International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 09 Issue 10 ǁ 2021 ǁ PP. 24-31

Utilization of MANOVA in investigation of the alteration of serum AST, ALT level and AST/ALT ratio among Libyan hepatitis B and C patients with or without ESRD

Hafsa A. Alemam[1], Abdounasser Albasher Omar[2]*, SamiaEmhemmed Abadi[3], Milud Ahmed Salem[4], Basher MansorZwali[2], Laila Elghawi[5], Abdulla Bashein[6]

[1]*Biotechnology Research Center, Tripoli, Libya.*
[2]*Department of Chemistry, Faculty of Science-Gharyan, University of Gharyan, Libya.*
[3]*Department of Statistics, Faculty of Science-Gharyan, University of Gharyan, Libya.*
[4]*Department of Zoology, Faculty of Science-Gharyan, University of Gharyan, Libya.*
[5]*Department of Genetic Engineering, School of Engineering and Applied Sciences, The Libyan Academy, Libya.*
[6]*Department of Biochemistry, Faculty of Medicine, University of Tripoli, Libya.*
**Email:abdounasseromar@yahoo.com*

Abstract
Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are among the common biomarkers which have been investigated in serum of patients suffering from end-stage renal disease (ESRD) and infections of hepatitis B virus (HBV) and hepatitis B virus (HBC). The present study aimed to statistically investigate whether serum levels of those enzymes and their ratio (AST/ALT) vary between hepatitis B and C patients with or without ESRD. Serum levels of AST and ALT were measured in 228 subjects of both genders, and those subjects were included in these groups: HBV and HCV patients, patients suffering from comorbidity of HBV/ESRD and HCV/ESRD as well as healthy subjects representing a control group. Means of serum level of ALT and AST for both genders in all patient groups were higher than that of control group and higher than the upper limit of ALT and AST reference intervals. MANOVA results revealed that group effect on the three variables, AST, ALT and AST/ALT, was significant ($p < 0.05$). The highest mean difference for ALT, AST and AST/ALT variables were, respectively, control and HBV/ESRD comorbidity groups; control and HCV/ESRD comorbidity groups; control and HBV infection groups. Moreover, it was found that the effect of the interactions between the three independent variables (group, age and gender) was not significant in all patient groups. Patients suffering from viral hepatitis and kidney failure disease will suffer from severe liver failure in an early time.

Keywords:*AST, ALT, ESRD, HCV, HBV, MANOVA.*

Date of Submission: 23-09-2021 Date of acceptance: 07-10-2021

I. INTRODUCTION

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are among the common biomarkers which have been investigated by researchers in serum of patients suffering from end-stage renal disease (ESRD) [1-4] and infections of hepatitis B virus (HBV) [5-7] and hepatitis C virus (HCV) [8-10].

ESRD is the last stage of chronic kidney disease (CKD) which is diagnosed by a persisting kidney failure for over 3 months or a decrease of glomerular filtration rate (GFR) to < 60 mL/min/1.73m2[11], and as kidney functions deteriorate more, GFR declines more till it reaches <15 mL/min/1.73 m2 in ESRD condition [12] at which patients should undergo kidney transplantation or dialysis due to total kidney failure [11]. The prevalence of dialysis in Libya, a North African country where this study was conducted, is 624 per million population (pmp) [12, 13] which is greater than in other neighboring countries [14]. In addition to that, the number of patients on dialysis in Libya was 2417 in 2009, and it is expected to increase to 7667 in 2024 [15].

Hepatitis B infection is caused by hepatitis B virus (HBV), which is a partially double-stranded DNA virus and belongs to the Hepadnaviridae family [16, 17], while hepatitis C is caused by hepatitis C virus (HCV), which is a single-stranded RNA and belongs to Flaviviridae family [18, 19]. The prevalence of HBV infection in Libya is 2.2% [20], which is a little higher than that of HCV infection (1.3%) and in both infections the prevalence in Libya is lower compared to neighboring countries [21].

AST and ALT are found mainly in liver [22, 23], and while AST is, also, present in appreciable levels in other organs such as kidneys and heart, ALT is present only in low concentrations in other organs such as

kidney [22]. Serum reference interval for ALT is 7–41 U/L, whilst for AST it is 12–38 U/L [2] and the alteration of their serum values are used mainly to monitor liver diseases including viral hepatitis. ALT and AST catalyze the transfer of an amino group from alanine or aspartate, respectively, to 2-ketoglutarate generating glutamate in both reactions in addition to pyruvate from alanine and oxaloacetate from aspartate [22, 24-26], and both of those products represent crucial contributors to Krebs cycle [22].

To the best of our knowledge, the alteration of AST and ALT serum levels has not been assessed in Libyan patients suffering from HCV and HBV infections, HBV/ESRD and HCV/ESRD comorbidities. Thus, the present study aimed to statistically investigate whether serum levels of those enzymes and their ratio (AST/ALT) vary between those patients. This was achieved by measuring AST and ALT serum levels in healthy people (control group) and patients suffering from the mentioned diseases, then the obtained data were statistically analyzed using descriptive statistics and multivariate analysis of variance (MANOVA).

II. MATERIALS AND METHODS

2.1 Subjects:

This study included 228 subjects of both genders and was conducted at Al-Zawia Educational Hospital and Ghadames General Hospital. Subjects were HBV and HCV patients, patients suffering from comorbidity of HBV/ESRD and HCV/ESRD as well as healthy subjects (Table 1) representing a control group who were selected on the basis that they were physically fit and free from any disease that may alter AST and ALT levels in serum.Oral consent was obtained from all participants after they were informed of the nature of the study.

Table (1): Number of participants in each group

Group	Total	Males	Females
Control group (C Group)	60	30	30
Hepatitis B infection group (HB group)	49	29	20
Hepatitis C infection group (HC group)	42	20	22
ESRD with Hepatitis B infection group (E-HB group)	35	20	15
ESRD with Hepatitis C infection group (E-HC group)	42	27	15

2.2Blood Samples Collection:

Ten mL of venous blood was withdrawn from all subjects (before dialysis for patients suffer from ESRD), then each sample was placed in a sterilized plain polystyrene tube. The tubes were incubated in a water bath at 37°C for 15 min and centrifuged for 10 min at 3000 rpm. Finally, the resulting serum of each sample was separated and analysed for AST and ALT levels.

2.3Analysis of AST and ALT:

AST and ALT levels in all samples were measured using kits purchased from Biolabo SAS, Maizy, France and the analysis was performed according to the kit instructions by using semi-automatic biochemistry analyzer Kenza Max BioChemistry (Biolabo Diagnostics, Kenza Biochemistry TM, France). The principle of both test is as follows: ALT catalyzes the reaction of L-Alanine and 2-oxoglutarate forming pyruvate and L-glutamate, then pyruvate is reduced to L-lactate by lactate dehydrogenase (LDH) with concurrent oxidation of reduced form of nicotinamide adenine dinucleotide to its oxidized form (NADH to NAD). The decrease in the absorbance, due to oxidation of NADH, is measured at 340 nm, and the measured value is proportional to ALT level. On the other hand, AST catalyzes the reaction of L-Aspartate and 2-oxoglutarate producing oxaloacetate and L-glutamate, then Oxaloacetate is reduced to L-malate by malate dehydrogenase (MDH) with concurrent oxidation of NADH to NAD. The rate of NADH oxidation is proportional to the AST activity in the specimen, which is measured at 340 nm [27].

2.4Statistical analyses:

Statistical analyses were performed using SPSS 25 (SPSS Inc., Chicago, IL, USA) and Minitab 17 (Minitab Inc., State College, Pennsylvania, US). The following tests were used: descriptive statistics including; maximum and minimum values (Max and Min), mean, standard deviation (StDev), coefficient of variation (CV%) to measure the relative dispersion; Shapiro-Wilk test (test of normality) and this test was considered significant if $p > 0.05$); multivariate analysis of variance (MANOVA) to study the effect of two or more independent variables (patient groups) measured (ordinal or nominal) on AST, ALT and AST/ALT variables; Levene's test for variance (significant if $p < 0.05$); Wilks' Lambda test to test whether there were statistically significant differences between two or more groups in two or more dependent variables, and this test was significant if $p < 0.05$); Least Significant Difference (LSD test) was applied to compare the significant differences between any two averages in the experiment when the value of F is significant, and this test is significant if $p < 0.05$.

III. RESULTS AND DISCUSSION

2.1 Descriptive statistics of age:

The mean of age, its standard deviation (StDev), its minimum (min) and maximum (max) values in all group are shown in table 2 as well as the number of subjects (N) and p-values (p) of normality test.Table (2) shows that C group had the lowest average and highest standard deviation compared to patient groups. Furthermore, Shapiro–Wilk test for normality showed that age follow normal distribution (significant at $p > 0.05$) in all groups.

Table (2): Descriptive statistics for age in all groups

Group	N	Min	Max	Mean	StDev	p
C	60	26	60	42.98	8.34	0.066
HB	49	39	59	48.41	5.22	0.051
HC	42	41	58	49.48	4.59	0.147
E-HB	35	43	58	49.37	4.09	0.236
E-HC	42	38	60	48.79	5.14	0.861

2.2Descriptive statistics of AST, ALT and AST/ALT variables:

Table (3) shows descriptive statistics of the three dependent variables (serum levels of AST and ALT and AST/ALT ratio) for the independent variables (control and patient groups). Means of AST/ALT ratio and serum level of both enzymes were greater in all patient groups compared to the control group. The orders were: for ALT serum levels: HB < HC < E-HC < E-HB; for AST serum levels: HB < HC < E-HB < E-HC; for AST/ALT ratio: E-HB < HC < E-HC < HB.

The rise of AST/ALT ratio and serum level of AST and ALT could be attributed to developing of liver cirrhosis among some patients, and/or of chronic viral hepatitis progress, which may have led to a change in the rate of removal of these enzymes from the circulation [28, 29].

Table (3): Descriptive statistics for serum values of ALT, AST and their ratio (AST/ALT) in all groups

Variable	Group	Min	Max	Mean	StDev	CV%	p*
ALT	C	10.06	25.31	17.03	3.93	23.09	0.143
	HB	58.99	90.67	73.98	8.21	11.10	0.067
	HC	78.45	99.92	88.31	4.91	5.56	0.880
	E-HB	83.56	98.09	92.21	3.16	3.43	0.125
	E-HC	86.74	98.12	91.06	2.89	3.17	0.000
AST	C	10.65	25.99	16.33	3.71	22.7	0.022
	HB	63.23	93.34	77.57	7.48	9.64	0.212
	HC	80.37	94.87	87.78	3.05	3.48	0.977
	E-HB	85.23	95.34	90.26	2.18	2.42	0.185
	E-HC	88.56	96.12	92.56	1.74	1.88	0.231
AST/ALT	C	0.56	1.26	0.97	0.17	16.94	0.010
	HB	0.94	1.14	1.05	0.04	3.58	0.004
	HC	0.93	1.08	0.99	0.04	3.64	0.177
	E-HB	0.95	1.02	0.98	0.02	1.87	0.005
	E-HC	0.96	1.05	1.02	0.02	2.18	0.001

* Significant at $P > 0.05$ level

Standard deviation (StDev) of ALT and AST variables were greater in HB group compared to other groups considering each dependent variable, indicating that serum levels of both enzymes were more spread for this group.

For coefficient of variation (CV%) of each dependent variable, the highest values were in C group in the order: ALT > AST > AST/ALT, reflecting that data sets of C groups were more dispersed (less homogenous) compared to patient groups. The less dispersed (most homogenous) data set was of AST/ALT variable for E-HB group, as the lowest CV% value (1.87%) was recorded for this group.

Furthermore, The Shapiro-Wilk test showed that all serum levels of ALT and AST follow normal distribution (significant at $p > 0.05$) with only one exception for both variables, which were E-HC and C groups, respectively. In the case of AST/ALT ratio, the results were dissimilar as all groups did not follow the normal distribution except HC group.

Table (4) shows the descriptive statistics of the three variables with regard to gender. Shapiro-Wilk test revealed that most data sets of the AST, ALT and AST/ALT variables follow the normal distributions as $p > 0.05$. While the means of each variable for both genders were close to each other in the control group, there were obvious differences of each variable for both genders in patient groups.

Table (4): Descriptive statistics for ALT, AST and AST/ALT variables with regard to gender.

Variable	Group	Gender	Min	Max	Mean	CV %	p*
ALT	C	Male	10.06	24.63	17.06	24.62	0.363
		Female	10.84	25.31	17.01	21.87	0.308
	HB	Male	58.99	89.01	73.07	12.16	0.153
		Female	63.39	90.67	75.31	9.49	0.209
	HC	Male	78.45	95.61	87.49	5.68	0.534
		Female	81.09	99.92	89.05	5.46	0.574
	E-HB	Male	88.34	95.81	91.89	2.43	0.345
		Female	83.56	98.09	92.64	4.47	0.240
	E-HC	Male	86.74	98.12	91.44	3.57	0.001
		Female	88.15	94.22	90.37	2.17	0.149
AST	C	Male	11.23	25.99	17.38	22.31	0.101
		Female	10.65	22.09	15.27	21.41	0.114
	HB	Male	63.23	93.34	76.43	11.34	0.172
		Female	72.34	88.08	79.20	6.41	0.034
	HC	Male	82.12	94.87	88.19	3.83	0.905
		Female	80.37	93.12	87.41	3.15	0.594
	E-HB	Male	87.45	93.12	89.91	1.59	0.263
		Female	85.23	95.34	90.73	3.19	0.783
	E-HC	Male	88.56	96.12	92.86	1.93	0.271
		Female	90.14	95.34	92.02	1.691	0.074
AST/ALT	C	Male	0.56	1.26	1.04	17.23	0.016
		Female	0.75	1.21	0.91	12.86	0.002
	HB	Male	1.01	1.11	1.05	2.11	0.168
		Female	0.94	1.14	1.06	5.03	0.063
	HC	Male	0.97	1.08	1.01	3.23	0.082
		Female	0.93	1.06	.983	3.58	0.423
	E-HB	Male	0.95	1.02	.979	1.72	0.049
		Female	0.95	1.02	.979	2.11	0.097
	E-HC	Male	0.96	1.05	1.02	2.43	0.003
		Female	0.98	1.05	1.02	1.69	0.237

* Significant at P > 0.05 level

In the case of ALT variable, the means of its serum level were lower in males than in females in all groups except for E-HC group. On the other hand, the means of AST variable were lower in males than in females in HB and E-HB groups. This may be due to several factors such as hepatocellular injury progressions due to infection with viral hepatitis, dialysis conditions, and possibly physiological failure in other organs such as hypertension, as a study conducted in China has indicated that ALT level was significantly associated with hypertension only in females [30]. This difference has also been observed between both genders in other studies [31, 32].

2.3Comparison with control group and reference interval:

As depicted in figure (1) and figure (2), means of serum level of ALT and AST for both genders in all patient groups were higher than that of C group, which was also the case for any individual value in all patient groups.

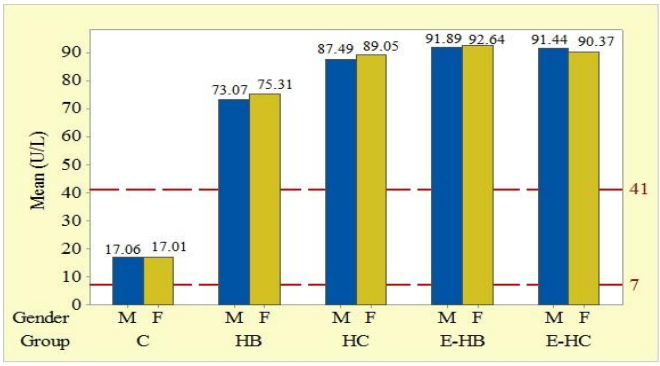

Figure (1): ALT level means in each group (males (M) and females (F)) and the upper and lower limits of ALT reference interval (the two horizontal dashed lines).

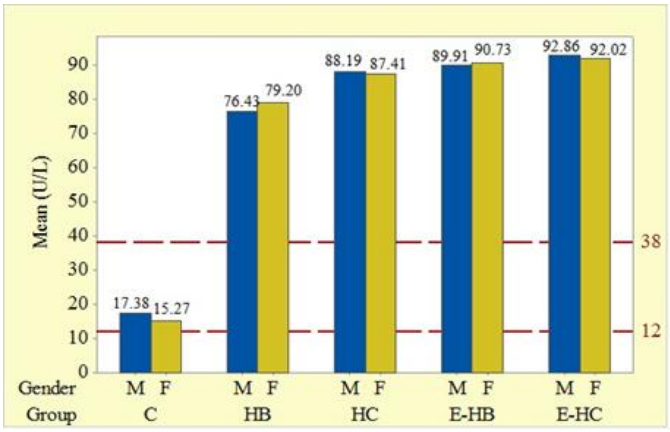

Figure (2): AST level means in each group (males (M) and females (F)) and the upper and lower limits of AST reference interval (the two horizontal dashed lines).

Compared to the reference interval, means of both enzyme levels for C group (both genders) were within the reference intervals, while enzyme level means in all patient groups (both genders) were higher than the upper limit of AST and ALT reference intervals, which was also the case for any individual value in all patient groups. This pointed out that serum level of ALT and AST increased in all patient groups regardless of gender. Since all groups of patients were infected with at least one of viral hepatitis, so it might be that all groups were in an ongoing liver disease process, resulting in elevated of serum AST and ALT levels among both genders [33].

2.3Multiple analysis of variance (MANOVA):

To achieve the main aim of this study which was to investigate the effect of the three independent variables (namely: age, gender, group) on ALT, AST and AST/ALT variables, multiple analysis of variance (MANOVA) was performed after converting age variable into ordinal variable. Although the results of homogeneity test (Levene's test) for ALT, AST and AST/ALT variables revealed that data of each variable was not homogeneous ($p < 0.05$), MANOVA was used because there is no an alternative nonparametric test. In addition to that, there are many studies which have proved that the results of analysis of variance does not change significantly when the data failed to meet its assumptions [34, 35].

Table (5): Wilk's Lambda multivariate tests

Effect	Value	F	p*
Group	0.019	157.488	0.000
Gender	0.983	1.206	0.318
Age	0.970	2.170	0.097
Group-gender	0.856	2.407	0.001
Group-age	0.981	0.455	0.912
Gender-age	0.999	0.050	0.985
Group-gender-age	0.986	0.485	0.819

* Significant at $P < 0.05$ level

From the data results of Wilk's Lambda test (table (5)), it was found that p-value was < 0.05 for group variable and for the interaction between group-gender variables. Therefore it was expected that each of these two variables had a significant effect on one or more of the dependent variables (ALT, AST and AST/ALT), which was confirmed from the results of tests of between subjects effect in table (6).

This study outcomes showed that group variable had a significant influence on the three dependent variables (ALT, AST and AST/ALT) ($p < 0.05$), indicating that disease type had significant effect on those variables. This finding was compatible with previous studies [36, 37]. Another finding was that age and gender variables, both individually, had no effect on the three dependent variables, which was compatible with a study of Esmaeelzadeh et al., [38] but in contradiction with a study performed by Mera et al. [39]. Also, it was found that the effect of the interactions between the three independent variables (group, age and gender) was significant ($p < 0.05$) only for group-gender interaction in AST/ALT dependent variable, while it was non-significant with respect to other interactions. Specifically, the mean difference of AST/ALT variable was significant ($p < 0.05$) only for C group, for which the mean in males was higher than in females. Elevation of AST/ALT ratio in males might be because of alcoholic liver disease, and muscle inflammation due to dermatomyositis [40].

Table (6): Tests of between subjects effect

	Source	F	p*
ALT	Group	1109.023	0.000
	Gender	0.002	0.847
	Age	2.008	0.134
	Group –gender	0.247	0.785
	Group-age	0.212	0.848
	Age - gender	0.020	0.888
	Group-gender-age	0.938	0.391
AST	Group	1708.785	0.000
	Gender	0.222	0.698
	Age	4.520	0.135
	Group-gender	0.900	0.511
	Group-age	0.748	0.500
	Age - gender	0.033	0.857
	Group-gender-age	1.160	0.316
AST/ALT	Group	5.013	0.001
	Gender	3.056	0.073
	Age	0.000	0.987
	Group –gender	4.175	0.003
	Group-age	0.149	0.934
	Age - gender	0.062	0.804
	Group-gender-age	0.074	0.929

* Significant at P < 0.05 level

In case of ALT serum level, results of post hoc comparisons between groups using the less significant difference (LSD test) are shown in table (7).Except for the mean difference of serum ALT levels between E-HB and E-HC, all mean differences between control and patients groups, as well as between patient groups to each other's, were significant ($p < 0.05$). The highest mean difference was between C and E-HB groups, where the mean difference was 75.18, indicating that ESRD/HBV comorbidity had the largest effect on ALT serum level.

Table (7): LSD test between groups in the ALT variable

(I) Group	(J) Group	Mean Difference (I-J)	p
C	HB	-56.95*	0.000
C	HC	-71.27*	0.000
C	E-HB	-75.18*	0.000
C	E-HC	-74.03*	0.000
HB	HC	-14.32*	0.000
HB	E-HB	-18.23*	0.000
HB	E-HC	-17.08*	0.000
HC	E-HB	-3.90*	0.001
HC	E-HC	-2.75*	0.013
E-HB	E-HC	1.1520	0.321

* Mean difference is significant at P < 0.05 level

For AST serum level, the LSD test (table (8)) showed that mean differences between C and patient groups were statistically significant ($p < 0.05$), with the highest difference was between C and group E-HC, where the mean difference was 76.23, suggesting that ESRD/HCV has the biggest effect on AST serum level. Because liver cirrhosis (LC) is an important problem in patients with ESRD [41], we predict that exacerbation of LC among patients with ESRD/viral hepatitis comorbidity led to rise of serum AST and ALT levels.

Table (8): LSD test between groups in the AST variable

(I) group	(J) group	Mean Difference (I-J)	p
C	HB	-61.24*	0.000
C	HC	-71.45*	0.000
C	E-HB	-73.93*	0.000
C	E-HC	-76.23*	0.000
HB	HC	-10.22*	0.000
HB	E-HB	-12.69*	0.000
HC	E-HB	-2.48*	0.011
HC	E-HC	-4.78*	0.000
E-HB	E-HC	-2.30*	0.018

* Mean difference is significant at p < 0.05 level

Table (9): LSD test between groups in the AST/ALT variable

(I) Group	(J) Group	Mean Difference (I-J)	p
C	HB	-0.0770*	0.000
C	HC	-0.0220	0.192
C	E-HB	-0.0049	0.785
C	E-HC	-0.0417*	0.014
HB	HC	0.0551*	0.002
HB	E-HB	0.0722*	0.000
HB	E-HC	0.0353*	0.045
HC	E-HB	0.0171	0.372
HC	E-HC	-0.0198	0.279
E-HB	E-HC	-0.0369	0.055

* Mean difference is significant at $p < 0.05$ level

The results of LSD test for AST/ALT ratio in table (9) showed that mean differences were not significant in all cases, and the highest significant difference was between HB group and C group, (mean difference = 0.0770). All mean differences between HB group and all other groups were significant as $p < 0.05$, reflecting that HBV infection has a different effect (higher AST/ALT ratio for HB group) on AST/ALT ratio compared to other diseases. AST/ALT ratios less than 1.0 are typical of chronic viral hepatitis, though ratios slightly more than 1.0 likely present in chronic viral hepatitis, especially when progression to fibrosis and cirrhosis is present [10, 28].

IV. CONCLUSION

Elevated liver AST and ALT result from inflammation of the liver or damage to its cells, as the affected liver cells cause the enzymes to be secreted into the bloodstream in larger quantities than usual, instead of using these enzymes in the vital functions assigned to them. Through this study, significant differences in the concentration of AST and ALT were observed between patient groups. Patients with more than one disease have higher levels of serum enzymes than the other cases and the amount of enzymes increased in patients with viral hepatitis and kidney failure disease more than in the case of patients with viral hepatitis only. Accordingly, patients suffering from viral hepatitis and kidney failure disease will suffer from severe liver failure in an early time. Further studies are needed to investigate other biochemicals such as Gamma-glutamyltransferase and bilirubin.

V. LIMITATION of the STUDY

This study did not include a group of patients who suffers only from ESRD. AST and ALT levels in such a group might have provided more useful information if it was included.

ACKNOWLEDGMENT

We would like to thank all the subjects who agreed to participate in this study.

REFERENCES

[1]. Sette LH, Lopes EP. The reduction of serum aminotransferase levels is proportional to the decline of the glomerular filtration rate in patients with chronic kidney disease. Clinics. 2015 May;70(5):346-9.
[2]. Ray L, Nanda SK, Chatterjee A, Sarangi R, Ganguly S. A comparative study of serum aminotransferases in chronic kidney disease with and without end-stage renal disease: Need for new reference ranges. International Journal of Applied and Basic Medical Research. 2015 Jan;5(1):31.
[3]. Liberato IR, Lopes EP, Cavalcante MA, Pinto TC, Moura IF, LoureiroJúnior L. Liver enzymes in patients with chronic kidney disease undergoing peritoneal dialysis and hemodialysis. Clinics. 2012;67(2):131-4.
[4]. Yasuda K, Okuda K, Endo N, Ishiwatari Y, Ikeda R, Hayashi H, Yokozeki K, Kobayashi S, Irie Y. Hypoaminotransferasemia in patients undergoing long-term hemodialysis: clinical and biochemical appraisal. Gastroenterology. 1995 Oct 1;109(4):1295-300.
[5]. Zeng LY, Lian JS, Chen JY, Jia HY, Zhang YM, Xiang DR, Yu L, Hu JH, Lu YF, Zheng L, Li LJ. Hepatitis B surface antigen levels during natural history of chronic hepatitis B: a Chinese perspective study. World Journal of Gastroenterology: WJG. 2014 Jul 21;20(27):9178.
[6]. Lin CS, Chang CS, Yang SS, Yeh HZ, Lin CW. Retrospective evaluation of serum markers APRI and AST/ALT for assessing liver fibrosis and cirrhosis in chronic hepatitis B and C patients with hepatocellular carcinoma. Internal Medicine. 2008;47(7):569-75.
[7]. Abdo AA, Al-Jarallah BM, Sanai FM, Hersi AS, Al-Swat K, Azzam NA, Al-Dukhayil M, Al-Maarik A, Al-Faleh FZ. Hepatitis B genotypes: relation to clinical outcome in patients with chronic hepatitis B in Saudi Arabia. World Journal of Gastroenterology: WJG. 2006 Nov 21;12(43):7019.
[8]. Anderson FH. An assessment of the clinical utility of serum ALT and AST in chronic hepatitis C./Anderson, FH, Lecheng, Z., Rock, NR and Yoshida. EM//Hepatology Research. 2000;18:63-71.
[9]. Assy N, Minuk GY. Serum aspartate but not alanine aminotransferase levels help to predict the histological features of chronic hepatitis C viral infections in adults. The American journal of gastroenterology. 2000 Jun 1;95(6):1545-50.

[10]. Park GJ, Lin BP, Ngu MC, Jones DB, Katelaris PH. Aspartate aminotransferase: alanine aminotransferase ratio in chronic hepatitis C infection: is it a useful predictor of cirrhosis?. Journal of gastroenterology and hepatology. 2000 Apr;15(4):386-90.

[11]. Omar AA, Salem N, Sagar S, Hamid A, Diab H, Alnnas MM. Abnormal Serum Concentrations of Creatinine, Phosphorous, Triglycerides and Total Cholesterol in Males with End-Stage Renal Disease: A Comparative Study. Libyan Journal of Medical Research, 1(12).

[12]. Omar AA, Aboud RR, Albakoush W, Anwesre RA. Effect of ESRD on concentration of serum creatinine, urea and glucose in male patients. MAYFEB Journal of Chemistry and Chemical Engineering. 2016 Aug 16;1.

[13]. Alemam H, Omar A, Abadi S, Salem M, Elghawi L, Bashein A. A comparative study of alkaline phosphatase level in serum of patients with end-stage renal disease, viral hepatitis (C), and (B). The 4th Annual conference on theories and applications of basic and biosciences. 2020; 291-298.

[14]. Goleg FA, Kong NC, Sahathevan R. Dialysis-treated end-stage kidney disease in Libya: epidemiology and risk factors. International urology and nephrology. 2014 Aug 1;46(8):1581-7.

[15]. Akkari KB. Projecting Requirements for End Stage Renal Disease Services in Libya 2014-2024. Ibnosina Journal of Medicine & Biomedical Sciences. 2013 Nov 1;5(6).

[16]. Salem MA, Elnifro EM, Alshuwen F. Molecular analysis of hepatitis B virus isolates in Libya: predominant circulation of hepatitis B virus genotype D. Journal of gastroenterology and hepatology Research. 2012 Aug 21;1(7):119-21.

[17]. Liang TJ. Hepatitis B: the virus and disease. Hepatology. 2009 May;49(S5):S13-21.

[18]. Dubuisson J, Cosset FL. Virology and cell biology of the hepatitis C virus life cycle–An update. Journal of hepatology. 2014 Nov 1;61(1):S3-13.

[19]. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nature Reviews Immunology. 2005 Mar;5(3):215-29.

[20]. Alawaini KA, Omar NA, Alhush HS, Alazabi EA. Carried hepatitis B among Libyan patients who attended the Al Zawia street hospital. GSC Biological and Pharmaceutical Sciences. 2020;13(1):062-6.

[21]. Elzouki AN, Smeo MN, Sammud M, Elahmer O, Daw M, Furarah A, Abudher A, Mohamed MK. Prevalence of hepatitis B and C virus infections and their related risk factors in Libya: a national seroepidemiological survey. EMHJ-Eastern Mediterranean Health Journal, 19 (7), 589-599, 2013. 2013.

[22]. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. Cmaj. 2005 Feb 1;172(3):367-79.

[23]. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. New England Journal of Medicine. 2000 Apr 27;342(17):1266-71.

[24]. McGill MR. The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI journal. 2016;15:817.

[25]. Nsiah K, Dzogbefia VP, Ansong D, Akoto AO, Boateng H, Ocloo D. Pattern of AST and ALT changes in relation to hemolysis in sickle cell disease. Clinical medicine. Blood disorders. 2011 Jan;4:CMBD-S3969.

[26]. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008 Mar 20;245(3):194-205.

[27]. Wilkinson JH, Baron DN, Moss DW, Walker PG. Standardization of clinical enzyme assays: a reference method for aspartate and alanine transaminases. Journal of Clinical Pathology. 1972 Nov;25(11):940.

[28]. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. Gastroenterology.1988;95:734-9.

[29]. Sheth SG, Flamm SL, Gordon FD. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol. 1998 Jan;93(1):44-8.

[30]. Lei W, Yao H, Bin J, Miao L, Shanshan Y, Yiyan W, Jing Z, Yao Y, Jianhua W. Gender difference in the association between aminotransferase levels and hypertension in a Chinese elderly population. Medicine. 2017 May;96 (21):e6996.

[31]. Zechini B, Pasquazzi C, Aceti A. Correlation of serum aminotransferases with HCV RNA levels and histological findings in patients with chronic hepatitis C: the role of serum aspartate transaminase in the evaluation of disease progression. Eur J GastroenterolHepatol. 2004 Sep;16(9):891-896.

[32]. de Oliveira VO, Oliveira JP, de Franca EV, Brito HL, Nascimento TV, Franca A. Advaced Liver Injury in Patients with Chronic Hepatitis B and Viral Load Below 2,000 IU/mL. Rev. Inst. Med. Trop. Sao Paulo 2016;58:65.

[33]. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepathology. 2008 Jan;47(4):1363-1370.

[34]. Tan WY, Tabatabai MA. Some robust ANOVA procedures under heteroscedasticity and nonnormality. Communications in Statistics-Simulation and Computation. 1985 Jan 1;14(4):1007-26.

[35]. Babu GJ, Padmanabhan AR, Puri ML. Robust one-way ANOVA under possibly non-regular conditions. Biometrical Journal: Journal of Mathematical Methods in Biosciences. 1999 Jun;41(3):321-39.

[36]. Gan T, Cheng N, Ding J, Cheng Z, Zhang D, Li H, *et al*. Effects of hepatitis B virus infection, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase on prediabetes and diabetes mellitus: A cohort study. Annals of Hepatology. 2020 March;19(2): 197-203.

[37]. Fabrizi F, Lunghi G, Andrulli S, Pagliari B, Mangano S, Faranna P, Pagano A, Locatelli F. Influence of hepatitis C virus (HCV) viraemia upon serum aminotransferase activity in chronic dialysis patients. Nephrol Dial Transplant. 1997 Jul;12(7):1394-1398.

[38]. Esmaeelzadeh A, Saadatina H, Memar B, Amirmajdi EM, Ganji A, Goshayeshi, Meshkat Z, Pasdar A, Vosoughinia H, Farzanehfar M, Tehranian S, Ghaffarzadehgan K, Rajabzadeh F, Ahadi M. Evaluation of serum HBV viral load, transaminases and histological features in chronic HBeAg-negative hepatitis B patients. GastroenterolHepatol Bed Bench. 2017 Winter;10(1): 39-43.

[39]. Mera JR, Dickson B, Feldman M. Influence of Gender on the Ratio of Serum Aspartate Aminotransferase (AST) to Alanine Aminotransferase (ALT) in Patients with and Without Hyperbilirubinemia. Dig Dis Sci. 2008;53:799-802.

[40]. Moussavian SN, Becker RC, Piepmeyer JL, Mezey E, Bozian RC. Serum gamma-glutamyltranspeptidase and chronic alcoholism. Influence of alcohol ingestion and liver disease. Dig Dis Sci. 1985 Mar; 30 (3): 211-214.

[41]. Kim AJ, Lim HJ, Ro H, Jung JY, Lee HH, Chung W, Chang JH. Liver cirrhosis leads to poorer survival in patients with end-stage renal disease. Korean J Intern Med. 2016;31(4):730-738.