

Prevalence of elevated levels of homocysteine (hyperhomocysteinemia) in patients with chronic kidney disease undergoing hemodialysis

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ABSTRACT

Background: Chronic kidney disease is a disease with kidney damage of more than 3 months. Chronic kidney disease has five stages, in which in the fifth stage the patient requires renal replacement therapy. Hemodialysis replaces kidney function to get rid of waste substances, this process can also reduce the substances that the body may still need such as vitamins that can be wasted. Even the metabolism in the body will be disrupted, one of which causes the balance of homocysteine metabolism to be disrupted. This causes homocysteine levels to increase in the plasma.

Objectives: To measure the prevalence of elevated homocysteine levels (hyperhomocysteinemia) in patients with chronic kidney disease who undergo hemodialysis.

Methods and subjects: This is a cross sectional study with number of respondents in this study amounted to 91 people who all had chronic kidney disease undergoing hemodialysis at Bethesda Hospital and Panti Rapih Hospital in Yogyakarta. The selection of subjects used the consecutive sampling method. The data analysis used is univariate analysis.

Results: Of the total 91 subjects with chronic kidney who undergo hemodialysis, 28.6% of them were elderly patients with an average age of 51.78 years, with men 59 subjects (64.8%) and women 32 subjects (35.2%). In all subjects got a history of hypertension and anemia 78 respondents (85.7%), diabetes 31 subjects (34.1%) and cancer 1 subjects (1.1%). The prevalence of subjects with hyperhomocysteinemia was 80 subjects (87.9%) and 11 were not (12.1%). In hyperhomocysteinemia patients (freq=80), 23 of them were elderly patients (88.5%), with men 55 subjects (93.2%) and women 25 subjects (78.1%). The history of the disease patients with hyperhomocysteinemia is anemia and hypertension 69 respondents (88.5%), diabetes 28 subjects (90.3%) and cancer 1 subject (100%).

Conclusion: The prevalence of hyperhomocysteinemia in patients with chronic kidney disease undergoing hemodialysis is 87.9%.

Keywords: chronic kidney disease, homocysteine, hyperhomocysteinemia

ГЕМОДИАЛИЗДЕГІ СОЗЫЛМАЛЫ БҮЙРЕК ЖЕТІСПЕУШІЛІГІМЕН АУЫРАТЫН ПАЦИЕНТТЕРДЕ ГОМОЦИСТЕИННІҢ (ГИПЕРГОМОЦИСТЕИНЕМИЯ) ЖОҒАРЫЛАҒАН ДЕҢГЕЙІНІҢ ТАРАЛУЫ

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ТҰЖЫРЫМДАМА

Кіріспе: Созылмалы бүйрек жеткіліксіздігі – бұл 3 айдан астам уақыт бойы бүйректің зақымдалу ауруы. Созылмалы бүйрек жеткіліксіздігінің бес сатысы бар, олардың ішіндегі бесінші сатысы пациент үшін алмастырушы бүйрек терапиясын жүргізуді талап етеді. Гемодиализ уытты заттардан тазалау бойынша бүйрек қызметін алмастырады, сондай-ақ бұл процесс уытты болуы ықтимал дәрумендер секілді ағзаға қажетті заттарды азайта алады. Тіпті ағзадағы метаболизм тоқтайды, оның бір себебі гомоцистеин метаболизмінің теңгерімі болып табылады. Бұл плазмадағы гомоцистеин деңгейінің артуына алып келеді.

Мақсаты: Гемодиализдегі созылмалы бүйрек жетіспеушілігімен ауыратын пациенттерде гомоцистеиннің (гипергомоцистеинемия) жоғарылаған деңгейінің таралуын айқындау.

Материал және әдістері: Көпденең болып табылады, Джокьякартадағы Бетесда және Панти Рапих ауруханаларында гемодиализден өтуші респонденттер саны созылмалы бүйрек жеткіліксіздігімен ауыратын 91 адам құрады. Зерттеу субъектілерін таңдау кезінде тізбекті таңдама әдісі қолданылды. Деректерді талдау үшін бір факторлы талдау қолданылды.

Нәтижелері: Гемодиализдегі созылмалы бүйрек жеткіліксіздігімен ауыратын пациенттердің жалпы санынан (91) зерттеуге 59 ер адам (64.8%) мен 32 әйел адам (35.2%) қатысты, 28,6% егде тартқан кісілер болды, олардың орташа жасы 51.78 жасты құрады. Барлық пациенттер арасында, 78 респонденттер (85.7%) сыртартқысында гипертензия мен анемия, 31 (34.1%) пациентте диабет, және 1 (1.1%) субъектіде обыр болған. Гипергомоцистеинемия жағдайларының таралуы 87.9% (80 пациент) құрады, 11 (12.1%) пациентте айқындалған жоқ. Гипергомоцистеинемиямен ауыратын пациенттер арасында (жиілігі=80), 23 пациент егде тартқандар (88.5%), 55 ер адам (93.2%) және 25 әйел (78.1%). Гипергомоцистеинемиямен ауыратын пациенттердің 69-ның сыртартқысында анемия және гипертензия бар, 28 пациент (90.3%) диабетке және 1 пациент (100%) обырға шалдыққан.

Қорытынды: Гемодиализдегі созылмалы бүйрек жетіспеушілігімен ауыратын пациенттер арасында гипергомоцистеинемияның таралуы 87.9% құрады.

Негізгі сөздер: созылмалы бүйрек жетіспеушілігі, гомоцистеин, гипергомоцистеинемия

РАСПРОСТРАНЕННОСТЬ ПОВЫШЕННОГО УРОВНЯ ГОМОЦИСТЕИНА (ГИПЕРГОМОЦИСТЕИНЕМИЯ) У ПАЦИЕНТОВ С ХРОНИЧЕСКОЙ ПОЧЕЧНОЙ НЕДОСТАТОЧНОСТЬЮ НА ГЕМОДИАЛИЗЕ

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РЕЗЮМЕ

Введение: Хроническая почечная недостаточность - это заболевание с повреждением почек в течение более 3 месяцев. Существует пять стадий хронической почечной недостаточности, при которых пятая стадия требует проведение заместительной почечной терапии для пациента. Гемодиализ замещает функцию почек по избавлению от токсичных веществ, а также данный процесс может уменьшить все еще необходимые организму вещества, такие как витамины, которые могут быть токсичны. Даже метаболизм в организме прерывается, одной из причин чего является баланс метаболизма гомоцистеина. Это приводит к повышению уровня гомоцистеина в плазме.

Цель: Определить распространенность повышенного уровня гомоцистеина (гипергомоцистеинемия) у пациентов с хронической почечной недостаточностью на гемодиализе.

Материал и методы: Настоящее исследование является поперечным, количество респондентов в котором составило 91 человек с хронической почечной недостаточностью, проходящих гемодиализ в больницах Бетесда и Панти Рапих в Джокьякарте. При выборе субъектов исследования использовали метод последовательной выборки. Для анализа данных использовали однофакторный анализ.

Результаты: Из общего числа пациентов (91) с хронической почечной недостаточностью на гемодиализе в исследовании приняло участие 59 мужчин (64.8%) и 32 женщины (35.2%), 28,6% были пожилые пациенты, средний возраст которых составил 51.78 лет. Среди всех пациентов, у 78 респондентов (85.7%) в анамнезе была гипертензия и анемия, диабет у 31 (34.1%) пациента, и рак у 1 (1.1%) субъекта. Распространенность случаев гипергомоцистеинемии составила 87.9% (80 пациентов), у 11 (12.1%) пациентов не обнаружено. Среди пациентов с гипергомоцистеинемией (частота=80), 23 пациента пожилого возраста (88.5%), 55 мужчин (93.2%) и 25 женщин (78.1%). Среди случаев гипергомоцистеинемии 69 пациентов (88.5%) в анамнезе имели анемию и гипертензию, 28 пациентов (90.3%) диабет и 1 пациент (100%) рак.

Заключение: Распространенность гипергомоцистеинемии среди пациентов с хронической почечной недостаточностью на гемодиализе составила 87.9%.

Ключевые слова: хроническая почечная недостаточность, гомоцистеин, гипергомоцистеинемия

Introduction

Chronic kidney disease is considered an impairment of excreting waste products of body metabolism by the kidney. Patients diagnosed with chronic kidney disease experienced kidney damage (urine albumin concentration of >30 mg/g) and/or reduction in kidney function (reflected by glomerular filtration rates/GFR of <60 ml/min/1.73 m²) that persists longer than 3 months [6].

According to the Global Burden of Disease Study in 2010, chronic kidney disease was found to be the 18th leading cause of death worldwide having mortality rates of 16.3 per 100,000 population. In a meta-analysis involving 44 countries, the prevalence of chronic kidney disease was 13.4% with stage 3 being the most prevalent among all stages [4].

A set of etiologies and risk factors influence the development of chronic kidney disease. Those risk factors can trigger worsening of chronic kidney disease towards end-stage renal disease (ESRD) and increase the risk of cardiovascular diseases as well as mortality. Several sociodemographic characteristics are considered the risk factors of chronic kidney disease which include gender in which the prevalence is higher amongst men compared to women (3.2% vs. 1.6%), age where it most occurs between 49 to 65 years of age (6.0%). Health problems are also considered to play a role in the development of chronic kidney disease, namely obesity,

hypertension, diabetes mellitus, hypertriglyceridemia, and hypercholesterolemia [9].

Chronic kidney disease can result in elevation of plasma homocysteine level due to impaired kidney function that is responsible to excrete this metabolite. Homocysteine is an amino acid with sulfhydryl group produced between the process of methionine and cysteine amino acids biosynthesis [10]. Almost 70% of daily homocysteine production are excreted through kidney and elevation in plasma homocysteine level is most commonly found among patients with kidney failure. Several comorbidities include renal failure, neoplasms, and diabetes, cigarette smoke exposure, alcohol, coffee, older age and menopause are also considered to increase homocysteine level [3]. Elevation in homocysteine level is followed by an increase in cardiovascular risk.

A previous study involving 138 patients with chronic kidney disease in a Nigerian hospital showed the prevalence of hyperhomocysteinemia of 57.9% [1]. In Indonesia, particularly in Yogyakarta, the prevalence of hyperhomocysteinemia has never been studied thus far therefore, the authors intended to conduct this study. We hope this study could be a basis for future studies and to elevate our knowledge regarding the increase of homocysteine level among patients with chronic kidney disease who undergo hemodialysis therapy.

Methods

This study was conducted using cross-sectional design. The subjects included patients with chronic kidney diseases undergoing hemodialysis at Bethesda and Panti Rapih Hospitals, Yogyakarta. The study was conducted from August to October 2018. The inclusion criteria include patients aged over 18 years old who were clinically diagnosed with chronic kidney disease and undergoing hemodialysis at Bethesda and Panti Rapih Hospitals. The exclusion criteria include those without complete laboratory data available. The data were obtained retrospectively through patients medical records that had gotten ethic approval previously.

The sample size was calculated using sample size formula for cross-sectional study with 95% confidence and 5% error where it resulted in a minimum sample size of 91 subjects. Secondary data were collected from patients who met inclusion and exclusion

criteria by recording their laboratory results (for homocysteine) and other characteristics. Data analysis was done using IBM SPSS Statistics version 22.0. Chi-square test was used for testing the significance of frequency of hyperhomocysteinemia.

Results

The age of the study patients ranged from 19 to 82 years with the mean age of 51.78 years. Among all, there were 59 (64.8%) male and 32 (35.2%) female patients. Of 91 patients, 26 (28.6%) were elderly and the remaining 65 (71.4%) were middle-aged individuals. In regard to comorbidities, a total of 78 (85.7%) patients also suffered from hypertension and anemia, 31 (34.1%) patients suffered from diabetes mellitus, and 1 (1.1%) patient was also diagnosed with cancer. Patients characteristics are shown in Table 1 and 2.

Table 1 Baseline characteristics of patients with chronic kidney disease who underwent hemodialysis

| Variables | Criteria | Frequency (n=91) | % |
|-----------|-----------|------------------|-------|
| Older age | ≥60 years | 26 | 28.6% |
| | <60 years | 65 | 71.4% |
| Sex | Male | 59 | 64.8% |
| | Female | 32 | 35.2% |

Table 2 Characteristics of study population in terms of comorbidity

| Variables | Criteria | Frequency (n=91) | % |
|-------------------|----------|------------------|-------|
| Cancer | Yes | 1 | 1.1% |
| | No | 90 | 98.9% |
| Diabetes Mellitus | Yes | 31 | 34.1% |
| | No | 60 | 65.9% |
| Anemia | Yes | 78 | 85.7% |
| | No | 13 | 14.3% |
| Hypertension | Yes | 78 | 85.7% |
| | No | 13 | 14.3% |

Of a total of 91 patients, 80 (87.9%) patients showed elevated plasma homocysteine level and the remaining 11 was not found (12.1%) patients. Of these 80 patients with hyperhomocysteinemia, 23 (88.5%) of them were elderly while the remaining 57 (87.71.3%) patients aged below 60 years old. Among those with hyperhomocysteinemia, 55 (93.2%) were men and 25 (78.1%) were

women. Among those 80 patients, their history of other illnesses that were associated with increased plasma homocysteine level was also recorded. It was noted that the most prevalent comorbid illnesses were hypertension and anemia in which each was found in 69 (88.5%) patients, followed by diabetes in 28 (90.3%) and cancer in 1 (100%) patient. The results are presented in Table 3.

Table 3 Characteristics of patients with and without hyperhomocysteinemia

| Variables | Criteria | Hyper-homocysteinemia | | Non hyper-homocysteinemia | | p-value | Total Freq (n=91) |
|-------------------|-----------|-----------------------|-------|---------------------------|-------|---------|----------------------|
| | | Freq (n=80) | % | Freq (n=11) | % | | |
| Older age | ≥60 years | 23 | 88.5% | 3 | 11.5% | 0.010 | 26 |
| | <60 years | 57 | 87.7% | 8 | 12.3% | | 65 |
| Sex | Male | 55 | 93.2% | 4 | 6.8% | 4.449 | 59 |
| | Female | 25 | 78.1% | 7 | 21.9% | | 32 |
| Cancer | Yes | 1 | 100% | 0 | 0% | 0.139 | 1 |
| | No | 79 | 87.8% | 11 | 12.2% | | 90 |
| Diabetes Mellitus | Yes | 28 | 90.3% | 3 | 9.7% | 0.257 | 31 |
| | No | 52 | 86.7% | 8 | 13.3% | | 60 |
| Anemia | Yes | 69 | 88.5% | 9 | 11.5% | 0.155 | 78 |
| | No | 11 | 84.6% | 2 | 15.4% | | 13 |
| Hypertension | Yes | 69 | 88.5% | 9 | 11.5% | 0.155 | 78 |
| | No | 11 | 84.6% | 2 | 15.4% | | 13 |

We also reviewed the history of folic acid supplementation that was thought to affect homocysteine level. From 91 patients studied, 82 patients were consuming folic acid supplement. Of 80

patients with hyperhomocysteinemia, 72 (87.8%) patients were consuming folic acid supplement and only 8 (88.9%) of them who did not take any folic acid supplement (Table 4).

Table 4 History of folic acid supplementation among study patients

| Variable | Criteria | Hyper-homocysteinemia | | Non hyper-homocysteinemia | | Total (n=91) |
|----------------------------|----------|-----------------------|-------|---------------------------|-------|-----------------|
| | | Freq (n=80) | % | Freq (n=11) | % | |
| Folic acid supplementation | Yes | 72 | 87.8% | 10 | 12.2% | 82 |
| | No | 8 | 88.9% | 1 | 11.1% | 9 |

Discussion

A total of 91 patients with chronic kidney disease undergoing hemodialysis were included in the analyses and were characterized based on their age, sex, and history of illnesses (cancer, diabetes, anemia, and hypertension). In general, the study participants consisted of more male than female patients who were 59 (64.8%) and 32 (35.2%) patients, respectively. A vast majority of these patients aged below 60 years old (mean age of 51.78 years) which were 65 (71.4%) patients, while the remaining 26 (28.6%) patients aged over 60 years (Table 1).

Hypertension and diabetes are known to be the two most important key factors for the development of chronic kidney disease [9]. In this study, of 91 patients included, we found 78 (85.7%) patients with a history of hypertension and 31 (34.1%) with diabetes mellitus. One of the most substantial functions of human's kidneys is to produce erythropoietin that is in charge in the synthesis of erythrocytes; however, this function is impaired in patients with chronic kidney disease and predisposes to the occurrence of anemia [5]. In this set of patients, we found 78 (85.7%) patients who suffered from anemia (Table 2).

Increased risk of cardiovascular events has been studied rigorously in relation to elevated homocysteine level among patients with chronic kidney disease. An increase in plasma homocysteine level beyond its normal range may lead to vascular endothelial dysfunction that predisposes to cardiovascular diseases. From 91 patients included in the analyses, a number of 80 (87.9%) patients showed elevated plasma homocysteine level with the mean level of 23.17 mol. This results showed an even higher prevalence of hyperhomocysteinemia as compared to a study before [1] where the prevalence was only 57.9%. This study suggested that plasma homocysteine level would increase substantially among patients with chronic kidney disease (particularly those who received renal replacement therapy through hemodialysis). Among these 80 hyperhomocysteinemic patients, 69 (86.3%) of them had a history of hypertension that is also considered to influence the level of plasma homocysteine.

Several factors are known to increase homocysteine level, particularly in those with chronic kidney disease who undergo hemodialysis. Impaired kidney function will subsequently lead to alteration of overall body metabolism, one of which is homocysteine metabolism. Among those chronic kidney disease sufferers, deficiency of vitamin B12 and folic acid, important cofactors for homocysteine metabolism, are frequent. The lack of this vitamin could result from impaired kidney function, syndrome of malnutrition or impaired nutrients reabsorption which occurred primarily in patients with renal failure who undergo hemodialysis [2]

Increase in homocysteine levels in chronic kidney patients are caused by the incidence of folic acid deficiency, vitamin B12 and

vitamin B6. This state of vitamin deficiency is triggered because vitamin B complexes include vitamins that are soluble in water and are often wasted as hemodialysis therapy progresses. Other conditions such as the diet of patients are reducing meat, while the meat is a source of vitamin folic acid [8].

Reported other potential factors contributing to the increase in plasma homocysteine level among patients with chronic kidney disease which include malignancy, diabetes mellitus, and older age [3]. In this study, we found that in 80 patients with hyperhomocysteinemia, 1 (100%) patient with a recorded history of cancer. Hyperhomocysteinemia patients who also had recorded history of diabetes mellitus were 28 (90.3%) patients, while 23 (88.5%) were in >60-year age group (Table 3).

Table 4 shows that from 80 hyperhomocysteinemic patients, 72 (87.8%) of them consumed oral folic acid supplementation. Of those without hyperhomocysteinemia (11 patients), 10 (12.2%) of them also took folic acid supplement. The data indicate that albeit the patients consume adequate oral folic acid supplement, plasma homocysteine levels still tend to increase.

Supplementation of vitamin B12 or folic acid in chronic kidney disease patients with hyperhomocysteinemia may potentially reduce homocysteine level. The reduction would be optimal if the patients take effective dose of these supplements for at least 4 weeks. The study also reported that daily supplementation of 1 mg folic acid for longer than 4 weeks reduced homocysteine level for as much as 19.2%. Therefore, our results could be due to suboptimal dose of folic acid supplementation, shorter duration the treatment course, and oral administration (the optimal route of administration is parenteral) [7].

Conclusion

From the analysis, the prevalence of hyperhomocysteinemia in patients with chronic kidney disease undergoing hemodialysis was 87.9%.

Recommendation

The high percentage of hyperhomocysteinemia cases among of chronic kidney disease patients undergoing hemodialysis could be prevented by optimal supplementation of vitamin B12 and folic acid in order to reduce the risk of cardiovascular disease.

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References

1. Afeaje B Olorok, Ikechukwu L.O., Peter F Ugbodaga. Hyperhomocysteinemia in Chronic Kidney Disease Patients in a Teaching Hospital in Nigeria. *British Journal of Medicine & Medical Research*. 2016; 18(9):1-7. <https://doi.org/10.9734/BJMMR/2016/28282>
2. Friedman A.N., Bostom A.G., Selhub J., Levey A.S., Rosenberg I.H. The Kidney and homocysteine metabolism. *J Am Soc Nephrol*. 2001; 12:2181-2189.
3. Dinavahi R., Falkner B. Relationship of homocysteine with cardiovascular disease and blood pressure. *J Clin Hypertens*. 2004; 6(9): 494-8. <https://doi.org/10.1111/j.1524-6175.2004.03643.x>
4. Hill N.R, Fatoba S.T, Oke J.L. Global Prevalence of Chronic Kidney Disease - A systematic Review and Meta-Analysis. *Plos One*. 2016; 11:e0158765. <https://doi.org/10.1371/journal.pone.0158765>
5. Kelly C., Jaime L. The Netter Collection of Medical Illustrations: Urinary System, the Second edition. Saunders: Elsevier 2012. Available from: <http://clinicalkey.com> [Accessed 28 Agustus 2018].
6. Kennedy F., Fred Ferri. Ferri's Clinical Advisor 2018. Philadelphia: Elsevier 2018. Available from: <http://clinicalkey.com> [Accessed 28 Agustus 2018].
7. Manns B., Hyndman E., Burgess E, et al. Oral Vitamin B12 and High-Dose Folic Acid in Hemodialyss patient with Hyperhomocyst(e)inemia. *Kidney International*. 2001; (59):1103-1109. <https://doi.org/10.1046/j.1523-1755.2001.0590031103.x>
8. Menon Vandana, Wang Xuelei, Greene Tom, et al. Homocysteine in Chronic Kidney disease : Effect of low protein diet and repletion with B vitamins. *Kidney International*. 2005; 67:1539-1546. <https://doi.org/10.1111/j.1523-1755.2005.00234.x>
9. Sulistiowati E., Idaiani S. Risk Factors of Chronic Kidney Disease Based on Cross-Sectional Analysis Baseline Cohort Study Non-Communicable Disease at Population 25-65 years old in Kebon Kelapa, Bogor 2011. Jakarta: Pusat Teknologi Terapan Kesehatan dan Epidemiologi Klinik; 2015.
10. Stanley M.D, James C., Veith F., et al. Current therapy in vascular and endovascular surgery. Philadelphia: Elsevier 2014. Available from: <http://clinicalkey.com> [Accessed 28 Agustus 2018].

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