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Ceramide 24 Level in Hepatitis C Virus- Patients and Healthy Persons

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ABSTRACT

Background: There are 170 million people in the globe living with hepatitis C virus (HCV) infection, which causes inflammation and hepatic fibrosis in various degrees. A portion of these patients will develop cirrhosis and other end-stage liver disease problems over the course of twenty to forty years. Low levels of serum ceramide 24 (Cer24) are linked to severe liver fibrosis and poor response to antiviral therapy in those with chronic HCV infection.

Objective: This study was designed to assess level of ceramide 24 in chronic HCV and normal individuals.

Subjects and Methods: The study was carried out as a case control study at Tropical Medicine Department, Clinical Pathology Department in Zagazig University Hospitals and at Viral Hepatitis Treatment Unit in Al-Ahrar Teaching Hospital. The study included 60 individuals who were divided into 2 groups. Group 1 included 30 case and group 2 was 30 healthy subjects as control. All patients were clinically evaluated, had routine laboratory investigations and measurements of circulating levels of ceramide 24. Abdominal ultrasonography was done.

Results: Serum ceramide 24 level in HCV patients (cases) is 15.16 ± 6.93 while its level in normal individuals (control) is 65.01 ± 65.84. **Conclusion:** It was found that serum ceramide 24 level was significantly reduced in case group.

Keywords: HCV, Serum ceramide 24

INTRODUCTION

170 million people around the world are infected with the hepatitis C virus (HCV), which causes inflammation and hepatic fibrosis in various degrees. Patients with chronic infection are more likely to develop cirrhosis and other end-stage liver disease complications over the next 20 to 40 years, depending on their age [(1)]. HCV incidence in Egypt was the highest in the world, with the majority of cases being caused by genotype 4, which has been shown to be highly related with severe fibrosis and cirrhosis. This has led to a rise in hepatocellular carcinoma as the most common cancer diagnosis in the country [(2, 3)].

The current HCV treatment includes a cocktail of antivirals known as direct acting antivirals (DAAS) that work together. Non-nucleoside NS3/4A Protease Inhibitors (PIs), nucleoside and nucleotide NS5B and NS5A polymerase inhibitors [(4)].

A group of lipid compounds known as the ceramides includes both ceramides and ceramides-related substances [(5)]. Signal transduction involves the production of quickly and transiently degradable lipid second messengers, such as ceramides, in response to certain stimuli. They may also influence the membrane characteristics to govern cellular functions [(6)].

Ceramide influences cellular and molecular physiology in both cell autonomous systems and complex organisms, and it is both a structural and functional unit of sphingolipid metabolism. The effects of Cer on cell destiny have been demonstrated to be largely dependent on the subcellular compartment of synthesis and metabolism, as well as the chain length of the fatty acid attached to the sphingosine backbone, despite its initial role as an antiproliferative and proapoptotic mediator. Serum Cer levels may be used as indicators in insulin resistance, diabetes mellitus, the metabolic syndrome, Alzheimer's disease, and acute phase responses, according to recent studies [(7)].

Membrane organisation and the development of lipid microdomains are both aided by ceramide, which plays a key role**.** In order for HCV entry factors to be properly located, sphingolipids may be crucial. Sphingomyelin hydrolysis enriched plasma membranes in ceramide, which inhibited HCV penetration [(8)].

It's vital to know that the hepatitis C virus has lipid components since they affect the virus' infectiousness and virion-building ability [(9)]. Reservoirs of HCV replication complexes are seen in fatty tissue [(10)]. Cholesterol and glycosphingolipids make up the majority of lipid raft composition.

Hepatitis C virus replication complex formation and localization as well as HCV RNA-dependent RNA polymerase activity are both dependent on sphingomyelin, according to research [(11)]. Chronic HCV infection reduces blood Cer24 concentrations markedly, and low serum Cer24 levels are linked to advanced liver fibrosis, poor antiviral medication response, ascites, and a bad prognosis in cirrhotic HCV patients with decompensated liver cirrhosis [(2)].

This study was designed to assess level of ceramide 24 in chronic HCV and normal individuals.

SUBJECTS AND METHODS

Between March 2019 and March 2020, researchers at Zagazig University Hospitals' Tropical Medicine Department, Clinical Pathology Department, and the Viral Hepatitis Treatment Unit at Al-Ahrar Teaching Hospital conducted this case control study. The attendance rate of hepatitis C patients at Al-Ahrar viral hepatitis treatment unit was 10 patients/month. The

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total number of subjects included in the study was 60. They were divided into 2 groups. Group 1 included 30 case and group 2 was 30 healthy persons as control.

Ethical approval:

As long as all of participants signed informed consent forms and submitted them to Zagazig University's Research Ethics Committee, the study was allowed (ZU-IRB#6198/15-11-2020). We followed the World Medical Association's ethical code for human experimentation, the Helsinki Declaration.

Inclusion criteria:

Patient's ≥ 18 years, patients of both sexes, patients HCV positive with Child-Pugh A, and patients who received the full course of HCV-DAAS treatment.

Exclusion criteria:

Patients <18 years, HBsAg +ve patients, patients who refused to give consent to participate in the study, Child-Pugh B and C, total bilirubin more than 5 mg/dl and platelets less than 50.000 $x10^3$/cmm, patients with malignancy e.g. hepatocellular carcinoma, cholangiocarcinoma, lymphoma, etc., and associated diseases as cardiac disease, renal disease, chronic obstructive pulmonary disease (COPD) and uncontrolled diabetes.

The data of all patients were collected at two times, the data included:

1. **Medical history taking and clinical examination.**
2. **Routine laboratory investigations:**
   Complete blood picture using Sysmex ***XS*** fully automated hematology analyser (sysmex diagnostic, Japan). Biochemical liver tests including serum albumin, bilirubin, ALT, AST on (Synchron CX5 auto-analyzer of Beckman***).*** Coagulation profile mainly prothrombin time in seconds and international randomization ratio (INR) on (Synchron CX5 auto-analyzer of Beckman). Kidney function tests including blood urea and serum creatinine using (auto-analyzer cobas-Roch diagnostic). HBsAg by ELISA. Random blood sugar and HbA1C for diabetic patients**.** Serum alpha-fetoprotein (AFP). HCV RNA by quantitative polymerase chain reaction (PCR) for positive hepatic C viral antibody patients before and 3 months after DAAS.

   Human serum ceramide 24 (Cer 24) by ELISA before and 3 months after treatment. It was also done to the control group.

   Kits: Human ceramide 24, Daxing Industry Zone, Beijing, China.

   Spacemen requirement:

   Centrifugation at a speed of 2000-3000 rounds per minute for 20 minutes after serum coagulation takes 10-20 minutes at room temperature. Remove the supernatant, and then centrifuge the system once more if any precipitation appears.

   Principle of the assay:

   Human Cer 24 is measured using the kit, which coats microtiterplate wells with purified human Cer 24 antibody, creating a solid-phase antibody. Cer 24 is added to the wells, and the horseradish peroxidase (HRP)-labeled human Cer 24 antibody is combined with the unlabeled Cer 24 antibody, resulting in a complex of antibodies, antigens, enzymes, and antibodies after thorough washing. This reaction is halted by adding a sulphuric acid solution, and the colour change is detected spectrophotometrically at a wavelength of 450 nanometers. The 3,3',5,5'-tetramethylbenzidine (TMP) substrate turns blue when exposed to HRP enzyme-catalyzed HRP reaction. Comparing the optical density (O.D.) of sample to the standard curve allows us to calculate the concentration of human Cer 24 in each sample. (All the part in blue is not needed in this paper. It's better to delete it and to write that you followed the instructions with the kit)
3. **Pelvi-abdominal Ultrasonography.**
4. **Child-Pugh classification.**

Statistical Analysis

SPSS 22.0 for Windows was used to gather, tabulate, and statistically analyse all of the data (SPSS Inc., Chicago, IL, USA). In order to ensure that the data were normal, the Shapiro Wilk test was used. Quantitative data were presented as means, standard deviation (SD), median, and range and were compared by independent t-test if regularly distributed and by Mann–Whitney U test if non-normally distributed. Qualitative data were expressed as frequency and percentage and were compared by Chi-square test. Study parameters were analysed for relationships using the Spearman's rank correlation coefficient (r). All of the experiments used a two-sided design. P-values below 0.05 was considered significant.

RESULTS

Demographic data of the 2 studied groups are shown in table 1. Cases were significantly older than control.

Table (1): Demographic data of studied groups

Basic characteristics	Cases (N=30)		Control (N=30)		Test	p-value (Sig.)
	No.	%	No.	%		
Sex						
Male	18	60%	19	63.3%	0.071‡	0.791 (NS)
Female	12	40%	11	36.7%		
Age (years)						
Mean ± SD	53.36 ± 12.37		33.50 ± 10.90		**-5.026•**	**<0.001 (HS)**
Median (Range)	55 (30 – 70)		31 (21 – 54)			
Smoking						
No	20	66.7%	22	73.3%	0.318‡	0.573 (NS)
Yes	10	33.3%	8	26.7%		

‡ Chi-square test, • Mann Whitney U test, Sig.: Significance, NS: Not significant, HS: Highly significant.

Symptoms and signs of the studied cases are shown in table 2.

Table (2): Clinical presentations of cases

	Cases (N=30)	
	No	%
Asymptomatic	13	43.3%
Symptomatic		
Fatigue	4	13.3%
Nausea	3	10%
Headache	5	16.7%
Gastrointestinal upsets	2	6.7%
Gastritis	3	10%
Signs		
1-Liver		
Normal	13	43.3%
Enlarged	17	56.7%
2-spleen		
Normal	23	76.7%
Enlarged	7	23.3%
3-Pallor	5	16.7%
4-Skin rash	7	23.3%

Level of hemoglobin was significantly higher in cases than control, while level of platelets was significantly lower in cases (Table 3)

Table (3): Complete blood picture of the studied groups

Complete blood picture	Cases (N=30)	Control (N=30)	Test	p-value (Sig.)
Hemoglobin (g/dl)				
Mean ± SD	13.83 ± 1.12	12.89 ± 0.44	**-4.279•**	**<0.001 (HS)**
WBCs (x10^9/l)				
Mean ± SD	8.09 ± 2.68	7.39 ± 1.24	-0.888•	0.375 (NS)
Plt count (x10^3/cmm)				
Mean ± SD	217.90 ± 50.86	283.33 ± 50.24	**-5.013***	**<0.001 (HS)**

• Mann Whitney U test, * Independent samples Student's t-test, Sig.: Significance, NS: Not significant, HS: Highly significant.

As regard liver functions tests, level of ALT, AST, PT, INR, and AFP was significantly higher in cases than in control, while serum albumin was significantly lower in cases compared to control group (Table 4).

Table (4): Liver function tests of the studied groups

Liver function tests	Cases (N=30)	Control (N=30)	Test•	p-value (Sig.)
ALT (u/l)				
Mean ± SD	27.56 ± 8.33	20.13 ± 7.12	**-3.406**	**0.001 (S)**
AST (u/l)				
Mean ± SD	26.60 ± 8.53	20.03 ± 6.87	**-3.328**	**0.001 (S)**
Serum albumin (g/dl)				
Mean ± SD	3.93 ± 0.36	4.58 ± 0.41	**-5.366**	**<0.001 (HS)**
Serum bilirubin (mg/dl)				
Mean ± SD	0.76 ± 0.19	0.64 ± 0.19	-1.448	0.148 (NS)
PT (second)				
Mean ± SD	14.40 ± 1.87	12 ± 0.01	**-7.321**	**<0.001 (HS)**
INR				
Mean ± SD	1.09 ± 0.19	1 ± 0.01	**-3.003**	**0.003 (S)**
AFP (ng/l)				
Mean ± SD	34.83 ± 3.71	7.06 ± 1.43	**-6.681**	**<0.001 (HS)**

• Mann Whitney U test, Sig.: Significance, S: significant, NS: Not significant, HS: Highly significant.

- RBS was significantly higher in cases than control group. There were 23 cases with Child score of 5 and 7 with Child score of 6 in
- case group (Table 5).

Table (5): Kidney function tests and other laboratory findings of the studied groups

Kidney function tests and other laboratory findings	Cases (N=30)	Control (N=30)	Test•	p-value (Sig.)
S. creatinine (mg/dl)				
Mean ± SD	0.63 ± 0.08	0.63 ± 0.08	0	1 (NS)
RBS (mg/dl)				
Mean ± SD	114.50 ± 17.85	92.20 ± 9.98	**-5.468**	**<0.001 (HS)**
HbA1C (%)				
Mean ± SD	4.32 ± 0.32	4.31 ± 0.26	-0.122	0.903 (NS)
Child sore				
Score 5	23			
Score 6	7			

• Mann Whitney U test, Sig.: Significance, NS: Not significant, HS: Highly significant.

Ultrasound showed that 17 patients had enlarged hyperechopattern liver (table 6).

Table (6): Pelvi-abdominal ultrasound of patients

Abdominal ultrasound	Cases (N=30)	
	No.	%
Liver		
Size		
• Normal	13	43.3%
• Enlarged	17	56.7%
Echopattern		
• Normal	13	43.3%
• Hyperechopattern	17	56.7%
PV diameter (mm)		
Mean ± SD	11.36 ± 0.49	
(Range)	(11 – 13)	
Spleen	**No.**	**%**
• Enlarged	17	56.7%
• Mild	10	
• Moderate	7	
Normal	13	43.3%

Serum ceramide 24 in case group was higher than in control group (Table 7).

Table (7): Serum Ceramide 24 of the studied groups

Serum Ceramide 24 (ng/ml)	Cases (N=30)	Control (N=30)	Test•	p-value (Sig.)
Mean ± SD	15.16 ± 3.93	65.01 ± 5.84	**-6.506**	**<0.001 (HS)**

• Mann Whitney U test. p< 0.05 is significant. Sig.: Significance.

There was no significant correlation between serum ceramide 24 and HCV PCR in case group with r= -0.417 and P= 0.06.

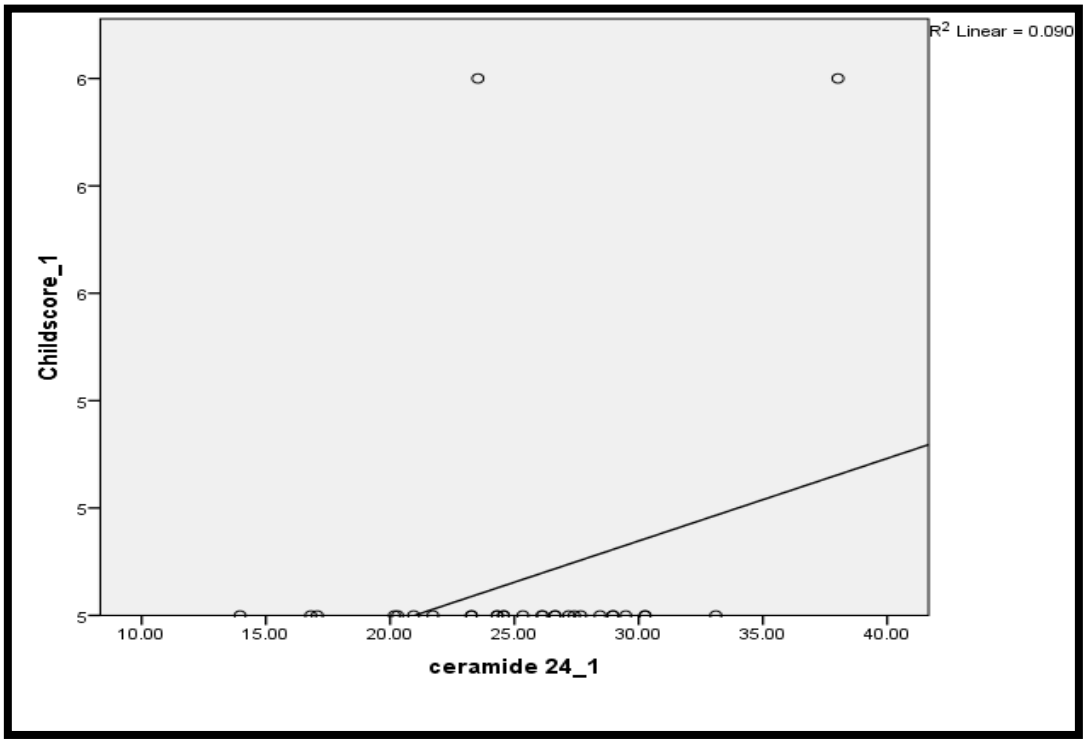

Figure (1): Correlation between serum ceramide 24 and HCV PCR in patients of the study

DISCUSSION

This study shows that there was high statistically significant difference as regard age. **El-Ghitany *et al.* [12]** did study on 12169 people and found that the most common age group affected was >15 years old**.**

This study found that there was highly significant decrease as regard platelets in cases compared to control. **Bano *et al.* [13]** observed in study included 30 patients of HCV positive, that thirteen of the patients had platelet levels lower than normal**.** 43.3 percent of those who took the test had thrombocytopenia. Also, **Sumit *et al.* [14]** observed in study included 50 HCV patients that thrombocytopenia prevalence ranged from 0.16 percent to 45.4%; more than half of these studies found a prevalence of 24 percent or higher**.**

In our study, there was a significant reduction in ceramide 24 level in case group. This finding is consistent with **Grammatikos *et al.* [2]** who found that long-term HCV infection reduces serum ceramide 24 levels and lowers serum C24 levels. Cer levels are linked to severe liver fibrosis, poor antiviral medication response, and ascites and poor overall mortality in cirrhotic HCV patients with decompensation of liver cirrhosis [2]. Also we found that there was a negative correlation between ceramide 24 and Child score. This finding is in agreement with that of **Pugh *et al.* [15]** who reported that when Child score increases, the liver status is not good**.** Also, they reported that the Child score is used for prognosis of chronic liver disease**.** This finding also agrees with **Grammatikos *et al.* [2]**. According to their research, severe liver fibrosis is linked to low serum C24Cer levels **[2].**

In our study, there was no significant correlation between ceramide 24 and viral load. Despite the fact that **Voisset *et al.*** found that ceramide enrichment in target cell plasma membranes significantly reduced HCV penetration**.** Ceramide enrichment of the plasma membrane resulted in internalization of CD81, which decreased its level on the cell surface. This internalization of CD81 by ceramide is most likely responsible for ceramide's ability to prevent HCV invasion. **[16].** Indeed, a three-fold drop in CD81 cell-surface expression results in a 100-fold reduction in HCV entrance, according to the research **[17].** It has been postulated that increasing the plasma membrane ceramide concentration reduces the quantity of CD81, an important molecule for HCV entrance, on the cell surface [18-20] .

Our study had some limitations. This may be due to small size of patients with Child-Pugh A included in the study.

CONCLUSION

Serum ceramide 24 had a negative significant connection with child score. Level of Ceramide 24 is higher in the control group than in the case group.

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