Original Research Article

Assessing the effect of homocysteine compound levels and biological parameters on subjects undergoing hemodialysis

BH. V. K. Praveen Varma[1], P.C. Mishra[2], M.K. Sagar 3*, D.S.S.K. Raju4

[1]Associate Professor, Department of General Medicine, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

[2]Professor and Head, Department of General Medicine, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

[3]Professor, Department of General Medicine, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India(corresponding author)

[4]Assistant Professor, Department of Biochemistry, Maharajah's Institute of Medical, Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

Received: 24-11-2021 / Revised: 28-12-2021 / Accepted: 15-01-2022

Abstract

Background: In subjects with renal disease, levels of homocysteine are raised leading to its excretion in urine. This correlation of raised homocysteine levels to renal disease can be attributed to the possible hypothesis that raised homocysteine can lead to renal disease or correlation of homocysteine to Glomerular Filtration Rate (GFR) where only a small fraction of less than 1% is only excreted. **Aim:** The present study was conducted to assess the effect of Homocysteine levels and biological parameters in subjects undergoing hemodialysis. **Methods:** In 120 subjects including both males and females with 57 controls and 63 cases. These subjects were within the age range of 19 years to 80 years and had a mean age of 46.6±4.22 years. Homocysteine and Vitamin B6 in the serum were assessed using the technology of ELISA using spectrophotometer, whereas, for evaluating urea and creatinine, electroluminescence was used with cobasd e411. The collected data were subjected to the statistical evaluation. **Results:** Homocysteine was 8.59±0.96 µmol/l in controls and was 22.97±4.77 µmol/l in the test group study subject ($p<0.0001$). Vitamin B6 levels were significantly lower in test subjects with 159.47±14.75 compared to the controls. Urea levels were significantly higher in test subjects having the renal disease with $p<0.0001$. Creatinine was significantly higher in test subjects having the renal disease. Creatinine value in controls was 0.45±0.12 and in test, subjects were 8.04±2.07 with $p<0.000$. B6 showed a negative correlation with homocysteine. With creatinine levels, homocysteine showed a positive correlation with an r-value of 0.187 and a p-value of 0.123. A similar positive correlation was seen with the urea levels with an r-value of 0.034 and a p-value of 0.792. **Conclusion:** The present study concludes that the levels of homocysteine, urea, and creatinine levels are raised in subjects having renal disease undergoing hemodialysis compared to the control subjects without renal disease. Also, Vitamin B6 and hemoglobin levels were significantly lower in subjects with renal disease undergoing hemodialysis compared to the subjects without renal disease.

Keywords: Hemodialysis, homocysteine, renal disease, renal failure, urea, vitamin B6

Introduction

Hemodialysis is done in subjects with compromised renal status and in subjects with renal failure using an artificial machine system to remove extracorporeal waste products like urea and creatinine. The basic mechanism of hemodialysis is to allow blood passage via a thin membrane having minute blood channels. Unwanted and waste products from the blood pass through the membrane via diffusion into the dialyzing fluid. Hemodialysis can be nocturnal which is done by patients themselves 5-6 times/week during sleep and conventional hemodialysis which is conducted at a clinical setup for 4 hours and for 3 times a week[1]. Hemodialysis is considered an effective and safe treatment for subjects with renal failure. However, various complications are being associated with the hemodialysis procedure such as restless leg syndrome, anaphylaxis, muscle cramps, cardiac arrhythmia, and/or hypotension[2].

These complications can be prevented to a large extent with the earliest treatment and appropriate monitoring these complications can be prevented or managed well. Also, adequate glycemic control leads to more survival in subjects with diabetic end-stage renal disease undergoing hemodialysis. Homocysteine is an amino acid that contains sulphur and is linked to the metabolism of methionine[3]. It is metabolically produced by the SAM (Sadenosylmethionine) dependent transmethylation pathway via a series of reactions in most of the cells and tissues. The most prominent site for SAM-dependent transmethylation is the liver, where maximum homocysteine is produced.

Vitamin status and dietary intake primarily assess homocysteine levels physiologically. An increase in homocysteine levels can be associated with folate or Vitamin B12 deficiency. Homocysteine can be metabolized via transsulphuration to cysteine with a cofactor as vitamin B6[4]. In subjects with renal disease, levels of homocysteine are raised leading to its excretion in urine. This correlation of raised homocysteine levels to renal disease can be attributed to the possible hypothesis that raised homocysteine can lead to renal disease or correlation of homocysteine to Glomerular Filtration Rate (GFR) where only a small fraction of less than 1% is only excreted. Another possible explanation can be the uptake of homocysteine by renal tubules and its metabolization to cysteine via the transsulphuration pathway which is not usually seen in subjects with normal renal

*Correspondence
Dr. D.S.S.K. Raju
Assistant Professor, Department of Biochemistry, Maharajah's Institute of Medical, Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India.
E-mail: dsskraju@gmail.com

functions[5]. However; the data assessing homocysteine compound levels in hemodialysis subjects are scarce in the literature. Hence, the present study was conducted to assess the effect of Homocysteine levels and biological parameters in subjects undergoing hemodialysis.

Materials and methods

The present study was conducted to assess the effect of Homocysteine levels and biological parameters in subjects undergoing hemodialysis. The study was carried out at Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India after obtaining clearance from the concerned Ethical committee. The study population was comprised of the subjects visiting the Department of Urology of the Institute. The study included a total of 120 subjects having both males and females with 57 controls and 63 cases. These subjects were within the age range of 19 years to 80 years and had a mean age of 46.6±4.22 years.

The inclusion criteria for the study were subjects having renal failure and who were willing to participate in the study. The exclusion criteria for the study were subjects with Hepatitis A in addition to renal failure, subjects of age less than 18 or more than 80 years, and subjects who were not willing to participate in the study. After explaining the detailed study design, informed consent was taken from all the study subjects in verbal as well as written form.

After the final inclusion of the study subjects, detailed medical history and demographics were recorded for all the study subjects including gender, age, and hemodialysis number per week. The blood sample was collected in aseptic and sterile conditions. The collected 5ml of venous blood was kept in a tube gel. This was followed by the separation of serum via centrifugation. The centrifugation was done at 4000 rpm for 10 minutes. After centrifugation, the serum was distributed in four fractions. These fractions were kept in eppendrof tubes which were then stored at -20^0 C.

Homocysteine and Vitamin B6 in the serum were assessed using the technology of ELISA using spectrophotometer, whereas, for evaluating urea and creatinine, electroluminescence was used with cobasd e411.

The collected data were subjected to the statistical evaluation using SPSS software version 21 (Chicago, IL, USA) and one-way ANOVA and t-test for results formulation. The data were expressed in percentage and number, and mean and standard deviation. The level of significance was kept at $p<0.05$.

Results

The present study was conducted to assess the effect of Homocysteine levels and biological parameters in subjects undergoing hemodialysis. The study included a total of 120 subjects having both males and females with 57 controls and 63 cases. These subjects were within the age range of 19 years to 80 years and had a mean age of 46.6±4.22 years.

The study results showed that the value of homocysteine was 8.59±0.96 µmol/l in controls and was 22.97±4.77 µmol/l in the test group study subjects. This difference was statistically significant with $p<0.0001$. These results showed that the levels of homocysteine were raised moderately in subjects undergoing hemodialysis. For Vitamin B6 the levels were significantly lower in test subjects with 159.47±14.75 compared to the controls where vitamin B6 value was 98.52±29.11. This difference was also statistically significant with $p<0.0001$ (Table 1).

Table 1: Levels of Homocysteine and Vitamin B6 in control and cases group subjects

Parameters	N	Mean±S.D	p-value
Homocysteine			
Control	57	8.59±0.96	<0.0001
Tests	63	22.97±4.77	
Vitamin B6			
Control	57	159.47±14.75	<0.0001
Tests	63	98.52±29.11	

It was seen that urea levels were significantly higher in test subjects having a renal disease where the urea levels in controls were 20.19±4.24 and in the test group was 140.75±35.50. This difference was statistically significant with $p<0.0001$. Concerning creatinine, the study results showed that the levels of creatinine were significantly higher in test subjects having the renal disease. Creatinine value in controls was 0.45±0.12 and in test, subjects were 8.04±2.07. This difference was also statistically significant with $p<0.0001$ as shown in Table 2.

Table 2: Levels of Urea and Creatinine in control and cases group subjects

Parameters	N	Mean±S.D	p-value
Urea			
Control	57	20.19±4.24	<0.0001
Tests	63	140.75±35.50	
Creatinine			
Control	57	0.45±0.12	<0.0001
Tests	63	8.04±2.07	

On assessing the hemoglobin in the study subjects, it was seen that hemoglobin in gm/dl was significantly higher in control subjects with the value of 14.24±1.13 gm/dl compared to test subjects with the renal disease where hemoglobin values were 9.69±1.85 gm/dl in mean values. This difference was statistically significant with $p<0.0001$ as depicted in Table 3.

Table 3: Levels of Hemoglobin in control and cases group subjects

Parameters	N	(Mean±S.D)	p-value
Hemoglobin			
Control	57	14.24±1.13	<0.0001
Tests	63	9.69±1.85	

The present study also assessed the correlation coefficients of homocysteine to other parameters considered in the study. The results showed that B6 showed a negative correlation with homocysteine with r and p-values as -0.388 and 0.001 respectively. With creatinine levels, homocysteine showed a positive correlation with an r-value of 0.187 and a p-value of 0.123. A similar positive correlation was seen with the urea levels with an r-value of 0.034 and a p-value of 0.792. Hemoglobin also showed a positive correlation with homocysteine levels in the study with r and p values of 0.026 and 0.825 respectively as summarized in Table 4.

Table 4: Correlation coefficient of Homocysteine with various other parameters of the study

Homocysteine with	r value	p-value	Inference
B6	-0.388	0.001	Negative correlation
Creatinine	0.187	0.123	Positive correlation
Urea	0.034	0.792	Positive correlation
Hemoglobin	0.026	0.825	Positive correlation

Discussion

The present study was conducted to assess the effect of Homocysteine levels and biological parameters in subjects undergoing hemodialysis. The study included a total of 120 subjects having both males and females with 57 controls and 63 cases. These subjects were within the age range of 19 years to 80 years and had a mean age of 46.6±4.22 years. The study results showed that the value of homocysteine was 8.59±0.96 µmol/l in controls and was 22.97±4.77 µmol/l in the test group study subjects. This difference was statistically significant with p<0.0001. These results showed that the levels of homocysteine were raised moderately in subjects undergoing hemodialysis. For Vitamin B6 the levels were significantly lower in test subjects with 159.47±14.75 compared to the controls where vitamin B6 value was 98.52±29.11. This difference was also statistically significant with p<0.0001. These results were in agreement with the studies of Schalinske KL[6] in 2009 and Smith AD et al[7] in 2016 where authors have reported higher values of homocysteine and lower levels of Vitamin B6 were seen in subjects having the renal disease.

Urea levels in the present study were significantly higher in test subjects having a renal disease where the urea levels in controls were 20.19±4.24 and in the test group was 140.75±35.50. This difference was statistically significant with p<0.0001. Concerning creatinine, the study results showed that the levels of creatinine were significantly higher in test subjects having the renal disease. Creatinine value in controls was 0.45±0.12 and in test, subjects were 8.04±2.07. This difference was also statistically significant with p<0.0001 as shown in Table 2. On assessing the hemoglobin in the study subjects, it was seen that hemoglobin in gm/dl was significantly higher in control subjects with the value of 14.24±1.13 gm/dl compared to test subjects with the renal disease where hemoglobin values were 9.69±1.85 gm/dl in mean values. This difference was statistically significant with p<0.0001. These results were consistent with the studies of Kraus MA et al[8] in 2016 and Yeh EL et al[9] in 2018 where authors reported significantly higher levels of urea, creatinine, and hemoglobin in subjects having renal disease and undergoing hemodialysis.

On assessing the correlation coefficients of homocysteine to other parameters considered in the study. The results showed that B6 showed a negative correlation with homocysteine with r and p-values as -0.388 and 0.001 respectively. With creatinine levels, homocysteine showed a positive correlation with an r-value of 0.187 and a p-value of 0.123. A similar positive correlation was seen with the urea levels with an r-value of 0.034 and a p-value of 0.792. Hemoglobin also showed a positive correlation with homocysteine levels in the study with r and p values of 0.026 and 0.825 respectively. These results were comparable with the studies of Ghali L[10] in 2020 and Katko M et al[11] in 2018 where the correlation of homocysteine to urea, creatinine, hemoglobin, and vitamin B6 was comparable to the present study.

Conclusion

Within its limitations, the present study concludes that the levels of homocysteine, urea, and creatinine levels are raised in subjects having renal disease undergoing hemodialysis compared to the control subjects without renal disease. Also, Vitamin B6 and hemoglobin levels were significantly lower in subjects with renal disease undergoing hemodialysis compared to the subjects without renal disease. However, the present study had a few limitations including a small sample size, shorter monitoring period, and geographical area biases. Hence, more longitudinal studies with larger sample size and longer monitoring period will help reach a definitive conclusion.

References

1. Voinova, M., Repin, N., Sokol, E., Tkachuk, B. and Gorelik, L. Physical Processes in Polymeric Filters Used for Dialysis. Polymers. 2019;11:389.
2. Macdougall IC, Bircher AJ, Eckardt KU, et al. Iron management in chronic kidney disease: conclusions from a "kidney disease: Improving Global Outcomes" (KDIGO) Controversies Conference. Kidney Int 2016;89:28-39.
3. Hahr A, Molitch M. Management of diabetes mellitus in patients with chronic kidney disease. Clin Diabetes Endocrinol. 2015;1:2.
4. Gao, J.; Cahill, C.M.; Huang, X.; Roffman, J.L.; Lamon-Fava, S.; Fava, M.; Mischoulon, D.; Rogers, J.T. S-Adenosyl Methionine and Transmethylation Pathways in Neuropsychiatric Diseases Throughout Life. Neurotherapeutics **2018**;15:156–75.
5. Cianciolo G, De Pascalis A, Di Lullo L, Ronco C, Zannini C, La Manna G. Folic acid and homocysteine in chronic kidney disease and cardiovascular disease progression: which comes first. Cardiorenal Med. 2017;7:255–66.
6. Schalinske KL. Hepatic sulfur amino acid metabolism. Glutathione Sulfur Amin Acids Hum Heal Dis eds Masella R, Maz G Hoboken, NJ John Wiley Sons, Inc. 2009;73–90.
7. Smith AD, Refsum H. Homocysteine, B vitamins, and cognitive impairment. Annu Rev Nutr. 2016;36:211–39.
8. Kraus MA, Fluck RJ, Weinhandl ED, Kansal S, Copland M, Komenda P, et al. Intensive Hemodialysis and Health-Related Quality of Life. Am J Kidney Dis. 2016;68:33–42.
9. Yeh EL, Huang YC, Tsai SF, Yu TM, Wu MJ, Chen CH. Relationship between plasma levels of homocysteine and the related B vitamins in patients with hemodialysis adequacy or inadequacy. Nutrition. 2018;53:103–8.
10. Ghali L. Evaluation of urea and creatinine concentration in chronic kidney failure Iraqi patients after dialysis Ministry of higher education & scientific research Asoul Al-dean University College Medical laboratory technology Evaluation of urea and creatinine con. 2020.
11. Katko M, Kiss I, Karpati I, Kadar A, Matyus J, Csongradi E, et al. Relationship between serum nickel and homocysteine concentration in hemodialysis patients. Biol Trace Elem Res. 2008;124:195–205.

Conflict of Interest: Nil Source of support: Nil