with glomerulus filtration rate (GFR) more than 90 mL/min/1,73 m^2 and the other group consist of children with GFR less than 90 mL/min/1,73 m^2. Both group will be given the same dose of Vitamin D, which is 2000 IU for 6 weeks. Measurements of vitamin D, hepcidin and ferritin level will be before and after Vitamin D supplementation.

Introduction:

Chronic kidney disease (CKD) is a significant global health issue characterized by a gradual decline in kidney function over time. Defined by the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, CKD involves abnormalities in kidney structure or function lasting more than three months, which can lead to complications such as anemia. In children, CKD can adversely affect growth patterns and is closely linked to anemia due to reduced erythropoietin production, which disrupts iron homeostasis essential for oxygen transport in the body.

Anemia in CKD patients is primarily driven by iron deficiency, with studies indicating that the incidence ranges from 7.2 to 13.96 per 1,000 people annually, contributing to approximately 800,000 deaths globally. In pediatric populations, the prevalence of anemia among children with CKD is notably high, affecting 36-58% of patients in regions like the United States and Germany. The increasing incidence of CKD in children is likely related to longer life expectancy and improved treatment options, although data on early-stage CKD remain limited.

Vitamin D deficiency is another critical issue associated with CKD, particularly in children. Research shows that a significant percentage of patients undergoing hemodialysis exhibit low vitamin D levels, which can exacerbate anemia by lowering hemoglobin levels. Vitamin D plays a vital role in erythropoiesis, and its deficiency correlates with increased serum hepcidin levels—a hormone that regulates iron metabolism and can hinder iron absorption and release from stores.

Given the interconnectedness of vitamin D levels, hepcidin concentration, and anemia in children with CKD, research is warranted to explore the effects of vitamin D supplementation on serum hepcidin and ferritin levels. There is currently a lack of studies addressing this relationship,

especially in Indonesia. This research aims to fill the gap in knowledge regarding vitamin D's potential benefits for managing anemia and iron homeostasis disorders in pediatric CKD patients.

Objectives of Trial:

1. **Primary Objective**
   a. To assess the effect of Vitamin D supplementation on hepcidin and ferritin serum level in children with CKD
2. **Secondary Objective**

   a. To assess vitamin D, hepcidin, and ferritin level in children with CKD and GFR less than 90 ml/min/1,73 m^2 before vitamin D3 supplementation
   b. To assess vitamin D, hepcidin, and ferritin level in children with CKD and GFR more than 90 ml/min/1,73 m^2 before vitamin D3 supplementation
   c. To assess vitamin D, hepcidin, and ferritin level in children with CKD and GFR less than 90 ml/min/1,73 m^2 after vitamin D3 supplementation
   d. To assess vitamin D, hepcidin, and ferritin level in children with CKD and GFR more than 90 ml/min/1,73 m^2 after vitamin D3 supplementation
   e. To compare vitamin D, hepcidin, and ferritin level in children with CKD and GFR less than 90 ml/min/1,73 m^2 before and after vitamin D3 supplementation
   f. To compare vitamin D, hepcidin, and ferritin level in children with CKD and GFR more than 90 ml/min/1,73 m^2 before and after vitamin D3 supplementation
   g. To compare vitamin D, hepcidin, and ferritin level between children with GFR less than 90 ml/min/1,73 m^2 and children with GR more than 90 ml/min/1,73 m^2 before vitamin D3 supplementation
   h. To compare vitamin D, hepcidin, and ferritin level between children with GFR less than 90 ml/min/1,73 m^2 and children with GR more than 90 ml/min/1,73 m^2 after vitamin D3 supplementation

Hypothesis:

1. Serum hepcidin level is lower after vitamin D3 supplementation in children with CKD
2. Serum ferritin level is lower after vitamin D3 supplementation in children with CKD
3. Serum hepcidin level is lower in pediatric CKD patients with GFR more than 90 ml/min/1,73 m^2 compare to GFR less than 90 ml/min/1,73 m^2
4. Serum ferritin level is lower in pediatric CKD patients with GFR more than 90 ml/min/1,73 m^2 compare to GFR less than 90 ml/min/1,73 m^2

Study Design:

Interventional study

Study Population:

Number of samples:

43 subjects

Inclusion criteria:

- Patientsa aged 2 to 18 years diagnosed with chronic kidney diseases at Dr. Wahidin Sudirohusodo hospital
- Parents have signed their child's consent to participate in the study

Exclusion criteria:

- History of blood transfusion 3 months prior
- Liver dysfunction
- Sepsis
- Routine consumption of Vitamin D
- Routine consumption of iron supplement
- Patients diagnosed with iron deficiency anemia

Product Description:

Vitamin D3 with the dose of 2,000 IU

Outcomes:

The primary outcomes being measured in this study are serum vitamin D levels, serum hepcidin levels and serum ferritin levels. These outcomes will be measured in children with CKD before and after 6 weeks of vitamin D3 supplementation. We plan to compare these levels in two groups of CKD patients - those with glomerular filtration rate (GFR) <90 mL/min/1.73 m^2 and those with GFR ≥90 mL/min/1.73 m^2 . Measurement will be done by collecting 3 ml of blood to measure the level of vitamin D, hepcidin and serum ferritin.

Statistical analysis will utilize unpaired T test or Mann-Whitney test will be used to compare vitamin D, hepcidin and ferritin serum level in subjects according to the distribution of the data. Paired T Test or McNemar test will also be use to compare proportion of individually matched event for the two groups before and after vitamin D3 supplementation. Lastly, Pearson or Spearman test will be done to assess the correlation between vitamin D level and hepcidin and ferritin serum level.

Conduct of Trial:

This study will be conducted as a clinical trial involving children with chronic kidney disease (CKD). Participants will be divided into two groups based on their glomerular filtration rate (GFR): those with GFR <90 mL/min/1.73 $m^2$and those with GFR ≥90 mL/min/1.73 m^2. All participants will undergo initial blood tests to measure serum levels of vitamin D, hepcidin, and ferritin. Following these baseline measurements, the children will receive vitamin D3 supplementation for a specified period. After the supplementation period, blood tests will be repeated to measure the same parameters. We will then compare the pre- and post-supplementation levels of vitamin D, hepcidin, and ferritin within each GFR group, as well as between the two GFR groups. This approach will allow us to assess the effect of vitamin D3 supplementation on serum hepcidin and ferritin levels in children with CKD, and to determine if the effect differs based on the severity of kidney dysfunction as indicated by GFR.

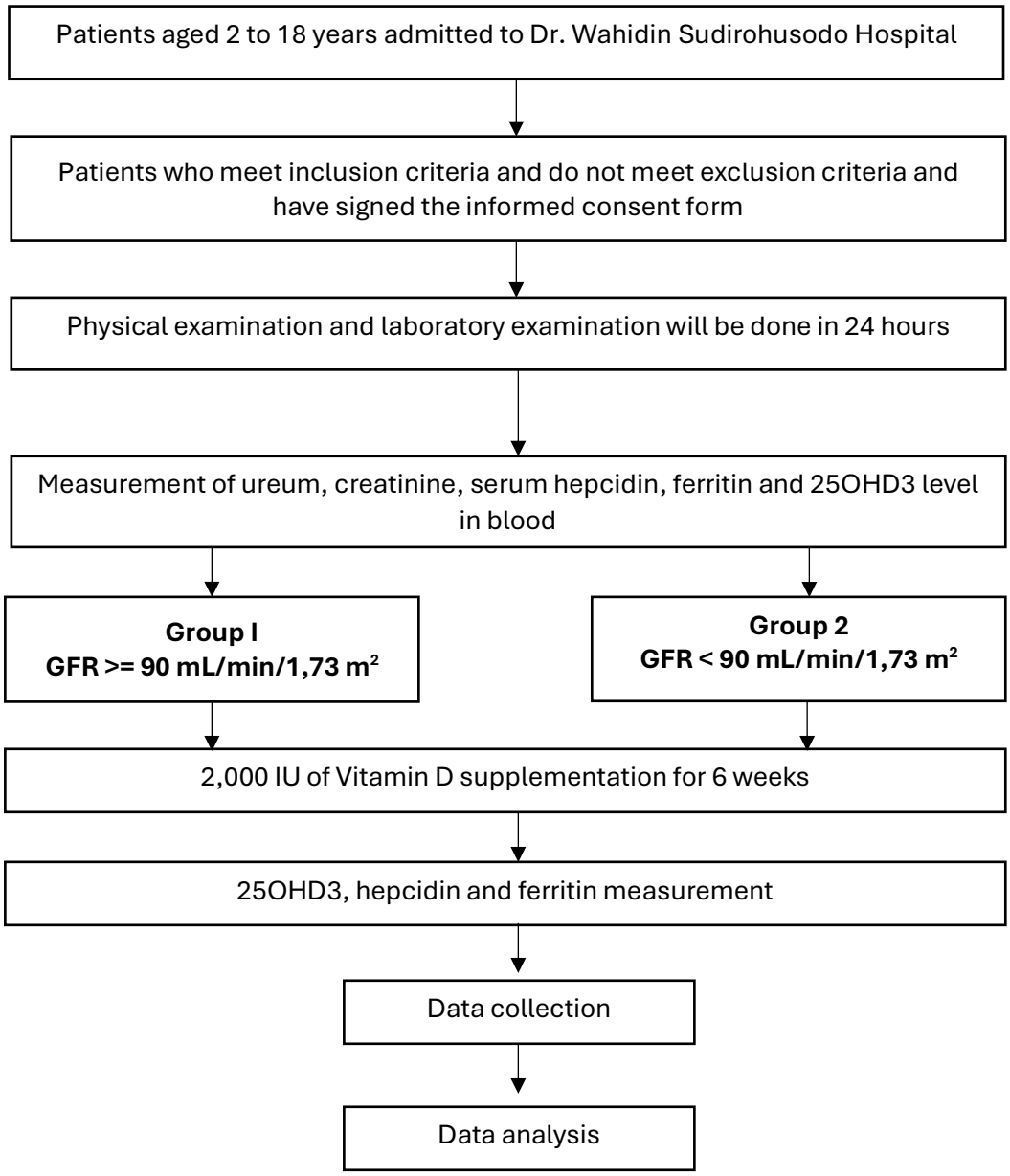

Figure 1. Study Procedure

Statistical Aspects and Considerations:

This study employs a comprehensive statistical approach to analyze the effect of vitamin D3 supplementation on serum hepcidin and ferritin levels in children with chronic kidney disease (CKD). We will utilize both descriptive and inferential statistics to examine their data. Descriptive statistics will be used to summarize the categorical data as counts and ratios, while quantitative

data will be presented as mean values with standard deviation. For inferential statistics, the study will employ Pearson's correlation test to differentiate between groups and assess statistical significance. The significance level is set at $P < 0.05$ for statistical significance and $P < 0.01$ for high significance. The statistical analysis will be conducted using SPSS version 20.0. Important considerations for this study include ensuring an adequate sample size to detect clinically meaningful differences, which will be determined through a sample size calculation. We will account for potential confounding variables, assess whether assumptions are met for the planned statistical tests, handle any missing data appropriately, and conduct sensitivity analyses to evaluate the robustness of results. By addressing these statistical aspects and considerations, our study aims to produce valid and reliable results regarding the effects of vitamin D3 supplementation on serum hepcidin and ferritin levels in children with CKD, comparing outcomes between groups with different glomerular filtration rates (GFR < 90 mL/min/1.73 $m^2$and GFR ≥ 90 mL/min/1.73 m^2).

References

Altemose, K. E., Kumar, J., Portale, A. A., Warady, B. A., Furth, S. L., Fadrowski, J. J., & Atkinson, M. A. (2018). Vitamin D insufficiency, hemoglobin, and anemia in children with chronic kidney disease. Pediatric Nephrology, 33(11), 2131–2136. https://doi.org/10.1007/s00467-018-4020-5

Amanullah, F., Malik, A. A., & Zaidi, Z. (2022). Chronic kidney disease causes and outcomes in children: Perspective from a LMIC setting. PLoS ONE, 17(6 June). https://doi.org/10.1371/journal.pone.0269632

Avner, E. D., Harmon, W. E., Niaudet, P., Yoshikawa, N., Emma, F., & Goldstein, S. L. (Eds.). (2016a). Pediatric Nephrology. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-43596-0

Avner, E. D., Harmon, W. E., Niaudet, P., Yoshikawa, N., Emma, F., & Goldstein, S. L. (Eds.). (2016b). Pediatric Nephrology. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-43596-0

Becherucci, F., Roperto, R. M., Materassi, M., & Romagnani, P. (2016). Chronic kidney disease in children. In Clinical Kidney Journal (Vol. 9, Issue 4, pp. 583–591). Oxford University Press. https://doi.org/10.1093/ckj/sfw047

Chen, T. K., Knicely, D. H., & Grams, M. E. (2019). Chronic Kidney Disease Diagnosis and Management: A Review. In JAMA - Journal of the American Medical Association (Vol. 322, Issue 13, pp. 1294–1304). American Medical Association. https://doi.org/10.1001/jama.2019.14745

Ganz, T., & Nemeth, E. (2016). Iron Balance and the Role of Hepcidin in Chronic Kidney Disease. In *Seminars in Nephrology* (Vol. 36, Issue 2, pp. 87–93). W.B. Saunders. https://doi.org/10.1016/j.semnephrol.2016.02.001

Gois, P. H. F., Wolley, M., Ranganathan, D., & Seguro, A. C. (2018). Vitamin D deficiency in chronic kidney disease: Recent evidence and controversies. In *International Journal of Environmental Research and Public Health* (Vol. 15, Issue8). MDPI AG. https://doi.org/10.3390/ijerph15081773

Iyengar, A., Kamath, N., Reddy, H. v., Sharma, J., Singhal, J., Uthup, S., Ekambaram, S., Selvam, S., Rahn, A., Fischer, D. C., Wan, M., & Shroff, R. (2022). Determining the optimal cholecalciferol dosing regimen in children with CKD: A randomized controlled trial. *Nephrology Dialysis Transplantation*, *37*(2), 326–334. https://doi.org/10.1093/ndt/gfaa369

KDIGO 2012. (2012). *Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease*. www.publicationethics.org

Lv, J. C., & Zhang, L. X. (2019). Prevalence and Disease Burden of Chronic Kidney Disease. In *Advances in Experimental Medicine and Biology* (Vol. 1165, pp. 3–15). Springer New York LLC. https://doi.org/10.1007/978-981-13-8871-2

Nalado, A. M., Olorunfemi, G., Dix-Peek, T., Dickens, C., Khambule, L., Snyman, T., Paget, G., Mahlangu, J., Duarte, R., George, J., & Naicker, S. (2020). Hepcidin and GDF-15 are potential biomarkers of iron deficiency anaemia in chronic kidney disease patients in South Africa. *BMC Nephrology*, *21*(1). https://doi.org/10.1186/s12882-020-02046-7

Roemhild, K., von Maltzahn, F., Weiskirchen, R., Knüchel, R., von Stillfried, S., & Lammers, T. (2021). Iron metabolism: pathophysiology and pharmacology. In *Trends in Pharmacological Sciences* (Vol. 42, Issue 8, pp. 640–656). Elsevier Ltd. https://doi.org/10.1016/j.tips.2021.05.001

Romagnani, P., Remuzzi, G., Glassock, R., Levin, A., Jager, K. J., Tonelli, M., Massy, Z., Wanner, C., & Anders, H. J. (2017). Chronic kidney disease. In *Nature Reviews Disease Primers* (Vol. 3). Nature Publishing Group. https://doi.org/10.1038/nrdp.2017.88

Ueda, N., & Takasawa, K. (2018). Impact of inflammation on ferritin, hepcidin and the management of iron deficiency anemia in chronic kidney disease. In *Nutrients* (Vol. 10, Issue 9). MDPI AG. https://doi.org/10.3390/nu10091173 USTUNER, B., BEK, S., EREN, N., BAKİRDOGEN, S., & KALENDER, B. (2021). Hepcidin and Vitamin D Levels: Mutual Effects in Anemia of Chronic Kidney Disease. *Kocaeli Üniversitesi Sağlık Bilimleri Dergisi*. https://doi.org/10.30934/kusbed.661316

Vahdat, S. (2020). Vitamin D and Kidney Diseases: A Narrative Review. *International Journal of Preventive Medicine*, *11*, 195. https://doi.org/10.4103/ijpvm.IJPVM

Whyte, D. A., & Fine, R. N. (2008). Chronic kidney disease in Children. *Pediatrics in Review*, *29*(10), 335–341. https://doi.org/10.1542/pir.29-10-335

Wong, M. M. Y., Tu, C., Li, Y., Perlman, R. L., Pecoits-Filho, R., Lopes, A. A., Narita, I., Reichel, H., Port, F. K., Sukul, N., Stengel, B., Robinson, B. M., Massy, Z. A., & Pisoni, R. L. (2021). Anemia and iron deficiency among chronic kidney disease Stages 3-5ND patients in the chronic kidney disease outcomes and practice patterns study: Often unmeasured, variably treated. *Clinical Kidney Journal*, *13*, 613–624. https://doi.org/10.1093/CKJ/SFZ091