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EVALUATION OF HEPATIC ENZYMES IN LIBYAN CHRONIC KIDNEY DISEASE PATIENTS: NEED FOR LOWER STANDARD "NORMAL" RANGE.

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Abstract:-

Chronic Kidney disease (CKD) patients on hemodialysis treatment are at higher risk of hepatitis C infection and they showed lower serum levels of aminotransferases than general population. These hepatic enzymes aid in the diagnosis, monitoring, and treatment of hepatic diseases. The present study aimed to estimate the changes in serum ALT, AST and ALP levels in both predialysis (PreHD-CKD) and hemodialysis (HD-CKD) patients in comparison to normal controls and to find out the association between hepatic enzyme changes and estimated Glomerular Filtration Rate (eGFR). The present study included 53 HD-CKD patients, 61 PreHD-CKD patients, and 50 healthy controls. A questionnaire that included variables was answered by all the participants, and Blood samples were collected and estimated for blood urea, creatinine, AST, ALT, and ALP using standard methods afterward, the data was statistically analyzed using SPSS software. Our results revealed lower AST and ALT levels in HD-CKD patients than both PreHD-CKD patients and healthy controls. On the contrary, ALP was significantly higher in both groups of CKD patients when compared to healthy controls. In addition, no significant correlation has been found between eGFR and hepatic enzymes. Serum ALT and AST levels tend to be reduced in CKD patients on hemodialysis treatment, a finding that enforces the urgent need for the establishment of separate reference ranges of hepatic enzymes for CKD patients in order to facilitate the diagnosis, monitoring, and treatment of liver diseases, especially hepatitis C infection, an establishment that may play a role in decreasing the mortality in CKD patients.

Key words:- *Chronic kidney disease, Hemodialysis, Hepatitis c, Aminotransferases, Mortality.*

INTRODUCTION:

Chronic kidney disease (CKD) results from inability of the kidneys to filter waste products (e.g. Urea and creatinine) from the circulation. CKD is considered as a major health problem worldwide [1]. Diabetes, hypertension, and obesity are the most implicated risk factors for CKD in the Middle East [2]. Glomerular filtration rate (GFR) lower than 60ml/min per 1.73m2 is the cut-off value for the diagnosis of CKD, which is usually presented with symptoms of uremia [3]. Regardless of the cause of kidney damage, once a critical level of renal functional deterioration is reached, progression to end stage renal failure (ESRD) is inevitable. ESRD is a term reserved, when hemodialysis or kidney transplant is required [4]. ESRD incidence rate in Libya is one of the highest in the world. An estimation of 2417 adult ESRD patients registered in Libya. The prevalence rate of ESKD undergoing hemodialysis was 624 per million population [5].

The serum levels of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are markers of aggression against hepatocytes [6]. Serum ALT and AST levels are elevated in several diseases, such as chronic viral hepatitis, non-alcoholic fatty liver disease, autoimmune hepatitis, hemochromatosis, and alcoholic liver disease. These enzymes assist in diagnosis, follow-up and response to treatment because they reflect inflammatory activity in liver parenchyma [7].

The prevalence of hepatitis C virus (HCV) infection is significantly higher in hemodialysis (HD) patients than in the general population [8]; this disease is associated with an increased mortality rate primarily due to hepatocellular carcinoma and liver cirrhosis [9, 10]. The prevalence of HCV infection in HD patients varies greatly between regions of the world and even within the same country [11]. The prevalence rates of hepatitis C are 31.1% for dialysis patients in Libya, 5.5% for dialysis patients in Brazil, 14.4% for those in the United States, and 68% for those in Saudi Arabia [11-14]. Although HCV infection results in an increase in ALT, these levels are generally lower in hemodialysis patients. Interestingly, some studies have shown that patients with chronic kidney disease (CKD) on HD may have lower serum levels of liver enzymes than those with normal renal function for reasons that remain unclear [15]. This profile may adversely affect the diagnosis, clinical management, and treatment of liver disease in these patients. It was hypothesized that the reduction in aminotransferase enzymes could be caused by several factors including elevated serum lactate concentration, and deficiency of the vitamin cofactor involved in transaminase biosynthesis [16, 17].

On the other hand, plasma ALP levels can originate from liver, bone, intestine and placenta. In general, the isoenzymes from liver and bone contribute to the majority of the circulating enzyme levels. Therefore, in a patient of liver disease serum ALP level is an important marker for screening and monitoring. However, in a CKD patient, renal osteodystrophy could result in a significant increase in the bone isoenzyme of ALP contributing to high serum ALP level. In fact, higher ALP has been associated with increased mortality in predialysis CKD as well as patients on maintenance hemodialysis [18].

The present study is the first of its kind in Libya, and it was undertaken to estimate the changes in serum alanine aminotranferase, aspartate aminotransferase, and alkaline phosphatase levels in both predialysis and hemodialysis chronic kidney disease patients in comparison to normal healthy control, and to find out the assosiasion between hepatic enzyme changes and eGFR, body weight loss, duration of disease, and duration of hemodialysis in CKD patients.

Subjects and methods:

The present case control study included 110 chronic kidney disease patients (53 patients were undergoing to maintenance hemodialysis and 61 patients were in pre-dialysis stages) recruited from the Center of Kidney Diseases Services – Benghazi and 50 age and sex- matched healthy controls selected from Al-Saleem laboratory- Benghazi.

Informed consent was obtained from all participants before the study. The selection of patients was based on previous diagnosis with chronic kidney disease, and that diagnosis was dependent on KDIGO guidelines (CKD is defined as kidney damage marked by albuminuria and GFR less than 60 mL/min per 1.73 m^2 for $\leq$ 3 months) [19].

Clinical information and medical history were obtained through the review of patient medical files and patients' interviews. Face-to-face interview were based on a questionnaire that included variables such as age, sex, date of the diagnosis, cause of the disease, blood pressure, weight, height, duration of hemodialysis with exclusion of patients undergoing hemodialysis for less than 6 months, times of hemodialysis/week, CKD treatments and any health problems or prescriptions.

Patients suffering from any disease other than CKD that could affect their metabolic status and the parameters studied such as malignancy, chronic viral hepatitis, non-alcoholic fatty liver disease, autoimmune hepatitis, hemochromatosis, and alcoholic liver disease were excluded from the study. Pregnant and lactating women were excluded, and patients with a history of recent surgery, smoking or alcohol intake were also excluded. The history of medication was recorded and patients taking any drugs that could affect liver function were excluded. The control group consisted of non-alcoholic healthy subjects with no history of inherited or acquired kidney or liver diseases.

Venous blood samples were collected from all participants in plain tubes. Serum was obtained by centrifugation of clotted blood and stored at -20 C^0 until the assays were performed. The measurements of blood urea nitrogen (BUN), serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were done

using an automated routine chemistry analyzer (Roche Cobas Integra 400 plus, Roche Diagnostics Limited, Switzerland) with commercial kits (Roche Cobas packs, Roche Diagnostics Limited, Switzerland) according to manufacturer's protocol.
The glomerular filtration rate was estimated by using Cockcroft-Gault formula [20]:
Men: (140− age in years) (weight in Kg)
$72 \times serum\ Cr$
Women: (140− age in years) (weight in Kg) × 0.8
$72 \times serum\ Cr$

The data were analyzed using the statistical package for the social sciences (SPSS version 17). Descriptive characteristics of the study participants were calculated as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to determine the differences in subject's characteristics. Pearson's correlation coefficient determination was done to evaluate the degree of association between hepatic enzymes changes and clinical and biochemical parameters. *P* value < 0.05 was considered as statistically significant.

Results:
The mean age and standard deviation (SD) of the hemodialyzed CKD patients selected for this study was 53.2 ± 13, and the male: female ratio was 5:4. The age range was 18- 95 years with duration of disease ranged from 1- 32 years, and duration of hemodialysis ranged from 1- 16 years. The mean age and SD of the pre-dialyzed CKD patients included in the study was 51.5 ± 14.8, and the male: female ratio was 1:3. The age range was 25- 90 years with duration of disease ranged from 1- 13 years. The mean age and SD of the control subjects was 47.4 ± 12.4, and the male: female ratio was 1:3. The age range was 19- 67 years. Blood urea and creatinine levels were significantly higher in predialyzed CKD (PreHDCKD) patients than controls ($p < 0.05$) (Table 1).

Table 1: Mean±SD of blood urea and serum creatinine in predialyzed CKD patients and control subjects.

Parameters	PreHD-CKD patients N= 61	Controls N= 50	*P* value
BUN (mg/dl)	107.4 ± 39.3	25 ± 8.7	$p < 0.05$
S. Creatinine (mg/dl)	3.78 ± 2.3	0.75 ± 0.2	$p < 0.05$

Hemodialyzed CKD patients had a significantly lower AST concentration when compared to predialyzed CKD patients ($p = 0.04$), and normal healthy controls ($p = 0.01$). The difference in AST levels between predialyzed CKD patients and healthy controls was statistically nonsignificant ($p = 0.6$), (Figure 1). Moreover, Alanine aminotransferase levels were significantly lower in hemodialyzed CKD patients than predialyzed CKD patients ($p = 0.00$), and normal healthy controls ($p = 0.00$). No significant difference in ALT levels have been shown between predialyzed CKD patients and normal healthy controls ($p = 0.3$), (Figure 2).

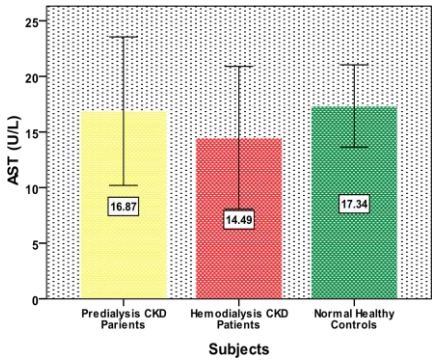

Figure 1: Mean ± SD of Aspartate Aminotransferase (AST) in CDK patients and healthy controls.

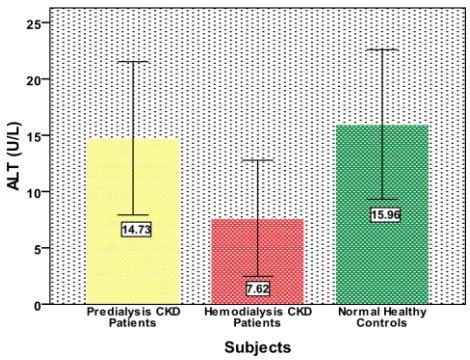

Figure 2: Mean ± SD of Alanine Aminotransferase (ALT) in CDK patients and controls.

On the other hand, the mean level of alkaline phosphatase was significantly higher in both groups of CKD patients when compared to normal healthy controls (predialyzed CKD patients vs. controls (p = 0.00); hemodialyzed CKD patients vs. controls (p=0.04).

Predialyzed CKD patients showed higher levels of ALP than hemodialyzed CKD patients, and the difference was statistically significant (p = 0.01) (Figure 3).

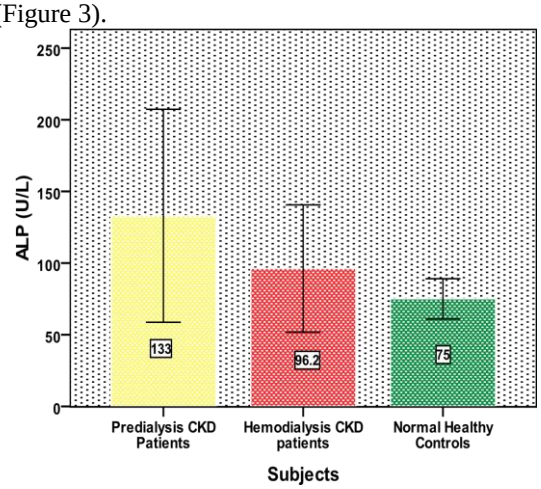

Figure 3: Mean ± SD of Alkaline Phosphatase in CDK patients and control subjects.

In addition, there were non-significant correlations between hepatic enzymes changes (AST, ALT, ALP) and age, duration of disease, or duration of hemodialysis in CKD patients. In pre-dialyzed CKD patients, AST showed a significant negative correlation with blood urea (r = - 0.285, p= 0.03) (Figure 4), but non-significant correlation with serum creatinine (r =- 0.076, p= 0.5) and eGFR (r = 0.04, p= 0.8). On the other hand, no significant correlations have been shown between either ALT or ALP and urea (ALT vs. urea (r = - 0.09, p= 0.5); ALP vs. urea (r = - 0.14, p= 0.3), creatinine (ALT vs. creatinine (r = 0.13, p= 0.33); ALP vs, creatinine (r = - 0.19, p= 0.2), or eGFR. ALT vs. eGFR (r = - 0.06, p= 0.7); ALP vs. eGFR (r = 0.25, p= 0.2).

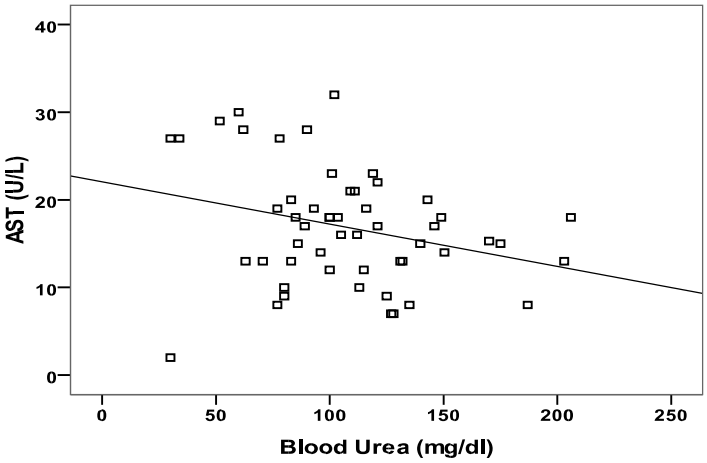

Figure 4: Correlation between blood urea and AST:

Discussion:

Hemodialysis affects various substances in the blood circulation by elevating or reducing their concentration or eliminating them. Chronic kidney disease patients undergoing hemodialysis are at higher risk of developing hepatitis C infection than general population. In 2011, Hrstić I and Ostojić R, concluded that major chronic liver diseases associated with chronic kidney disease are hepatitis B and C [21]. Diagnosis and monitoring of liver damage as well as, clinical management and treatment of liver disease in those patients require the continuous assessment of liver function, so we carried out this study to evaluate hepatic enzymes in order to find out possible effects of CKD upon the levels liver function enzymes. The present case control study revealed significantly lower aspartate aminotransferase and alanine aminotransferase levels in hemodialyzed CKD patients compared to both healthy controls and predialyzed CKD patients. Our results are in agreement with other studies which revealed a reduced aminotransferase levels in CKD patients undergoing hemodialysis [22, 23]. In accordance with our work, a study by Ray and colleagues (2015) included 100 CKD patients with ESRD, 100 CKD patients without ESRD, and 100 healthy individuals, showed that serum AST and ALT levels were significantly lower in CKD patients without ESRD and CKD patients with ESRD compared to controls [24]. Aminotransferase levels were also significantly lower in CKD patients with ESRD compared to CKD patients without ESRD. Furthermore, a study done in Italy demonstrated lower AST and ALT levels among hemodialyzed CKD patients

compared to predialyzed CKD patients, in addition to the lower level of aminotransferases has been found in both groups of CKD patients compared to healthy individuals [25].

The finding of Lower aminotransferases levels CKD patients compared to predialyzed patients demonstrated that the decrease in aminotransferases is concomitant with the progression of renal disease [15]. It was suggested that the reduction in aminotransferase enzymes in hemodialyzed may be due to various factors including the elimination of aminotransferases during the HD session; the elevated lactate levels in blood, which, during biochemical dosages, would rapidly consume nicotinamide adenine dinucleotide phosphate (NADPH) and cause decreased aminotransferases concentration; the existence of uremic factors that would inhibit enzyme activity; and, finally, decreased aminotransferases levels may result from pyridoxine deficiency, a cofactor needed for the biosynthesis of the transaminases [17, 18]. In accordance, Ono et al., (1995) reported that patients with CKD on HD with pyridoxine deficiencies have lower serum aminotransferase levels than those without vitamin deficiencies [16]. On the other hand, Lopes et al. observed hemodilution and found that weight loss during HD was associated with an increase in serum concentration of ALT. The possibility of hemodilution is corroborated when the mean values of AST and ALT in the same 40 patients are compared in the samples collected before and after the HD session. There was a significant increase in both the AST and the ALT serum levels after the dialysis session. Moreover, a significant increase in the hematocrit after the HD session was also observed, which provided support for the hypothesis that patients with CKD retain water before HD [26, 27]. Huang et al. evaluated the serum homocysteine levels of 145 patients undergoing HD and found that they were negatively related to AST. Thus, AST serum levels might reflect the high metabolic activity of homocysteine and influence its serum levels [28]. Importantly, these hypotheses concern patients with CKD under HD; however, they cannot be rejected with regard to patients during predialysis stages [27].

Some researchers suggested that the aminotransferase serum levels were not related to the dialysis method. Hung et al. investigated 90 patients on peritoneal dialysis and healthy adults and found an average ALT level of 15 IU/L in the peritoneal dialysis patients compared with 22 IU/L in the healthy control [29]. These data is in agreement with the hypothesis that CKD patients have lower levels of ALT regardless of the dialysis method. Subsequently, some researchers have suggested that the aminotransferase serum levels could be reduced even during conservative treatment at earlier stages of CKD prior to hemodialysis treatment [25, 30]. In contrast to our findings, studies of Sette et al. [27], and Fabrizi et al. [25], have found a significant inverse relationship of AST and ALT levels with serum creatinine, and a direct relationship with eGFR, observing the declining of aminotransferase levels in proportion to CKD progression.

On the contrary, alkaline phosphatase concentration was significantly higher in both groups of CKD patients than controls, and the difference between hemodialyzed CKD patients and predialyzed CKD patients was statistically significant. Moreover, a significant negative correlation has been shown between blood urea and aspartate aminotransferase levels in CKD predialyzed group. On the other hand, no significant correlation has been observed between aminotransferases and duration of disease, duration of hemodialysis, eGFR, or serum creatinine.

Kovesdy and his colleagues [18], concluded that serum ALP is an important parameter in the assessment of liver function and aids in diagnosing the type of jaundice in patients without CKD. However, this diagnostic importance of ALP is masked in CKD patients, as it is a well-recognized fact that serum ALP level increases in patients with CKD. In fact, in CKD patients without liver disease serum ALP can be elevated in high-turnover bone disease [31, 32]. In spite of this knowledge, we included ALP in our study to reiterate the non-specificity of serum ALP in CKD, further underlining the importance of correct interpretation of serum AST and ALT levels in CKD patients, more so in patients with CKD on HD. Moreover, higher levels of serum ALP are associated with increased mortality in CKD patients [18, 33].

In most normal individuals, approximately 95% of the total ALP activity is derived from bone and liver sources in 1:1 ratio [34]. Polyacrylamide gel electrophoresis is the most reliable method of determination of the tissue origin of ALP. Bone derived ALP is heatlabile whereas liver-derived ALP is not. Therefore, exposure of the serum to elevated temperatures can determine the tissue source of elevated levels of ALP. Since neither polyacrylamide gel electrophoresis nor heat-testing of serum ALP are routinely used in a clinical laboratory setting, determination of the tissue source of high ALP in CKD patients with suspected hepatobiliary dysfunction is difficult [24].

The major limitations of the present study included small sample size, reference intervals variations as no studies were done to establish reference intervals in our population, undetermination of serum pyridoxine, homocysteine, and hematocit in both hemodialyzed and predialyzed CKD patients, the impact of Age, weight, underlying causes of CKD, and drugs on aminotransferase levels was not studied.

Conclusion: serum ALT and AST levels tend to be reduced in CKD patients, particularly in those on maintenance hemodialysis treatment. Accordingly, the usage of standard normal intervals of transaminases to help detect liver disease becomes less beneficial in hemodialysis patients. These findings enforce the urgent need for establishing lower "standard normal" reference values of liver function tests in CKD patients in order to facilitate the diagnosis, monitoring, and treatment of liver diseases, especially hepatitis C infection, an establishment that may play a role in decreasing the mortality in CKD patients.

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