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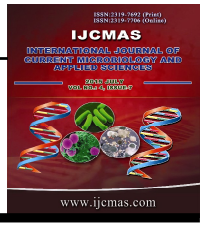


Original Research Article

Evaluation of Some Novel Biomarkers in Renal Failure Patients on Hemodialysis

Aseel Ghassan Daoud*, Wassan Abdul-Kareem Abbas, Haethem Qassim Mohammed

Clinical Laboratory Science Department/College of Pharmacy/
University of Al-Mustansiriyah, Iraq
**Corresponding author*

Keywords

Hemodialysis, Cystatin-C, Beta-2-microglobulin, Tumor necrosis factor-α

A B S T R A C T

The purpose of the present study was to determine the effects of hemodialysis on serum levels of β2-MG, Cys-C and TNF-α in patients with renal failure pre- and post-hemodialysis session. Thirty ESRD HD patients were enrolled in this study. Serum levels of β2-MG, Cys-C an/d TNF-α were measured before and after dialysis and compared to healthy subjects. There was a significant difference in the level of β2-MG in healthy group when compared to pre-dialysis group and post-dialysis group with (P=0.0001). The difference in the mean serum level of Cys C was also observed to be very highly significant in control group compared to pre-dialysis group and post-dialysis group with (P=0.0001). This study also observed no significant difference in the mean serum concentration of TNF-α among pre-dialysis , post-dialysis and control groups with (P= 0.73). Hemodialysis showed only a little non-significant increasing effect on serum activities of TNF-α. Whereas it had no significant effect on serum levels of Cys C and β2-MG which were considerably higher in those patients compared to healthy controls.

Introduction

Chronic kidney disease (CKD) is a condition in which there is a progressive decline in kidney function over a period of time. There are many causes of CKD, it can be caused by hypertension, diabetes mellitus or by other factors. Rapid diagnosis and management may decrease the progression of CKD. When kidney disease progresses, it may lead to kidney failure, which requires dialysis or a kidney transplant to maintain life (NKF, 2015). ESRD is the last stage of CKD (stage 5) in which the kidneys cannot supply the body with essential requirements (Scott Miller, 2013). In ESRD patients the immune system is impaired. In these patients, there is a pre-activation of monocytes and overproduction of cytokines such as tumor necrosis factor-alpha (TNF-α) (Lang, 2013). In CKD patients there are chronic elevations in inflammation markers which will get worse as the disease is progressed and start of hemodialysis (Dungey, 2013).

There are two lines in treatment of end-stage kidney disease including dialysis and kidney transplant(Christine DiMaria, 2012). There are two principle types of dialysis: "hemodialysis" and "peritoneal dialysis." In hemodialysis, the most common method,

excess waste products and water are removed from the body through a special filter (William, 2014).

The diagnosis of CKD using creatinine measurement cannot predict the early defect in kidney function especially in patients with low muscle mass. However, there is another marker for the estimation of kidney function which is newer and more precise than creatinine called cystatin C (Cys C) which is becoming more widely used (Karin Rush-Monroe, 2013). Human cystatin C (HCC) is a protein of low molecular weight (120 amino acid residues, 13,343 Da. It is found in all body fluids especially in cerebrospinal fluid (CSF) and it is a reversible inhibitor of cysteine proteases (Janowski, 2004). Cystatin C is freely filtered through the glomeruli of the kidney since it has a low molecular mass so GFR can determine its blood concentration (Medicine Net, 2014).

Human β2-microglobulin (β2M) is a protein constituent of Class-I major histocompatibility (MHC-I) complexes displayed on the surfaces of human cells. β2M will be accumulated as insoluble amyloid precipitates in the joints of CKD patients receiving hemodialysis leading to Dialysis- Related Amyloidosis (DRA) (Kumar, 2014). DRA is usually developed within 3-5 years after initiation of dialysis treatment and it is affected by several factors like patient's age and dialysis duration (Traut, 2007).

TNF is a transmembrane protein arranged in stable homotrimers and about 212-amino acid chain length (Tang P, 1996). In CKD patients, TNF-α level is increased. TNF-α is mainly produced by monocytes, macrophages, and T cell (Vázquez-Huerta DI, 2014). When there is an infection, it acts by damaging the stimuli and therefore containing the infection to elicit the immune response but when the infection cannot be resolved or when there is a defect in anti-inflammatory response it will lead to chronic inflammation which is harmful and may result in end organ and vascular damage(Dungey M,2013).

Materials and Methods

This study was performed on 30 patients with ESRD who were undergoing hemodialysis (using glucose-free dialysate) and 25 healthy subjects as control (11 males and 14 females). The samples of patients were collected from AL-Shifaa Dialysis Center at AL-Yarmook Teaching Hospital in Baghdad, Iraq at the period from October to December 2014. Cystatin-C, β2-microglobulin (β2M) and TNF-α were assayed using enzyme linked immune sorbent assay ((Elabscience, China). The patients included in this study were 13 males and 17 females within the age range of (15-73) years old without hypertension or diabetes mellitus, live in the city, and with duration of dialysis between 2 months and 8.5 years.

Statistical analysis

The Statistical Analysis System- SAS (2012) was used to see the effect of different factors on the study parameters. Least significant difference –LSD test was used to significantly compare among means in this study(SAS. 2012).

Results and Discussion

The present study found that the difference in the mean serum level of Beta-2 MG in control group (1.62 ± 0.08) was very highly significant when compared to pre-dialysis group (21.39 ± 3.79) and post-dialysis group (20.83 ± 3.35) with (P=0.0001). While the difference in the mean serum level of Beta-2

MG was non-significant between pre- and post-dialysis groups as shown in figure 1 and table 1. The difference in the mean serum level of Cys C was also observed to be very highly significant in control group (35.26 ± 1.53) compared to pre-dialysis group (57.12 ± 2.94) and post-dialysis group (57.54 ± 3.16) with (P=0.0001), whereas there was no significant difference between pre- and post-dialysis groups as shown in figure 2 and table 1. This study also observed no significant difference in the mean serum concentration of TNF-α among pre-dialysis (145.82 ± 44.33), post-dialysis (194.22 ± 46.77) and control (161.98 ± 46.38) groups with (P= 0.7392) as described in figure 3 and table 1.

ESRD patients were on hemodialysis for years, this duration of dialysis differed from patient to patient. The duration of dialysis was divided into 3 groups as illustrated in table 2.

The present study found no significant difference in the mean serum level of β2-MG among the three duration groups as shown in table 2. In case with Cys C, the difference in the mean serum level was very highly significant in the < 2 years dialysis group (66.62 ± 5.02) when compared to that of 2-4 years dialysis group (53.62 ± 2.39) and that of > 4 years dialysis group (51.50 ± 2.54) with (P< 0.01).

The present study observed a very high significant difference in the mean serum concentration of TNF-α in the < 2 years dialysis group (314.89 ± 78.28) in comparison with each of 2-4 years dialysis group (122.42 ± 32.19) and of > 4 years dialysis group (61.57 ± 44.37) where (P< 0.01).

In long term hemodialysis, β2MG may form insoluble amyloid deposits and this is called dialysis-related amyloidosis (DRA) (Torbeev, 2013). There was a study reported that as the duration of dialysis increases, serum level of β2MG also increases where it reaches plateau after 5-10 years of HD then start to decrease after about 18 years of HD(Celik G, 2013). However other studies reported that β2MG level decreases after about 8 weeks on HD and the risk of mortality increases as the blood level of β2MG increases (El-Wakil HS, 2013). These studies are not agreed with the present study where it was found that mean serum level of β2MG was not affected by duration of dialysis (P = 0.4) (Table 2).

The current study found that there was no significant statistical difference in the mean serum level of β2MG when compared between pre- and post-dialysis ESRD patients as elucidated in figure 1 and table 1. This agreed with a study which was conducted to see the effect of HD on the removal of large molecules including β2MG where they found that these large molecules would be highly resistant to be removed by 4 hours HD while it seemed that its removal would be increased by about 81.2% by increasing dialysis period to about 8 hours (Heaf, 2013). A recent study demonstrated that β2MG pre-dialysis serum level was considerably high in HD patients when compared to healthy controls (El-Wakil, 2013). This result was approved by the present study where it was found that serum level of β2MG in dialysis group was very high compared to healthy controls.

There were some studies performed that Cys C elimination would be more efficient in high-flux-high-efficiency HD and there were a significant reduction in its level compared to using low-efficiency HD where it was found a paradoxical increase in post-dialysis serum level of Cys C, this was thought to be due to Cys C hemoconcentration between extravascular and intravascular spaces (Huang, 2011).

Table.1 The difference in serum level of Beta-2 MG, Cys C and TNF-α among the study groups

Parameters	Mean ± SD			LSD value	P-value
	Control	Pre-Dialysis	Post-Dialysis		
Beta2 MG (ng/ml)	1.62 ± 0.08 b	21.39 ± 3.79 a	20.83 ± 3.35 a	8.747 **	0.0001
Cystatin C (ng/ml)	35.26 ± 1.53 b	57.12 ± 2.94 a	57.54 ± 3.16 a	7.785 **	0.0001
TNF-α (pg/ml)	161.98 ± 46.38 a	145.82 ± 44.33 a	194.22 ± 46.77 a	129.74 NS	0.7392
** (P<0.01), NS: (Non-significant)					

Table.2 Effect of duration of dialysis on the parameters measured in the study

Parameters	Mean ± SD			LSD value	P-value
	Less than 2 yr.	2-4 yr.	More than 4 yr.		
Beta2 MG (ng/ml)	16.81 ± 3.57	21.65 ± 3.60	27.13 ± 7.30	13.754 NS	0.400
Cystatin C (ng/ml)	66.62 ± 5.02 a	53.62 ± 2.39 b	51.50 ± 2.54 b	11.070 **	0.013
TNF-α (pg/ml)	314.89 ± 78.28 a	122.42 ± 32.19 b	61.57 ± 44.37 b	164.29 **	0.0081
** (P<0.01), NS: Non-significant.					

Figure.1 Difference in serum level of β2-MG among study groups

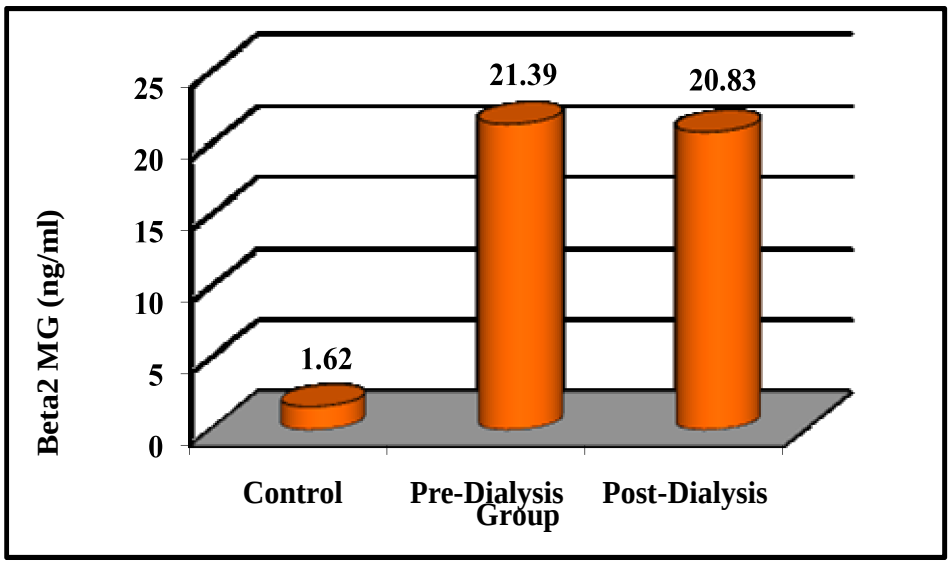


Figure.2 Difference in serum level of Cys C among study groups

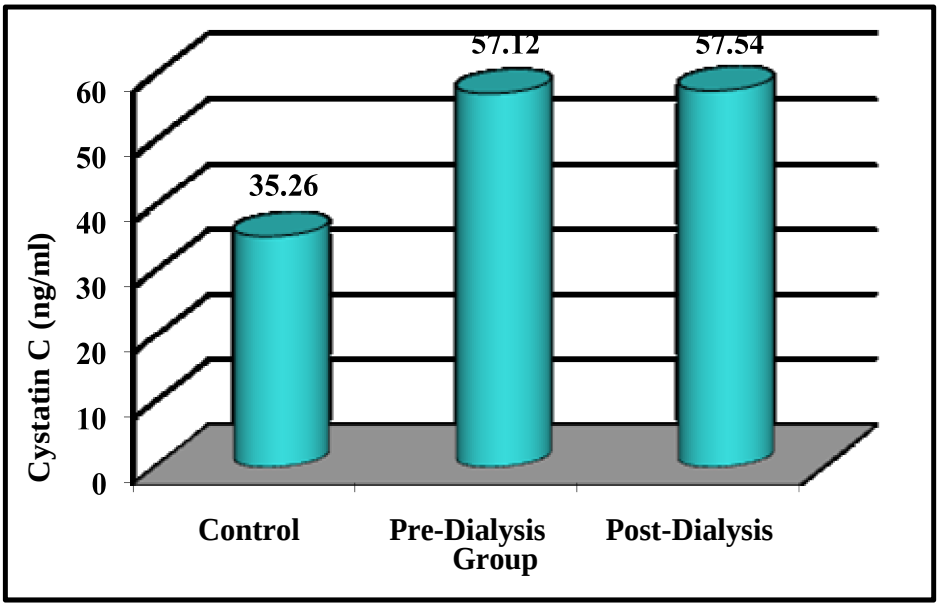
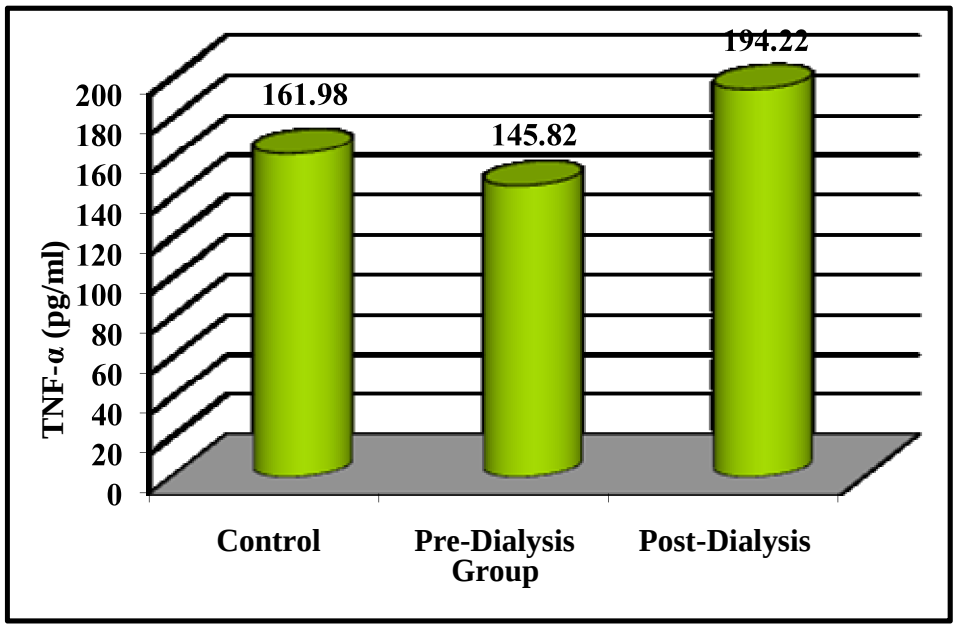


Figure.3 Difference in serum level of TNF-α among study groups



These studies were not agreed with the current study where it was found there was no significant difference in the mean serum level of Cys C pre- and post-dialysis whereas there was a very high significant difference when compared ESRD-HD patients with control subjects where its level seemed to be very high in those patients (Figure 2 and Table 1). This persistent high post-dialysis serum Cys C level might be attributed to the use of low efficient HD or it might be due to genetic factors. However, there was a previous study observed that elimination of Cys C would be more efficient using hemodiafiltration instead of low-flux HD (Lindström, 2008). On the

other hand, a recent study demonstrated that urinary elimination of Cys C and β2MG would be increased as GFR decreased due to their tubular reabsorption saturation (Donadio, 2014). The current study also demonstrated that serum level of Cys C decreased as the duration of dialysis increased (Table 2).

In the present study, there was no significant difference observed in the mean serum level of TNF-α when compared pre- and post-hemodialysis patients with healthy controls. However, there was a slight increase in its level following HD session although statistically non-significant and it seemed to be little higher than in healthy controls (Figure 3 and Table 1). This result had an agreement with a previous study which performed those serum levels of pro-inflammatory cytokines, including TNF-α, showed higher values following HD session (Cohen, 2010). Elevation in the serum level of pro-inflammatory cytokines, including TNF-α, in ESRD HD patients increased mortality risk, such elevations are multifactorial, it may be caused by dialysis procedure itself (which causes inflammation), impaired renal excretion of cytokines or infections (Heng-Jung Hsu,2014). Another study was in agreement with the current study in that serum level of TNF-α was higher in ESRD patients on maintenance HD compared to normal subjects as a result of micro-inflammation that associated with dialysis procedure (Ren, 2013). There was a study which demonstrated that TNF-α serum levels would be significantly increased in HD patients compared to healthy controls (Shi, 2013). This increase in the TNF-α serum level was also seen in the present study but it was statistically non-significant. This is approved by another study which also found that serum level of TNF-α was increased in HD patients compared to healthy controls and was statistically non-significant (Babaei, 2014). However, other study detected a decrease in the serum level of TNF-α following HD session (Rosales, 2015). In the present study, a very high significant statistical difference was detected in the mean serum TNF-α level among HD patients with different durations on HD. It was demonstrated that serum level of TNF-α decreased as the duration of HD increased with years (Table 2).

There was no significant effect of hemodialysis on serum β2MG and Cys C level where it was still higher than in healthy controls. TNF-α serum activity seemed to be not significantly affected in ESRD patients on HD compared to healthy controls, although it showed a little increase following HD session compared to its pre-dialysis activity.

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