Full Length Research Paper

Hematological changes before and after hemodialysis

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Renal failure is a situation in which kidney fails to function adequately. There are two forms of renal failure: acute and chronic. End-Stage renal disease is the final stage of chronic renal failure where there is a progressive, irreversible deterioration in renal function which can be substituted by renal replacement therapy, haemodialysis (HD), peritoneal dialysis, or transplantation. The aim of this study was to investigate changes in various hematological parameters that occur in renal failure patients before and after HD as compared to the control group. The potential effect of dialysis during hematological procedures that occur in renal failure patients was also investigated. The study result showed that most of the hematological parameters measured before and after HD in renal failure patients were either elevated or lowered as compared to the control group. Furthermore, it was observed that most of hematological parameters elevated after HD. More significantly, PT, APTT and fibrinogen were found to increase post-HD while there was a concurrent decrease of platelet counts. The present investigation might help clinicians to initiate precautions before and after dialysis procedures. Therefore, it is recommended that all patients are screened appropriately before and after dialysis to avoid complications.

Key words: Hemodialysis, renal failure, hematological profile.

INTRODUCTION

Over 1.1 million patients are estimated to have renal failure worldwide with an annual increase at a rate of 7%. In the USA, the incidence and prevalence counts are expected to increase by 44 and 85%, respectively, from 2000 to 2015 the incidence and prevalence rates per million populations by 32 and 70% (Gilbertson et al., 2005). Awareness of the cause of chronic renal failure (CRF) helps the nephrologists to anticipate problems during renal replacement therapy and plan preventive measures for the community (Martins et al., 2006). In developing countries, the growth of CRF population has similar trends (Mahon, 2006). The average incidence of End-Stage Renal Disease (ESRD) in Middle East countries with similar renal care systems is more than 93 per million populations (Afshar et al., 2007). Similarly, the incidence and prevalence of hemodialysis (HD) patients in Saudi Arabia are increasing dramatically. Kidney disease is emerging as a serious health problem in this country, especially, the ESRD. The total number of patients with ESRD in the Middle East is almost 100 000, the mean prevalence being 430 per million population (Najafi, 2009). Unfortunately, in most of the Middle East countries, patients with renal disease present late and it is difficult to diagnose the cause of ESRD. Because of lack of proper follow up, these patients can only be evaluated while they are undergoing dialysis or enlisted for renal transplantation (Salahi et al., 2004). The prevalence of ESRD is high in Saudi Arabia and over a 20 year period, the prevalence of ESRD has increased from 20 to 25 to 270 per million populations per year with 11 year fold increase (Hejaili et al., 2009). HD increases longevity of patients with ESRD by removing the metabolic end products and excess of water (Costa et al., 2008).

According to the Ministry of Health reports, the prevalence of renal failure in Saudi Arabia has been on the increase over the last few years. There are almost 8,000 patients on dialysis in Saudi Arabia (Hejaili et al., 2009). Thus, HD is widely used worldwide but the effects of HD on patients' blood have not been investigated thoroughly in the Kingdom of Saudi Arabia. To our

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knowledge, the present study is the first of its kind in Saudi Arabia. This study was primarily conducted to investigate changes in various hematological parameters that may occur in renal failure patients after HD, to determine the differences between various hematological parameters that occur in renal failure patients before and after–HD as compared to the control group. The possible effect of the duration of dialysis on hematological parameters that may occur in renal failure patients was also investigated. Results from this study may enable us to determine whether precautions need be taken before and after HD sessions to avoid unnecessary complications. This study was therefore carried out to investigate the hematological changes before and after hemodialysis in renal failure patients compared to controls in Riyadh, Saudi Arabia.

MATERIALS AND METHODS

Study population

This study enrolled one hundred patients suffering from renal failure. Study was done on these patients before dialysis and after dialysis at Prince Salman Centre for Kidney diseases, Riyadh, Saudi Arabia, and sixty healthy subjects as control group. Among 100 patients included in the study, 61 and 39% were males and females. The mean age of the study population was 46.25 ±14.94 and 50 ± 12.67 years respectively. Written informed consent was obtained from each patient before participation and the study was approved by Institutional Review Board. The mean time period of these patients suffering from renal failure was 49.44 ± 33.25 months. Patients undergoing dialysis was thrice in a week for about 3 to 4 h per session at blood flow rates of 250 to 350 ml/min using polysulphone hollow-fiber filter.

Laboratory assays

In this study, a total of 160 blood samples were analyzed. Whole blood from participants was drawn by venipuncture into dipotassiumethylenediamine-tetraacetate or sodium citrate containing Vacutainer tubes (Becton Dickinson, FranklinLakes, NJ). All samples were processed for analysis immediately after collection. Peripheral blood baseline parameters were measured using Sysmex XE 2100 analyzer. Coagulation profile was performed using Multifibren® U, Prothombin® SL, Thromborel® S reagents on Behring coagulation Timer (BCT-2 Dade Begring) as per manufacturer's instructions. Blood smears were made as a reference for the differential WBC count, irrespective of the results from the analyzers. Preparation and staining was done according to the standard procedure routinely used in our laboratory. Double-blind test was performed; each of the observers counted 100 cells and screened for abnormal cells independently. Their results were averaged. Sample results from these 'first-pass' processes were reviewed and secondary processes (analyzer rerun and/or blood smear examination) were then initiated according to the resulting haematological profiles and instrument flags and alerts. All patients were Saudi's, above 18 years of age, and free from chronic degenerative diseases such as cancer or peritonitis. Pregnant women were not recruited into the study.

Statistical analysis

Data were analyzed with SPSS Version 16 statistical software.

Variables were presented as mean ± standard deviation (SD) or frequency. Descriptive statistics were analyzed by independent t-test and paired t-test. Pearson Correlation Coefficient was used to calculate correlations between dialysis duration and each of RBCs and RBCs indices, leucocytes and differential count, and coagulation profile in renal failure patients on regular HD sessions. All tests were two-tailed, and the level of significance was $P<0.05$.

RESULTS

Peripheral blood Erythrocytes data from pre and post- HD patients and healthy controls

Table 1 show the differences between the mean of RBCs, Hb, Hct, RDW indices that occurred in renal failure patients before after HD compared to the control group. A statistically significant ($p<0.05$) decrease in RBCs count was observed in pre- HD patients (4.13 ± 0.54) as compared to the control group (4.74 ± 0.64). The Hb concentration also showed a statistically significant decrease in pre-HD patients (11.70 ± 1.29) as compared to the control group (14.06 ± 1.39) ($P<0.05$). The Hct level similarly showed a statistically significant decrease ($P<0.05$) in pre-HD patients (35.14 ± 3.86) compared to control group (41.58 ± 4.05). The MCH level, similar to the indices mentioned above, showed a statistically significant decrease in pre-HD patients (27.28 ± 2.43) when compared to that of the control group (29.66 ± 1.00) ($P<0.05$). However, the MCV, MCHV and RDW levels did not show any statistically significant change in pre-HD patients compared to the control group ($p> 0.05$). Figure 1 shows differences between the mean of RBCs count, Hb, Hct, RDW, RBCs indices in renal failure patients before and after HD. All the measured indices showed a statistically significant increase in post- HD patients ($P<0.05$). The current study also showed that there were no significant correlations between the time period at which the patients have been on dialysis and each of RBCs count, Hb, Hct, MCV, MCH, MCHC, and RDW levels ($P>0.05$).

Peripheral blood leukocyte data from pre and post- HD patients and healthy controls

Compared to the control group, the renal failure patients before HD did not exhibit any significant differences between the mean number of leucocytes and differential count (Table 2). The result of the present study showed that there were statistically insignificant differences between mean number of leucocytes and differential count in renal failure patients before HD procedures and that of the control group ($P>0.05$). Table 2 shows the differences between the mean number of leucocytes and differential counts in renal failure patients after HD procedures, and that of the control group. The WBC counts showed statistically significant increase in post-

Table 1. Difference between mean of erythrocytes count before and after HD procedures in patients and healthy controls.

Variables (values are mean ± SD)	Pre-hemodialysis			Post-haemodialysis		
	Patients (n=100)	Controls (n=60)	T-test (p-value)	Patients (n=100)	Controls (n=60)	T-test (p-value)
RBCs X10^6/μL	4.13±0. 54	4.74±0.46	0.001	4.67±0.64	4.74±0.46	0.471
Hb g/dl	11.70±1.29	14.06±1.39	0.001	13.16±1.57	14.06±1.39	0.001
Hct %	35.14±3.86	41.58±4.05	0.001	39.49±4.70	41.58±4.05	0.005
MCV Fl	86.94±5.81	87.09±2.89	0.856	88.29±6.03	87.09±2.89	0.151
MCH Pg	27.28±2.43	29.66±1.00	0.001	28.29±2.18	29.66±1.00	0.001
MCHC g/dl	32.31±1.35	34.3±1.46	0.312	33.78±1.53	34.3±1.46	0.001
RDW %	12.93±1.14	13.16±0.50	0.143	13.22±0.68	13.16±0.50	0.558
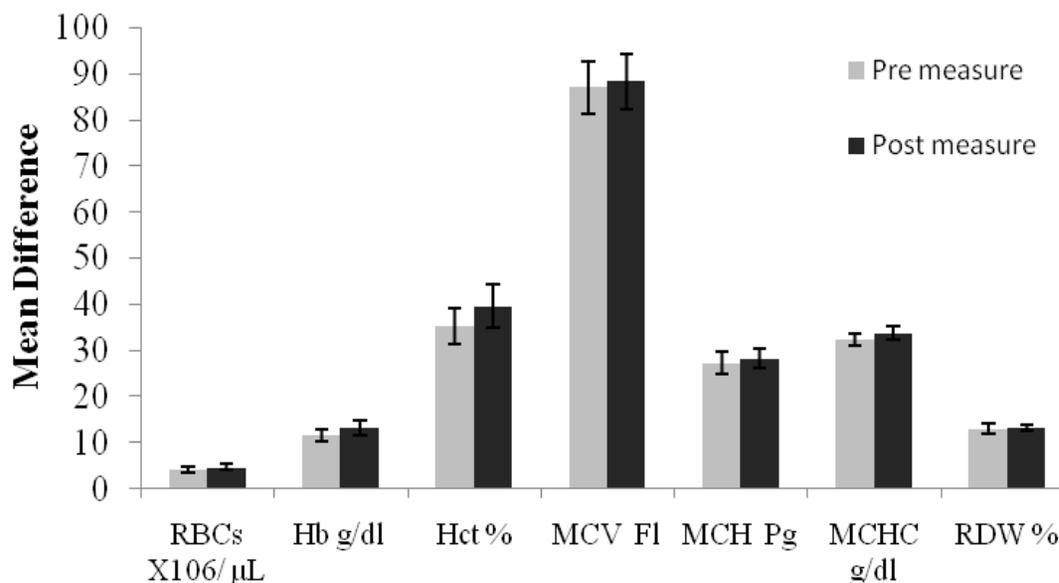
Figure 1. Difference between mean of Blood haemoglobin concentration, erythrocytes count from renal failure patients before and after HD procedure.

HD patients (7.46 ± 1.87) when compared to that of the counts in the control group (6.30 ± 1.88) (P<0.05). The neutrophil count increased significantly in post-HD patients (3.89 ± 1.31) as compared to the control group(3.40 ± 1.31) (P<0.05); the lymphocyte counts also increased significantly in post-HD patients (2.33 ± 0.76) when compared to control group (2.05 ± 0.63) (P<0.05); the monocyte counts significantly

Table 2. Difference between mean of leucocytes and differential count before and after HD procedures in patients and healthy controls.

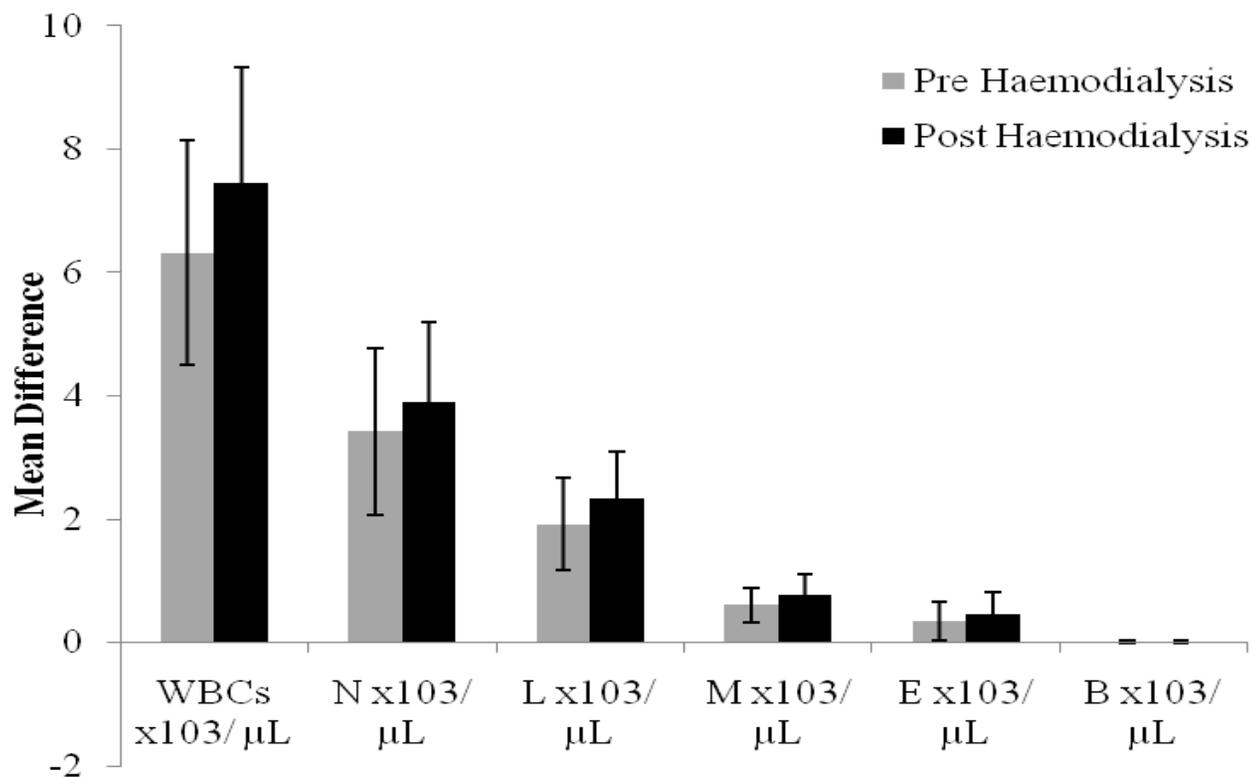| Variables (Values are Mean±SD)	Pre haemodialysis			Post-haemodialysis		
	Patients (n = 100)	Controls (n = 60)	T-test (p-value)	Patients (n = 100)	Controls (n = 60)	T-test (p-value)
WBCs X10^3/ µL	6.32±1.82	6.30±1.88	0.927	7.46±1.87	6.30±1.88	0.001
N X10^3/ µL	3.42±1.35	3.40±1.36	0.919	3.89±1.31	3.40±1.36	0.026
L X10^3/ µL	1.92±0.75	2.05±0.63	0.287	2.33±0.76	2.05±0.63	0.015
M X10^3/ µL	0.61±0.28	0.55±0.17	0.099	0.76±0.34	0.55±0.17	0.001
E X10^3/ µL	0.35±0.31	0.30±0.31	0.172	0.46±0.35	0.30±0.13	0.001
Bx10^3/ µL	0.01±0.03	0.01±0.02	0.072	0.01±0.03	0.01±0.02	0.072

increased in post-HD patients (0.76 ± 0.34) compared to the counts in control group (0.55 ± 0.17) (P<0.05); the eosinophil counts showed a statistically significant increase in post-HD patients (0.46 ± 0.35) compared to that of the control group (0.30 ± 0.13) (P<0.05). However, the basophils count did not show any statistically significant difference in post-HD patients compared to control group (P>0.05). The WBCs count showed statistically significant increase in post-HD (7.46 ± 1.87) when compared to the pre-HD (6.32 ± 1.82) (P<0.05). The neutrophil counts showed significant increase post-HD (3.89 ± 1.31) when compared to that of the pre-HD (3.42 ± 1.35) (P<0.05); the lymphocyte counts increased significantly in post-HD (2.33 ± 0.76) when compared to the pre-HD count (1.92 ± 0.75) (P<0.05) ; the monocyte counts significantly increased post-HD (0.76 ± 0.34) when compared to the pre-HD count (0.61 ± 0.28) (P<0.05); the eosinophil counts increased in post-HD (0.46 ± 0.35) when compared to the pre-HD count (0.35 ± 0.31) (P<0.05). The basophil counts did not show statistically significant differences in post-HD when compared to pre-HD count (P>0.05) (Figure 2).The findings of the present study revealed that there were insignificant correlations between the

time period the patients have been on dialysis and each of leucocytes and differential counts (P>0.05).

Peripheral blood coagulation profile from pre and post- HD patients and healthy controls

The difference between mean of coagulation profile that occurred in renal failure patients before, after HD procedures, and control group is shown in Table 3.The platelets count showed statistical significant decrease in pre-HD patients (199.19 ± 56.74) when compared to that of the control group (262.3 ± 48.00) (P<0.05). The increase of fibrinogen level in pre-HD patients (3.54 ± 0.73) was also statistically significant when compared to that of the control group (2.21 ± 0.43) (P<0.05).However there were no statistically significant differences between the PT, APTT levels in pre-HD patients compared to controls (P> 0.05). Similarly, the platelet counts showed statistical significant decrease in post-HD patients (176.86 ± 56.08) when compared to control group (262.32 ± 48.00) (P<0.05), However, the PT levels showed significant increase in post-HD patients (23.10 ± 5.69)

compared to the control group (12.54 ± 0.82) (P<0.05). Significant increase was also seen in the APTT level of post-HD patients (64.64 ± 13.61) when compared to the control group (33.67 ± 3.99) (P<0.05). The fibrinogen levels increase significantly in post-HD patients (6.85 ± 1.10) when compared to the control group (2.21 ± 0.43) (P<0.05). The difference between the mean of coagulation profile that occurred in renal failure patients before and after HD sessions were calculated (Figure 3). The platelet counts showed statistical significant decrease post-HD (176.86 ± 56.08) when compared to that of the pre-HD (199.19±56.74) (P<0.05). The PT levels on the other hand, showed statistical significant decrease post-HD (23.10±5.69) when compared to the pre-HD levels (12.91±3.19) (P<0.05). Likewise the APTT levels increased significantly post-HD (64.64 ± 13.61) when compared to the pre-HD levels (33.67 ± 3.99) (P<0.05). The fibrinogen levels also showed a statistically significant increase post-HD (6.85 ±1.10) when compared to those of the pre-HD (3.54 ± 0.73) (P<0.05). Data from the current study also showed that there were significant positive correlation between dialysis duration and each of fibrinogen, APTT and PT levels while, there was a negative

Figure 2. Difference between mean of leucocytes and differential count in renal failure patients before and after HD procedure.

Table 3. Difference between mean of coagulation profile before and after HD procedures in patients and healthy controls.

Variables (values are mean ± SD)	Pre haemodialysis			Post-haemodialysis		
	Patients (n=100)	Controls (n=60)	T-test (p-value)	Patients (n=100)	Controls (n=60)	T-test (p-value)
Plts x10³/ µL	199.19±56.74	262.32±48.00	0.001	176.86±56.08	262.32±48.00	0.001
PT second	12.91±3.19	12.54±0.82	0.379	23.10±5.69	12.54±0.82	0.001
INR	1.04±0.30	1.00±0.7	0.279	1.97±0.52	1.00±0.7	0.001
APTT second	33.67±3.99	32.93±5.85	0.390	64.64±13.61	32.93±5.85	0.001
FIB g/L	3.54±0.73	2.21±0.43	0.001	6.85±1.10	2.21±0.43	0.001
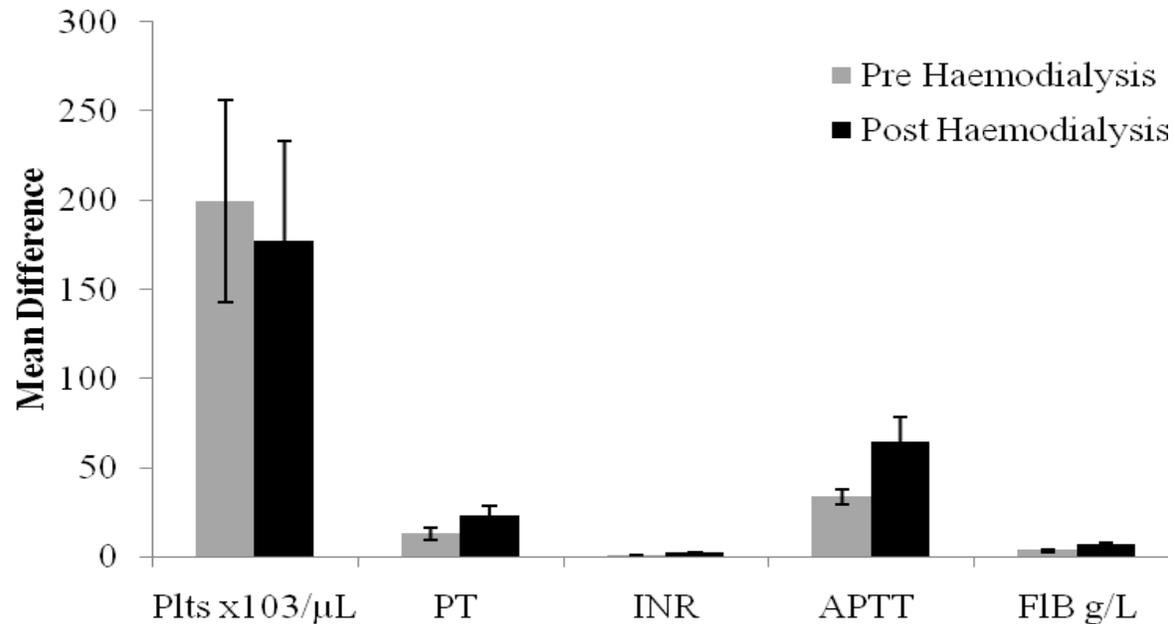
Figure 3. Difference between mean of coagulation profile in renal failure patients before and after HD procedure.

significant correlation between dialysis duration and platelets count.

DISCUSSION

The results of the present study showed that patients with renal diseases on regular HD display various degrees of changes in hematological parameters. The RBCs count, Hb and Hct levels in these patients, although often within the normal range, were significantly lower when compared to the levels in healthy controls. There were no statistically significant differences between means of MCV, MCHC, RDW levels in renal failure patients before HD when measured against the control group. On the other hand, the present study indicated that the mean of each RBCs count, Hb, Hct, MCH, MCHC and RDW levels show a statistically significant increase in renal failure patients' post-HD when compared to pre-HD levels. The increase of each RBCs count, Hb, Hct levels post-HD were explained by the fact that before HD, patients are usually hypervolemic and the values of each RBCs count, Hb, Hct levels are also lower. The findings of this study also indicated that there were statistically insignificant differences between the mean number of leucocytes and differential counts in renal failure patient's pre-HD when compared to the counts of the control group. Similar results were reported by Pereira et al. (2010) which showed that chronic kidney disease patients presented a significant decrease neutrophil and monocyte expression. After hemodialysis, a statistically significant increase in certain monocyte surface markers and a decrease in

neutrophil expression was found showing that neutrophils and monocytes are activated these patients. On the other hand, the result of this study showed that the mean of WBCs, neutrophiles, lymphocytes, monocytes, and eosinophils count were significantly increased in renal failure patients post-HD when compared to that of the control group, while, there were statistically insignificant differences between the mean of basophils count in renal failure patients post-HD sessions and those in the control group. These findings are to some extent congruent with the study conducted by Mohamed et al. (2008) who reported that the time period has been on dialysis did not affect the hematological parameters.

This study has also used a control group. Although this is generally not considered in these types of studies, it gave us a general idea of the variations in hematological parameters in the general population. Such results will help in forming guidelines for treatment patterns to be followed in this population. Therefore, controls were used as a standard against which experimental observations may be evaluated. This was primarily important because such there is a paucity of literature regarding these studies from the Saudi Arabian population.

It was also observed that the mean of leucocytes counts and the mean count of each, neutrophils, lymphocytes, monocytes and eosinophils, with the exception of basophils, showed statistically significant increase in renal failure patients' after HD when compared to before HD procedures. The increase of leukocytes and differential counts after-HD were explained by the fact that at the beginning of HD, patients are usually hypervolemic and the values of the

leucocytes and differential counts are lower. Findings of the present study revealed that there were insignificant correlations between the time period the patient have been on dialysis and each of leucocytes and differential counts. Similarly, and in support to our study (Mohamed et al., 2008) reported that the time period the patients have been on dialysis did not affect the leucocytes count. This study also found that leukocyte and platelet counts were increased slightly after hemodialysis.

Thrombocytes are nucleate fragments of mega karyocytes that are involved in the formation of blood clots (Sunitha et al. 2008). The present study revealed that there was a statistically significant decrease in the mean platelet counts, though still within the normal range, failure patients pre- and post-HD when compared to the results of the control group. The mean platelet counts in the current study showed a significance decrease in patients' after-HD when compared to pre-HD procedures. This finding is in agreement with Yenicerioglu et al. (2000), who reported a significant decrease in circulating platelets after-HD when compared to the before-HD procedures. The present study also indicated that there was a significant negative correlation between the time period the patients have been on dialysis and platelets counts. This observation can be explained by the finding that mega karyocytopoiesis decreases with years on HD; possibly contributing to their annual decreases in platelet counts (Ando et al., 2001). In contrast to our results, Mohamed et al. (2008) did not find any correlation between blood platelets count and the time period patients are on dialysis. They found that most of the hematological parameters as well as prothrombin and partial thromboplastin times increased after a dialysis session.

In the present study, conventional haemostasis parameters (PT and APTT) were significantly increased after-HD when compared to the before-HD levels. This finding can be explained by using systemic anticoagulation (heparin) during conventional HD for extracorporeal procedures which binds to the enzyme inhibitor antithrombin III. This result in the inactivation of thrombin and other proteases involved in blood clotting, most notably FXa (Wardle, 2002). Another possible factor that could account for the increased heaemostasis parameters would be the increase in the level of TFPI (potent inhibitor of the extrinsic coagulation pathway) and the reduced activity of several coagulation factors during HD, including factor II, IX, X, and XII (Maderna et al., 1999; Naumnik et al., 2002). These finding were consistent with the work of Mohamed et al. (2008) who found a statistically significant increase of PT and a highly significant increased of APTT after-HD. On the other hand, the current study showed that there were statistically insignificant differences between the mean of PT and APTT in renal failure patients' before-HD and those of the control group. This finding was in agreement with the results obtained by Erdem et al. (1996) and

Ulusoy et al. (2004). It has been shown that the levels of plasma fibrinogen increase significantly in patients with the renal failure after-HD when compared to the before-HD levels (Martinez et al., 1999; Ciaccio et al., 2008) and the result of the current study support these report. This study also clearly showed that there was statistically significant increase in the mean of fibrinogen levels in renal failure patients before and after HD procedures when compared to the normal health controls. Findings of the present study revealed that there were significant positive correlations between the time periods the patients that have been on dialysis and fibrinogen levels. However, contrasting results have been presented by Tzanatos et al. (2006) who did not find any significant correlation between the time period the patients have been on dialysis and fibrinogen levels.

In a nutshell, the results of this study indicate that most of the hematological parameters measured in HD patients, pre- or post-HD were either elevated or lowered compared to the control groups. Furthermore, it was noticed that there were changes in these parameters between post-HD and pre-HD levels. More significantly, PT, APTT and fibrinogen were found to increase post-HD compared to pre-HD procedures while there was a concurrent decrease of platelet counts. A significant positive correlation was noticed between the time period the patients have been on dialysis and each of fibrinogen, APTT and PT levels. However there was a significant negative correlation between the time periods the patients have been on dialysis and platelets count. Thus, the longer the time period the patients are on dialysis, the more the coagulation profile is affected, that is, fibrinogen, APTT and PT levels increase with longer duration of HD whereas the platelet count decreases with longer duration of HD. Therefore, it is recommended that all patients be screened appropriately before and after dialysis to avoid complications. The present investigation might help clinicians to initiate precautions before and after dialysis procedures. Thus, it is recommended that all patients be screened appropriately before and after dialysis to avoid complications.

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