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Homocysteine and Hematological Indices in Hemodialysis Patients

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Abstract

Objectives: To explore the relationship between homocysteine and various hematological indices in hemodialysis patients. **Patients and Methods:** This case-control study comprised 60 hemodialysis patients and 60 healthy controls matched for gender and age. Hemodialysis duration was 3.2±2.9 year at frequency of 2.6±0.6/week. Hypertension and diabetes were the most common self-reported disorders among the hemodialysis patients. **Results:** Serum homocysteine was significantly higher in hemodialysis patients than in controls (50.8±9.7 vs. 13.1±3.7 µmol/l, P=0.000). White blood cell (WBC) and platelet (PLT) counts were significantly higher in hemodialysis patients than in controls [(7.18±2.37 x10^9/L and 266.3±111.9 x10^9/L vs 5.95±1.37 x10^9/L and 222.0±54.1 x10^9/L) with P=0.017 and P=0.045, respectively]. In contrast, red blood cell (RBC), hemoglobin, and hematocrit were significantly lower in hemodialysis patients (3.1±0.5 x10^{12}/L, 8.9±1.5 gm/dl and 26.3±4.6%) than in controls (4.0±0.4 x10^{12}/L, 12.8±1.6 gm/dl and 45.0±4.6%) with P=0.000. Prothrombin time (PT) and international normalized ratio (INR) were significantly higher in hemodialysis patients compared to controls (16±3 sec and 1±0 vs. 14±0 sec and 1.0±0.1, P=0.000), whereas activated partial thromboplastin time (APTT) was significantly decreased in hemodialysis patients (25±5 vs 33±2 sec, P=0.000). Homocysteine correlated directly with WBC count (r=0.338, P=0.008) and PLT count (r=0.369, P=0.000) whereas inverse correlations were found between homocysteine and RBC count (r=-0.648, P=0.000), hemoglobin (r=-0.733, P=0.000) and hematocrit (r=-0.836, P=0.000). In addition, homocysteine showed direct correlations with PT (r=0.564, P=0.000) and INR (r=0.657, P=0.000) and inverse correlation with APTT (r=-0.690, P=0.000). **Conclusion**: Serum homocysteine was significantly higher in hemodialysis patients compared to controls. Homocysteine correlated directly with WBC count, PLT count, PT and INR, and inversely with RBC count, hemoglobin, hematocrit and APTT.

Key words: Homocysteine, Hematological indices, Hemodialysis patients, Gaza Strip.

Introduction

Chronic kidney disease (CKD), defined as progressive loss in renal function over a period of months or years, may lead to one or more well recognized complications such as cardiovascular disease, anemia or pericarditis (1,2). CKD is a well-known risk factor for end-stage renal disease (ESRD) (3). The number of patients being treated for ESRD globally was estimated to be 2,786,000 at the end of 2011 and, with a 6-7% growth rate, continues to increase at a significantly higher rate than the world population. Of these 2,786,000 ESRD patients, approximately 2,164,000 were undergoing dialysis treatment and around 622,000 people were living with kidney transplants. In the USA, Japan and the European Union, dialysis patient population growth rates between 2010 and 2011 were in a range of 1-4% and, as such, were significantly lower than growth rates in regions such as Asia, Latin America, the Middle East and Africa (4). The Palestinian health annual report showed that renal failure constitutes one of the ten leading causes of death in the Gaza strip with mortality rate of 2.8% (5). Hemodialysis is the most common treatment option for ESRD patients with dialysis being typically administered using a fixed schedule of three times per week.

Hyperhomocysteinemia, defined as serum homocysteine greater than 15 mmol/L, has been strongly associated with the pathogenesis of CVD, and correspondingly has been identified as a contributing factor in four main disease mechanisms including thrombosis, vascular oxidative stress, apoptosis and cellular proliferation (6,7). In fact, ESRD patients die more from CVD than from accumulation of toxin *per se*. In addition, among the common complications seen in persons with ESRD, are anemia mainly due to loss of erythropoietin production (8-10), abnormalities in WBC and PLT functions (11,12). Recently, hyperhomocysteinemia has been linked to different stages of CKD including ESRD (13-15). The present work is the first to explore correlation between homocysteine and hematological indices in hemodialysis patients in Gaza Strip.

Patients and Methods

Subjects

The study was approved by the Ethical Review Board and all participants gave an informed consent. Sixty patients (34 males) with ESRD who were undergoing maintenance hemodialysis treatment at Al-Shifa Hospital, Gaza strip of Palestine were included. Sixty healthy individuals (34 males, 26 females) served as controls. Controls and patients were matched for age and sex. Exclusion criteria included pregnancy and hepatitis.

Sampling and processing

Blood samples were collected by a well-trained nurse from all subjects; before hemodialysis sessions for the patients. Nine milliliter blood were obtained from each subject and divided into EDTA tube (2 ml) for CBC analysis, Sodium citrate 3.2% tube for PT and APTT determination in plasma (3 ml) and vacutainer plain tube (4 ml) that was left for a while to allow blood to clot. Then, serum samples were obtained by centrifugation at 3000 rpm for 15 minutes for homocysteine determination. Blood samples were processed by an automatic counter for hemoglobin concentration and other whole blood component concentrations (Cell Dyn 1800, USA).

Laboratory Assays

Prothrombin time was measured semi-automatically by pipetting 50 µl of plasma into a cuvette and prewarming at 37°C for 3 min. Then 100µl of prewarmed hemostat thromboplastin reagent was added and the time taken for clot formation was detected automatically by HumaClotJuniour Coagulometer. The results of PT (Sec) and INR were displayed. Similarly, APTT was measured by initially pipetting 50 µl plasma in a cuvette and prewarming for 2 minutes. 50 µl Hemostat aPTT-EL was then added to the plasma and incubated for an additional 5 min. Finally, 50 µl of prewarmed 0.02 mol/l calcium chloride was added and the time taken to clot formation was detected using the coagulometer. Serum homocysteine was determined by enzymatic colorimetric method for the quantitative determination of homocysteine (Globe diagnostics kit, Italy. National Institute of Standards and Technology (NIST) standardized study shows 15 µmol/l as the cut-off value for normal level of homocysteine for adults.

Data analysis

Data were analyzed using SPSS/ PC statistical package (Statistical Package for the Social Science Inc. Chicago, Illinois USA, version 18.0). Simple distribution of the study variables and the cross tabulation were applied. The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between hemodialysis patients and controls homocysteine levels.

Table 1. Clinical data of the sixty hemodialysis patients studied*	
Age (year)	53.9 ± 10.1
Gender: men/women (number (%))	34 (56.7) / 26 (43.3)
Hemodialysis duration (Year) *(min-max)*	3.2 ± 2.9 (0.5-10.0)
Hemodialysis frequency/week *(min-max)*	2.6 ± 0.6 (1-3)
Other disorders: Yes/ No	53 (88.3) / 7 (11.7)
Details of co-morbid conditions:	
Hypertension	49 (81.7)
Diabetes mellitus	32 (53.3)
Cardiovascular disease	6 (10.0)
Asthma	2 (3.3)

** Data are presented as mean ± SD (min-max range) or number (%)*

Table 2. Hematological parameters of the study population. Data are expressed as mean ± SD (minimum-maximum range).

Parameter	Patients	Controls	Percentage difference	t	P-value
WBCx10^9/L	7.18 ± 2.37 (2.0-14.1)	5.95 ± 1.37 (3.9-9.0)	18.7	2.468	0.017
RBC x10^{12}/L	3.12 ± 0.54 (2.3-4.7)	4.03 ± 0.37 (3.2-5.0)	25.4	7.689	0.000
Hb (gm/dl)	8.9 ± 1.5 (6.8-12.7)	12.8 ± 1.6 (10.2-15.0)	36.0	9.559	0.000
Hct (%)	26.3 ± 4.6 (20.4-37.0)	45.0 ± 4.6 (38.0-53.0)	52.4	15.743	0.000
PLT x10^9/L	266.3 ± 111.9 (118.0-618.0)	222.0 ± 54.1 (146.0-340.0)	18.1	2.052	0.045

WBC: White Blood Cell, RBC: Red Blood Cell, Hb: Hemoglobin, Hct: Hematocrit, PLT: Blood Platelet. P<0.05: Significant.

Table 3. Hemostasis parameters of the study population of 60 patients and 60 controls. Data are expressed as mean standard deviation (minimum-maximum range).

Parameter	Patients	Controls	Percentage difference	t	P-value
PT (Sec)	16.2 ± 2.6 (13.5-26.8)	13.5 ± 0.4 (13.0-14.1)	18.2	5.733	0.000
APTT (Sec)	25.3 ± 5.3 (17.0-40.0)	32.6 ± 2.1 (30.0-37.0)	25.2	6.930	0.000
INR	1.23 ± 0.17 (1.0-1.9)	0.97 ± 0.07 (0.9-1.1)	23.6	7.745	0.000

PT: Prothrombin Time, APTT: Activated Partial Thromboplastin Time, INR: International Normalized Ratio. P<0.05: Significant.

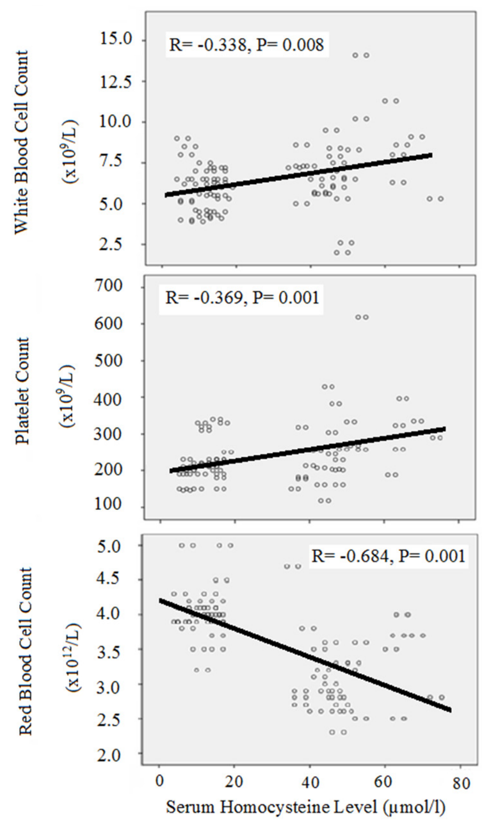

Figure 1. The correlation between homocysteine level and white blood cell (WBC) count (upper), platelet (PLT) count (middle) and red blood cell (RBC) count (lower). Correlation coefficient (r value) and significance level (p value) is indicated for each relationship individually.

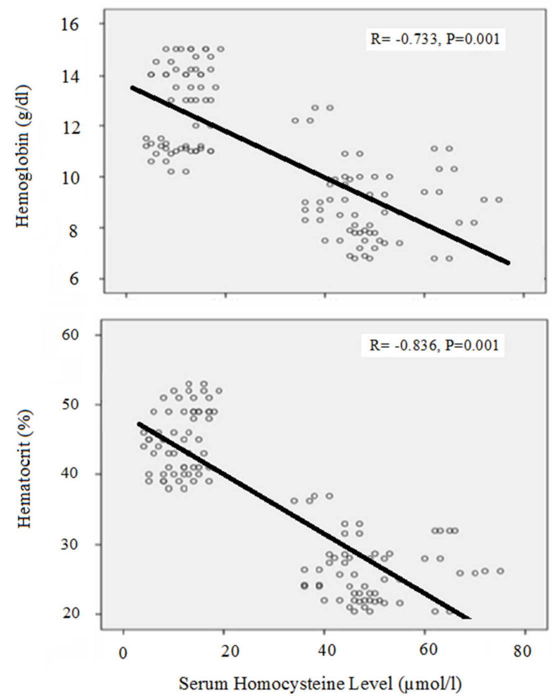

Figure 2. The correlation between homocysteine level and hemoglobin (Hb) content (upper), hematocrit (Hct) (lower). The correlation coefficient (r value) and significance level (p value) is indicated for each relationship individually.

Pearson's correlation test was applied. The results were accepted as statistical significant when the p-value was less than 5% ($p<0.05$). The percentage difference was calculated according to the formula: Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100. Percent difference = [(V1 - V2) | / ((V1 + V2)/2)] X 100.

Results

Clinical characteristics of the hemodialysis patients are presented in table 1. The most common self-reported disorders among the hemodialysis patients were hypertension and diabetes mellitus. The mean level of homocysteine was significantly higher in the hemodialysis patients than in the matched healthy controls (50.8±9.7 (37-75) versus 13.1±3.7 (7-19) μmol/l; $p<0.001$). The hematological parameters of the study population are summarized in table 2. The mean counts of WBC and PLT were greater in the hemodialysis patients group than in the controls. The means of RBC count, hemoglobin and hematocrit were lower in the hemodialysis patients. Hemostasis parameters are presented in table 3. The mean PT and INR were significantly higher in hemodialysis patients than controls. In contrast, the mean APTT was significantly decreased in hemodialysis patients compared to controls. Homocysteine correlated positively with WBC count (Figure 1, upper) and PLT count (Figure 1, middle). Negative correlations were found between homocysteine and RBC count (Figure 1, lower), hemoglobin (Figure 2, upper) and hematocrit values (Figure 2, lower). In addition, homocysteine showed direct correlations with PT (Figure 3, upper) and INR (Figure 3, middle) and inverse correlation with APTT (Figure 3, lower).

Discussion

Although the number of hemodialysis patients in the Gaza Strip has been doubled in the last decade with mortality rate of 2.8%, the published data on the ESRD are very few and restricted to annual reports produced by the Palestinian Ministry of Health. The available biochemical test of ESRD is limited to routine traditional kidney function including urea and creatinine tests. This necessitated further assessment of other biochemical and hematological parameters such as homocystine and hemostatic parameters

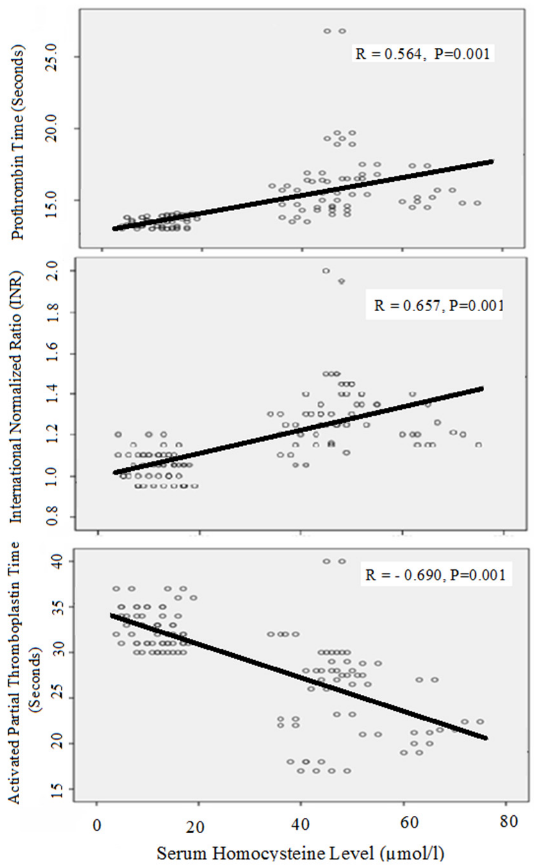

Figure 3. The correlation between homocysteine level and prothrombin time (PT) (upper), international normalized ratio (INR) (middle) and activated partial thromboplastin time (APTT) (lower). The correlation coefficient (r value) and significance level (p value) is indicated for each relationship individually.

which were recently linked to ESRD (16,17). The mean duration of hemodialysis among patients was 3.2±2.9 year and the mean frequency of hemodialysis was 2.6±0.6 sessions/week. The most common self-reported disorders among the hemodialysis patients were hypertension and diabetes, a finding coincides with the fact that high blood pressure and diabetes play a key role in the progression of renal failure and/ or cause of CKD (18). As indicated in the present study, there is a significant elevation of homocysteine level in hemodialysis patients compared to controls. This means that high homocysteine levels are found in ESRD. Such finding concur with that demonstrated in other studies (16,19,20).

Hyperhomocysteinemia recorded in hemodialysis patients has been attributed to 1) cessation of homocysteine disposal in the kidneys and 2) impaired extrarenal homocysteine metabolism (13). White blood cell and PLT counts were significantly increased in hemodialysis patients compared to controls. Leukocytosis recorded in the present study is in concurrent with that obtained by other authors (21-23). It is known that hemodialysis patients suffer inflammation which is associated with increased number of WBCs (22-24). When related to homosycteine level, results revealed that the higher the homosycteine, the higher the WBC. This positive correlation was previously reported (25, 26). There was platelet count in hemodialysis patients was significantly greater in patients than compared to controls. This finding is in agreement with that demonstrated in previous studies (23,27,28). A significant direct correlation of platelet count with serum homocysteine was found. Similar result was obtained and the author reported that in hemodialysis patients high homocystiene levels make the PLT more likely to clump and cause clots and contributes to the possibility of thrombotic events among these patients (27). Red blood cell count, hemoglobin and hematocrit values were significantly lower in hemodialysis patients compared to controls. This indicates that hemodialysis patients are more likely to be anemic. Such results agreed with that reported in earlier studies (16,24,29-31). Anemia in hemodialysis patients may be due to many factors including blood loss, shortened red cell life span, vitamin deficiencies, the “uremic milieu,” renal erythropoietin deficiency due to kidney failure, iron deficiency, and inflammation (16,32,33). Person’s correlation test showed negative significant correlations of homocysteine with RBC count, hemoglobin, and hematocrit values. Various studies recorded such negative correlations (16,25,31) and this supported the idea that homocysteine is a suitable biomarker of ESRD, where most patients suffers hematological disorders.

The mean PT and INR were significantly higher in hemodialysis patients than in controls. In contrast, the mean APTT was significantly decreased in hemodialysis patients. These results are in agreement with those reported in earlier studies (34-36). Possible interactions of homocysteine with endothelial cells, blood PLT, plasmatic fibrinogen and plasminogen, as the important major components of hemostasis were postulated (37). These indicate that hemostasis is impaired in hemodialysis patients. Disorders of hemostasis were previously reported to be associated with chronic kidney disease (17). The hemostatic abnormalities described in ESRD involved both intrinsic and extrinsic pathways implicating defects of coagulation factors and PLT dysfunction (38,39). The direct significant relationship of homocysteine levels with PT and INR and significant inverse relationship recorded between homocysteine and

APTT observed in the present study is agreement with results reported by other workers (27).

In conclusion, serum homocysteine was significantly higher in hemodialysis patients than in controls. Homocysteine correlated directly with WBC count, PLT count, PT and INR, and inversely with RBC count, hemoglobin, hematocrit and APTT. Therefore, perhaps homocysteine could be introduced as an indicator of ESRD. Further research on the relation of homocysteine with clotting factors and the role of homocysteine in fibrinolysis are needed.

Conflict of interest: The authors have no relevant conflicts of interest to declare.

References

1. Nurko S. Anemia in chronic kidney disease Causes, diagnosis, treatment. CCJM 2006;73(3):289-97.
2. Herzog C, Asinger R, Berger A, Charytan D, Dı´ez J, Hart R, Eckardt K, Kasiske B, McCullough P, Passman R, DeLoach S, Pun P, Ritz, E, et al. Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney ISN 2011.10.1038/ki .223.
3. Iseki k, Ikemiya Y, Iseki C and Takishita S. Proteinuria and the risk of developing end-stage renal disease. Kidney Int 2003;63(4):1468-74.
4. Fresenius Medical Care. End stage renal disease patients in 2011. A Global Perspective 2011; Germany.
5. Ministry of Health. Health annual report, Health Status in Palestine 2009, Ministry of Health 2010; Palestine.
6. Al-Obaidi- M K, P J Stubbs, R Amersey, M I M Noble. Acute and convalescent changes in plasma homocysteine concentrations in acute coronary syndromes. Heart 2001;85:380-84.
7. Agoston-Coldea L, Mocan T, Gatfosse M, Lupu S and Dumitrascu D.L. Plasma homocysteine and the severity of heart failure in patients with previous myocardial infarction. Cardiol J 2011;18(1):55-62.
8. Jonathan J. Anemia and chronic kidney disease: What's the connection?. J fam pract 2010;59(1):14-18.
9. Lovcić V, Vujić J, Basić-Jukić N, Kurtović I, Janković RI, Lovcić P, Dzapo M et al. Treatment of renal anemia in hemodialysis patients in General Hospital Bjelovar from 2007 to 2010. Acta Med Croatica 2011;65(3):49-53.
10. Portolés J, Gorriz JL, Rubio E, de Alvaro F, García F, Alvarez-Chivas V, Aranda P, Martinez-Castelao A et al. The development of anemia is associated to poor prognosis in NKF/KDOQI stage 3 chronic kidney disease. BMC Nephrol 2013;14(2):1-9.
11. Kaw D and Malhotra D. Platelet dysfunction and end-stage renal disease. Semin Dial 2006;19(4):317–22.
12. Turkmen K, Guney I, Yerlikaya F.H and Tonbul HZ. The relationship between neutrophil-to-lymphocyte ratio and inflammation in end-stage renal disease patients. Ren Fail 2010;34(2):155-9.
13. Van Guldener C. Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering? Nephrol Dial Transplant 2006;21(5):1161–66.
14. Vieira C, Baptista A, Malho A, Pinho A, Silva A.P, Bernardo I.C, Neves P.L et al. Homocysteine is a risk factor in predialysis patients when associate with malnutrition and inflammation. Int J Nephrol;2010:10.4061/957645.
15. Paterson J. Prevalence of hyperhomocysteinemia in patients with predialysis chronic kidney disease after folic acid food fortification of the canadian food supply. Department of Nutritional Sciences University of Toronto 2011:133.
16. Anees M, Mumtaz A, Ibrahim M, Shaheen, M and Asghar, A. Effect of anemia and hyperhomocysteinemia on mortality of patients on hemodialysis. IJKD 2010;4(1):60-65.
17. Jalal D, Chonchol M and Targher G. Disorders of hemostasis associated with chronic kidney disease. Semin Thromb Hemost 2010;36(1):34-40.
18. Etemadi J, Zolfaghari H, Firoozi R, Ardalan M.R, Toufan M, Shoja M.M, Ghabili K et al. Unexplained pulmonary hypertension in peritoneal dialysis and hemodialysis patients. Elsevier España, Rev Port Pneumol 2012;18(1):10-14.
19. Koning L and Hu F. Homocysteine lowering in end stage renal disease: Is there any cardiovascular benefit?. Circulation 2010;(121):1379-81.
20. Chao Wu C, Zheng C, Lin Y, Lo L, Liao M Cheng Lu K et al. Role of homocysteine in end-stage renal disease. Clin Biochem 2012;45:1286–94.
21. Nasri H. Association of white blood cell count with left ventricular hypertrophy and ejection fraction in stable hemodialysis patients. Saudi J Kidney Dis Transpl 2007;18(1):31-63.
22. Wei Hsu C, Lin J, Lin-Tan D,Yen T, Chen K et al. White blood cell count predicts all-cause, cardiovascular disease-cause and infection-cause one-year mortality of maintenance hemodialysis patients. Ther Apher Dial;14(6):552-9.

23. Molnar M, Streja E, Kovesdy C, Budoff M, Nissenson A, Krishnan M, et al. High platelet count as a link between renal cachexia and cardiovascular mortality in end-stage renal disease patients. Am J ClinNutr 2011;(94):945–54.
24. Afshar R, Sanavi S, Salimi J and Ahmadzadeh M. Hematological profile of chronic kidney disease patients (CKD) in Iran, in predialysis stages and after initiation of hemodialysis. Saudi J Kidney Dis Transpl 2009;20(1):368-71.
25. Nasri H and Baradaran A. Association of serum homocysteine with anemia in maintenance hemodialysis patients. Pakistan J Nut 2005;4(6):414-7.
26. Guerra-Shinohara E, Morita O, Pagliusi R, Avila V, Allen R, Stabler S et al. Elevated serum S-adenosylhomocysteine in cobalamin-deficient megaloblastic anemia. Metabolism 2007;56(3):339-47.
27. Nasri H. Influence of serum homocysteine on platelet count instable hemodialysis patients. Pak J Physiol 2006;2(2):5-7.
28. Alghythan K and Alsaeed H. Hematological changes before and after hemodialysis. Sci. Res. Essays 2012;7(4):490-7.
29. Mohsin S, Aslam M, Anees M, Hussain S, Ahmed T, Qamar U et al. Platelets dysfunction in patients of end stage renal disease. Biomedica 2010;26(14):157-61.
30. Suresh M, reddy M.N, Singh S.M, Hari Krishna. BandiHK, keerthi S.G, Chandrasekhar M et al. Hematological changes in chronic renal failure. IJSRP journal 2012;2(9).
31. Poudel B, Kumar Yadav B, Jha B, Raut K and Pandeya D. Prevalence and association of anemia with CKD: A hospital based crosssectional study from Nepal. Biomed Res 2013;24(1):99-103.
32. Locatelli F, Pozzoni P and Del Vecchio L. Recombinant Human Epoietin beta in the Treatment of Renal Anaemia. TherClin Risk Manag 2007;3(3):433-9.
33. Shittu A, Chijioke A, Biliaminu S, Makusidi A, Sanni M, Abdul-Rahman M, Abdul-Azeez I et al. Haematological profile of patients with chronic kidney disease in Nigeria. JNRT 2013;5(1):2-10.
34. Brophy D, Martin E, Gehr T, Best AM, Paul K and Carr ME JR et al. Thrombin generation time is a novel parameter for monitoring enoxaparin therapy in patients with end-stage renal disease. J Thromb Haemost 2006;(4):372-6.
35. Ali M, Babiker M, Merghani L, Ali F and Abdulmajeed M. Hematological changes post-hemo and peritoneal dialysis among renal failure patients in sudan. Saudi J Kidney Dis Transpl 2008;19(2):274-9.
36. Park Y, Waldrum M and Marques M. Platelet Count and Prothrombin Time Help Distinguish Thrombotic Thrombocytopenic Purpura–Hemolytic Uremic Syndrome From Disseminated Intravascular Coagulation in Adults. Am J Clin Pathol 2010;133(3):460-5.
37. Karolczak K and Olas B. Mechanism of action of homocysteine and its thiolactone in hemostasis system. Physiol. Res. 2009;58(5):623-33.
38. Rios D.RA, Carvalho M.G, Lwaleed B.A, Silva A.CS, Borges K.BG, Duss L.MS et al. Hemostatic changes in patients with end stage renal disease undergoing hemodialysis. Clin Chim Acta 2010;411(3-4):135-9.
39. Mannucci P and Tripodi A. Hemostatic defects in liver and renal dysfunction. Hematology Am Soc Hematol Educ Program 2012;168-73.