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A Comparative Study of Alkaline Phosphatase Levels in the Serum of Patients with End-Stage Renal Disease, and Viral Hepatitis At Dhi Qar, Iraq

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ABSTRACT

In recent years, the extent of liver damage by hemodialysis with hepatitis C is critical interest and debate. In this research, Patients on hemodialysis and those with normal renal function had their biochemical profile, liver histology, and symptoms of the hepatitis C virus examined and analyzed. Alkaline phosphatase enzyme (ALP), is linked to high levels of hepatitis and end-stage renal disease. In this study, patients with Hepatitis B virus (HBV) and thirty end-stage renal disease (ESRD) patients with Hepatitis C virus (HCV) were included. Gender, anticipated period of infection and age of infection were matched among ESRD patients. The aim of study is to measure and compare the serum levels of alanine aminotransferase (ALT), aspartate lipase (ALP), and aspartate aminotransferase (AST) in four groups: ESRD patients without hepatitis B, ESRD patients with hepatitis C, and healthy controls. The results demonstrated that hepatitis C patients with ESRD exhibited increases in their blood ALT and AST levels in contrast to hepatitis B patients with ESRD and the control group. Moreover, compared to chronic kidney disease (CKD) patients without hepatitis, individuals with ESRD had noticeably lower levels of these enzymes. The levels of Serum ALP were considerably greater in the hepatitis and ESRD groups than in the control group. Patients with HBV/HCV coinfection they often more vulnerable to liver-related morbidity and mortality compared to ESRD, HBV, or HCV patients.

Keywords: CKD, ESRD, ALP, AST, ALP, HCV, HBV

1 INTRODUCTION

Chronic kidney disease (CKD) is a disorders by an irregular glomerular filtration rate (GFR) and a gradual deterioration in kidney function [1,2]. Newly published recommendations from the National Kidney Foundation classify estimated glomerular filtration rate (eGFR) based chronic kidney disease into five distinct phases. When the eGFR is 90 mL/min/1.73 m^2 or above, together with other symptoms of renal impairment such abnormal blood, chronic proteinuria and urine chemistry, it is referred to as Stage 1. Stages 2, 3, and 4 are associated with eGFR values ranging from 60–89 mL/min/1.73m2, 30–59 mL/min/1.73m2, and 15–29 mL/min/1.73m2, respectively. The slow down the progression of CKD is the main goal of treatment throughout the four stages of CKD while simultaneously managing any complications that may occur, such as evaluating and treating the risk of cardiovascular disease. CKD stage 5 is characterised by an eGFR below 15 mL/min/1.73 m2. ESRD is a commonly used term for this condition. Death may occur due to the accumulation of electrolytes, fluids, and toxins, if renal replacement therapy, such as kidney transplantation or dialysis, is not administered at this stage [3,4].

Hepatitis B and C are the most common chronic liver illnesses among Patients with chronic liver disease (CKD) [5,6] that 1.4% tested positive for hepatitis B virus (HBV), whereas 5.9% tested positive for hepatitis C virus (HCV). 3.7% of the patients had dual infections(7,8). Different studies found that whereas the prevalence of HBV infection ranged from 1.3% to 14.6%, the prevalence of HCV infection among dialysis patients varied from 0.7% to 18.1% in various Asia-Pacific countries. The added burden

of alcoholic liver disease is on top of this. Due to the occurrence of concomitant liver diseases, persons with chronic kidney disease (CKD) generally need regular monitoring of their liver function, particularly the blood levels of liver enzymes [7,8].

Hepatitis C is the primary factor responsible for chronic liver damage in individuals suffering from end-stage renal disease (ESRD). The incidence of hepatitis C virus antibody (anti-HCV) is significantly elevated, ranging from 3.4% to 32.1%, among individuals undergoing hemodialysis (HD) treatment and those who are prospective candidates for kidney transplantation [9]. Although there has been a decline in the number of newly acquired HCV infections in recent years, this may be attributed to the effective serologic HCV testing used in blood transfusions and the use of erythropoietin to treat anaemia. However, it is important to note that HCV contamination still occurs in HD units [10,11].

2 MATERIALS AND METHODS

2.1 Study subjects and samples collection

Study Design: For this research effort, a case-control study was conducted with patients at Al Hussein Teaching Hospital. This research was conducted in the College of Dentistry, Dhi Qar University, Dhi Qar, Iraq. Thirty ESRD patients, thirty ESRD patients with hepatitis B, and thirty ESRD patients with hepatitis C in the 30- to 70-year-old age range were selected from Al Hussein Teaching Hospital after undergoing hospitalised health assessments. Forty controls in the same age range who had no prior history of CKD were also selected. Records of people with a history of liver illness and control subjects who did not take medications that alter liver enzyme levels were not included in the research. Each file yielded the following information: Serum values for ALT, AST, ALP, serum urea, serum creatinine, age, and sex.

Blood samples were centrifuged at 3000 rpm for 10 to 15 minutes in order to separate serum. The samples were held at -80 °C until they were needed for analysis. Following written informed permission and in accordance with the guidelines established by the university's scientific and ethical council, blood samples were taken from every patient. Using Proforma, comprehensive demographic, anthropometric, and other pertinent data was collected. Each participant had four millilitres of peripheral blood drawn, which was then examined to determine the aforementioned biochemical characteristics.

2.2 Kits

The kits that had been used in this study are shown in Table 1.

Table 1. Kits (Companies and Countries of Origin) used in the present study.

No	Kit	Company	Country
1	Urea kit	Biosystem	Spain
2	Creatinine kit	Biosystem	Spain
3	Alanine transaminase kit	Biosystem	Spain
4	Aspartate aminotransferase kit	Biosystem	Spain
5	Alkaline Phosphatase kit	Biosystem	Spain

2.3 Statistical analysis

Microsoft Office Excel 2013 and GraphPad Prism 9.2.0 were used to gather, analyse, and present the data. Numbers were used to represent categorical data, while mean Standard Error of Mean was used to convey numerical data. An unpaired t-test and a one-way ANOVA were used to compare the mean values across the different groups for variables that were regularly distributed. Chi-square analysis was performed on the qualitative data. Bivariate correlation was carried out using Pearson's correlation coefficient. When the P-value was less than 0.05, it was deemed significant.

3 RESULTS

The purpose of the research was to evaluate alkaline phosphatase levels in the serum of patients with end-stage renal disease and viral hepatitis. ESRD patients had assessments for urea, creatinine, alanine transaminase, aspartate aminotransferase, and Alkaline phosphatase. The results were contrasted with the values obtained for the control group (individuals in the same age range). In all, 30 ESRD patients, 30 ESRD patients with hepatitis B, 30 ESRD patients with hepatitis C, and 40 controls participated in the research. Gender-wise, there were male 10 (56%) and female 13 (44%) patients with ESRD, male 25 (83%) and female 5 (17%) patients ESRD with Hepatitis B, male 20 (67%) and female 10 (33%) patients ESRD with Hepatitis C, and male 20 (50%) and female 20 (50%) subjects out of 40 controls. Baseline characteristics of patients and controls are presented in Table 2

3.1 Biochemical analysis

3.1.1 Kidney function tests

There was a significantly higher ($P < 0.001$) increase in urea levels in both ESRD (50.76 ± 5.479), ESRD with Hepatitis B (39.26 ± 1.875), and ESRD with hepatitis B (36.81 ± 2.758) and as compared to controls (27.31 ± 2.015). There was also a significantly higher ($P < 0.001$) increase in creatinine levels in both ESRD with Hepatitis C (2.961 ± 0.7204), ESRD with Hepatitis B (2.705 ± 0.8374), and ESRD (2.105 ± 0.6124) and as compared to controls (0.6345 ± 0.4235).

Table 2. Baseline characteristics of T1DM patients and control group.

Baseline characteristics		Control	ESRD	ESRD with Hepatitis B	ESRD with Hepatitis C
		n=40	n=30	n=30	n=30
Age		49.92 (30-70)	51.31(30-70)	55.63 (30-70)	54.2(30-70)
Gender	Male	20 (50 %)	17 (56 %)	25 (83 %)	20 (67 %)
	Female	20 (50 %)	13 (44 %)	5 (17 %)	10 (33 %)
BMI (kg/m2)		22.21±2.17	28.22±2.12	23.21±2.17	24.24±5.34

Table 3. Comparison of mean values of the studied biomarkers among the control group and patients Liver Function Test Kidney function tests.

Characteristic	Control	ESRD	ESRD with Hepatitis B	ESRD with Hepatitis C	P value
	n=40	n=30	n=30	n=30	
Serum Urea levels (mg/dl)					
Range	25.06 - 33.6	42.1 - 58.6	37.06 - 43.6	23.6 - 41.2	<0.0001
Mean ± SD	27.31 ± 2.015	50.76 ± 5.479	39.26 ± 1.875	36.81 ± 2.758	***
Serum Creatinine levels (mg/dl)					
Range	0.191 - 1.904	1.199 - 3.004	1. 035 - 3.731	1.425 - 3.945	<0.0001
Mean ± SD	0.6345 ± 0.4235	2.105 ± 0.6124	2.705 ± 0.8374	2.961 ± 0.7204	***

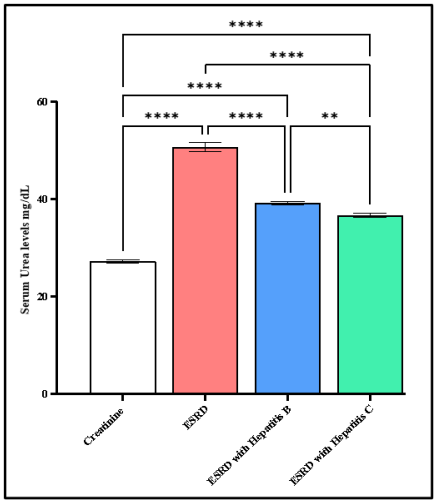

Fig. 1. Estimation of serum Urea levels (mg/dl).

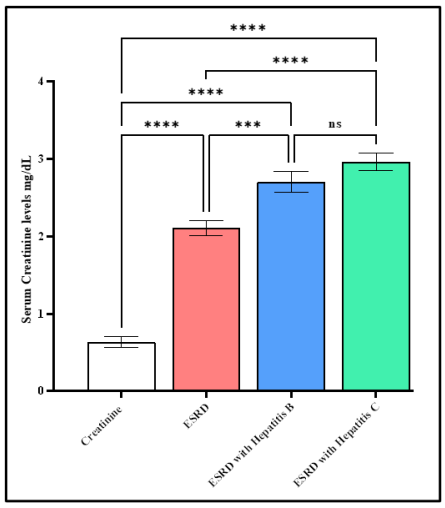

Fig. 2. Estimation of serum creatinine levels (mg/dl).

3.1.2 *Liver function test*

There was a significantly higher (P < 0.001) increase in serum alanine transaminase levels(U/L) in both ESRD with Hepatitis C (61.38 ± 6.242), ESRD with Hepatitis B (38.13 ± 6.242), and ESRD (35.15 ± 11.37) and as compared to controls (18.13 ± 6.242). There was a significantly higher (P < 0.001) increase in serum aspartate aminotransferase levels(U/L)in both ESRD with Hepatitis C (51.13 ± 6.242), ESRD with Hepatitis B (49.85 ± 6.083), and ESRD (48.88 ± 11.4) and as compared to controls (21.85 ± 6.645). There was a significantly higher (P < 0.001) increase in serum aspartate aminotransferase levels(U/L)in both ESRD with Hepatitis B (208.2 ± 18.8), ESRD (204.2 ± 24.27), and ESRD with Hepatitis C (202.4 ± 15.1) and as compared to controls (83.07 ± 17.46).

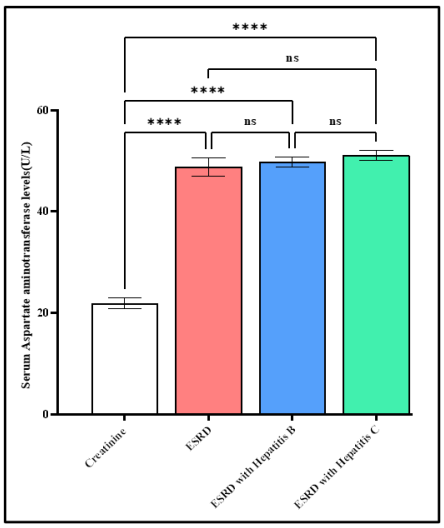

Fig. 3. Estimation of serum concentrations of Aspartate aminotransferase levels(U/L).

Table 4. Comparison of mean values of the Liver Function Test among the control group and patients.

Characteristic	Control	ESRD	ESRD with Hepatitis B	ESRD with Hepatitis C	P value
	n=40	n=30	n=30	n=30	
Serum Alanine transaminase levels(U/L)					
Range	11.3 - 31.8	22.48 - 64.62	31.3 - 51.8	54.55 - 75.05	<0.0001
Mean ± SD	18.13 ± 6.242	35.15 ± 11.37	38.13 ± 6.242	61.38 ± 6.242	***
Serum Aspartate aminotransferase levels(U/L)					
Range	13.92 - 35.08	35.58 - 77.69	42.77 - 63.79	44.3 - 64.8	<0.0001
Mean ± SD	21.85 ± 6.645	48.88 ± 11.4	49.85 ± 6.083	51.13 ± 6.242	***
Serum Alkaline Phosphatase levels(U/L)					
Range	40.29 - 100.7	179.9 - 303.2	182 - 244	181.8 - 243.9	<0.0001
Mean ± SD	83.07 ± 17.46	204.2 ± 24.27	208.2 ± 18.8	202.4 ± 15.1	***

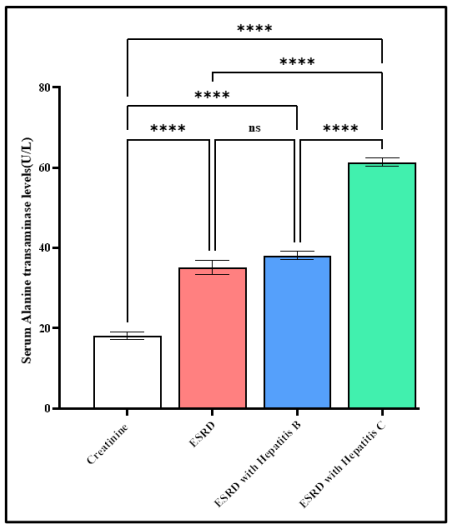

Fig. 4. Estimation of serum concentrations of Alanine transaminase levels(U/L).

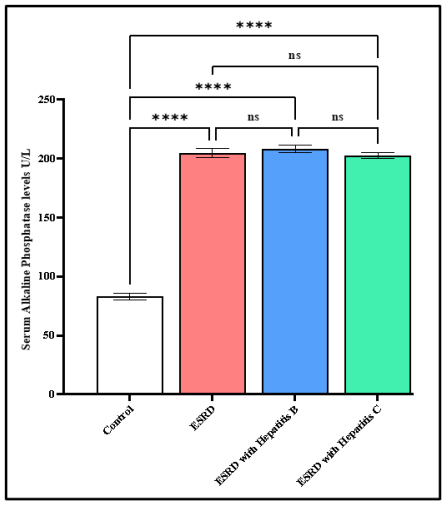

Fig. 5. Estimation of serum concentrations of Alkaline Phosphatase levels(U/L).

4 DISCUSSION

Patients with chronic renal disease represent a significant subset of patients with ongoing co-morbidities who need ongoing laboratory testing. Depending on the various phases of CKD, there are notable variations in the biochemical environment of individuals within this group. Patients with chronic kidney disease (CKD) often have hepatic co-morbidities, including hepatitis B and hepatitis C [12–14]. In this case, liver function tests specifically, serum liver enzyme tests are crucial for both diagnosing and tracking the patients' liver impairment. In order to highlight the urgent need for new reference ranges of these enzymes in CKD patients, we conducted the study to determine whether there is any significant difference between serum AST, ALT, and ALP levels among four groups: ESRD with Hepatitis C, ESRD with Hepatitis B, CKD patients with ESRD, and healthy controls.

According to our research, ESRD patients with hepatitis C or hepatitis B had considerably higher blood AST and ALT levels than controls. In addition, CKD patients with ESRD had considerably lower levels of these two enzymes than ESRD with Hepatitis C and ESRD with Hepatitis B. Serum ALT levels are also lower in CKD patients than in those with good renal function, according to a number of recent investigations [15–17]. The findings of those studies likewise indicated a lower AST level in CKD patients compared to controls, despite the fact that fewer authors have addressed AST levels in patients with CKD [18,19].

Additionally, a research conducted in Italy showed that dialysis patients had lower levels of AST and ALT than predialysis patients with CKD, in addition to CKD patients having lower levels of aminotransferases than healthy people [20,21]. Our findings largely agree with those of the research that were previously cited. Still, only few research have examined AST and ALT levels together. To the best of our knowledge, only one study that from Italy has made the comparison of enzyme levels between CKD patients with and without ESRD and healthy controls, as we have done in our investigation [22].

Because of this, the current study which is the first of its sort in the iraqpopulation shows a comparable decline in serum aminotransferases as CKD severity rises. Addition-

Table 5. Comparison with the control group and other groups.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Serum Urea levels (mg/dl)				
Creatinine vs. ESRD	-23.45	-25.40 to -21.50	****	<0.0001
Creatinine vs. ESRD with Hepatitis B	-11.95	-13.90 to -9.998	****	<0.0001
Creatinine vs. ESRD with Hepatitis C	-9.5	-11.45 to -7.548	****	<0.0001
ESRD vs. ESRD with Hepatitis B	11.5	9.548 to 13.45	****	<0.0001
ESRD vs. ESRD with Hepatitis C	13.95	12.00 to 15.90	****	<0.0001
ESRD with Hepatitis B vs. ESRD with Hepatitis C	2.45	0.4979 to 4.402	**	0.0074
Serum Creatinine levels (mg/dl)				
Creatinine vs. ESRD	-1.471	-1.858 to -1.084	****	<0.0001
Creatinine vs. ESRD with Hepatitis B	-2.071	-2.458 to -1.684	****	<0.0001
Creatinine vs. ESRD with Hepatitis C	-2.327	-2.714 to -1.940	****	<0.0001
ESRD vs. ESRD with Hepatitis B	-0.6	-0.9868 to -0.2132	***	0.0005
ESRD vs. ESRD with Hepatitis C	-0.856	-1.243 to -0.4692	****	<0.0001
ESRD with Hepatitis B vs. ESRD with Hepatitis C	-0.256	-0.6428 to 0.1308	ns	0.3174
Serum Alanine transaminase levels(U/L)				
Creatinine vs. ESRD	-17.02	-21.58 to -12.47	****	<0.0001
Creatinine vs. ESRD with Hepatitis B	-20	-24.56 to -15.44	****	<0.0001
Creatinine vs. ESRD with Hepatitis C	-43.25	-47.81 to -38.69	****	<0.0001
ESRD vs. ESRD with Hepatitis B	-2.975	-7.531 to 1.581	ns	0.3293
ESRD vs. ESRD with Hepatitis C	-26.23	-30.78 to -21.67	****	<0.0001
ESRD with Hepatitis B vs. ESRD with Hepatitis C	-23.25	-27.81 to -18.69	****	<0.0001
Serum Aspartate aminotransferase levels(U/L)				
Creatinine vs. ESRD	-27.03	-31.62 to -22.44	****	<0.0001
Creatinine vs. ESRD with Hepatitis B	-28.01	-32.60 to -23.41	****	<0.0001
Creatinine vs. ESRD with Hepatitis C	-29.28	-33.87 to -24.69	****	<0.0001
ESRD vs. ESRD with Hepatitis B	-0.9755	-5.567 to 3.616	ns	0.946
ESRD vs. ESRD with Hepatitis C	-2.253	-6.845 to 2.339	ns	0.5806
ESRD with Hepatitis B vs. ESRD with Hepatitis C	-1.278	-5.870 to 3.314	ns	0.8879
Serum Alkaline Phosphatase levels(U/L)				
Control vs. ESRD	-121.2	-132.3 to -110.0	****	<0.0001
Control vs. ESRD with Hepatitis B	-125.2	-136.3 to -114.0	****	<0.0001
Control vs. ESRD with Hepatitis C	-119.3	-130.4 to -108.1	****	<0.0001
ESRD vs. ESRD with Hepatitis B	-3.988	-15.14 to 7.164	ns	0.7895
ESRD vs. ESRD with Hepatitis C	1.88	-9.272 to 13.03	ns	0.9718
ESRD with Hepatitis B vs. ESRD with Hepatitis C	5.868	-5.284 to 17.02	ns	0.5222

ally, our research is unique in that it examined the serum aminotransferase levels of individuals with CKD who do not have ESRD. In this case, it is essential to establish new reference ranges for serum aminotransferases in patients with chronic kidney disease (CKD) with and without end-stage renal disease (ESRD) in order to avoid missing the diagnosis of hepatic dysfunction in CKD.

As shown in Table 2, the means of ALP level in all patient groups were higher than that of C group, and the highest mean (190.1 U/L) was recorded for the EBC group. Similar studies have also revealed that serum ALP level is higher in patients with ESRD [19], HBV [23] and HCV [24] compared to controls. Figure 5 shows that means of ALP level for both genders, in each patient group, were higher than the upper limit of ALP reference interval and the mean of ALP level in the control group, indicating that ALP level increased in all patient groups regardless of gender. Concerning the HB group, the reason for increasing ALP serum mean could be due to their unhealthy lifestyles, such as smoking, which are more common, thus it is possible that were more seropositive of HBeAg which serves as an indicator of HBV infection severity and this might have led to increasing the ALP level [25]. In the case of the HC group, it has been reported that vitamin D deficiency increases ALP level [26]; this could be the reason for increasing of ALP serum mean in this group, as vitamin D deficiency is more common among Libyan females compared to Libyan males [27, 28]. As age and gender were not found to independently affect ALP level mean, it was statistically safe to proceed to analyze the mean difference regarding group type and calculate the post hoc.

5 CONCLUSION

Therefore, our research supports the notion that serum aminotransferase levels are higher in Hepatitis C patients with ESRD and likely to stay lower in ESRD patients relative to the general population. Therefore, in individuals with end-stage renal disease (ESRD), a serum aminotransferase number within the current normal reference range does not rule out hepatobiliary pathology. Compared to ESRD, HBV, or HCV patients, patients with HBV/HCV

coinfection may be more susceptible to liver-related morbidity and death. Based on our findings, physicians may use ALP as a risk estimator for ESRD patients who co-occur with HB and HCV to identify those with the greatest mortality risk.

Conflict of Interest: The authors declare no conflict of interest.
Financing: The study was performed without external funding.
Ethical consideration: The study was approved by University of Thi-Qar, Thi Qar, Iraq.

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