Evaluation of Serum C24 Ceramide as a Predictor of Hepatic Decompensation in Patients with Liver Cirrhosis

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Abstract

Background: It was found that there is a significant progressive decrease in the serum level of C24 ceramide with increasing severity of cirrhosis. Our aim was to evaluate the efficacy of C24 Ceramide (C24Cer) as a predictor of decompensation in cirrhotic patients at Suez Canal University hospital, Ismailia, Egypt. *Subjects and Methods:* Patients with viral related liver cirrhosis were consecutively confronted in the outpatient and inpatient sections. They were classified according to Child-Pugh into A, B, C groups. A control group (13 individuals) was compared with each group. Clinical assessment and Liver profile were performed. The serum level of C24Cer was measured using 20μL extracted serum with methanol: chloroform: HCl (15:83:2), using liquid chromatography coupled to tandem mass spectrometry. *Results:* Patients with Child-A cirrhosis showed a significantly higher mean C24Cer level compared to Child-C patients (p <0.001). Pairwise comparisons showed a statistically significant stepwise decrease in the mean C24Cer level from control group to Child-A, B then C cirrhotic patients respectively (p-value: 0.029, <0.001, and <0.001). There was a highly significant negative correlation between C24Cer and each of total bilirubin, PT, ascites grade, hepatic encephalopathy grade, and Child score (p<0.001 for each) as well as a highly significant positive correlation with albumin level (p<0.001). *Conclusion:* a low serum C24Cer is associated with hepatic decompensation and is good with a sensitivity of 100% and a specificity of 73%. Further studies are needed to elucidate the real-life significance of C24Cer as a non-invasive mortality predictor in cirrhosis.

Keywords: liver cirrhosis, Child-Pugh classification, Ceramide 24

Introduction

Chronic hepatitis C virus (HCV) infection affects more than 170 million persons worldwide and responsible for the development of liver cirrhosis in many cases[1]. Due to the frequently asymptomatic clinical presentation, cirrhosis remains often unsuspected until clinical complications become apparent. Progression of cirrhosis is often preventable and since it is linked with increased mortality rates especially when decompensated, early diagnosis and adequate monitoring of the disease are indispensable[2]. The Child-Pugh and Model for End-Stage Liver Disease (MELD) scores are the main clinical tools widely deployed to define short term prognosis of affected patients[3,4], but they do not provide evidence on disease progression and dynamic stage of cirrhosis[5]. Incorporation of novel non-invasive markers of disease progression in

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the currently clinically used scores would improve the identification of severely ill patients and enable a more accurate organ allocation in patients with end-stage liver disease. Ceramides are a family of waxy lipid molecules composed of sphingosine and a fatty acid. Ceramides are found in high concentrations within the cell membrane of cells[6]. They are one of the component that make up sphingomyelin, one of the major lipids in the lipid bilayer. Contrary to previous assumptions that ceramides and other sphingolipids are found in cell membrane were purely supporting structural elements, ceramide can participate in a variety of cellular signaling, examples include regulating differentiation, proliferation, and programmed cell death (PCD) of cells[6] Roles for ceramide and its downstream metabolites have also been suggested in a number of pathological states including cancer[7],neurodegeneration[8], obesity associated diabetes[9], microbial pathogenesis and inflammation[10]. There is a tight interaction between variations in serum sphingolipid levels and progression of liver fibrosis as well as responsiveness to antiviral therapy[11], particularly, sphingosine, sphinganine, and C24ceramide (C24Cer) that appeared as promising novel biomarkers in chronic HCV infection and should be further evaluated in the context of noninvasive prediction of liver fibrosis[12]. A significant decrease of long and very long chain ceramides, particularly of C24Cer in patients with increasing severity of cirrhosis was found. Additionally, hydropic decompensation, in the form of ascites, significantly correlated to low C24Cer level as well as hepatic decompensation[13]. Poor overall survival was observed in those with low serum concentration of C24Cer as well[13]. So, our aim was to evaluate serum level of C24Cer as a predictor of hepatic decompensation in patients with HCV-related liver cirrhosis in Suez Canal area.

Subjects and Methods

Patients with HBV and/ or HCV-related liver cirrhosis were consecutively confronted in the outpatient clinic and inpatient department of internal medicine department of the Suez Canal university hospital in Ismailia city and they were classified according to Child-Pugh classification to Child A, B and C. A control group of normal population was compared. Accordingly, the study population was divided into 4 groups as follow: Group1: normal population, Group2: Child A, group3: Child B and group 4: Child's C, with each group including 13 patients. Clinical assessment, abdominal ultrasonography, Liver profile, serum creatinine, CBC, HCV RNA and HBV-DNA were performed to all groups. Serum level of C24Cer was investigated using 20μL extracted serum with methanol: chloroform: HCl (15:83:2). Amount of C24 was analyzed by liquid chromatography coupled to tandem mass spectrometry. We excluded cirrhotic patients with evidence of malignancy, solid organ transplantation and those with autoimmune disease or on immunosuppressive therapy.

Results

Our results showed no statistically significant difference in socio-demographic background among various study groups as shown in Tables1&2. We found a statistically significant difference among the study groups regarding the ceramide 24 level. In Table 3-A; patients with Child-A cirrhosis showed a significantly higher mean C24Cer level compared to Child-C patients (p <0.001) as shown in Figure 1. In Table 3-B; Pairwise comparisons showed a statistically significant stepwise decrease in the mean C24Cer level from control group to Child-A, B then C cirrhotic patients respectively (p-: 0.029, <0.001, and <0.001).

Table 1: Comparison of socio-demographic data among different Child's classes					
	Control	Class A	Class B	Class C	Total	p-value
Age (Mean ± SD)	58.46 ± 9.04	54.92 ± 3.43	53.77 ± 9.82	61 ± 8.73	57.04 ± 8.42	0.213
Male n (%)	3 (23.1)	8 (61.5)	11 (84.6)	8 (61.5)	30 (57.7)	0.08
Rural residence	6 (46.2)	2 (15.4)	8 (61.5)	7 (53.8)	23 (44.2)	0.091

In this study, we found that a low serum C24Cer concentration correlated with a higher frequency of occurrence of hepatic decompensation parameters; as our data showed a highly significant negative correlation between C24Cer and total bilirubin, PT, ascites grade, hepatic encephalopathy grade, and Child score (p<0.001 each) as well as a highly significant positive correlation with albumin level (p<0.001) (Table 4).

Table 2: Comparison of the clinical background among different Child's classes				
Group*	Control N=13	Class A N=13	Class B N=13	Class C N=13
HBV	0	0	2 (15.4)	2 (15.4)
HCV	0	10 (77)	5 (38.5)	8 (61.5)
H. of Bilharziasis	0	1 (7.7)	6 (46.2)	2 (15.4)
Hypertension	1 (7.7)	1 (7.7)	1 (7.7)	1 (7.7)
Diabetes mellitus	0	1 (7.7)	2 (15.4)	3 (23.1)
Pregnancy	1 (7.7)	0	0	0
Other disorders	0	0	0	3 (23.1)

Figure 2 shows a scatterplot for ceramide 24 level in relation to both serum albumin and total bilirubin levels. In addition, the univariate analysis revealed a significant association between a low serum level of C24Cer and the presence of ascites (p<0.001) and hepatic encephalopathy (p=0.003) as shown in Table 5. While Figure 3 shows the ROC curve parameters of hepatic decompensation in relation to serum ceramide 24 level. It shows that serum ceramide 24 level can be used as a good indicator of hepatic decompensation with a sensitivity of 100% and specificity of 71.8%. Our results showed that C24Cer can be used as a good indicator for hepatic decompensation with a sensitivity of 100% and a specificity of 73%.

Table 3A: Comparison of the mean serum C24Cer level among different Child's classes				
Group	Control	Class A	Class B	Class C
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
C24Cer level	6417.54 ±2451.41	3506.69 ±150.57	2132.69 ±123.27	1126.85 ±87.28
A significant difference was found among the study groups regarding C24Cer level. (<0.001)				

Discussion

We aimed in this study at assessing the serum concentration of C24 ceramide in a series of patients with different stages of liver cirrhosis as well as evaluating its predictive potential regarding disease progression, hepatic decompensation, and overall

survival. We found that patients with Child-A cirrhosis showed significantly higher concentration of ceramide 24 compared to those with Child-C ($p < 0.001$). Pairwise comparisons of category regarding ceramide 24 showed a statistically significant difference between Child-C cirrhotic patients and each of Child-B, Child-A patients, and controls ($p = 0.029$, < 0.001, and < 0.001 respectively).
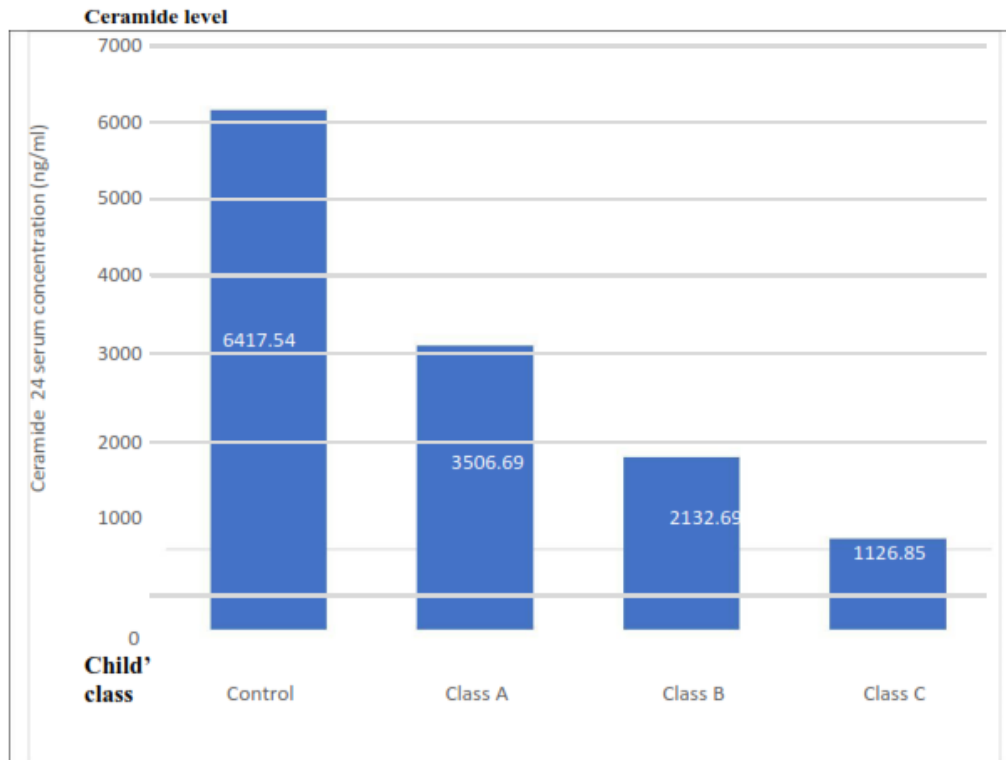
Ceramide level

Figure 1: Comparison of the mean Ceramide 24 level according to the Child class

These results were consistent with previous studies. For instance, in their study to evaluate serum sphingolipid profile of 244 patients with cirrhosis prospectively followed for a median period of 228±217 days *via* mass spectrometry, Grammatikos et al[13] demonstrated that patients with Child-A had significantly higher concentrations of long and very long chain Cer's compared to patients with Child-C cirrhosis ($p < 0.001$).

Table 3B: Pairwise comparisons of different Child's classes regarding C24Cer		
Sample 1	Sample 2	p-value
Class C	Class B	**0.029**
Class C	Class A	**<0.001**
Class C	Control	**<0.001**
Class B	Class A	**0.012**
Class B	Control	**<0.001**

Furthermore, comparisons between Child-B cirrhosis and Child-A or Child-C cirrhosis revealed marked differences as well. In the current study there was a significant variation of serum C24Cer levels observed in patients with cirrhosis caused by HCV (p=0.017) which is similar to the results of the previous studies. One study which compared 72 healthy volunteers to 69 patients with non-alcoholic fatty liver disease and 69 patients with chronic hepatitis C virus infection identified C24Cer being significantly decreased in chronic HCV infection as compared to healthy individuals and patients with NAFLD[12]. Similarly, another study demonstrated a significantly decreased serum C24Cer in patients with HCV-induced liver cirrhosis as compared to cirrhosis induced by other chronic hepatopathies[8]. Moreover, in this study we found that low serum Cer concentration correlated with higher rates of hepatic decompensation; as a significant strong negative correlation was found between serum ceramide 24 and total bilirubin, PT, ascites, hepatic encephalopathy grade, and Child score (p<0.001 for each respectively) as well as a significant strong positive correlation was found with serum albumin level (p<0.001).
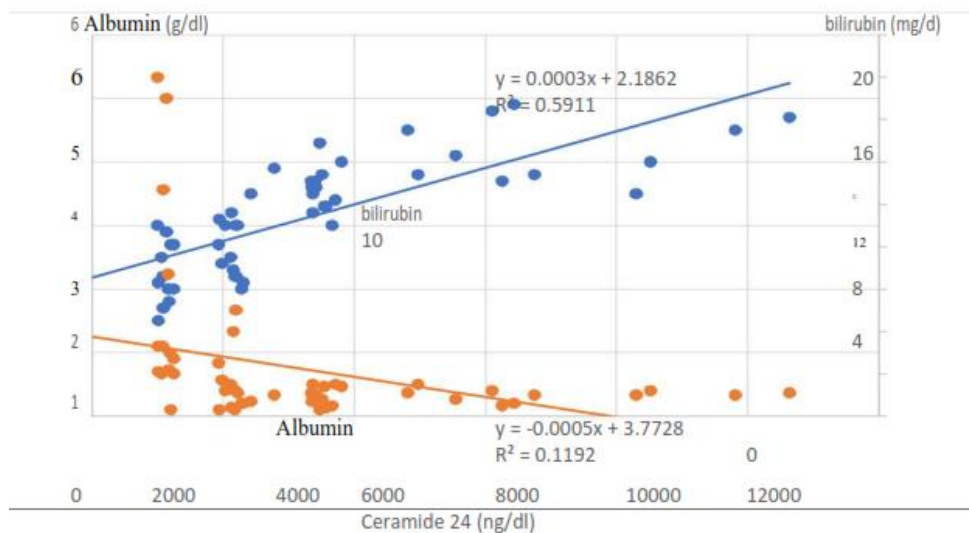

Figure 2: Correlation of the serum Ceramide 24 level with both serum total bilirubin and albumin

Additionally, the univariate analysis revealed a significant association between the serum C24Cer and ascites (p<0.001) as well as hepatic encephalopathy (p=0.003). In line with these findings, the study of Grammatikos et al. demonstrated decreased serum concentrations of long and very long chain ceramides associated with the presentation of major clinical complications defining hepatic decompensation such as ascites, spontaneous bacterial peritonitis, hepatic encephalopathy and hepatorenal syndrome with ascites occurrence being only associated with serum concentration of C24Cer and dhC24Cer (p<0.001 and p<0.01 respectively). Another study demonstrated a decreased C24Cer (P=0.028) were associated with severe liver fibrosis (F3- F4) [7].

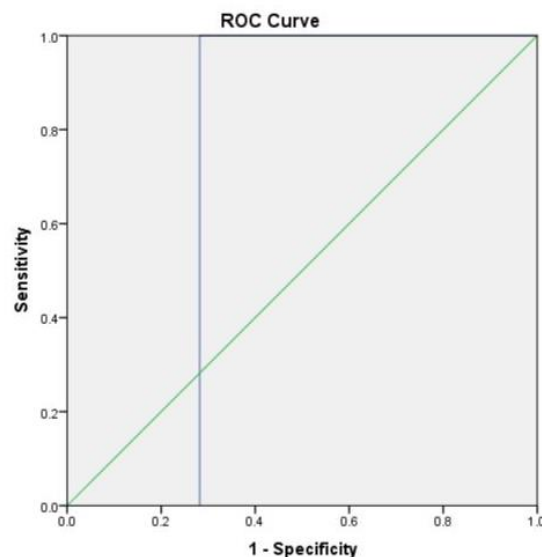| Table 4: Correlation between serum Ceramide 24 and different parameters of hepatic decompensation in the study population					
Variable	Child's grade	S. Bil.	S. Alb.	PT	H.E. grade	Child's Score
Ceramide 24 (R)	-0.93	0.520	0.834	-0.738	-0.549	0.887

In another study, assessing variations in serum sphingolipid levels associated with liver fibrosis progression and poor treatment outcome in HCV, it was observed serum C24Cer associated significantly with severity of liver fibrosis in HCV patients[10,11]. Additionally, when included in a multivariate logistic regression model after adjustment for other variables, also in univariate analysis, it is associated with liver fibrosis progression within the HCV patient cohort. Our results showed that serum C24Cer can be used as a good indicator for hepatitis infection with a sensitivity of 100% and specificity of 71.8%.

Table 5: Univariate analysis of Different variables in association with serum ceramide 24

Variable	P value
− Sex	0.024
− HCV	0.017
− DM	0.018
− Bilirubin	0.002
− Albumin	<0.001
− PT	0.001
− Ascites	<0.001
− HE	0.003
− Score	<0.001
− Hepatitis	<0.001

Area	p-value	Cut-off point	Sn	Sp	PPV	NPV	LR+	LR-	DOR	A
71.8	**0.02**	3068.5	100	71.8	33	15.4	-1.41	-1.38	1.02	28.8

Sn = Sensitivity (%), Sp = Specificity (%), PPV = Positive-predictive value, NPV = Negative predictive value, LR+ = Likelihood ratio positive, LR- = Likelihood ratio negative, DOR = Diagnostic odds ratio, A = Accuracy (%)

Figure 3: ROC curve of the serum ceramide 24 level as an indicator hepatic Decompensation

Additionally, it can be used as an indicator for decompensation with a sensitivity and specificity of 100%. Despite all these promising findings, this study has some limitations, as a clinical association study, it is difficult to ascertain causation and to establish the causal relationship between SL metabolism and cirrhosis. Thus, future prospective cohort studies are suggested to better understand this relationship. In addition, data regarding the survival are lacked in our study and they could have been beneficial to effectively assess the predictive ability of the serum C24Cer of survival. Moreover, since serum Cer's are bound to lipoproteins such as low-density lipoprotein (LDL), high density lipoprotein (HDL) and very low-density lipoprotein (VLDL) it cannot be excluded that the observed differences are due to changes of the mentioned lipoproteins in patients with cirrhosis. Unfortunately, serum levels of these lipoproteins were not available in our patients.

Conclusion

our study identified that C24Cer is associated with progression of the hepatic decompensation. Certainly, further studies are needed to elucidate the underlying mechanism as well as the role of serum SL's and particularly C24Cer in the non-invasive mortality prediction in patients with cirrhosis.

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