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Evaluation of Complement Function in Children with Chronic Liver Disease

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Abstract: Chronic liver diseases have numerous complications in the children. Low serum complement level is a risk factor for the most serious complication especially spontaneous bacterial peritonitis. This study aimed on evaluating the complement function in patients with Chronic liver disease. This descriptive study was carried out on 23 patients under the age of 14 admitted in the gastroenterology ward of Ghaem hospital. A 3cc blood sample was examined with an auto-analyzer by Radial Gel immune-diffusion method for each patient. The collected data (age, sex, complement levels, etc) were analyzed by Mann-Witney test. 23 children were enrolled in the study during one year. One refused sampling for the test. From the 22 remaining, 12 were female and 10 male. 9 cases had a low C3 level and 3 had low C4. In the 9 patients with spontaneous bacterial peritonitis, 3 had low complement levels. According to the results low serum complement level in chronic liver disease significantly increases the risk of spontaneous bacterial peritonitis.

Key word: Chronic liver disease · Complement · Children

INTRODUCTION

Chronic liver disease is one of the main reasons of admission in the children age group. Although the number of such cases is not high but because of the chronic and often incurable course of most cases, extra care and attention should be paid to their complications, prevention and life quality improvement.

Chronic liver disease has numerous types in the children age group and some of its causes are potentially curable.

One of the most important and serious complication of these diseases is spontaneous bacterial peritonitis (SBP) which significantly increases the rate of morbidity and mortality in these patients [1]. SBP is also the most serious complication in ascetic patients and occurs in 10-20% of cases with cirrhosis or ascites [2]. In any cirrhotic patient whom suddenly develops a major general condition, SBP is taken into consideration. In definition, SBP is a peritoneal infection which occurs in the absence of gastrointestinal organs perforation and with the only etiology of bacteremia. SBP is diagnosed by its clinical signs and symptoms, positive ascetic fluid culture or a polymorphonuclear cell count over 250 in each 3mm of the ascetic fluid.In general, it is accepted that SBP in ascetic patients is caused by bacteremia and mainly with a GI source.

Chronic liver disease patients have several immunologic deficiencies which predisposes them to frequent and elongated bacteremia; they include complement system deficiency, neutropenia, neutrophil quality impairments and disturbed opsonophagosytosis.

In a retrospective study, it was shown that patients with low C3 and C4 complement levels are in a higher risk of SBP [3].

Kourilsky *et al*. showed serum complement levels in 53 patients with various liver diseases.The total serum complement level,C4 and C3 was raised in 28,normal in 12 and low in 13 patients [4].

Previous studies showed that significantly lower C3 and C4 levels found is patients with chronic active hepatitis than in controls [5].

Potter *et al*. showed that, the mean C4 concentration was reduced in all types of chronic disease studies [6].

In 1992 schlesinger published that the level of C4 was slightly lower than that of the normal controls [7].

Different studies have suggested that low serum complement levels, specially C3 and C4 can be the underlying cause in SBP incidences, therefore we aimed our study on evaluating the complement system function in chronic liver disease in the children age group, which is a solvable element of the innate immune system and is composed of a group of enzymes and regulatory proteins which are activated in a cascade pattern with specific

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receptors and their overall function results in cell lysis; and to determine whether there is any relation between the serum levels of these plasma proteins with the occurrence of abdominal infection.

In order to evaluate the complement system we can measure its components, individually. CH50 test which is also called the total complement hemolytic activity is based on the compatibility of C1 to C2 complement in the lyses of antibody-coated red blood cells.

In congenital disorders with C1 to C8 deficiency, CH50 level is equal to zero. In C4 deficiency it decreases to 1/2. Reduction in C3 and C4 serum concentrations shows the activation of the classic pathway. A reduced C3 level and a normal C4 level cause an increase in the susceptibility rate towards pneumococcal, meningococcal and pyogenic infections.

The main goal of this study was evaluating the complement system in patients with chronic liver disease; the other aims included determining the relation between serum complement level and the occurrence of bacterial peritonitis and also the relevancy of different chronic liver disease with serum complement levels.

Considering previous reports on the decrease in serum and ascetic fluid complement levels of some liver disease patients, we aimed on evaluating C3, C4 and CH50 complement levels in the children under the age of 14 with chronic liver disease.

METHODS AND MATERIALS

This descriptive study was carried out on 23 patients under the age 14 admitted in the gastero enterology ward of Ghaem Hospital of Mashhad University of Medical Sciences (North east of Iran). The study was conducted between March 2007 and March 2008. One refused sampling for the test. From the 22 remaining had the signs of chronic liver disease including icterus, hepatosplenomegaly, ascites and hemorrhage from esophageal varices which were also documented according to positive laboratory findings or a positive liver biopsy were enrolled in the study.

The different types of liver disease included in this study were as following:

- Hepatic cirrhosis with an unknown or cryptogenic cause
- Congenital hepatic fibrosis
- Autoimmune hepatitis
- Neonatal hepatitis with an unknown cause
- Galactosemia
- Bile duct atresia
- Progressive intrahepatic familial cholestasis (PFIC) or byler disease
- Hemosidrosis secondary to the thalassemia
- Wilson's disease
- Glycogen storage disease

After taking an informed consent from the child's parents a 3cc blood sample was taken. Centrifuged and C3, C4 and CH50 complement levels analyzer by Radial Gel immune-diffusion and analyzed based on Mann-Whitney test.

Laboratory tests including liver enzymes serum albumin and bilirubin, alkaline phosphatase, ANA and SMA antibodies, Hida scan, liver, spleen and abdominal sonography, Doppler sonography of portal vessels for studying portal hypertension, liver biopsy, 24hour urine copper level, serum ceruloplasmin, ophthalmologic examination for Kaser-Flescher ring were performed on all the studied cases and included in the research questionnaire.

RESULTS

A total of 22 patients with 10 different types of chronic liver disease, with the partial prominence of autoimmune hepatitis and cryptogenic cirrhosis were studied.

The patients' average age and weight was 4-6 years and 21.6kg, CH50 level was measured in 16 cases and C3 and C4 levels were examined in all, due to economical problems. The average level of C3, C4 and CH50 were 75.5mg/dl, 23 mg/dl and 78 mg/dl, respectively.

Among whole cases 13 had normal C3 and C4 levels whereas decreased levels were detected in the other, In other words the C3 level was normal in 13(59.1%) and low in 9(40.9%) cases. Also 14 cases (89.4%) had normal and in 3(13.6%) C4 level was located in abnormal status, respectively.

Serum C3 and C4 level was evaluated in all patients with bacterial peritonitis and the average C3 level of these cases was 51mg/dl and it was 75mg/dl in total where the difference is statistically significant according to Mann-Whitney test, (Z=-2.431, P=0.012).

The average C4 level was 14.8mg/dl in SBP patients and 23.86 mg/dl in total, (Z=-2.551, P=0.009), whereas the difference of CH50 average serum level was not statistically significant in these two groups, (Z=-1.687, P=0.111).

Table 1: Distribution of cases based or the type of their chronic liver disease

Diagnosis	No	percentage
Autoimmune hepatitis	5	22.7
Glycongen storage disease	2	9.15
Galactosemia	1	4.55
Congenital hepatic fibrosis	1	4.55
Bile duct atresia	1	4.55
Progressive intrahepatic familial cholestasis (PFIC) or byler disease	1	4.55
Idiopathic Neonatal Hepatitis	2	9.15
Wilson's disease	3	13.6
Hemosidrosis secondary to the thalassemia	1	4.5
Cryptogenic hepatic cirrhosis	5	22.7
Total	22	100

Table 2: Comparing the mean, Standard deviation, min and max of factors including age, weight, C3, C4 and CH50 levels

	No	Min	Max	Mean	SD
Age (y)	22	0.2	13	6.3909	3.86768
Weight (kg)	21	4.4	53	21.5762	13.17057
C3 level (mg/dl)	22	28	165	75.5455	30.50484
C4 level (mg/dl)	22	11	60	23.8636	11.33177
CH50 level (mg/dl)	16	16	105	77.8125	23.54066

Table 3: Normal C3 and C4 level

	Male	Female	
Normal C3 and C4 level	7	6	13
Percentage including the complement	53	47	100
Percentage including age	70	50	40
Low C3 and C4 levels	3	6	9
Percentage including the complement	33	67	100
Percentage including age	30	50	60
Total	10	12	22

Table 4: Association between complement system and spontaneous Bacterial peritonitis

Spontaneous Bacterial peritonitis		C3	C4	CH50
Negative cases	Mean	82.8235	26.5294	85.3846
	No	17	17	13
	SD	29.61468	11.56567	10.54782
Positive cases	Mean	50.800	14.8	45
	No	5	5	3
	SD	19.66469	2.48998	38.74274
Total	Mean	75.5455	23.8636	77.8125
	No	22	22	16
	SD	30.50484	11.33177	23.54066

Table 5: Studying the relation between SMA and ANA autoantibodies with the complement level

ANA and SMA autoantibodies		C3	C4	CH50
Negative cases	Mean	73.4706	25.6471	77.0833
	No	17	17	12
	SD	33.84361	12.03090	25.91405
Positive cases	Mean	82.6	17.8	80
	No	5	5	4
	SD	14.94323	5.97495	17.32051
Total	Mean	75.5455	23.8436	77.8125
	No	22	22	16
	SD	30.50484	11.33177	23.54066

Table 6: Studying the relation between SMA and ANA autoantibodies with the complement level

	C3-C4 level		
Diagnosis	Normal	Low	Total
Autoimmune hepatitis	3	2	5
Glycongen storage disease	2	0	2
Galactosemia	1	0	1
Congenital hepatic fibrosis	1	0	1
Bile duct atresia	0	1	1
Progressive intrahepatic familial cholestasis (PFIC) or byler disease	1	0	1
Idiopathic Neonatal Hepatitis	1	1	2
Wilson's disease	0	3	3
Hemosidrosis secondary to the thalassemia	1	0	1
Cryptogenic hepatic cirrhosis	3	2	5
Total	13	9	22

However, the average serum level in autoimmune hepatitic patients with a positive antinuclear antibody (ANA) and ant smooth muscle antibody (SMA) was lower than those without these antibodies.

Frequency distribution of serum complement level and the specific diagnosis of the studied cases have been shown in Table 6.

According to this Table, 9 patients had low and 13 had normal complement levels whereas its interesting point was the low complement levels in all Wilson's patients and bile duct atresia, which opens a door to further researches on a larger group of Wilson's disease cases for assessing their serum complement level.

In Summery, C3 complement level was low in 9 out of 22 patients. C4 was low in only 3 patients who spontaneously had a low C3 level. 4 out of the 16 mentioned cases 4 had a low CH50 level, 3 of these patients had low C3 levels and 2 had low C4 while just 1 patient had low CH50 level with normal C3 and C4.

DISCUSSION

One of the most important complications of chronic liver disease is ascites, which was also present in 63% of our cases.

Knowing that ascites is an appropriate environment for bacterial growth, accordingly, SBP which is the infection of the ascitic fluid, is a potential cause of morbidity and mortality in such cases.

C3 and C4 complement levels were evaluated for all patients but the CH50 level, because of the high expenses, was exclusively studied in 16 cases.

In a study performed by Larcher *et al*. in 1985, 12 occurrences of SBP were studied in 11 cirrhotic patients, they all had ascites. Their clinical signs and symptoms

included abdominal distention, high fever, abdominal pain, gastrointestinal abnormalities and finally hepatic encephalopathy.

The most common organism in the ascitic fluid culture was streptococcus pneumonia (pneumococcus) which was found in 9 cases, 2 cases of klebsiella and 1 hemophilus influenza were also detected. Blood and ascitic fluid cultures were similar in 9 patients. 7 patients died due to severity of the disease. During the 3 months before the occurrence of SPB, a drop in C4 level in 9 and C3 level in 8 patients was recorded. In contrast, only 8 out of the 59 cirrhotic cases without SBP had a low C3 level, where as 35 had low C4. The mentioned study suggested that low complement levels might be responsible in the pathogenesis of SBP, but it is not enough, particularly for C4 [3].

In another study taking place in 1985 Nagura showed that C3 and C4 are exclusively produced in the liver tissue and only hepatocytes are capable of doing so. Whether hepatocellular injury causes a fall in C3 and C4 serum levels was not proved in this study [8].

Such *et al*. in 1988 in Spain, proved that low C3 levels in the ascitic fluid causes an increase in the incidence of SBP. 33cirrhotic patients were enrolled in the study, SBP had occurred more than once in 7 cases. C3 complement level of the ascitic fluid in these cases was significantly lower that those without SBP (9+2.67<18.26+8.11), P<0.01. They concluded that low C3 level increases the risk of SBP [9].

In a similar study by Zhoghua *et al*, C3 and C4 levels of the serum and the ascitic fluid in patients with SBP were significantly lower than those with no SBP (P<0.05). All the mentioned patients were cirrhotic. Cases with a lower C3 and C4 level have a worse prognosis based on Child- Pugh scoring. 69 cirrhotic patients took part in this study. C3 level in child-Pugh score, stage A was 1.06_0.21 g/d whereas in Child – Pugh stage B, it was 0.78+0.24g /d. C4levels were also the same [10].

Buumann *et al*. in 2004 in Germany proved that C3 and C4 serum levels have a prognostic value in cirrhotic patients [11].

Homann *et al*. showed that C3 and C3 concentrations and the hemolytic complenent activity of the alternative pathway were decreased in decompensate cirrhotic patients compared with controls (P<0.01) [12].

In another study in 1991 showed that C3 and C4 were lower in children with sever liver disease compared with controls [13].

In a study conducted by Bilic *et al*. in Taiwan, ascitic fluid total protein, albumin, globulin and complement levels in SBP patients was significantly lower than those with sterile ascites. They concluded that all such factors can play a role in protecting the patient against SBP.

In other studies it was proven that IL-6 and fibronectin levels in the ascetic fluid also affect the incidence of SBP [14, 15].

It is of importance to note that firstly, most of the studies carried out so far have been performed on cirrhotic patients in the adult age group, therefore knowing the difference in the complement level of adults and children; it was essential to do a similar study on children. Secondly, the studied cases so far have been exclusively cirrhotic patients.

In our study patients with several types of disease including autoimmune hepatitis, Wilson's disease, congenital hepatic fibrosis, galactosemia and glycogen storage disease took part.

CONCLUSION

Regarding the fact that low complement level increases the risk of infection and that one of the most serious infections in chronic liver disease is spontaneous bacterial peritonitis, special attention was paid to these patients. Unfortunately most of these cases had received antibiotics before visiting the hospital and therefore a positive blood and ascetic fluid culture was not reported in them, but ascetic fluid analysis in most patients had positive signs of infection (polymorphonuclear >250 in the ascetic fluid).

Statistically, serum C3 level in patients with SBP was significantly lower than the other cases of the study group (P=0.11). This statistically significant difference was also proven for C4 (P=0.009).

Therefore, we can say that SBP has a higher prevalence in patients with lower C3 and C4 levels. Based on the similar studies performed up to now, streptococcus pneumonia has been reported as the most common organism in SBP patients in Iran. We suggestion:

- Evaluating C3 and C4 levels in all chronic liver disease and ascitic patients for screening those high risks for SBP.
- Anti-pneumococcal vaccination in chronic liver disease and ascitic patients
- Antibiotic prophylaxis in high risk patients (with a low complement level).
- Evaluating complement levels in Wilson's patients and finding out whether it can be used as a diagnostic factor.

- Increasing the number of studied cases in order to achieve a more definite result on the complement level in chronic liver disease patients.
- Conducting further studies in the future on whether antibiotic prophylaxis should be carried out on all patients or only on those with recurrent peritonitis.

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