

Synthesis of Three Unsymmetrical Bridged Terphthaloyl p-Substituted Acetophenone Oxime Esters

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Abstract: Unsymmetrical bridged terphthaloyl p-substituted acetophenone oxime esters 1-3 have been synthesized throughout an esterification reaction between three different para-substituted acetophenone oximes and the terphthaloyl chloride in a molar ratio of (2:1) under mild basic conditions. Spectroscopic techniques, such as IR, HNMR and mass spectrometer, were utilized to confirm the structures of these oxime esters. The yields of the synthesized oxime esters ranged from 70% to 89%. **Keywords:** unsymmetrical, bridged, para-substituted, oximes, esters, esterification, spectroscopic.

Introduction:

Oximes and their corresponding esters could be found in various bioactive molecules. Oxime derivatives show a wide range of activities, such as antimicrobial, antiinflammatory, antioxidant and cytotoxic activities [1]. Additionally, they have been involved as building blocks into the synthesis of photosensitive materials [2]. Oxime esters have significantly been considered as useful building units for obtaining a number of nitrogen containing compounds such as amines, amides, nitriles, aliphatic heterocycles and aromatic heterocyclic compounds like pyrroles, pyridines, quinolones, etc [1,2]. Transforming the carbonyl compounds into oximes has intensively been considered for years as an efficient method for both characterization and the purification of carbonyl compounds. Because of the nucleophilic character of oximes, they have widely been used for the preparation of several nitrogen containing molecules such as amides, nitrones and nitriles [1]. The organic synthetic significance of a number of functional groups is due to their ability to be converted into oximes [3]. The importance of the oxime esters comes from their wide range of useful activities such as anti-microbial, anti-inflammatory, fungicidal, antidepressant, antiulcer, analgesic, anti-HIV in addition to their involvement in the production of agrochemicals [1-6]. Oxime esters are interestingly useful for the photopolymerization of polymerizable molecules containing a C=C bond [7]. Herein, a synthetic approach towards the synthesis of three unsymmetrical bridged terphthaloyl acetophenone oxime esters is explained.

Experimental:

Materials:

The 4-methylacetophenone oxime, 4-aminoacetophenone oxime and 4-hydroxyacetophenone oxime were obtained by following a literature procedure [8]. Terphthaloyl chloride, anhydrous sodium sulphate, triethyl amine and chloroform. These chemicals were P. K. Park and used without further purification.



Instrumentation:

Melting points were measured on a Barnstead Electrothermal IA 9100. ¹HNMR spectrum was recorded on a JEOL ECA-300 II spectrometer. Residual proton signal from the deuteriated solvent was used as reference [DMSO (¹H, 2.50 ppm), whereas coupling constants were measured in hertz (Hz)]. Infrared spectrum was recorded on Jasco FT/IR-4100 Fourier transform infrared spectrometer. Mass spectrum was recorded on a Micromass Autospec M spectrometer.

Synthesis of 1-(p-aminoacetophenone imino)-4-(p-methylacetophenone imino) phenyl dicarboxylate 1:

An adapted literature procedure [8] was followed to synthesize the oxime ester 1. In a round-bottomed flask, a solution of terphthaloyl chloride (0.203 g, 1 mmol) in chloroform (50 cm³) was added dropwise to a solution of the 4-methylacetophenone oxime (0.149 g, 1 mmol) in chloroform (20 cm³) and in the presence of triethyl amine (0.252 g, 2.5 mmol) while stirring at 0 - 5 °C. The 4-aminoacetophenone oxime (0.150 g, 1 mmol) solution in chloroform (10 cm³) was then added dropwise. The reaction mixture was left stirring for 1 hour at 0-5 °C and then the reaction was stirred at room temperature for 2 hours. A distilled water (30 cm³) was added to the reaction mixture and stirred for further 10 min. The organic layer was extracted, dried over anhydrous Na₂SO₄ and filtered. The solvent was evaporated in vacuo to obtain the desired oxime ester 1 in a very good yield (0.323 g, 0.703 mmol, 70%) as an off-white solid. The product was recrystallized from diethyl ether. mp 143 °C, IR v_{max} (cm⁻¹) 3662 (NH₂), 1730 (2 × C=O, ester), 1600 (C=N), 1405 (C=N), 1521 (NO₂). ¹HNMR (DMSO-d6, 300 MHz) δ 7.96 (4H, d, J = 6.0, 4 × Ar-CH), 7.70 – 7.55 (4H, m, $4 \times Ar-CH$), 7.50 – 7.30 (4H, m, $4 \times Ar-CH$), 4.43 (2H, br s, NH₂), 2.37 (3H, s, CH₃), 2.29 (3H, s, CH₃), 1.64 (3H, s, CH₃). Mass spec m/z (C₂₅H₂₁N₃O₆, MWt 459.46) 459 (20%), 413 (19%), 368 (22%),149 (100%), 132 (17%), 118 (40%), 94 (38%).

Synthesis of 1-(p-hydroxyacetophenone imino)-4-(p-methylacetophenone imino) phenyl dicarboxylate 2:

An adapted literature procedure [8] was followed to synthesize the oxime ester **2**. In a round-bottomed flask, a solution of terphthaloyl chloride (0.203 g, 1 mmol) in chloroform (50 cm³) was added dropwise to a solution of the 4-methylacetophenone oxime (0.149 g, 1 mmol) in chloroform (20 cm³) and in the presence of triethyl amine (0.252 g, 2.5 mmol) while stirring at 0 - 5 °C. The 4-hydroxyacetophenone oxime (0.151 g, 1 mmol) solution in chloroform (10 cm³) was then added dropwise. The reaction mixture was left stirring for 1 hour at 0 - 5 °C and then the reaction was stirred at room temperature for 2 hours. A distilled water (30 cm³) was added to the reaction mixture and stirred for further 10 min. The organic layer was extracted, dried over anhydrous Na₂SO₄ and filtered. The solvent was evaporated in vacuo to

obtain the desired oxime ester **2** in a good yield (0.303 g, 0.704 mmol, 71%) as an off-white solid. The product was recrystallized from diethyl ether. mp 230 °C, IR v_{max} (cm⁻¹) 3675 (OH), 1741 (2 × C=O, ester), 1611 (C=N), 1405 (C=N). HNMR (DMSO-d6, 300 MHz) δ 8.90 (1H, s, OH), 8.02 – 7.75 (4H, m, 4 × Ar-CH), 7.60 – 7.40 (4H, m, 4 × Ar-CH), 7.37 – 7.25 (4H, m, 4 × Ar-CH), 2.35 (3H, s, CH₃), 2.30 (3H, s, CH₃), 1.50 (3H, s, CH₃). Mass spec m/z (C₂₅H₂₂N₂O₅, MWt 430.46) 430 (15%), 413 (10%), 282 (70%),148 (90%), 118 (20%), 104 (100%), 93 (25%), 91 (16%).

Synthesis of 1-(p-aminoacetophenone imino)-4-(p-hydroxyacetophenone imino) phenyl dicarboxylate 3:

An adapted literature procedure [8] was followed to synthesize the oxime ester 3. In a round-bottomed flask, a solution of terphthaloyl chloride (0.203 g, 1 mmol) in chloroform (50 cm³) was added dropwise to a solution of the 4-hydroxyacetophenone oxime (0.151 g, 1 mmol) in chloroform (20 cm³) and in the presence of triethyl amine (0.252 g, 2.5 mmol) while stirring at 0-5 °C. The 4-aminoacetophenone oxime (0.150 g, 1 mmol) solution in chloroform (10 cm³) was then added dropwise. The reaction mixture was left stirring for 1 hour at 0-5 °C and then the reaction was stirred at room temperature for 2 hours. A distilled water (30 cm³) was added to the reaction mixture and stirred for further 10 min. The organic layer was extracted, dried over anhydrous Na₂SO₄ and filtered. The solvent was evaporated in vacuo to obtain the desired oxime ester 3 in an excellent yield (0.384 g, 0.890 mmol, 89%) as an off-white solid. The product was recrystallized from diethyl ether. mp 156 °C, IR v_{max} (cm⁻¹) 3500 (OH), 3300 (NH₂), 1731 (2 × C=O, ester), 1600 (C=N), 1400 (C=N). ¹HNMR (DMSO-d6, 300 MHz) δ 9.22 (1H, s, OH), 7.98 (2H, d, J = 6.0, 2 × Ar-CH), 7.89 (2H, d, J = 6.0, 2 × Ar-CH), 7.70 (2H, d, J = 6.0, 2 × Ar-CH), 7.50 (2H, d, J = 6.0, $2 \times Ar-CH$), 7.42 - 7.34 (4H, m, $4 \times Ar-CH$), 4.45 (2H, br s, NH_2), 2.25 $(3H, s, CH_3), 1.53 (3H, s, CH_3).$ Mass spec m/z $(C_{24}H_{21}N_3O_5, MWt 431.45) 431$ (11%), 298 (7%), 282 (43%), 150 (26%), 149 (100%), 93 (13%).

Results and Discussion:

The 4-methylacetophenone oxime and 4-aminoacetophenone oxime or 4-hydroxyacetophenone oxime were reacted with the terphthaloyl chloride in the ratio of (2:1 mole/mole) under mild basic conditions at 0 °C to room temperature. The oxime esters 1 and 2 were obtained in good yields as an off-white solids. Similar approach was followed when 4-aminoacetophenone oxime and 4-hydroxyacetophenone were reacted with the terphthaloyl chloride in the ratio of (2:1 mole/mole) under the same conditions, the oxime ester 3 was resulted in an excellent yield as an off-white solid (Scheme 1).



Oxime: X and Y may be CH₃ or NH₂ or OH

Reagents & reaction conditions: (i) Et₃N, CHCl₃, 0 - 5 °C, 30 min, then rt, 2 hrs

Scheme 1: synthesis of the unsymmetrical bridged terphthaloyl p-substituted acetophenone oxime esters 1-3

The IR data showed the presence of the imino group (C=N) in all obtained oxime esters 1-3 appearing as two rather weak sharp absorption bands. The ¹HNMR collected data of the resulting oxime esters 1-3 revealed the formation of these oxime esters as the expected chemical shifts for all protons appeared in the spectra. The mass spectrometer provided a further prove on the formation of the oxime esters 1-3. The molecular ion peaks for the obtained oxime esters 1-3 were observed at 459, 430 and 431 m/z along with other molecular fragments that were in an agreement with the expected theoretical fragmentation patterns.

Conclusion:

The synthetic approach of three unsymmetrical bridged terphthaloyl p-substituted acetophenone oxime esters has been illustrated. An esterification reaction between three p-substituted acetophenone oximes and the terphthaloyl chloride under mild basic conditions led to the production of the targeted three oxime esters 1 - 3. The yields of the obtained oxime esters were ranging from 70% to 89% respectively.

Acknowledgments:

Authors would like to thank Dr. Huda S. Elgobbi and Mr. Waleed A. Tanashi at the bioresearch office, Misurata University for their assistance throughout this research project.

References:

[1] Jadhav A., Gade E., Angarkhe B. and Durrani A. "An Efficient One Pot Synthesis Of Oxime By Classical Method" International Journal of Chemical and Physical Sciences, 2018, 7, 2319 – 6602.



- [2] Vessally E., Saeidian H., Hosseinia A., Edjlali L. and Bekhradnia A. "A Review on Synthetic Applications of Oxime Esters" Current Organic Chemistry, 2017, 21, 249 271.
- [3] Guo J., Jin T., Zhang S. and Li T. "TiO2/SO4²-: An Efficient and Convenient Catalyst for Preparation Of Aromatic Oximes" Green Chemistry, 2001, **3**, 193 –195.
- [4] Damljanovic' I., Vuckovic' M. and Rastko D. "A Simple Synthesis Of Oximes" Monatshefte fur Chemie, 2006, **137**, 301 305.
- [5] Harikrishna A., Kumar K. and Elias G. "Oxime Esters As Potential Pharmacological Agents— A Review" IJPCBS, 2017, 7, 128 137.
- [6] Bawa R. A. and Friwan M. M. "Synthesis and Antifungal Study of Some Acetophenone oximes and their terphthaloyl oxime esters" Academic Journal of Chemistry, 2019, 5, 96 101.
- [7] Komiyama Y., Oishi T., Ariyoshi T., Tamachi T., Ikaga T. and Sato N. "Oxime Ester Compound And Photopolymerization Initiator Containing Said Compound" U.S. Patent Application, 2019, **10**,189–847.
- [8] Bawa R. A. and Friwan M. M. "Synthesis of Some Acetophenone Oximes and Their Corresponding Bridged Terphthaloyl Oxime Esters" Academic Journal of Life Sciences, 2019, 5, 87 92.



A comparative study of alkaline phosphatase level in serum of patients with end-stage renal disease, viral hepatitis (C) and (B)

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Abstract:

Alkaline phosphatase (ALP) enzyme level, which is routinely measured at clinical laboratories, increases in end-stage renal disease (ESRD) and hepatitis patients. This study investigated the difference in ALP level among ESRD and hepatitis patients. ALP level was measured in sera of patients suffering from ESRD, HCV and HBV infections, as well as patients suffering from comorbidity of these diseases, then the obtained values of ALP level were statistically compared to a control group. The results of three-Way ANOVA revealed that the mean of ALP level increased significantly (P-value<0.05) in all types of diseases compared to the control group, with the highest increase in case of ESRD patients infected with Hepatitis B and C. Also, it was found that the interaction of group-gender significantly (P-value<0.05) altered ALP level in patients suffering from HCV or HBV infections, while the interaction of group-age, gender-age, group-gender-age were found not to significantly alter it. In conclusion, ESRD patients with HBV/HCV coinfection may have a higher risk of liver-related morbidity and mortality than ESRD or HBV or HCV patients.

Keywords: ALP, ESRD, HCV, HCB, three-way ANOVA, comparison.

Introduction

Measuring the level of alkaline phosphatase (ALP), a metal-containing enzyme, is one of the routine tests which are carried out at clinical laboratories to assess functions of body organs. ALP presents in most tissues, mainly in bones and liver [1] and it has an important role in bone mineralization by promoting the hydrolysis of phosphates at alkaline pH [2]. Its level may increase physiologically or pathologically. Physiological causes include pregnancy in third trimester, adolescents and benign familial, while pathological causes include primary biliary cirrhosis, primary sclerosing cholangitis, adult bile ductopenia, metastatic liver disease and bone disease [3]. On the other hand, ALP level may decrease in hypothyroidism, pernicious anaemia, zinc deficiency and congenital hypophosphatasia [1].

Chronic kidney disease (CKD) is defined as either a kidney damage lasts for over 3 months or a decline of glomerular filtration rate (GFR) to lower than 60 ml/min/1.73 m² [4]. Based on GFR value, CKD is divided into five stages and at the fifth stage, known as end-stage renal disease (ESRD), GFR decreases to lower than 15 ml/min/1.73 m² [5]. At this stage, the patient should undergo kidney transplantation or dialysis to compensate for losing kidney functions [4]. In Libya, a



developing North African country of approximately 6 million people, the prevalence of dialysis patients is 624 per million population (pmp) [6] which is higher than those reported for other Arab countries and the Mediterranean countries [7]. Moreover, it has been reported that the number of patients on dialysis in Libya is increasing, and that it reached 2417 in 2009, with estimations that it will rise to 7667 in 2024 [8]. Many diseases are associated with CKD including hepatitis B and C [9] which are caused by Hepatitis B virus (HBV) and Hepatitis C virus (HBC); respectively. Whereas Hepatitis B virus (HBV) is a partially double-stranded DNA virus and belongs to the Hepadnaviridae family [10, 11], hepatitis C virus (HBV) is a singlestranded RNA and belongs to Flaviviridae family [12, 13]. HCV infection could develop into chronic active hepatitis, cirrhosis, and hepatocellular carcinoma and it is associated with cryoglobulinemia and polyarteritis nodosa [14]. HCV is transmitted through blood product transfusion, haemodialysis, occupational injury, injection drugs use, organ transplantation, sexual transmission, and vertical transmission [15]. Complications of HBV infection include chronic hepatitis, cirrhosis and hepatocellular carcinoma [10]. It is transmitted through contact with body fluids of infected patients and the modes of transmission are perinatal, sexual and parenteral/percutaneous transmission [16]. The prevalence of HBV infection in Libya is 2.2%, while that of HCV infection is lower (1.3%). This makes Libya a country with a low prevalence of HCV and HVB infections compared to North African countries [17].

As it has been reported that ALP level in blood increases in ESRD [9] and viral hepatitis [18] patients, the present study aimed to statistically investigate if ALP level in serum varies between ESRD and viral hepatitis patients as well as patients suffering from comorbidity of these diseases. This was achieved by measuring ALP level in the serum of healthy people (control group) and patients suffering from the above-mentioned diseases, then the obtained data were statistically analysed using descriptive statistics and three-way ANOVA.

Material and Methods

Subjects:

This study was carried out at Al-Zawia Educational Hospital and Ghadames General Hospital from September 2018 to March 2019. Including the control group, the present study involved 323 subjects (males and females) aged 27-57 years. ESRD, HBV and HCV patients, as well as patients suffering from the comorbidity of these diseases, were diagnosed by physicians working at the above-mentioned hospitals. Healthy subjects were chosen taking into account that they are physically fit and do not suffer from any disease that may alter the level of the enzyme ALP. All participants were divided into seven groups as shown in Table 1.

Table (1) Number of participants in each group

Group	Total	Males	Females
Control group (C Group)	60	30	30
ESRD group (E Group)	40	20	20
Hepatitis B infection group (HB group)	49	29	20
Hepatitis C infection group (HC group)	42	20	22

ESRD with Hepatitis B and C infection group (EBC group)	55	35	20
ESRD with Hepatitis B infection group (EB group)	35	20	15
ESRD with Hepatitis C infection group (EC group)	42	27	15

Blood Samples Collection: Ten ml of blood was withdrawn from each participant after area sterilization, and each sample was placed in a clean, sterilized plain tube made of polystyrene. These tubes were then incubated in a water bath at 37°C for 15 min, then centrifuged for 10 min at 3000 rpm. The serum of each sample was transferred to 1.5 ml tubes. Then, ALP level was measured in the obtained serum.

Measurement of ALP level: ALP enzyme level was 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). Briefly, ALP enzyme exists in blood sample catalyses the hydrolysis of p-nitrophenyl phosphate into p-nitrophenol and phosphate, and p-nitrophenol formation rate, which is proportional to ALP level, is measured calorimetrically at 405 nm.

Statistical analysis: Statistical analysis was performed using SPSS 25 (SPSS Inc., Chicago, IL, USA) and Minitab 17 (Minitab Inc., State College, Pennsylvania, US).

RESULTS AND DISCUSSION

Descriptive statistics of Age:

This study included 6 patient groups and a control group which was comprised of healthy subjects. The mean of age, its standard deviation (StDev), its coefficient of variance (CV%), its minimum (min) and maximum (max) values in all groups are shown in table 1 as well as the number of subjects (N) and male/female ratio (M/F). StDev and CV% values of age in all groups were close to each other, in particular, patient groups to each other.

Table (1) Descriptive statistics of age for all groups.

Group	N	M/F	Mean	StDev	min	max	CV%
С	60	1	42.98	8.34	26	60	19.39
Е	40	1	48.35	5.531	37	57	11.44
НВ	49	1.45	48.41	5.337	39	59	10.78
НС	42	0.91	49.48	4.592	41	58	9.28
EB	35	1.33	49.37	4.095	43	58	8.29
EC	42	1.8	48.79	5.135	38	60	10.53
EBC	55	1.75	48.09	4.816	38	59	10.01

Descriptive statistics of ALP level:

Based on skewness (S) results of ALP level shown in table 2, the groups were divided into two sets. Skewness was negative in HB, HC, E and EC groups, suggesting that the distribution of ALP level in these groups was left-skewed, which implied that most values in these groups were higher than their means. On the other hand, the skewness was positive in C, EB and EBC groups, reflecting that the



distribution of ALP level of these groups was right-skewed and most values in these groups were lower than their means. Skewness results pointed out that the distribution of ALP level in E group was the closest to symmetry as it was the closest to zero, while that of HC group was the farthest from symmetry as it has the higher skewness value.

The kurtosis (K) for ALP level (table 2) was positive for HB, HC, EB, EC groups pointing out that distribution of those values was more peaked than the normal distribution, and most values of ALP level in these groups were close to each other. On the other hand, the kurtosis of ALP level for C, E and EBC groups was negative, suggesting that the distribution of ALP level in these groups was more spread than the ALP values in other groups, reflecting they were less close to each other.

Results of CV% in table 2 revealed ALP level values in C group were more variable than in the other groups as the highest CV% (14.4%) was recorded for this group, while ALP level values in EBC were less variable than the other groups (more homogenous) because the lowest CV% (0.8%) was recorded for this group.

Table (2) Descriptive statistics for ALF level in all groups.								
Group	N	Mean	StDev	Min	Max	S	K	%C.V
С	60	55.5	7.99	40.19	74.09	0.21	-0.71	14.4
Е	40	155.1	9.93	138.45	171.09	-0.07	-1.09	6.4
HB	49	172.5	8.55	148.95	189.23	-0.82	0.74	5
HC	42	185	7.77	158.92	192.63	-2.18	4.67	4.2
EB	35	189.6	2.13	185.22	195.01	0.18	0.87	1.1
EC	42	189.7	2.04	183.18	193.17	-0.76	1.32	1.1
EBC	55	190.1	1.58	187.19	193.37	0.36	-0.67	0.8

Table (2) Descriptive statistics for ALP level in all groups.

Comparison with control group and reference interval:

As shown in table 2, 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 EBC group. Similar studies have also revealed that serum ALP level is higher in patients with ESRD [19], HBV [20] and HCV [21] compared to controls. Compared to the reference interval of ALP (20-140 U/L), ALP level in all patient groups exhibited the same pattern. All of its values in patient groups (both genders) were higher than the upper limit of ALP reference interval.

Figure 1 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. Furthermore, figure 1 depicted that the mean of ALP level was higher in males than in females in E, EBC, EC and HB groups, while it was higher in females than in males in EB and HC groups, which resembles the case in the control group.

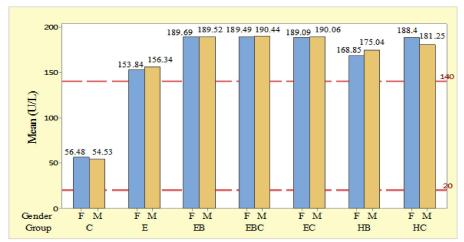


Figure (1) ALP level means in each group (males (M) and females (F)) and the upper and lower limit of ALP reference interval, the two horizontal dashed lines.

Three-way ANOVA:

To test the effect of group, age and gender factors on the ALP level, the parametric three-way ANOVA was performed after converting patients' age from scale variable into ordinal variable [22,23], and the results are shown in table 3.

Table (3) three-way ANOVA results of the effect of each factor and their interactions on ALP level.

	P-value	Partial Eta Squared
Group	0.000	0.979
Gender	0.808	0.000
Age	0.364	0.108
Group-gender	0.005	0.063
Group-age	0.274	0.041
Gender-age	0.260	0.009
Group-gender-age	0.483	0.022

In light of three-way ANOVA results, shown in table 3, it was found that the interactions between group-gender-age, group-age, gender-age were not found to significantly (P-value>0.05) alter ALP level, while group-gender interaction significantly (P-value<0.05) altered ALP level. In other words, only the group-gender interaction affected ALP level. To test the role of gender in each group, the Dunnett test was applied and the results are shown in table 4.

Table (4) Dunnett test results for gender role.

	Gender (I)	Gender (J)	Mean Difference (I-J)	P-value
C group	Female	Male	1.950	0.244
E group	Female	Male	-2.5	0.224
HB group	Female	Male	- 6.19	0.001
HC group	Female	Male	7.15	0.000
EB group	Female	Male	0.17	0.940
EC group	Female	Male	-0.97	0.644
EBC group	Female	Male	-0.95	0.601



Results of Dunnett test for gender role on ALP level revealed that the mean difference was significant (P-value<0.05) only in the case of HB and HC groups, while it was not significant in other groups. In HB group, the mean of ALP level in males was significantly higher than in females, whereas in HC group the mean of ALP level in females was significantly higher than in males.

Concerning HB group, the reason for increasing ALP serum mean in males more than in females could be due to their unhealthy lifestyles, such as smoking, which are more common in males than in females, thus it is possible that males were more seropositive of HBeAg that serves as an indicator of HBV infection severity and this might have led to increasing the ALP level [24].

In case of HC group, it has been reported that vitamin D deficiency increases ALP level [25]; this could be the reason for increasing of ALP serum mean in females than in males in this group as vitamin D deficiency is more common among Libyan females compared to Libyan males [26,27].

Three-Way ANOVA results (table 3) also revealed that the difference between ALP level means was significant (P-value<0.05) in case of group factor, and not significant (P-value>0.05) in case of gender or age factors (table 3) suggesting that these two factors did not significantly alter the ALP level in patient groups in contrast to group factor. The values of partial eta squared, which reflects effect size for each factor, was 0.979 in the case of the group factor indicating that 97.9% of ALP level change due to group type. Thus, all patient groups affected means of ALP level.

As age and gender were not found to independently affect ALP level mean, then it was statistically safe to proceed into analysing the mean difference regarding group type and to calculate the post hoc. This was done by performing Dunnett test [28] which its results are shown in table 5.

Table (5) results of Dunnett test for group role.

Group (I)	Group (J)	Mean Difference (I-J)	P-Value
E group	C group	99.58433	0.000
HB group	C group	117.00989	0.000
HC group	C group	129.49302	0.000
EB group	C group	134.09226	0.000
EC group	C group	134.21160	0.000
EBC group	C group	134.59247	0.000

Results of Dunnett test for group role revealed that the difference between ALP level means was significant (P-value<0.05) in all groups suggesting that each disease increased ALP level, with the highest increase in EBC group and the lowest increase in E group. And, according to the results of Dunnett test, the order of mean difference of ALP level between patient groups and control group was: EBC > EC > EB > HC > HB > E. This points out that comorbidity led to increasing ALP level, in particular in ESRD/HBV/HCV comorbidity. Patient groups with comorbidity in this study are expected to be at high threat for developing hepatocellular carcinoma [29].

CONCLUSION

ESRD patients with HBV/HCV coinfection may have an increased risk of liver-related morbidity and mortality than ESRD or HBV or HCV patients. Depending on our results, clinicians could use ALP as a risk estimation for ESRD patients with comorbidity of HB and HCV to recognize patients who are at the highest risk of mortality rate. Similar studies should be conducted in which other biochemicals are involved, such as alanine aminotransferase (ALT) enzyme and aspartate aminotransferase (AST) enzyme.

ACKNOWLEDGMENT

We would like to thank all the subjects who agreed to participate in this study after being informed of the nature of it.

REFERENCES

- 1- Thapa, B. R. and Walia, A. (2007) Liver function tests and their interpretation. The Indian Journal of Pediatrics, 74(7):663-71.
- 2- Sharma, U. Pal, D. and Prasad, R. (2014) Alkaline phosphatase: an overview. Indian Journal of Clinical Biochemistry, 29(3):269-78.
- 3- Limdi, J. K. and Hyde, G. M. (2003) Evaluation of abnormal liver function tests. Postgraduate medical journal, 79(932):307-12.
- 4- Omar, A. A. Salem, N. Sagar, S. Hamid, A. Diab, H. and Alnnas, M. M. (2018) 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).
- 5- Omar, A. A. Aboud, R. R. Albakoush, W. and Anwesre, R. A. (2016) Effect of ESRD on concentration of serum creatinine, urea and glucose in male patients. MAYFEB Journal of Chemistry and Chemical Engineering, 1:1-9.
- 6- Alashek, W. A. and McIntyre, C. W. and Taal, M. W. (2012) Epidemiology and aetiology of dialysis-treated end-stage kidney disease in Libya. BMC nephrology, 13(1):33.
- 7- Goleg, F. A. Kong, N. C. and Sahathevan, R. (2014) Dialysis-treated end-stage kidney disease in Libya: epidemiology and risk factors. International urology and nephrology, 46(8):1581-7.
- 8- Akkari, K. B. (2013) Projecting Requirements for End Stage Renal Disease Services in Libya 2014-2024. Ibnosina Journal of Medicine & Biomedical Sciences, 5(6):354-362.
- 9- Manju, M. Rajendran, S. Mishra, S. and Pavithra, M. (2019) Serum Liver and Pancreatic Enzymes in Chronic Kidney Disease with and without End-stage Renal Disease: A Comparative Study. Indian J Med Biochem. 23(1):207-212.
- 10-Liang, T. J. (2009) Hepatitis B: the virus and disease. Hepatology, 49(S5):S13-21.
- 11- Salem, M. A., Elnifro, E. M. and Alshuwen F. (2012) Molecular Analysis of Hepatitis B Virus Isolates in Libya: Predominant Circulation of Hepatitis B Virus Genotype D. Journal of Gastroenterology and Hepatology Research, 1(7):119-121
- 12-Dubuisson, J. and Cosset, F. L. (2014) Virology and cell biology of the hepatitis C virus life cycle–An update. Journal of hepatology, 61(1):S3-13.
- 13-Rehermann, B. and Nascimbeni, M. (2005) Immunology of hepatitis B virus and hepatitis C virus infection. Nature Reviews Immunology, 5(3):215-229.
- 14- Johnson, R. J. Gretch, D. R. Yamabe, H. Hart, J. Bacchi, C. E. Hartwell, P. Couser, W. G. Corey, L. Wener, M. H. Alpers. C. E. and Willson, R. (1993) Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. New England Journal of Medicine, 328(7):465-70.
- 15-Yen, T. Keeffe, E. B. and Ahmed, A. (2003) The epidemiology of hepatitis C virus infection. Journal of clinical gastroenterology, 36(1):47-53.
- 16-Hou, J. Liu, Z. and Gu, F. (2005) Epidemiology and prevention of hepatitis B virus infection. International journal of medical sciences, 2(1):50-57.



- 17- Elzouki, A. N. Smeo, M. N. Sammud, M. Elahmer, O. Daw, M. Furarah, A. Abudher, A. and Mohamed, M. K. (2013) Prevalence of hepatitis B and C virus infections and their related risk factors in Libya: a national seroepidemiological survey. East Mediterr Health J, 19(7):589-599
- 18-Hyder, M. A. Hasan, M. and Mohieldein, A. H. (2013) Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. European journal of experimental biology, 3(2):280-4.
- 19-Ray, L. Nanda, S. Chatterjee, A. Sarangi, R. Ganguly, S. (2015) A comparative study of serum aminotransferases in chronic kidney disease with and without end-stage renal disease: Need for new reference ranges. Int J App Basic Med Res, 5(1):31-35.
- 20-Al- Madany, N. A. and Sarhat, E. R. (2018) Determination of Some Biochemical Parameters of Patients with Hepatitis B in Kirkuk City. KUJSS, 13(2):139-148.
- 21- Yazdansetad, S. Nikoo, H. R. Azimi, S. M. Mohebbi, A. Niazi, M. and Ajorloo, M. (2019) Comparison of biomarkers between genotypes 1a and 3a in hepatitis C virus patients with control group. Biomed Res Ther, 6(4):3121-3130.
- 22- Babu G. J. Padmanabhan A. R. Puri M. L. (1999) Robust one-way ANOVA under possibly non-regular conditions. Biometrical Journal: Journal of Mathematical Methods in Biosciences, 41(3):321-39.
- 23-Tan W. Y. Tabatabai M. A. (1985) Some robust ANOVA procedures under heteroscedasticity and nonnormality. Communications in Statistics-Simulation and Computation, 14(4):1007-26.
- 24- Jia, J. Li, Y. Wei, C. Guo, R. Xu, H. Jia, Y. Wu, Y. Li, Y. Wei, Z. Qi, X. and Li, Z. (2019) Factors associated with disease progression and viral replication in patients with chronic hepatitis B virus infection. Exp Ther Med, 17(6):4730-4740.
- 25-Guo, G.Y. Shi, Y. Q. Wang, L. Ren, X. Han, Z.Y. Guo, C.C. Cui, L.N. Wang, J.B. Zhu, J. Wang, N. Zhang, J. Cai, Han, Y. X.-M. Zhou, Z.M. and Fan, D.M. (2015) Serum vitamin D level is associated with disease severity and response to ursodeoxycholic acid in primary biliary cirrhosis. Aliment Pharmacol Ther, 42(2):221–230.
- 26-Abumhdi, A.A. Azab, A. E. and Albasha, M.O. (2019) Evaluation of Vitamin D Status among Populations in Alejelat, Libya. East African Scholars J Med Sci, 2(11):666-673.
- 27-Omar, M. Nouh, F. Younis, M. Younis, M. Nabil, N. Saad, M. and Ali, M. (2017) Vitamin D Status and Contributing Factors in Patients Attending Three Polyclinics in Benghazi Libya. JAMMR, 24(5):1-13.
- 28-Lee, S. and Lee, D. K. (2018) What is the proper way to apply the multiple comparison test? Korean journal of anesthesiology, 71(5): 353-360.
- 29- Desikan, P. Rangnekar, A. Khan, Z. Panwalkar, N. Bosel, P. Gulwani, H. and Kaur, S. (2019) Sero-Occurrence of HBV/HCV Co-infection and Levels of Liver Enzymes among Patients at a Tertiary Care Hospital in Central India: a Pilot Study. CAJGH, 8(1):1-9.

مقارنة احصائية لمستوى انزيم الفوسفاتيز القلوي في مصل مرضى الفشل الكلوي المرحلة الأخيرة ومرض التهاب الكبد C-B

الملخص:

يتم قياس مستوى إنزيم الفوسفاتيز القلوي (ALP) بشكل روتيني في المختبرات، ومستوى هذا الإنزيم يزداد عند مرضى الفشل الكلوي المرحلة الأخيرة (ESRD) ومرضى إلتهاب الكبد. هذه الدراسة تقصت الفرق في مستوى ALP بين مرضى ESRD وإلتهاب الكبد. لقد تم قياس مستوى الإنزيم في مصل مرضى ESRD ومرضى إلتهابي الكبد B ، C ، B ومرضى يعانون من هذه الامراض معاً، وقد تم مقارنة القيم المتحصل عليها مع مثيلاتها التهابي الكبد B ، ALP قد إزداد بشكل في مجموعة الشاهد. أظهرت نتيجة إختبار تحليل التباين الثلاثي أن متوسط مستوى ASD قد إزداد بشكل معنوي في كل أنواع المرض مقارنة بمحموعة الشاهد، وكانت أكبر زيادة عند مرضى ESRD الذين يعانون في نفس الوقت من إلتهابي الكبد B و C. كما أظهرت النتائج أن التأثير المشترك لنوع المرض والجنس كان معنوياً عند مستوى معنوية أقل من 0.05، بينما كان التأثير غير معنوي في حالة التأثير المشترك لكل من: نوع المرض-العمر، الجنس-العمر، نوع المرض-الجنس-العمر. من خلال هذه الدراسة نستنتج أنه من المحتمل أن مرضى ESRD الذين يعانون في نفس الوقت من إلتهابي الكبد B ، C ، هم أكثر عرضة للوفاة ولامراض أخرى تتعلق بالكبد من المرضى الذبن يعانون فقط من مرض ESRD أو إلتهاب الكبد B أو إلتهاب الكبد C ، تحليل التباين الكلوي المرحلة الأخيرة، إلتهابي الكبد B و C، تحليل التباين الكلاثي، مقارنة