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The Effects of Vitamin B Combination Injection on Lymphocyte Count in Chronic Kidney Failure Patients

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Chronic kidney failure will lead to decline in immune system. Vitamin B considered has essential roles in immune system, including lymphocyte count. Measure the effects of vitamin B combination injection on lymphocyte count in chronic kidney failure patients. This was quasi-experimental study with one group pretest-posttest design from the period of August 2018 - October 2018 at Bethesda Hospital and Panti Rapih Hospital, Yogyakarta, Indonesia. Subjects received vitamin B combination injection (intravenous) after each hemodialysis. Lymphocyte count were measured 3 times namely visit I (before first hemodialysis), visit II (after second hemodialysis), and visit III (after third hemodialysis). The data were obtained from 115 chronic kidney failure patients, consist of 75 male and 40 female. The most common age group in this study was 40-59 years old. There were no significant improvements of lymphocyte counts, both on visit II compared to visit I (1285.713±475.9829/mm^3 vs 1504.219±1148.974/mm^3; p: 0.065) as well as visit III compared to visit I (1285.713±475.9829/mm^3 vs 1315.192±658.6673/mm^3; p: 0.766). Vitamin B combination injection has no significant effects on lymphocyte count in chronic kidney failure patients. Intravenous vitamin B was safe for chronic kidney failure patients.

Keywords: Vitamin B, Chronic Kidney Failure, Lymphocyte, Immune, Hemodialysis**.**

Chronic kidney failure is defined as abnormalities in renal structure or progressive decline in renal function for more than 3 months[1]. Globally, the prevalence of chronic kidney failure in 2016 was 13,4%[2]. Yogyakarta - along with East Nusa Tenggara, South Sulawesi, Lampung, West Java, Central Java, and East Java - has the third highest stroke cases among provinces in Indonesia[3].

Chronic kidney failure and immune system has reciprocal relationship[4]. Various kidney diseases are caused by immune disorders. These kidney diseases will lead to kidney failure. One of the pathomechanisms that strongly related to kidney diseases is deposit of immune complexes in kidneys which will cause inflammatory responses and damages to kidneys[5]. On the other hand, chronic kidney failure will lead to decline in immune system. This will make patients with chronic kidney failure more susceptible to infection than healthy population[4].

It has long been known that adequate immune system determined by adequate nutrition[6]. Vitamin B considered has essential roles in immune system, including lymphocyte count[7].

The aim of this study is measuring the effects of vitamin B combination injection on lymphocyte count in chronic kidney failure patients.

MATERIAL AND METHODS

Study Design

This was quasi-experimental study with one group pretest-posttest design. This study was conducted at Bethesda Hospital and Panti Rapih Hospital, Yogyakarta, Indonesia with the period of August 2018 - October 2018.

There were 115 chronic kidney failure patients who met the inclusion and exclusion criteria. Each subject received vitamin B combination injection (intravenous) after each hemodialysis. This vitamin B combination injection consists of 100 mg vitamin B1 (thiamin), 100 mg B6, (pyridoxine) and 500 mcg B12 (cobalamine). Lymphocyte count were measured 3 times namely visit I (before first hemodialysis), visit II (after second hemodialysis), and visit III (after third hemodialysis). Visit II took place 2 weeks after visit I, visit III took place 2 weeks after visit II. There was no control group in this study. Blood sample needed for each examination is as much as 5 mL, stored in a specimen tube containing clot activator.

Table 1. Subjects' characteristics and current medications

	Total	%
Sex		
Male	75	65.22%
Female	40	34.78%
Age		
19-39 years old	23	20%
40-59 years old	59	51.30%
60-85 years old	33	28.69%
Comorbid conditions		
Hypertension	97	8.34%
Diabetes	39	33.91%
Concomitant medications		
Calcium channel blocker (CCB)	67	68.26%
Angiotensin II receptor blocker (ARB)	51	44.34%
Diuretic	49	42.61%
Antidiabetic (Insulin)	9	7.83%
Calcium carbonate	79	68.69%
Folic acid	100	86.96%

Subject Selection

Subjects were recruited using consecutive sampling. The inclusion criteria of this study were: (i) Male or female over 18 years old, (ii) Diagnosed with chronic kidney failures and undergo routine hemodialysis (2 times per week) and (iii) Did not use any vitamin B supplementation before. The exclusion criteria were: (i) Hypersensitivity to vitamin B (ii) Participated in other studies and (iii) Subject was pregnant or planned to become pregnant during the study period.

Ethical approval number 906/C.16/FK/2019 was obtained from Duta Wacana Christian University, Yogyakarta, Indonesia.

Statistical Analysis

Subjects' characteristics, current medications, and lymphocyte count were evaluated in this study. Subjects' characteristics and current medications were presented on descriptive analysis. Wilcoxon test was used to analyze lymphocyte count and their relationship with subjects' current medications. Statistical significance was set at $p<0.05$.

RESULTS

One hundred and fifteen subjects in this study were dominated by male subjects (65.22%). The most common age group in this study was 40-59 years old (51.3%).

Hypertension was the most common comorbid conditions, found in 97 subjects (84.34%). The concomitant medications were

Table 2. Effects of concomitant medications on lymphocyte counts

Medication	*p* Visit I and II	Visit I and III
Calcium channel blocker (CCB)	0.03	0.802
Angiotensin II receptor blocker (ARB)	0.017	0.033
Diuretic	0.016	0.489
Insulin	0.058	0.646
Calcium cabonate	0.133	0.945

67 (68.26%) calcium channel blocker (CCB), 51 (44.34%) angiotensin II receptor blocker (ARB), 49 (42.61%) diuretic, 9 (7.83%) insulin, 79 (68.69%) calcium carbonate, and 100 (86.96%) folic acid.

Table 2 shows CCB has significant effect on lymphocyte count on visit II compared to visit I (p: 0.03). ARB has significant effect on lymphocyte count on visit II compared to visit I (p: 0.017), also on visit III compared to visit I (p: 0.033). Table 3 shows there were no significant improvements on lymphocyte count before and after vitamin B combination injection, both on visit II compared to visit I (1285.713±475.9829/mm^3 vs 1504.219±1148.974/mm^3; p: 0.065) as well as visit III compared to visit I (1285.713±475.9829/mm^3 vs 1315.192±658.6673/mm^3; p: 0.766).

DISCUSSION

Vitamin B effects on immune system are already known. However, the effects of vitamin B combination on immune system, especially lymphocyte count have not been much studied. This study is aimed to identify the effects of vitamin B combination injection on lymphocyte count in chronic kidney failure patients.

Male subjects dominated this study. Previous study showed similar results, there were 0.744 times more male hemodialysis patients than female hemodialysis patients[8]. The most common age group in this study was 40-59 years old (51.3%), consistent with previous study that showed most of hemodialysis patients were between 40-59 years old[9].

Hypertension is the most common comorbid conditions in this study. This is in line with a research in 2019 which found 85.7% with hypertension as comorbid conditions[10]. Hypertension leads to glomerular ischemia that will damage glomerular arteries and arterioles. This mechanism causes and worsens kidney diseases by decreasing glomerular blood flow, impairing kidneys' structures and functions. Hypertension also damages autoregulation system of kidneys[11].

Chronic kidney failure and immune system has reciprocal relationship. Various kidney diseases are caused by immune disorders and chronic kidney failure will lead to decline in immune system. Chronic kidney failure causes damage to kidney structure which causes the body to excrete protein. The excretion of proteins limits the quantity of materials required for composing immune system components, such as antibodies and enzymes[4].

Lack of micronutrients may lead to immune system dysregulation[12]. Vitamin B deficiency is common in patients with chronic kidney failure. Water-soluble vitamins, including vitamin B were wasted during hemodialysis. Other factors, such as minimizing meat consumption, one of vitamin B sources, will reduce vitamin B levels even further[13].

There were no significant improvements of lymphocyte counts, both on visit II compared to visit I (1285.713±475.9829/mm^3 vs 1504.219±1148.974/mm^3; p: 0.065) as well as visit III compared to visit I (1285.713±475.9829/mm^3 vs 1315.192±658.6673/mm^3; p: 0.766). Unlike this study, previous study in Wadsworth showed significant lymphocyte count improvement ($p<0.05$) in 8 hemodialysis patients after administration of vitamin B6 supplementation[14]. Another studies involving elderly persons and young women consuming a controlled diet showed

Table 3. Comparison of lymphocyte counts on every visits

	Visit I and II (pretest and posttest or before and after vitamin B combination injection) Mean (s.d)	*p*
Visit I (pretest)	1285.713 (475.9829)	0.065
Visit II (posttest)	1504.219 (1148.974)	
	Visit I and III (pretest and posttest or before and after vitamin B combination injection)	
Visit I (pretest)	1285.713 (475.9829)	0.755
Visit III (posttest)	1315.192 (658.6673)	

vitamin B6 supplementation has significant effects on lymphocyte proliferation[15,16]. A study in 2008 found significant improvement of lymphocyte percentage after vitamin B12 supplementation[17]. These differences might be due to only vitamin B6 and vitamin B12 has direct effects on lymphocyte counts, while vitamin B1 does not have direct effect on lymphocyte counts. Innate and adaptive immunity were affected by vitamin B6 deficiency, including T-lymphocytes proliferation and function. Furthermore, vitamin B6 associated with cellular immune activation mediated by IFN-gamma[18]. Immune sytem regulation, immunomodulator, and involved in cell division were 3 main roles of vitamin B in immune system. Decreased levels of vitamin B12 will decrease cytotoxic T cells levels[19]. Vitamin B12 also helps body use folate. Folate deficiency causes immunodeficiency with T-lymphocyte proliferation impairment[20]. Vitamin B1 main roles in immune response are its antioxidative effect and inhibit oxidative stress stimulation of NF- êB[7].

Concomitant medications might affect the identification of vitamin B combination injection effects on lymphocyte counts. There were 2 antihypertensive medications CCB has significant effect on lymphocyte count visit II compared to visit I (p: 0.03). ARB has significant effect on lymphocyte count on visit II compared to visit I (p: 0.017), also on visit III compared to visit I (p: 0.033). CCBs are known as inhibitor of phytohemagglutinin (PHA)-induced proliferation of human lymphocytes[21]. ARBs inhibit angiotensin II receptor (AGT1R) signaling, an important part for lymphocyte, especially T-lymphocyte activation[22]. Thus, antihypertensive medications effects work against vitamin B effects on immune system.

The limitations of this study were: there was no control group in this study and confounding factors did not well-evaluated.

CONCLUSIONS

Vitamin B combination injection has no significant effects on lymphocyte count in chronic kidney failure patients. Intravenous vitamin B was safe for chronic kidney failure patients.

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Conflict of interest

Nothing to declare.

REFERENCES

1. Dipiro J. T. Pharmacotherapy Handbook, 9th Edition. McGraw Hill, New York: 787 (2015).
2. Hill N. R, Fatoba S. T, Oke J. L, Hirst J. A, O'Callaghan C. A, Lasserson D. S, *et al*. Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis. *PLoS ONE,* **11**(7): e0158765 (2016).
3. Indonesian Ministry of Health. Basic Health Research. Health Research and Development Agency, Jakarta 2013: 95.
4. Imig J. D and Ryan M. J. Immune and inflammatory role in renal disease. *Compr Physiol,* **3**: 957-976 (2013).
5. Heeringa S. F and Cohen C. D. Kidney diseases caused by complement dysregulation: acquired, inherited, and still more to come. *Clin Dev Immunol*, 2012; 695131 (2012).
6. Chandra R. K. Nutrition and the immune system from birth to old age. *Eur J Clin Nutr,* **56**: S73-S76 (2002).
7. Spinas E, Saggini A, Kritas S. K, Cerulli G, Caraffa A, and Antinolfi P. Crosstalk between vitamin B and immunity. *Journal of Biological Regulators & Homeostatic Agents,* **29**(2): 283-288 (2015).
8. Hecking M, Bieber B. A, Ethier J, Kautzky-Willer A, Sunder-Plassmann G, Säemann M. D, *et al*. Sex-specific differences in hemodialysis prevalence and practices and male-to-female mortality rate: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *PLoS medicine,* **11**(10): e1001750 (2014).
9. Bayhakki B and Hasneli Y. Relationship between Length of Undergoing Hemodialysis and Inter-Dialytic Weight Gain (IDWG) in Hemodialysis Patients. *Jurnal Keperawatan Padjadjaran,* **5**(3): 242-248 (2017).
10. Pinzon R. T, Wijaya B. M, Pramudita E. A, Wuryaningsih N. S and Sujatno P. Prevalence of elevated levels of homocysteine (hyperhomocysteinemia) in patients with chronic kidney disease undergoing hemodialysis. *J Clin Med Kaz*, **3**(53): 21-25 (2019).
11. Abraham G, Arun K, Gopalakrishnan N, Renuka

S, Pahari D. K and Deshpande P. Management of hypertension in chronic kidney disease: Consensus statement by an expert panel of Indian nephrologists. *Journal of The Association of Physicians of India*, **65**(2): 6-22 (2017).

12. Bull S. J, Huezo-Diaz P, Binder E. B, Cubells J. F, Ranjith G, Maddock C, *et al*. Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. *Mol Psychiatry,* **14**: 1095-1104 (2009).
13. Menon V, Wang X, Greene T, Beck G. J, Kusek J. W, Selhub J, *et al.* Homocysteine in chronic kidney disease: Effect of low protein diet and repletion with B vitamins. *Kidney International,* **67**: 1539-1546 (2005).
14. Casciato D. A, McAdam L. P, Kopple J. D, Bluestone R, Goldberg L. S, Clements P. J, *et al*. Immunologic Abnormalities in Hemodialysis Patients: Improvement after Pyridoxine Therapy. *Nephron,* **38**: 9-16 (1984).
15. Talbott M. C, Miller L. T and Kerkvliet N. I. Pyridoxine supplementation: effect on lymphocyte responses in elderly persons. *The American Journal of Clinical Nutrition,* **46**(4): 659-664 (1987).
16. Kwak H, Hansen C. M, Leklem J. E, Hardin K and Shultz T. D. Improved Vitamin B-6 Status Is Positively Related to Lymphocyte Proliferation in Young Women Consuming a Controlled Diet. *The Journal of Nutrition*; **132**(11): 3308-3313 (2002).
17. Erkurt M. A, Aydogdu I, Dikilitaº M, Kuku I, Kaya E, Bayraktar N, *et al*. Effects of Cyanocobalamin on Immunity in Patients with Pernicious Anemia. *Med Princ Pract,* **17**: 131-135 (2008).
18. Christensen M. H. E, Pedersen E. K. R, Nordbø Y, Varhaug J. E, Midttun Ø, Ueland P. M, *et al*. Vitamin B6 Status and interferon-ã-mediated Immune Activation in Primary Hyperparathyroidism. *J Intern Med,* **272**(6): 583-591 (2012).
19. Mikkelsen K, Stojanovska L, Prakash M and Apostolopoulos V. The effects of vitamin B on the immune/ cytokine network and their involvement in depression. *Maturitas,* **96**: 58-71 (2017).
20. Kishimoto K, Kobayashi R, Sano H, Suzuki D, Maruoka M, Yasuda K, *et al*. Impact of Folate Therapy on Combined Immunodeficiency Secondary to Hereditary Folate Malabsorption. *Clin Immunol,* **153**(1): 17-22 (2014).
21. Weir M. R, Peppler R, Gomolka D and Handwerger B. S. Calcium channel blockers inhibit cellular uptake of thymidine, uridine and leucine: the incorporation of these molecules into DNA, RNA and protein in the presence of calcium channel blockers is not a valid measure of lymphocyte activation. *Immunopharmacology,* **25**(1): 75-82 (1993).
22. Tawinwung S, Petpiroon N and Chanvorachote P. Blocking of Type 1 Angiotensin II Receptor Inhibits T-lymphocyte Activation and IL-2 Production. *In Vivo,* **32**(6): 1353-1359 (2018).