Biological Forum – An International Journal **11(1): 248-254(2019)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Impact of Serum Interleukin 6 (IL-6) Level of Patients with Acute & Chronic Hepatitis B Virus

Ali A. Dawood
Department of Anatomy,
College of Medicine, University of Mosul, Mosul, Iraq

(Corresponding author: Ali A. Dawood)
(Received 06 March 2019, Accepted 08 June, 2019)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Hepatitis B infection is a global health problem. Hepatitis B virus infects liver and causes inflammation reaching to hepatocellular carcinoma. The human interleukin 6 (IL-6) is a multifunctional cytokine that interfere in the regulation, maturation and differentiation of immune response. The aim of this study is to demonstrate the interleukin 6 (IL-6) and correlates with ALT and AST levels in patients serum with hepatitis B virus. We evaluate 58 patients presumably with HBV in acute and chronic cases whom have HBsAg positive. AST and ALT chemical serum levels were detected using kits of an automated chemical analyzer. IL-6 serum level was detected using ELISA technique. Correlation between parameters were done using One-ANOVA and Analyse-it statistical software. IL-6 serum level is raised with acute hepatitis B patients more than of chronic hepatitis B with high concentration absorption. The AST and ALT levels were elevated with AHB group more than CHB group. The correlation between IL-6 level and AST is correlated significant value but is not significant value with ALT level. There is no correlation efficient between age parameter and case type of hepatitis. Our data indicated that serum levels of IL-6 elevated with the increase of AST levels in patients with acute hepatitis B. The serum levels of IL-6, AST and ALT varied in different courses of acute and chronic hepatitis B infections. We surmised that IL-6 level might indicate liver injury of patients with acute hepatitis B infections.

Key words: AHB, CHB, IL-6, HBsAg, AST.

How to cite this article: Dawood, Ali A. (2019). Impact of serum Interleukin 6 (IL-6) level of patients with acute & chronic Hepatitis B virus. *Biological Forum – An International Journal*, **11**(1): 248-254.

INTRODUCTION

Hepatitis B infection is a global health problem which is around 50-100 times more infectious than HIV. Approximately 400 million people are carriers of chronic liver disease, every year due to consequences of the disease (Horvat, 2011). Hepatocellular injury is generally accepted to be the result of attacks from the immune system. The immune response to HBV infection is by both B and T-cell derived. Antibodies (anti-HBs, anti-HBc, and anti-HBe) are produced and targeted towards their antigens (Alestig, 2011). Cytokines are important chemical mediators synthesized and secreted from immune cells which described as anti-inflammatory, including the Th2 cytokines (IL-4, IL-6, IL-10, IL-13 and TGF-b) (Qiao-ling, 2004). The human interleukin 6 (IL-6) is a multifunctional cytokine that interfere in the regulation, maturation and differentiation of immune response (Gora-G, 2003, Min, 2014, Marzieh, 2016). IL-6 gene is located on chromosome 7 and is composed of five exons and four introns (Tian, 2015). Serum IL-6 levels are correlated with disease severity, so that IL-6 may be a useful as indicator of disease activity and therapeutic efficacy in patients with hepatitis B (Tian, 2015). Biochemical tests are used for initial assessment of liver disease include measuring levels of serum Alanine and Aspartate aminotransferases ALT(GPT) and AST(GOT).

AST and ALT levels are a valuable aid primarily in the diagnosis of such liver diseases (Zing-Jiu, 2006). The purpose of this study is to demonstrate the interleukin 6 (IL-6) and correlates with ALT and AST levels in patients serum with hepatitis B virus. This study was done among populations who were infected or under suspicion infected persons with hepatitis B virus in Mosul city. The samples were collected from May 2018 to November 2018. Informed consent was obtained from every patient prior to sample collection which was performed according to standard protocols approved by the local health authority in Mosul city. A (43) of samples collected from Central blood bank and (30) samples from Hemodialysis patients in Ibn–Alatheer Teaching Hospital. Their ages ranged from (5-64) years at the mean of (33.36). This study intended to study the expression of human inflammatory cytokine in acute and chronic hepatitis B patients related to the severity of the infection.

II. OBJECTIVE

Current study was aimed to determine the serum level of the most important interleukin (IL-6) in patients with an acute and chronic HBV infection in comparison with the levels of these cytokines in healthy controls and to determine the correlation between the IL-6 level with the chemical parameters ALT and AST level.

III. MATERIALS AND METHODS

Current study is an analytical cross-section trial involving two disease groups' acute flare and chronic hepatitis B with one healthy control group. The ages of patients and healthy control donors were 5-64 years old which grouped into 3 classes A=5-24 years, B=25-44 years and C=45-64 years. Patients were recruited consecutively from the Central blood bank and Ibn–Alatheer teaching hospital. Blood samples were obtained from (73) patients and healthy individuals. The serum was separated and stored in multiple marked clean tubes at (-20ºC).

Detect HBsAg using ELISA: Detection of HBsAg positive sensitive was done using enzyme linked immunosorbent assay (ELISA) commercial kit from (DIALAB- Austria) purchased from according to the manufacturer's instruction.

Detect AST, ALT levels: Biochemical liver tests included of alanine aminotransferase ALT and a spartate aminotransferase AST were determined using an automated chemical analyzerkits form (BIOLABO, S.A.S-France) according to the manufacturer's instruction. The levels of serum ALT and AST were detected by velocity method using big biochemistry automatic analyzer (Olympus 2700, Japan).

Detection of IL-6 level using ELISA: Serum concentrations of IL-6 were measured in duplicate using a commercial human IL-6 platinum enzyme-linked immunosorbent assay kit from (Komabioteh). The OD $_{450}$nm were determined using an ELISA reader (Awreness-USA). The cytokine standards were also prepared and the concentration of IL-6 (pg. /mL) was determined using the standard curve. Samples were divided into 3 groups, acute hepatitis (AHB-22), chronic hepatitis (CHB-36) and control (CL-15). According to the age, all samples separated into 3 groups, A (5-24 years), B (25-44 years) and C (45-64 years) respectively. ELISA positive results for patients' samples and negative results for the healthy control group samples were confirmed and the results were evaluated. The demographic data and clinical history of patients were gathered based on a questionnaire completed.

Statistical analysis: The analysis of data was made by using SPSSInc. Chicago, IL, USA software version16.0 software especially for the calculation of mean values, standard deviations (SD) and standard error (SE) for serological and biochemical parameters. One- way ANOVA test and Analyse-it software were used for homogeneity and correlation between parameters. The results were compared significant value at $P < 0.05$. Microsoft office excel version 2010 was used to explain the values graphically.

RESULTS

Patients with acute and chronic hepatitis B virus got positive results for HBsAg with ELISA test while normal control group got negative results. According to the case types, the mean, standard deviations and standard errors for all parameters were revealed in table 1. Depending on the age groups, mean, slandered deviations and standard errors for all parameters were revealed in Table 2.

Table 1: Number, mean, standard deviations and standard errors for all parameters with case groups.

		N	Mean	Std. Deviation	Std. Error
lL-6 (pg/ml)	Cl	15	30.0933	44.28419	11.43413
	CHB	36	1.9798E2	230.50000	38.41667
	AHB	22	2.1538E2	239.39766	51.03975
	Total	73	1.6873E2	219.13753	25.64811
AST U/L	Cl	15	29.2067	11.91988	3.07770
	CHB	36	34.1861	12.33152	2.05525
	AHB	22	44.6409	11.78904	2.51343
	Total	73	36.3137	13.26872	1.55299
ALT U/L	Cl	15	41.7533	9.64875	2.49130
	CHB	36	39.0250	12.39865	2.06644
	AHB	22	44.5455	10.29233	2.19433
	Total	73	41.2493	11.38305	1.33229
Age (years)	Cl	15	1.4000	.63246	.16330
	CHB	36	2.2222	.59094	.09849
	AHB	22	1.5909	.66613	.14202
	Total	73	1.8630	.71327	.08348

Table 2: Number, mean, standard deviations and standard errors for all parameters with age groups.

		N	Mean	Std. Deviation	Std. Error
IL-6 (pg/ml)	A	24	1.1985E2	198.54918	40.52868
	B	35	1.9077E2	231.01470	39.04861
	C	14	1.9740E2	223.68707	59.78288
	Total	73	1.6873E2	219.13753	25.64811
AST U/L	A	24	35.5167	14.95831	3.05335
	B	35	38.7314	11.53342	1.94950
	C	14	31.6357	13.81496	3.69220
	Total	73	36.3137	13.26872	1.55299
ALT U/L	A	24	40.1667	11.75391	2.39926
	B	35	44.3086	8.64701	1.46161
	C	14	35.4571	14.63445	3.91122
	Total	73	41.2493	11.38305	1.33229
Case	A	24	2.0417	.95458	.19485
	B	35	2.1429	.60112	.10161
	C	14	2.0714	.47463	.12685
	Total	73	2.0959	.71033	.08314

AST serum level: Depending on the case groups, some of control samples (CL) were elevated level of AST while most acute cases (AHB) were presented high elevation contrariwise of chronic cases (CHB) with significant value $p < 0.05$. On the other hand, according to age groups, AST levels showed no significant value with $p > 0.05$ (Fig. 1-A and C) respectively.

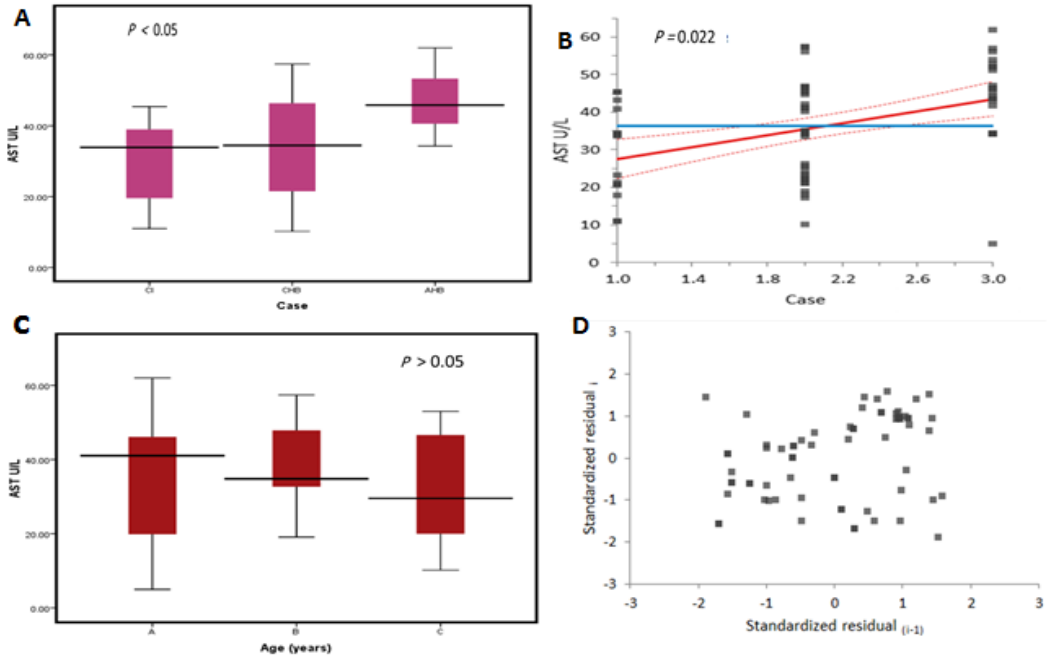

Fig. 1. Box-whisker-Line scattered plots. A: AST level in case groups (CI=control, AHB= acute hepatitis B, CHB= chronic hepatitis B); **B:** Correlation between AST level and case groups (A=5-24y, B= 25-44y, C= 45-64y)**; C:** AST level in age groups; **D:** Scatter scale of samples.

ALT serum level: Elevation of serum ALT level for most samples depending on the AHB and CL samples were more than that of CHB with no significant value $p > 0.05$. Moreover, there is a significant value $p < 0.05$ according to the age groups figure (2-A and C) respectively.

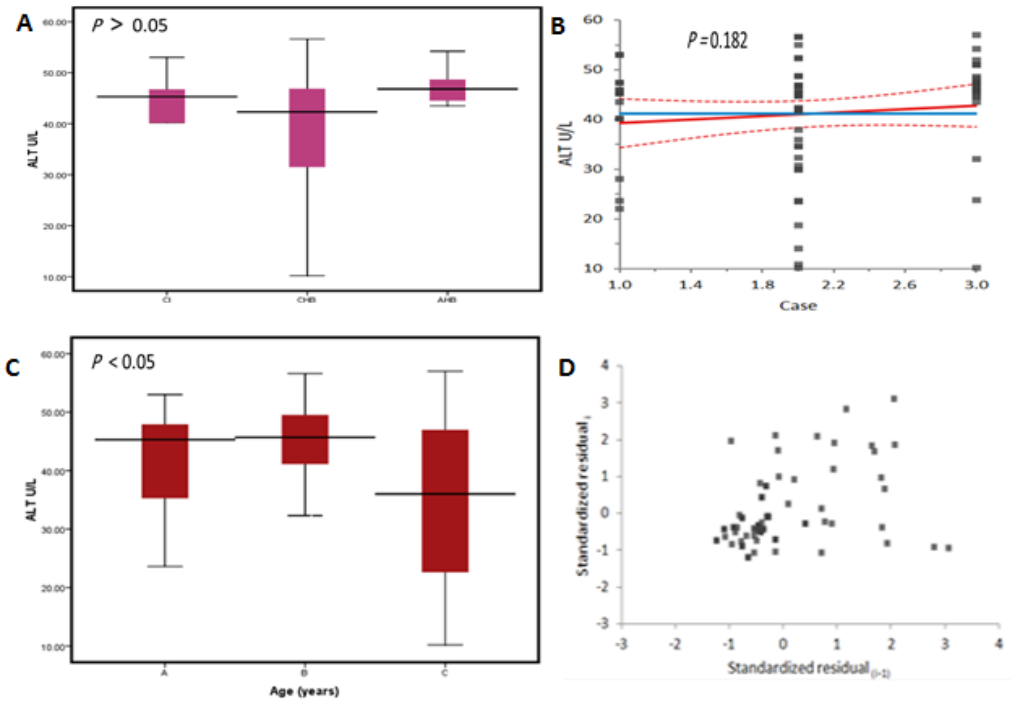

Fig. 2. Box-whisker-Line scattered plots. A: ALT level in case groups (CI=control, AHB= acute hepatitis B, CHB= chronic hepatitis B); **B:** Correlation between ALT and case groups; **C:** ALT level in age groups; **D:** Scatter scale of samples.

IL-6 serum level: AHB and CHB cases demonstrated elevation of serum IL-6 level though some control samples exhibited elevation of IL-6 with high significant value $p < 0.05$ (Fig. 3-A). IL-6 level was presented no significant value $p > 0.05$ according to the age groups (Fig. 3-C).

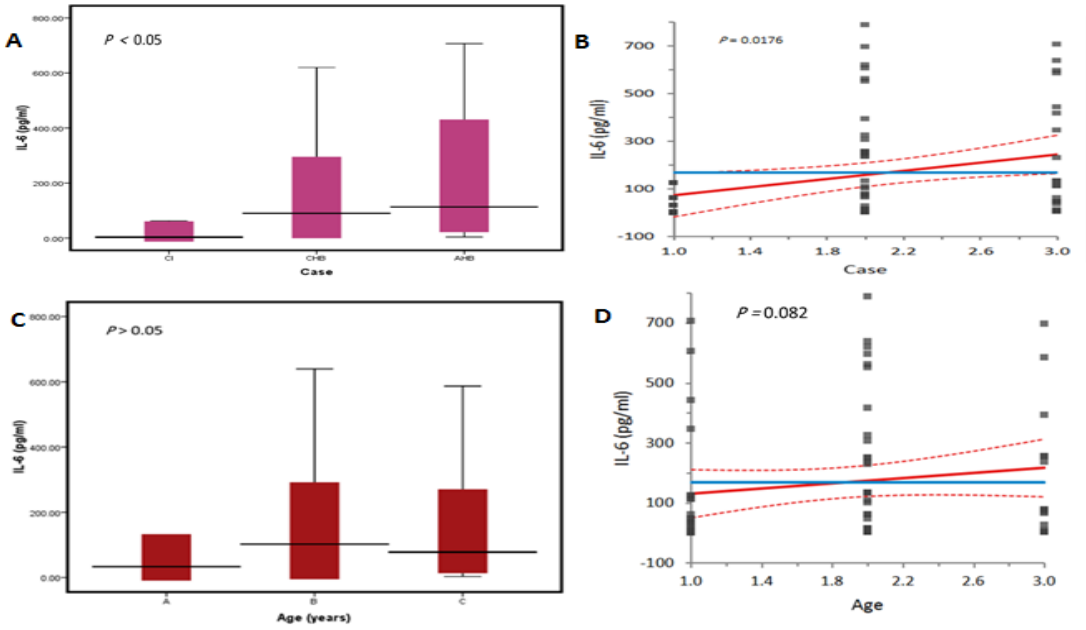

Fig. 3. Box-whisker-Line plots. A: IL-6 level in case groups (CI=control, AHB= acute hepatitis B, CHB= chronic hepatitis B); **B:** Correlation between IL-6 level and case groups**; C:** IL-6 level in age groups; **D:** Correlation between IL-6 level and age groups.

Current study was disclosed no significant value between age groups and cases groups with p = 0.242 that means there is no relative between age groups and case groups Fig. 4-A. Red and blue line is already at the same level and this meant no effect between fitted and null models Fig. 4-B.

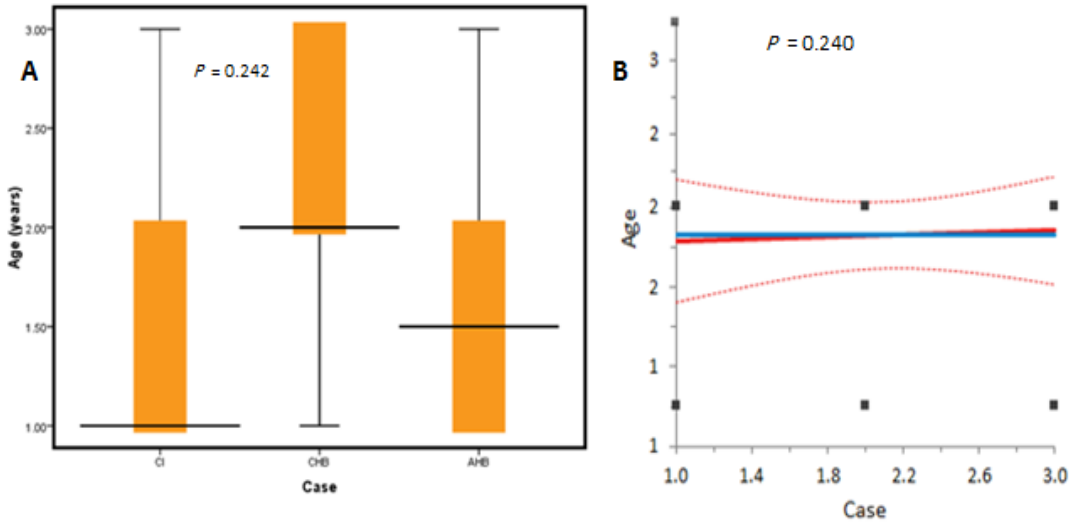

Fig. 4. Box-whisker-Line plots. A: Age groups in cases groups; **B:** Correlation between Age groups in case groups**.**

Correlation between parameters: Figure (5-A) is designated correlation between IL-6 level and ALT level which presented less noteworthy value with p = 0.0723. Dissemination of scattered dots nearby red line is elucidated the correlation between two parameters while the blue line is denoted null association. Fig. 5-B is described correlation IL-6 level and AST level which demonstrates high significant value with p = 0.031.Correlation between AST level and ALT level is detected no significant value with p = 0.16 in Fig. 5-C. Moreover, there is a high significant value between AST level and IL-6 level with value p = 0.031 Fig. 5-D.

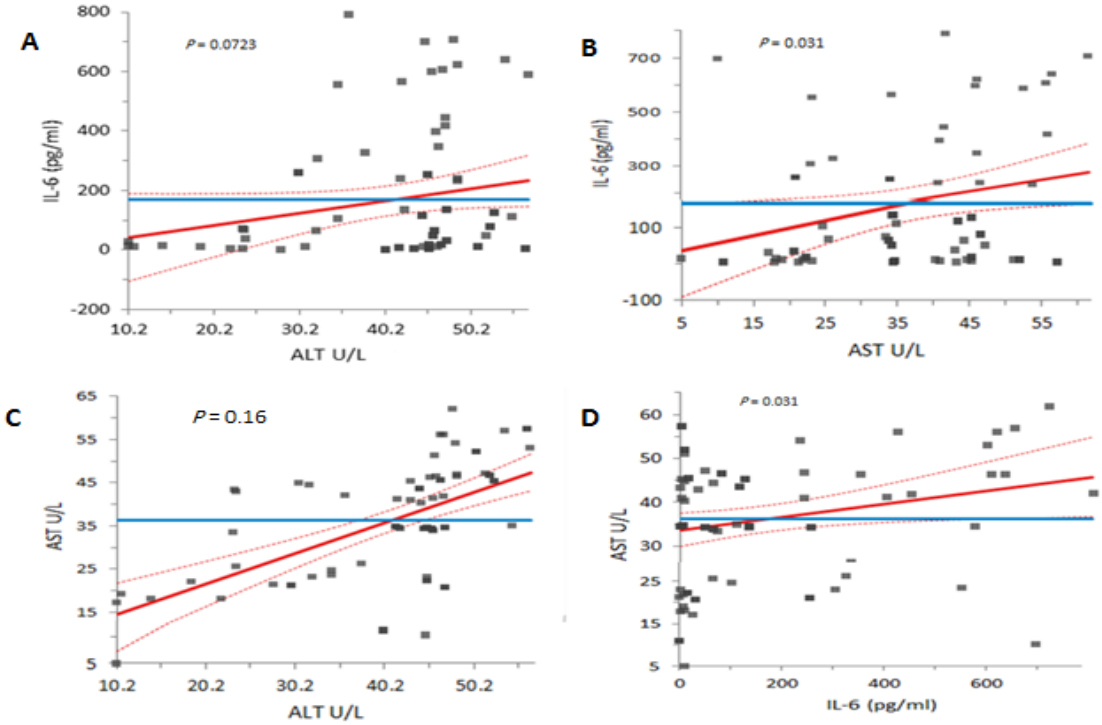

Fig. 5. Line plots. A: Correlation between IL-6 level and ALT level; **B:** correlation between Il-6 level and AST level; **C:** Correlation between AST level and ALT level; **D:** Correlation between AST level and IL-6 level.

According to the Analyse-it software, the scattered black dots between IL-6 level and both AHB, CHB are denoted as a high affinity between parameters in Fig. 6-A. Correlation coefficient between IL-6 level and case groups is a positive perfect linear association Fig. 6-B. The same thing is holed for IL-6 level and AST level in Fig. 6-C. Scattering scale for samples are detected in Fig. 6-D.

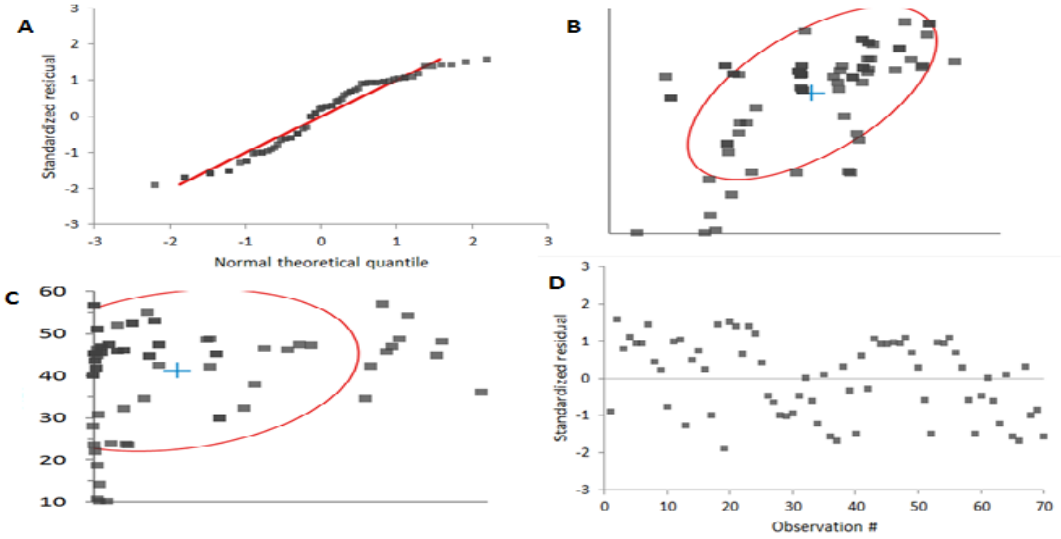

Fig. 6. Line and scattered plots. A: Linear scattered dots of IL-6 level and case groups; **B:** positive linear relationship of IL-6 level and case groups; **C:** positive linear relationship of IL-6 level and AST level; **D:** Scattering scale for samples.

DISCUSSION

Liver inflammation infection caused by hepatitis B virus is still a major health problem worldwide (Shu-Ling, 2016). The cytokines may be implicated in the pathogenesis of hepatitis disease beside more accentuated liver function and elevated markers (Genglin, 2016). The cytokines may stimulate inflammatory processes in both acute and chronic infections (Shubham, 2018). Interleukin 6(IL-6) is one of a multifunctional, heady, pleiotropic inflammatory cytokine which is a key of immune response regulator (Chih-Yung, 2006, Xing-Jiu, 2006, Marzieh, 2016 and Tian, 2016). IL-6 may be a key marker for monitoring disease activity and therapeutic efficacy of patients with hepatitis B (Lee, 2012, Behnaz, 2018). It has been demonstrated that IL-6 blocks HBV DNA replication through a moderate reduction of viral transcripts as IL-4 and TGF-β1 (Caixia, 2015). It is vital to bear in mind that neutralization of IL-6 may present a risk for hepatitis B patients (Caixia, 2015). The most cytokines such as (IL-1β, IL-6, IL-10, IL-21, IL-27, TNF-α) reached the highest expression in the acute aggravated group (Hong-Me, 2014, Ke, 2014, Genglin, 2016 and Kazumichi, 2016). It was stated that the activation of the IL-6 gene influence trigger of initial trials that lead to oncogenic transformation and this activation might affect chronic disease progression (Lamiaa, 2013). Some studies reported that elevated IL-10 levels have been associated with the increase of HBV DNA level (Hyodo, 2004, Roli, 2014 and Seyed, 2018). It has also been reported that serum levels of IL-33 and ST2 elevated with the increase of ALT levels in patients with CHB (Shu-Ling, 2016) and this is consistent with the our findings of this study.

We investigated that most chemical laboratory parameters AST, ALT levels were in normally range. On the other side, the AST and ALT levels were elevated with AHB and CL groups more than CHB group. The actual significant value with AST $p = 0.022$ but less significant with ALT $p = 0.182$ with case groups. On the other hand, above parameters is given significant on the contrary of case groups AST and ALT levels value $p > 0.05$, $p < 0.05$ respectively. There is no correlation efficient between AST and ALT levels $p = 0.16$ Fig. 5-C.

We enrolled that IL-6 serum level is raised with AHB group more than of CHB group with high concentration absorption and this is settled with previous study demonstrated that patients with various stages of chronic HBV infection exhibited different levels of serum IL-6 (Pisit, 2000). Our data provided evidence that correlation between IL-6 level and chemical parameters is disclosed that IL-6 level is correlated significant value with AST level $p = 0.031$ but less significant value with ALT level $p = 0.0723$ (Fig. 5-A, B and D. This result doesn't agree with previous study presented that the low IL-6 serum levels lack of correlation with biochemical and histopathological parameters of the chronic hepatitis (Gora-G, 2003). By contrast, our study publicized a positive linear correlation between serum IL-6 level and acute and chronic hepatitis Fig. 6-A, B. Moreover, a positive linear relationship of IL-6 level and AST level is shown in Fig. 6-C.

In our opinion, current study is enrolled no correlation between three groups of ages and registered cases AHB and CHB of participants with $p = 0.242$, $p = 0.240$ value respectively (Fig. 4-A and B). The major limitation of our study was the small sample size, which demands a further validation of the findings obtained, by carrying out studies on a larger set of population.

CONCLUSION

In conclusion, our data indicated that serum levels of IL-6 elevated with the increase of AST levels in patients with acute hepatitis B. The serum levels of IL-6, AST and ALT varied in different courses of acute and chronic hepatitis B virus infections. We surmised that IL-6 level might indicate liver injury of patients with acute hepatitis B infections. Moreover, negative correlation coefficient was emerged between chemical parameters and hepatitis cases according with age serial groups.

ACKNOWLEDGMENT

At the end of this study, we would like to thank blood bank center and Ibn-Al-Atheer teaching hospital for providing blood samples. All research finances were self-supporting.

REFERENCES

Alestig, E. (2011). Geographic and genetic diversity of hepatitis B., M.Sc. thesis in microbiology, University of Gothenburg.

Behnaz, R., Zahurin, M., Yamunah, A., Noor, S. and Rosmawati, M., (2018). Interleukin-6 gene variants are associated with reduced risk of chronicity in hepatitis B virus infection in a Malaysian population; *Biomedical Reports*, vol. **9**: 213-220.

Caixia, X., Yanning, L., Zhi, C. and Min, Z., (2015). Involvement of Interleukin 6 in Hepatitis B Viral Infection; *Cellular Physiology and Biochemistry*, vol. **37**: 677-686.

Chih-Yung, Y., Tzu-Hsing, K. and Ling-Pai, T., (2006). Human Hepatitis B Viral e Antigen Interacts with Cellular Interleukin-1 Receptor Accessory Protein and Triggers Interleukin-1 Response; *The journal of biological Chemistry*, vol. **281**, no. 45: 34525–34536.

Genglin, Z., Ting, Z., Qiyi, Z., Chan, X., Jing, L., Liang, P., Zhiliang G., (2016). Overproduction of IL-27 may play a pro-inflammatory role in HBV infected patients with severe liver and inflammation; *International Journal of Clinical and Experimental Medicine*, vol. **9**(3): 7000-7007.

Gora-G B., Anna L., Wies, A., Szyd, O., Wanda, B., Maria, K., (2003). Serum interleukin 6 and interleukin 12 levels in children with chronic hepatitis HBV treated with interferon alpha Magdalena. *Annals of Hepatology*, vol. **2**(2): 92-97.

Hong-Me, C., Hong-Le, L., Yu-Cong, Y., Xiao-Li, C., Yue-Fei, W., Fan-Fan, X. and Ying-Ren, Z., (2014). Serum IL-21 levels associated with chronic hepatitis B and hepatitis B-related liver failure; *Experimental and Therapeutic Medicine*, vol. **7**: 1013-1019.

Horvat, R.T. (2011). Diagnostic and Clinical Relevance of HBV Mutations. *Laboratory Medicine*, vol. **42**(8): 488-496.

Hyodo, N., Nakamura, I. and Imawari, M., (2004). Hepatitis B core antigen stimulates interleukin-10 secretion by both T cells and monocytes from peripheral blood of patients with chronic hepatitis B virus infection; *Clinical & Experimental Immunology*, vol. **135**: 462–466.

Kazumichi, A., Atsushi, T., Hiromichi, I., Manabu, H., Ken O., Yukiko, K., Hiroshi, W. and Hiromasa, O., (2016). Interleukin-21 plays a critical role in the pathogenesis and severity of type I autoimmune hepatitis; *Springer Plus*, vol. **5**: 777.

Ke, W., Zhe-bin, W., Yi-nong, Y., Jing, L., Geng-lin, Z., Yu-jie S., Hong-liang, H., Yu-bao, Z. and Zhi-liang, G., (2014). Plasma Interleukin-10: A Likely Predictive Marker for Hepatitis B Virus-Related Acute-on-Chronic Liver Failure; *Hepatology Monthly*, vol. **14**(7): e19370.

Lamiaa, N.H., Sahar, M.A., Fetouh, S.H., Walid, A.M. and Mona, F.S., (2013). Circulating IL-6, IL-17 and vitamin D in hepatocellular carcinoma: Potential biomarkers for a more favorable prognosis?. *Journal of Immunotoxicological*, vol. **10**(4): 380–386.

Lee, JK, Bettencourt, R., Brenner, D., (2012). Association between serum interleukin-6 concentrations and mortality in older adults: the Rancho Bernardo Study. *PLoS One*, Vol. **7**(4): e34218.

Marzieh, A., Saleh, S., Majid, S., (2016). Interleukin-6-174 Promoter Polymorphism and Susceptibility to Hepatitis B Virus Infection as a Risk Factor for Hepatocellular Carcinoma in Iran, *Asian Pacific Journal of Cancer Prevention*, vol. **17**: 2395-2399.

Min S., Jue, W., Jinbin D., Wenying, M., Jiali, M., Ting, W., Na, W. and Yugang, W., (2014). Interleukins-10 and 18 Genes Polymorphisms in Hepatitis B Virus Infected Saudi Patients*; Molecular Medicine Reports*, vol. **11**: 121-126.

Pisit T., Thosporn V., Apiradee T., Pinit K., Pongspeera S. and Yong P., (2000). Serum Interleukin-6 and Interferon-gamma Levels in Patients with Hepatitis B Associated Chronic Liver Disease; *Asian Pacific Journal of Allergy and Immunology*, vol.**18**: 109-114.

Qiao-Ling, S., Wei, R., (2004). Review of cytokine profiles in patients with hepatitis, *World Journal of Gastroenterol*ogy, vol. **10**(12): 1709-1715.

Roli, S., Yogesh, K.C., Indu, V. and Jyotdeep, K., (2014). Association of interleukin-10 with hepatitis B virus (HBV) mediated disease progression in Indian population; *Indian Journal of Medicine and Research*, vol. **139**: 737-745.

Seyed, H., Hosseini, K., Foroogh, N. and Mohammad, A. D., (2018). Serum Levels of Interleukin-4, Interleukin-10 and Interferon- in Patients with Chronic Hepatitis B Infection; *Hepatitis Monthly*, vol. **18**(4): e60377.

Shubham, S., Anupam, M., Ranjit, R., Ratna, R., (2018), Hepatitis C Virus Induces Interleukin-1 (IL-1)/IL-18 in Circulatory and Resident Liver Macrophages, *Journal of Virology*, vol. **87**: 12284–12290.

Shu-Ling, H., Ji-Guang Z., Zhen-Li W., Shuai G. and Kai W., (2016). Relevance of serum interleukin-33 and ST2 levels and the natural course of chronic hepatitis B virus infection; *BMC Infectious Diseases*, vol. **16**: 200: 1-8.

Tian L., Lei C., Long W. and Yu-Feng Y., (2015). IL-6 Plays a Crucial Role in HBV Infection; *Journal of Clinical and Translational Hepatology*, vol. **3**: 271–276.

Xing-Jiu H., Yang-Kyu., Hyung-Soon I., Oktay Y., Euisik Y. and Hak-Sung K., (2006). Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques, *Sensors*, vol. **6**: 756-782.