Interleukin 6 level in pediatric chronic liver diseases (CLD) hospital-based study

By

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Abstract:

Background: Chronic liver disease is a progressive destruction of the liver structures over a period greater than 6 months leading to fibrosis and cirrhosis and its consequence complication that lead to significant morbidity and mortality in pediatric patients. Interleukin 6 is one of pro-inflammatory cytokines involved in pathogenesis of acute and chronic liver diseases and plays an important role in liver regeneration and is one of cytokines that involved in pathogenesis of liver fibrosis and cirrhosis. **Aim:** To evaluate the level of IL-6 in chronic liver disease and the association between the level of IL-6 and severity of chronic liver disease. **Patients and Methods:**This cross sectional case_ control study that included seventy four patients with signs and symptoms of chronic liver disease from gastroenterology departments in children welfare teaching hospital from December 2019 till November 2020 considered as patients group and thirty healthy children as control group . Blood was aspirated from them and tested for serum level of IL_6 in each patients. This level was compared between them. **Results:** There was significant increase of IL-6 level in patients group but no difference between IL-6 level and the age and type of diagnosis. There was also significant increase of IL_6 level in patients who developed encephalopathy and positive correlation between IL-6 with child pugh score and pediatric end stage liver disease score. **Conclusions:** There significant increase of IL_6 and chronic liver disease and being higher in those with encephalopathy and but no relation between its level and gender of patients or diagnosis. IL_6 level increase with increase severity of liver disease.

Keywords: Interleukin 6 (IL-6), pediatric, chronic liver diseases (CLD), encephalopathy.

Introduction:

Chronic liver disease (CLD) in children represents a growing health problem with significant morbidity and mortality[(1)] The exact prevalence of pediatric CLD is unknown , though, in the United States, it has been reported that it is the cause of hospitalization of about 15,000 children every year[(2)]Pediatric liver diseases comprise a wide variety of disorders, including infections, developmental abnormalities, genetic, and metabolic disorders that ultimately result in progressive alterations in structures of liver and may end in cirrhosis and its consequences[(3).]

The CLD is a progressive deterioration of liver functions for more than six months, which includes synthesis of clotting factors, other proteins, detoxification of harmful products of metabolism and excretion of bile. however the presence of continued hepatic inflammation, as confirmed by clinical manifestations and laboratory studies, for a period of greater than 10 weeks usually excludes a self-limited hepatitis and implies chronicity[(4)].

In children, a wide range of causes of hepatocellular injury may result in cirrhosis. These include causes of cholestasis, where there is accumulation of hydrophobic bile acids toxic to hepatocytes (e.g., biliary secretory disorders or obstruction), as well as infections, toxins, metabolic, vascular, and nutritional disorders[(5)] , These cellular events combine to result in oxidant stress, release of cytokines, accumulation of collagens, increased turnover of components of the ECM, that lead to fibrosis[(5)] . Several profibrogenic cytokines and chemokines are found to be expressed in relation to pediatric liver fibrogenic, Many inflammatory cytokines such as IL-6 have been shown to play key roles in regulating liver fibrogenesis[(6)]. IL-6 promptly and transiently is produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase reactions, hematopoiesis, and immune reactions[(7)].send out a warning signal to the entire body[(8)] , The IL-6 synthesized at the initiation of the acute phase response, is considered responsible for signaling hepatocytes to produce acute phase proteins. It is widely posited that IL-6 is either delivered to the liver in an endocrine fashion from immune cells at the site of injury, or alternatively, in a paracrine manner by neighboring hepatic immune cells within the liver itself[(9)]. In the liver, IL-6 is an important inducer of the acute phase response and infection defense.

Aim of study: to define the relationship between IL-6 and severity of liver disease as measured by child pugh score and PELD score

Patients and methods

This is a prospective cross sectional case-control study. The study was conducted in the department of gastroenterology from December 2019 to November 2020 both in outpatient and inpatients departments of children welfare teaching hospital of medical city. About 74 patients included in this study have an age ranged between 6 months to 12 years old with signs and symptoms of chronic liver disease and chronicity was determined by either by duration of liver disease (typically >3-6 months) or by evidence of chronic hepatic decompensation (hypoalbuminemia, thrombocytopenia) or physical stigmata of chronic liver disease (clubbing, spider telangiectasia, hepatosplenomegaly). The severity was variable;

the affected child might have only biochemical evidence of liver dysfunction, might have stigmata of chronic liver disease, or can present in hepatic failure[(10)].

So according to the above criteria 74 patients of CLD were included in this study and were assessed by : CBC .liver function test (TSB .ALT, AST ,TSP , S. albumin) ,coagulation profile(PT, PTT ,INR) and Ultrasound.

To reach for the diagnosis of patients who presented with signs and symptoms of CLD during study period many of investigations were done for them to reach for the diagnosis Child pugh score which use ascites , encephalopathy , TSB level , albumin level , INR level for calculation .1 ml of serum was removed from each patient by using pyrogen /endotoxin free collecting tubes. IL-6 level was measured by ELISA procedure with the minimum detectable level was 2 pg/ml.

Statistical analysis

Data of current study were analyzed by using Chi-square (X2) test to compared between percentages. Measured sensitivity and specificity of diagnostic tests (detection the best test for diagnosis). Numeric date were described by (Mean ± SD). T test used to compare between two numeric variables, while F test (ANOVA) used to compared between three numeric variables or more. LSD used tom compared between means. Pearson correlation (R) accounted to explain type and strength of relationship between variables. A 2 level of significance of α=0.05 was applied to test. (SPSS v.22 and excel 2013) programs used to analyze current data.Notes: *= significant different ($p<0.05$); **= high significant different ($p<0.01$); ***= very high significant different ($p<0.001$)

Results

Table 1 shows demographic data of patients group , the age of patient divided in to three different age periods as14 patients (18.9%) below age of 1 year old , 17 (23%) patients at age between 1 to 5 years old, and 43(58.1%) patients at age between 6 to 12 years old with statistical significant as (p value 0.009)

Regarding the gender patients divided into 39 (52.7%) males and 35(47.3%) females without statistical significant as (p-value=0.62), 12(16.2%) patients diagnosed with Wilson disease, 27(36.5%) patients diagnosed with cholestatic liver disease, 9 (12.2%) patients with autoimmune hepatitis, 7 (9.5%) patients with chronic hepatitis B,C, others19 (25.7%)patients were undiagnosed. This was statistical significant as (p- value =0.001).

Patients divided into 3 groups according to child pugh score as 35(47.3%) patients with class A ,20(27%) patients with class B .19(25.7%) patients with class C and this was statistically significant as (p- value= 0.03) ,Regarding PELD score patients divided in to 55(74.3%)patients with score <6 ,10(13.5%) patients with score between7-17, 5(6.8%) patients with score between 18-27, 4(5.4%) patients with score >28. This was statistically significant as p value 0.001.From those 74 patients . 8(10.8%) patients developed encephalopathy and 66(89.2%) patients did not with statistical significant as p value 0.001.

Table (1) Demographic Data

	Value	Count	Percent	P value
Age periods	<1	14	18.9%	0.009**
	1-5	17	23.0%	
	6-12	**43**	**58.1%**	
Gender	Male	39	52.70%	0.62
	Female	35	47.30%	
Diagnosis	Wilson Diseases	12	16.2%	0.001***
	Cholestatic	**27**	**36.5%**	
	Undiagnosed	19	25.7%	
	Autoimmunehepatitis	9	12.2%	
	Viral infection	7	9.5%	
push-score	**Mild**	**35**	**47.3%**	0.03*
	Moderate	20	27.0%	
	Sever	19	25.7%	
PELD	**_11 to 6**	**55**	**74.3%**	0.001***
	7-17	10	13.5%	
	18-27	5	6.8%	
	>28	4	5.4%	
Encephalopathy	Yes	8	10.8%	0.001***
	No	**66**	**89.2%**	

Table 2 show the comparison between study groups in which IL-6 level was higher in 74 patients group in comparison to 30 children in control group with mean level 231.69 pg/ml in patients group and mean level 81.73 pg/ml in control group. This was statistically significant with p value 0.001.

Table (2) comparative IL-6 between study groups.

Groups		N	Mean	Std. Deviation	P value
IL_6	Patients	**74**	**231.69**	**118.77**	0.001***
	Controls	30	81.73	42.93	

Table 3 show the comparison of IL-6 level in patients according to age, It was statistically significant (p value 0.01)with mean level of IL-6 in 14 patients less than 1 year old 330.39 pg/ml higher than other age periods . mean level was 247.00 pg/ml in 17 patients with age between 1 to 5 years old and mean level was 193.53 pg/ml in 43 patients with age between 6-12 years old

Table (3) comparative IL-6 according to age periods of patients

age periods		N	Mean	Std. Deviation	P value
IL_6	**<1**	**14**	**330.29**	**151.61**	P= 0.01**
	1-5	17	247.00	136.05	
	6-12	43	193.53	74.33	

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Table 4 show the comparison of IL-6 level with 39 male patients with mean level 244.54 pg/ml in relation to 35 female patients with mean level 217.37pg/ml and this was not significant as p value about 0.32.

Table (4) comparative IL-6 according to gender of patients

Gender		N	Mean	Std. Deviation	P value
IL_6	Male	39	244.54	128.07	0.32
	Female	35	217.37	107.48	

Table 6 show the comparison of IL-6 level according to encephalopathy of patients in which 8 patients were diagnosed with encephalopathy with mean level of IL-6 was 456.25 pg/ml was higher than the level of IL-6 in 66 patients without encephalopathy and mean level was 204.47 . this was statistically significant with p value 0.001

Table (6) comparative IL-6 according to Encephalopathy of patients

Encephalopathy		N	Mean	Std. Deviation	P value
IL_6	Yes	8	456.25	17.68	0.001
	No	66	204.47	94.07	

Table 7 show the comparison of IL-6 level according to child pugh score with mean level 184.66pg/ml in 35 patients with class A . 252.8 pg/ml in 20 patients with class B .296.11pg/ml in 19 patients with class C. this was statistically significant with p value 0.002.

Table (7) comparative IL-6 according to pugh score of patients

pugh score		N	Mean	Std. Deviation	Statistics
IL_6	Mild(A)	35	184.66	76.42	P= 0.002**
	Moderate(B)	**20**	**252.80**	**136.35**	
	Sever(C)	19	296.11	131.81	

Discussion

Chronic liver disease (CLD) is a progressive deterioration of liver functions for more than six months duration. Cytokines such as IL_6 are key mediators in the pathophysiology of acute and chronic liver diseases, and essential molecules in hepatic regeneration and fibrosis[(11)].

In the present study the maximum incidence of CLD was in the age period between 6 to 12 years old with a percentage 58.1% higher than other age groups with percentages 23% in age periods between1 to 5 years old and 18.9% in children with less than 1 year old similar to that seen in (Dniele et al.2015) [(12)], with a percentage 48.9% and (sachin et al.2015) [(13)].this may be due to more obvious appearance of signs and symptoms with increasing of age and increase incidence of Wilson and autoimmune disease and chronicity of viral hepatitis in this age period.

No gender difference was noted (p value=0.62) with male percentage was (52.7%) and female percentage was (47.3%)

In this study, the cholestatic liver disease was the most common diagnosis with incidence 36.5% in relation to other disease, the same result was discovered (Snanto et al 2010) [(14)], (Ashraf et al 2019) [(15)] because inherited syndrome of intrahepatic cholestasis and biliary atresia and metabolic liver diseases are the most common causes of chronic liver disease and indication for liver transplant in pediatric age groups as mentioned in these studies and this may be due to using more advanced investigations to diagnose metabolic liver disease have been used as well as using genetic study to reach for diagnosis with more radiological orientation to diagnose biliary atresia.

Child pugh score classification of patients in the current study more commonly was in mild severity with class A percentage 47.3 % followed by 27% class B with moderate severity and finally 25.7% class C with high severity opposite to that mentioned in (Dehghani et al 2007) [(16)] in which class B was the most common one 53%, followed by class C 35% then class A 12%. But similar to that mentioned in (Dniele et

al.2015) [12] with class A was the most common one 57.1% followed by class B 34.3% then class C 8.5% .this is because most of patients in this study presented with mild severity.

According to PELD score most patients were in low grades of severity as 55 (74.3%) patients with PELD score ≤ 6 . 10(13.5 %) patients with PELD score (7-17) , 5 (6.8%) patients with PELD score (18-27), 4 (5.4%) patients with PELD score >28. This was statistically significant with p value 0.001

Eight (10.8%) patients developed encephalopathy in this study period .which is lower than mentioned in (Gulzar et al 2014) [17].(Anshu et al 2016) [18] with a percentage 29% and 50 % respectively this may be due to short study period and mild HE is more difficult to diagnose and given the difficulty of administering psychometric tests to children to and the absence of measures validated for use in this age range to diagnose mild hepatic encephalopathy. Children are usually diagnosed on the basis of clinical symptoms which are subtle in MHE.

In the present study, regarding serum interleukin-6 level, it was assessed in the studied groups and showed a statistically significant difference between patients with chronic liver disease and normal control subjects (p value=0.001) being higher in chronic liver disease groups with mean level 231.69 pg/ml in relation to mean level in control group 81.73 pg/ml . This was also detected by (Reiner et al 2011) [19] who stated that serum level of IL-6 increases significantly in chronic liver diseases and reaches its maximum concentration in decompensated cirrhosis. This may be due to its role in liver inflammation and regeneration and in diseased liver there is an imbalance between the productions pro-inflammatory cytokines and their clearance .also there is impairment of hepatic uptake of IL-6 in liver disease and the Kupffer cells of diseased liver involved in the increment secretion of cytokines. ALL of these are suggested to contribute to higher levels in these patients.

According to age groups classification. serum level of IL-6 was higher in patients with age less than 1 year old than other groups (p value 0.01) with mean level 330.29 pg/ml. this is most probably due to biliary atresia was the most common diagnosis in this age group and Serum level IL-6 are high in patients with BA because it is a dynamic process with ongoing inflammation as mentioned in (Amel et al 2011) [20]

In this study there was no significant difference (p value 0.32) in the level of IL_6 between males(244.54 pg/ml) and females (217.37pg/ml).

There is also no significant difference between IL-6 level and type of diagnosis as opposite to that mentioned in (Yousefi et al 2018)[21] this because almost all causes of chronic liver diseases lead cirrhosis and fibrosis irrespective to underlying etiology and also because small numbers of patients involved in the current study

In current study, serum level of IL-6 was significantly (p value=0.001) higher in patients with CLD who developed encephalopathy with (mean level 456.25 pg/ml) than CLD without encephalopathy with mean level was (204.47 pg/ml) .This was in agreement with (Ehab et al 2017)[22] in which there was significant difference between IL-6 levels in patients having cirrhosis with and without encephalopathy. This

is because serum level of IL-6 increase with increase severity of CLD as noted with child pugh score as well as there may be significant increase in brain receptor level of these cytokines in those who have hepatic encephalopathy[23].IL-6 might be involved in the mechanism by which ammonia contributes to the pathogenesis of OHE and there is also evidence of a potential synergistic interaction between proinflammatory cytokines and ammonia in the pathogenesis of OHE[24].

The serum level of IL-6 was significantly(p value 0.002) correlated with Child-Pugh classification as mean level was 296.11pg/ml in class C higher than class B with mean level 252.8pg/ml and class A with mean level 184.66 pg/ml .similar to that mentioned in (Ehab et al 2017) [22]. ,because pro- inflammatory circulating cytokines are known to be elevated in liver cirrhosis and in decompensated cirrhosis higher level of circulating IL-6 reported and was related to mortality and worse prognosis[25].

There is also significant correlation between IL_6 and PELD score (P value= 0.014) in which mean level was 335. pg/ml as PELD score degree reach 28 and more. The same result was reported in (Dniele et al.2015) [12], because as mentioned previously serum level of IL_6 increased when the severity of liver disease also increased

Conclusions:

1. CLD associated with significant increase in serum level of IL-6.
2. There was no significant difference between underlying etiology of liver disease or gender of patients with IL_6 level.
3. Serum level of IL_6 was very high in patients with encephalopathy and its increased with increase of severity of liver disease.
4. There was positive correlation between child pugh score and PELD score with IL-6 level.

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