Evaluation of Some Trace Elements in Biological Samples of Egyptian Viral Hepatitis Patients under Nutrition Therapy

T. Elnimr, R. Morsy, A. El fert, and A. Ismail

***Abstract*—**Hepatitis infection is the most serious liver diseases causing cirrhosis and liver cancer. Multifunctional trace elements possessing antioxidant and immunomodulatory activity could have clinical benefits against the development of cirrhosis or hepatocellular carcinoma. This study evaluated the effect of trace elements formulation as dietary supplements composed of Viusid, Pharma Nord, and SiliciumOrganiqe G5. The study was conducted on 149 Egyptian patients with hepatitis virus infection (Hospital of Tanta University, Tanta, Egypt). The patients were subdivided into three groups, acute hepatitis, chronic hepatitis, and post hepatitis cirrhosis. The patients received a formulation composed of Viusid, Pharma Nord, and SiliciumOrganiqe G5 (3 times per day) during 21 months. The scalp hair, nail, serum, RBC and blood were used to determine the level, efficacy and safety of trace elements, iron, copper, zinc and selenium, using ICP-MS spectroscopy. Statistical methods such as correlation coefficient and cluster analysis were used to analysis the data. The results exhibited significant changes in the iron, copper, zinc and selenium levels in the biological samples, hair, nail, RBC, serum and whole blood, of patients relative to those of healthy controls. The mean copper levels in patients with hepatitis virus were significantly higher than that of the control group whereas the mean iron, zinc, and selenium levels in the patients were significantly lower than those of the control group. Therefore, on the basis of this study, we identified the impact of natural supplements to improve the treatment of viral liver damage, using the level of some trace elements such as, iron, copper, zinc, and selenium, which might serve as biomarkers for increases survival and reduces disease progression.

***Keywords*:** ICP-MS, nutrition therapy, serum, trace element, viral hepatitis.

I. Introduction

HEPATITIS C virus (HCV) infection is one of the most problematic liver diseases due to its economic and medical problems. Medically, it can cause chronic liver disease such as cirrhosis and hepatocellular carcinoma (HCC) [1]. In last decades, World Health Organization statistics revealed increasing in affected people that exceeded 350 million people [7]. According to these statistics, Egypt is the first in the world in the spread of this disease, where more than 15 million Egyptian citizens affected by hepatitis viruses [8]. In Egypt, Medical treatment of chronic hepatitis C (CHC) is based only on using PEG-interferon plus ribavirin due to their proven efficacy [2]. However, the main drawback is that only 50 % of treated patients have a sustain responses against the virus. Various studies have reported various side effects during the treatment such as the risk of adverse events and discontinuations that may lead to reject their use in clinical practice [3,4]. The oxidative damage is the most serious effect that reported in effect with chronic HCV patients, however the whole diagnostic of liver injury pathogens still incomplete [5,6]. Trace elements as vital elements are of most related and important to the liver for two several reasons: 1) trace elements control many biological and biochemical activities of liver such as enzymatic functions, oxidative/anti-oxidative reactions and protein synthesis; 2) their concentrations are a function of types of liver disease where the liver is the position of their metabolism [9-10]. The relation between content of trace elements and different types of liver diseases results from their toxicity to liver cells or problems of liver functions [9]. Therefore, it became of important to develop integrated treatment strategies in particular for patients with poor response to antiviral therapy. These treatment strategies are based on administration of multifunctional compounds possessing antioxidant and immune modulatory activity. In clinical trials, Viusid is used extensively as an antioxidant and nutritional supplement containing the zinc as a trace element, ascorbic acid, and glycyrrhizic acid [11-13]. In this study, we will examine the effect of adding Viusid to Pharma Nord (Bio-Selenium + Zinc + Vitamin C & E + B6), dietary supplement, and SiliciumOrganiqe G5. Selecting this formulation depends on the individual characteristics; their components as reported by various studies have antioxidant and immune modulatory activities that can influence on the function and histology of affected liver in addition to halt the progression of HCV disease. Therefore, the aim of this study is to to investigate the efficacy of a formulation composed of Viusid, Pharma Nord and SiliciumOrganiqe G5 as multifunctional nutrition therapy possessing an antioxidant and an immune modulator activity in patients with hepatitis. Also, this study will provide practical up-to-date guidelines for trace element monitoring and therapy for adult long-term viral hepatitis patients under nutrition therapy based on the limited data available, and the quantification of trace element using inductively coupled plasma- mass spectrometry (ICP-MS).

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II. Methods

A. ICP-MS calibration

To test the possibilities and the accuracy of the quantitative analysis technology, three certified reference materials were used in this study. They were IAEA-A-13, Bowen's Kale, and GBWO7601. These were the only materials available with a reasonable accuracy on the specified concentrations. All these samples were prepared by recommended method of IAEA and were digested in triplicate and analyzed using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS: Finnigan element 2). Table I summarizes the concentrations found in this work together with the specified values.

Table I
Quantitative analysis results of three certified reference materials using ICP-MS

Element	IAEA-A-13(µg/g)		Bowen's Kale (µg/g)		GBWO7601 (µg/g)	
	Specified	This work	Specified	This work	Specified	This work
Br	22.00	22.46±0.5 1	24.00	23.28±0.44	--	-
Ca	286.00	291.43±8.73	4.14	4.22 ± 0.11	2.90	2.97 ± 0.07
Cd	0.07	0.07± 0.003	0.89	0.90 ±0.03	0.11	0.11 ±0.003
Cr	0.26	0.25 ±0.007	0.31	0.32±0.01	0.37	0.36 ±0.01
Cu	4.30	4.39 ±0.12	4.90	4.74±0.12	10.60	10.31±0.28
Fe	2400	2352.78 ±56.45	115.00	110.98±2.42	54.00	55.35 ±1.49
Hg	0.16	0.15 ±0.003	0.18	0.18 ±0.005	--	-
K	2500	2425.12 ±65.48	2.43	2.47±0.07	--	-
Mg	99.00	100.58 ±2.04	0.16	0.16 ±0.005	360.00	366.84±9.52
Mn	3.60	3.52 ±0.10	15.00	15.31 ±0.38	--	-
Na	12600	12247.21±252.89	0.23	0.22 ±0.01	--	-
Ni	1.00	1.03 ±0.02	1.08	1.11 ±0.03	0.83	0.81 ±0.03
P	940.00	909.45 ±25.45	0.45	0.46 ±0.01	--	-
Pb	0.18	0.18 ±0.004	---	-	8.80	8.58 ±0.26
Rb	2.30	2.34 ±0.06	52.00	50.02 ±1.52	--	-
S	6500	6292.02 ±163.59	----	-	--	-
Se	0.24	0.25 ±0.005	0.14	0.14 ±0.003	--	-
Zn	13.00	13.98 ±0.36	31.00	31.74 ±0.65	190.00	193.06± 4.63

B. Nutrition therapy doses

The patients received a formation composed of Viusid (Catalyis laboratory, Madrid, Spain) (3 sachets daily), whereas, antioxidant properties of Viusid shown in Table II [14], Pharma Nord, Bio-Selenium+Zinc + vitamin C, E and B6, (3 tablets daily), dietary supplement, and SiliciumOrganiqe G5 (LLR-G5, Golden Mile Ind. PK., Breaffy Rd., Castlebar, County Mayo, Irland). The ingredients of G5 is shown in Table III (3 time per day) during the duration of treatment, which depends on the types of hepatitis C , whereas, 90 week for chronic hepatitis, 24 week for acute hepatitis and 12 week for post hepatitis cirrhosis, while, no one of the healthy individuals supplemented with the formulation.

Table II
Ingredients of Viusid [14]

Malic acid	0.666 g	Ascorbic acid	0.020 g
Glycyrrhizic acid	0.033 g	Folic acid	66 mcg
Glucosamine	0.666 g	Cyanocobalamine	0.3 mcg
Arginine	0.666 g	Zinc sulfate	0.005 g
Glycine	0.333 g	Pyridoxal	0.6 mg
Calcium pantothenate	0.002 g		

C. Samples collection and acid digestion

The samples collected are blood, scalp hair, and nail. For blood samples, 6–8 mL of fasting blood samples was withdrawn from patient's vein twice, before and during nutrition therapy every three months. The samples were centrifuged at 3000 rpm for 10 min to separate serum and red blood cells (RBC), then, the samples were preserved in sterilized clean tube at -20 °C until digestion. For scalp hair, and nails samples gathered in the same moment the collection of blood samples and washed to remove the impurity then transferred in polythene bags separately and stored in cool and dry place until further digestion [15]. For digestion, the exactly weighed quantity of hair and nail samples (1.0 g) was taken in the digestion vessel. 10mL of HNO3 (E-Merck, Germany) was added in the hair, nail, blood, serum and RBC samples and left for 5 min, followed by the addition of 10mL of HClO4 (BDH, UK), with heating during which the samples were completely digested and dissolved [16]. Deionized water was used for the preparation of the samples and standards. ICP-MS spectrometry was used to quantitative evaluation of the digested samples under optimum analytical condition.

Table III
Ingredients of G5 for 90 ml

Monomethylsilanetriol (MMST)	34.75 mg
Silicon	10.35 mg
Potassium	20.25 mg
Phosphorus	13.5 mg

D. Data analysis

For distinguish between healthy and patients, the results were subjected to multivariate methods, the principal component analysis (PCA) [17-20]; correlation analysis using Pearson correlation coefficient; and the cluster analysis by using the Ward's Method for Hierarchical cluster analysis whereas the square Euclidean for the distance method.

II. Results and Discussion

A. Patients

The selected hepatitis patients were randomly assigned to the Viusid, Pharma Nord (Bio-Selenium + Zinc + vitamin C, E and B6), dietary supplement and SiliciumOrganiqe G5. Out of 149 volunteers, 38 patients were excluded from analysis during the experimental period because they had not one or more of the inclusion criteria. The patients enrolled in this study were clinically classified into 3 groups, using a simple grading system, which classifies into three grades as follows: acute hepatitis, Grade I, chronic hepatitis, Grade II, and post hepatic cirrhosis, Grade III. Table IV shows the trace elements analysis in patients and controls. The results revealed a significant increasing in the level of copper in patients' samples in comparison to control group. Both of iron, zinc and selenium have showed a significant decrease in patients' samples in comparison to control group. These results indicate that the trace elements, copper, iron, zinc and selenium, can be used as a biomarker for the diagnosis of hepatitis. Treatment started two weeks after the clinical evidence with appropriate therapy (Viusid + Pharma Nord (Bio-Selenium + Zinc + vitamin C, E and B6) and dietary supplement. SiliciumOrganiqe G5). All patients were medically examined to determine the health status and then were assigned to the treatment program. Table V, Table VI, and Table VIIshow the efficacy of the nutritional supplement on the level of biomarkers of trace elements in case of chronic hepatitis, acute hepatitis and post hepatitis cirrhosis, respectively. The results for chronic hepatitis exhibit a gradual increase in the concentrations of iron, zinc and selenium with increasing duration of use of nutritional supplements, while showing a gradual decrease in the concentration of copper for the same periods, we think that this is due to, the beneficial effects of a formation composed of viusid, Pharma Nord and Siliciumorganiqe G5 for patients with chronic hepatic. The results also show that the concentrations of all of the iron, zinc and selenium after the use of nutritional supplements for 90 weeks are still lower than the concentrations of the control group, this is may be due to the need for more treatment period, while, patients with acute hepatitis and post hepatitis cirrhosis, we have not any indication about any improvement, but their health worsened. In case of post hepatitis cirrhosis patients, we stopped providing the formation composed of viusid, Pharma Nord and Siliciumorganiqe G5 after 12 weeks and for acute hepatitis patients after 25 weeks.Also, We analyzed serum samples of some patients with chronic hepatitis treated with alpha interferon or the combination of alpha interferon and ribavirn without dealing with any nutritional supplements and at the same time with the group, which concern us and the results showed a continuing decrease in the concentrations of iron, zinc, selenium and copper concentration increase.

TABLE IV
MEAN CONCENTRATION OF SERUM, RBC, BLOOD, NAIL AND SCALP HAIR TRACE ELEMENTS IN VARIOUS KINDS OF VIRAL HEPATITIS PATIENTS IN COMPARISON TO CONTROL

Sample	Element	Control Group	Acute Hepatitis	Chronic Hepatitis	Posthepatitic Cirrhosis
Serum	Iron, *µg/L*	90.96 ± 8.76	64.32 ± 6.21	52.11 ± 5.10	45.85 ± 4.63
	Copper, *µg/mL*	0.96± 0.06	1.24 ±0.07	1.38± 0.07	1.47± 0.06
	Zinc, *µg/L*	972.52 ± 135.65	720.96 ±100.32	591.19 ± 90.22	514.33 ± 77.13
	Selenium, *µg/L*	304.82 ±41.54	227.48 ± 33.75	181.98 ± 26.14	158.33 ± 23.78
RBC	Iron, *µg/L*	92.74 ± 11.26	70.78 ± 7.75	57.51 ± 6.11	51.12 ± 5.34
	Copper, *µg/mL*	0.94 ± 0.06	1.25 ± 0.07	1.32 ± 0.08	1.47 ± 0.08
	Zinc, *µg/L*	1110.23±153.77	824.49 ± 123.98	653.69 ± 92.84	568.31 ± 87.42
	Selenium, *µg/L*	300.89 ± 46.33	229.99 ± 34.89	189.87 ± 25.10	168.43 ±26.67
Blood	Iron, *µg/L*	95.23 ± 11.23	70.23 ± 9.54	57.59 ± 6.89	46.70 ± 6.22
	Copper, *µg/mL*	0.96 ±0.07	1.30 ± 0.06	1.41 ± 0.08	1.49 ±0.07
	Zinc, *µg/L*	1678.43±251.25	1285.67±187.92	1105.32±160.17	908.65 ±142.11
	Selenium, *µg/L*	329.87 ± 50.34	250.52 ± 38.54	205.88 ± 30.11	176.37 ± 27.45
Scalp hair	Iron, *µg/g*	21.28 ± 2.67	16.3 1 ± 1.57	12.96 ± 1.32	11.40 ± 1.21
	Copper, *µg/g*	8.55 ± 1.56	13.58 ± 2.72	15.86 ± 3.34	17.76 ± 3.08
	Zinc, *µg/g*	111.35 ± 16.22	85.90 ± 12.98	70.55 ± 10.78	62.79 ± 8.93
	Selenium, *µg/g*	2.07 ± 0.31	1.5 1 ± 0.21	1.27 ± 0.18	1.12 ± 0.17
Nail	Iron, *µg/g*	21.28 ± 2.22	16.3 1 ± 1.73	13.45 ± 1.35	11.83 ± 1.20
	Copper, *µg/g*	6.82 ± 1.4	11.01 ± 1.97	13.09 ± 2.11	14.33 ± 2.34
	Zinc, *µg/g*	115.62 ± 17.34	85.90 ± 12.67	74.81 ± 11.21	68.07 ± 10.11
	Selenium, *µg/g*	2.06 ± 0.31	1.5 1 ± 0.23	1.30 ± 0.19	1.17 ± 0.17

TABLE V
THE EFFICACY OF THE NUTRITIONAL SUPPLEMENT ON THE LEVEL OF BIOMARKERS OF TRACE ELEMENTS IN CASE OF CHRONIC HEPATITIS

Sample	Element	Primary date	After 12 weeks	After 25 weeks	After 38 weeks	After 52 weeks	After 65 weeks	After 78 weeks	After 90 weeks
Serum	Iron *µg/L*	54.10±6.01	57.78±5.87	60.23±6.11	62.56±6.54	65.71±6.23	67.45±7.26	70.15±7.33	72.24±7.28
	Copper *µg/mL*	1.47±0.08	1.41±0.07	1.33±0.08	1.26±0.05	1.20±0.06	1.16±0.07	1.11±0..07	1.05±0.08
	Zinc *µg/L*	608.33±93.45	646.63±99.22	681.53±95.18	726.07±100.08	744.12±109.76	761.09±103.21	786.11±114.78	816.48±115.33
	Selenium *µg/L*	189.27±29.41	206.01±28.78	211.23±34.56	218.56±31.32	225.07±29.76	235.41±33.78	246.77±34.11	258.46±39.87
RBC	Iron *µg/L*	57.51 ± 6.11	61.39 ± 5.83	64.03 ± 4.61	66.50 ± 5.23	69.83 ± 4.22	71.70 ± 6.09	74.58 ± 5.43	76.81 ± 5.89
	Copper *µg/mL*	1.32 ± 0.08	1.27 ± o.11	1.19 ± 0.07	1.13 ± 0.09	1.08 ± 0.08	1.04 ± 0.10	1.00 ± 0.07	0.94 ± 0.09
	Zinc *µg/L*	653.69 ± 92.84	694.57 ± 87.04	732.20 ± 94.15	782.21 ± 79.91	799.91 ± 90.52	820.09 ± 88,99	843.35 ± 92.03	879.67 ± 96.44
	Selenium *µg/L*	189.87 ± 25.10	206.91± 24.73	211.34 ± 26.41	219.08 ± 23.67	226.04 ± 25. 22	236.11 ± 27.45	247.16 ± 28.01	260.09 ± 25.11
Blood	Iron *µg/L*	57.59 ± 6.89	61.74 ±7.32	64.25 ± 6.54	67.18 ± 6.95	70.28 ± 5.66	72.34 ± 8.01	76.04 ± 6.99	77.68 ± 8.23
	Copper *µg/mL*	1.41 ± 0.08	1.33 ± 0.07	1.28± 0.09	1.21± 0.07	1.15 ± 0.10	1.11 ± 0.08	1.06± 0.10	1.01 ± 0.09
	Zinc *µg/L*	1105.32±160. 17	1175.41± 158.79	1238.28± 167.33	1319.82±159. 87	1352.62±163. 11	1383.45±166. 78	1436.24±159. 08	1483.09±157. 23
	Selenium *µg/L*	205.88 ± 30.11	224.63 ± 29.19	233.12± 31.45	239.25 ± 32.75	247.56 ± 29.08	256.07 ± 30.67	268,74 ± 33.11	281.08 ± 30.23
Scalp hair	Iron *µg/g*	12.96 ± 1.32	13.67± 1.27	14.43 ± 1.35	14.99 ± 1.31	15.75 ± 1.33	16.16 ± 1.29	16.83 ± 1.30	1.75 ± 1.32
	Copper *µg/g*	15.86 ± 3.34	15.22 ± 3.37	14.27 ± 3.29	13.64 ± 3.28	12.94± 3.31	12.51± 3.36	11.97 ± 3.25	11.32 ± 3.61
	Zinc *µg/g*	70.55 ± 10.78	74.98± 11.10	79.08 ± 12.02	84.24± 9.87	86.33 ±10.23	88.35 ± 11.45	91.21 ± 11.33	94.69 ± 10.81
	Selenium *µg/L*	1.27 ± 0.18	1.38 ± 0.20	1.42 ± 0.17	1.45± 0.19	1.52 ± 0.21	1.58 ± 0.16	1.65 ± 0. 18	1.74 ± 0.20
Nail	Iron *µg/g*	13.45 ± 1.35	14.41± 1.38	14.98 ± 1.41	15.56 ± 1.39	16.40 ± 1.33	16.77 ± 1.38	17.44 ± 1.35	18.06 ± 1.36
	Copper *µg/g*	13.09 ± 2.11	12.43± 2.19	11.79 ± 2.14	11.13 ± 2.08	10.68 ± 2.13	10.33± 2.18	9.88± 2.16	9.29 ± 2.14
	Zinc *µg/g*	74.81 ± 11.21	80.09± 11.67	83.86 ± 12.23	89.34 ± 10.89	91.55 ± 13.11	93.67 ± 12,09	97.25 ± 12.11	100.99± 11.21
	Selenium *µg/g*	1.30 ± 0.19	1.42 ± 0.21	1.46 ± 0.20	1.50 ± 0.18	1.55± 0.19	1.62 ± 0.21	1.70 ± 0.22	1.78± 0.18

TABLE VI
THE EFFICACY OF THE NUTRITIONAL SUPPLEMENT ON THE LEVEL OF BIOMARKERS OF TRACE ELEMENTS IN CASE OF ACUTE HEPATITIS

Sample	Element	Primary date	After 12 weeks	After 25 weeks	Sample	Element	Primary date	After 12 weeks	After 25 weeks
Serum	Iron *µg/L*	64.32 ± 6.21	60.51±5.91	58.22±6.23	Blood	Zinc *µg/L*	1285.67±187.92	1166.12±169.33	1100.07±155.21
	Copper *µg/mL*	1.24 ±0.07	1.26±0.09	1.27±0.12		Selenium *µg/L*	250.52 ± 38.54	225.34±35.17	211.21±32.42
	Zinc *µg/L*	720.96 ±100.32	689.11±86.32	677.41±79.41	Scalp hair	Iron *µg/g*	16.3 1 ± 1.57	14.41±1.46	13.67±1.52
	Selenium *µg/L*	227.48 ± 33.75	205.09±29.43	200.43±31.07		Copper *µg/g*	13.58 ± 2.72	14.78±2.65	15.89±2.81
RBC	Iron *µg/L*	70.78 ± 7.75	62.23±8.11	61.01±9.32		Zinc *µg/g*	85.90 ± 12.98	72.19±14.06	69.54±15.12
	Copper *µg/mL*	1.25 ± 0.07	1.28±0.08	1.30±0.12		Selenium *µg/L*	1.5 1 ± 0.21	1.35±0.24	1.31±0.22
	Zinc *µg/L*	824.49 ± 123.98	802.34±111.32	789.22±102.67	Nail	Iron *µg/g*	16.3 1 ± 1.73	14.65±1.89	13.87±1.93
	Selenium *µg/L*	229.99 ± 34.89	210.00±33.98	202.03±29.77		Copper *µg/g*	11.01 ± 1.97	12.89±1.87	13.69±1.77
Blood	Iron *µg/L*	70.23 ± 9.54	62.21±11.21	60.00±12.09		Zinc *µg/g*	85.90 ± 12.67	73.61±13.22	70.05±15.05
	Copper *µg/mL*	1.30 ± 0.06	1.37±0.09	1.40±0.08		Selenium *µg/L*	1.5 1 ± 0.23	1.32±0.21	1.29±0.24

TABLE VII
THE EFFICACY OF THE NUTRITIONAL SUPPLEMENT ON THE LEVEL OF BIOMARKERS OF TRACE ELEMENTS IN CASE OF POST HEPATITIS CIRRHOSIS

Sample	Element	Primary date	After 12 weeks	Sample	Element	Primary date	After 12 weeks
Serum	Iron *µg/L*	45.85 ± 4.63	40.31±5.11	Blood	Zinc *µg/L*	908.65 ±142.11	866.10±79.23
	Copper *µg/mL*	1.47± 0.06	1.56±0.12		Selenium *µg/L*	176.37 ± 27.45	165.14±30.17
	Zinc *µg/L*	514.33 ± 77.13	489.31±66.52	Scalp hair	Iron *µg/g*	11.40 ± 1.21	10.21±1.06
	Selenium *µg/L*	158.33 ± 23.78	145.22±21.47		Copper *µg/g*	17.76 ± 3.08	19.92±2.75
RBC	Iron *µg/L*	51.12 ± 5.34	46.03±6.43		Zinc *µg/g*	62.79 ± 8.93	51.13±8.00
	Copper *µg/mL*	1.47 ± 0.08	1.58±0.13		Selenium *µg/L*	1.12 ± 0.17	1.05±0.14
	Zinc *µg/L*	568.31 ± 87.42	542.55±91.30	Nail	Iron *µg/g*	11.83 ± 1.20	10.55±0.89
	Selenium *µg/L*	168.43 ±26.67	152.32±31.45		Copper *µg/g*	14.33 ± 2.34	15.99±1.67
Blood	Iron *µg/L*	46.70 ± 6.22	42.01±7.01		Zinc *µg/g*	68.07 ± 10.11	58.73±6.22
	Copper *µg/mL*	1.49 ±0.07	1.57±0.10		Selenium *µg/L*	1.17 ± 0.17	1.02±0.11

B. Correlation analysis

Table VIII tabulates the correlation coefficients, calculated from Pearson correlation coefficient, to investigate the inter-relationships between the determined trace elements in serum of patients groups and health group. In serum samples of patients with acute hepatitis, the observed positive correlations between Cu and Se, Zn and Se are (r = 0.460), (r = 0.533), respectively, while, negative correlations are found between Fe and Cu, Cu and Zn to be (r = -0.452) and (r = -0.232), respectively. In serum samples of patients with chronic hepatitis, positive correlations are observed between Fe and Se (r = 511), while, negative correlations are found between Cu and Zn (r = -0.303), while, in serum samples of patients with post hepatitis cirrhosis, positive correlations are observed between Fe and Se (r=0.511), and negative correlations are found between Fe and Cu, Cu and Zn, (r = -0.465), (r = -0.351), respectively. In comparison with control group, strong positive correlations are found for Fe and Zn (r = 0.638), while, negative correlations are found between Fe and Cu, Zn and Se (r = -0.412), (r = -0.427), respectively.

TABLE VIII
CORRELATION COEFFICIENTS BETWEEN METALS IN SERUM OF PATIENTS GROUPS AND HEALTH GROUP

	Fe	Cu	Zn	Se	Fe	Cu	Zn	Se
	Control group				Acute Hepatitis			
Fe	1.000				1.000			
Cu	-0.412(**)	1.000			-0.452(**)	1.000		
Zn	0.638(**)	-.427(**)	1.000		0.019	-0.232(*)	1.000	
Se	0.055	0.012	0.022	1.000	0.036	0.460(**)	0.533(**)	1.000
	Chronic Hepatitis				Post hepatitic Cirrhosis			
	Fe	Cu	Zn	Se	Fe	Cu	Zn	Se
Fe	1.000				1.000			
Cu	-0.069	1.000			-0.003	1.000		
Zn	0.021	-0.303(*)	1.000		0.143	-0.351 (*)	1.000	
Se	0.5 11(**)	0.184	0.179	1.000	0.592(**)	0.010	-0.465(**)	1.000

** Correlation is significant at the 0.01 level.*correlation is significant at 0.05 level.

C. Cluster analysis

Fig.1 represents the determined trace elements apportion using the cluster analysis (CA). The clustering of the metals in serum of health group is represented in Fig.1 (a). Three main clusters of the metals are found in serum of health group. The first cluster includes Zn, the second include includes Se, while, the third strong cluster contains Cu- Fe. Fig 1(b, c and d) shows the clustering of the metals in serum of patients groups, acute, chronic, and post hepatitic cirrhosis, respectively. The cluster analysis revealed identical clusters for both of control group and acute hepatitis patients. The results of cluster analysis are similar between control group and acute hepatitis patients however the degree of association of Se and Cu, in control group is higher than acute hepatitis patients. Cluster analysis doesn't found different patterns of metal distribution in serum of acute hepatitis patients in comparison with control group. The cluster analysis revealed that for chronic hepatitis and Post hepatitis Cirrhosis patients, there are three main clusters of the metals are found. The first cluster contains Zn and the second cluster contains Se and Cu, while the third cluster contains Fe. These results may be concluded that significantly different distribution of trace metals concentration patterns including Cu, Se and Fe in the serum of chronic hepatitis and post hepatitis cirrhosis patients in comparison with health group. Se and Cu may be significantly associated with chronic hepatitis and post hepatitis cirrhosis patients.

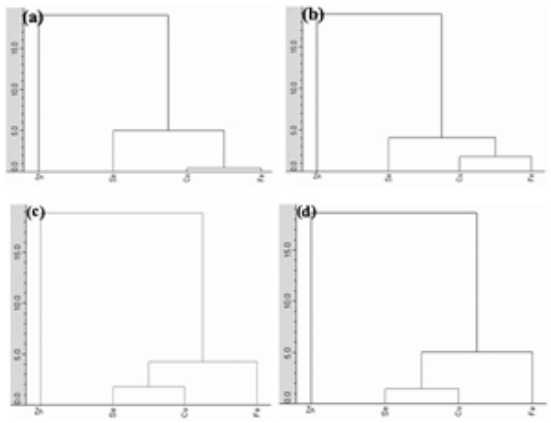

Fig.1 the clustering of the determined trace metal in serum of control group (a), acute hepatitis patients (b), chronic hepatitis (c) and post hepatitic cirrhosis (d).

The resulting dendrogram (Fig.2) Cluster analysis divided multiple duration of nutrient supplement into two different clusters has two major groups, they based on a similarity of eight parameters. The first group represents primary date, after 3, 6, 9 and 12 months. The second group represents after 15, 18 and 21 months). It is seen from the dendrogram that group 1 is characterized by the biggest Euclidean distance in comparison to the second group, which have high significance of clustering. The impact of natural supplements to improve the treatment of viral liver damage, using the level of some trace elements such as, iron, copper, zinc, and selenium is remarkable at three nutritional supplement durations after 15, 18 and 21 months. It indicates that the nutrition supplement after 21 months has a high significant improvement on the level of iron, copper, zinc, and selenium relative to the other durations.

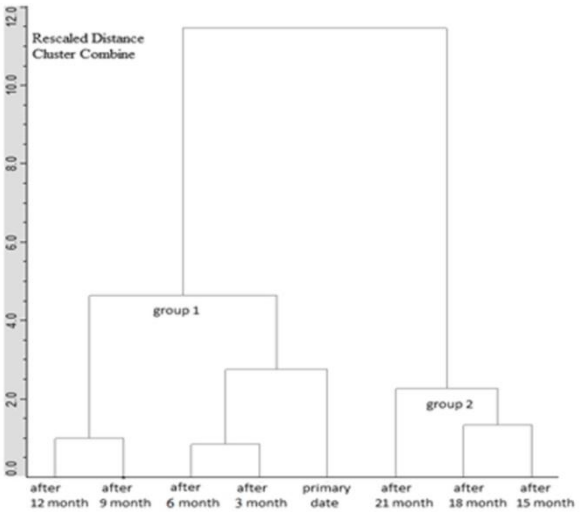

Fig. 2 cluster analysis of the duration supplement in serum of patient group.

III. Discussion

Hepatitis infection frequently leads to chronic hepatitis with increasing risk of developing liver cirrhosis and HCC. The essential micronutrients (Zn, Cu, Fe, and Se) might exacerbate liver disease in case of deficiency, imbalance, or toxicity [21]. Clinical studies reported that hepatitis C virus (HCV) related chronic liver disease patients at different stages of liver damage have impaired metabolism of trace elements [9]. The main aim of this study was to evaluate the efficacy and safety of Viusid, Pharma Nord (Bio-Selenium + Zinc + vitamin C, E and B6) and dietary supplement SiliciumOrganiqe G5 in patients who had a previous history or current evidence of clinical hepatic decompensation. Also, it aims to investigate the serum, RBC, blood, scalp hair and nail levels of trace elements (Cu, Fe, Zn and Se) in the patients with viral hepatitis before and after treatment by Viusid, Pharma Nord (Bio-Selenium + Zinc + vitamin C, E and B6) and dietary supplement SiliciumOrganiqe G5, and to find the relationship between the changes of these levels. The current study showed that the use of nutritional supplements containing trace elements for treatment hepatitis patients can enhance a clear improvement. This improvement may be due to the beneficial effects of Viusid to improve the health of the patients during the period of the disease based on the improvement in the level of the involved trace elements. On another hand, some studies have showed the effectiveness of Viusid for treating the patients with chronic hepatitis C where it can reduce the inflammation and fibrosis by controlling the production of anti-inflammatory cytokines and pro-inflammatory cytokines factors [14]. In addition, zinc as one of most important trace elements has exhibited antioxidant, anti-inflammatory activities and can induce immune modulatory effects [22]. The results in present study showed that Zn levels in serum, RBC, blood, scalp hair and nail of patients before treatment were statistically significant decreased, while after treatments they were statistically significant increased when, compared with before treatment and still they were statistically significant decreased when compared with control group. The behavior of zinc is in agreement with [9,23-25]. We think that the patients have a good response to treatment by Viusid + Pharma Nord (Bio-Selenium + Zinc + vitamin C, E and B6) and dietary supplement. SiliciumOrganiqe G5), where, we found an increase in the level of zinc, iron and selenium and a decrease in the level of copper in serum, RBC, blood, scalp hair and nail for patients under treatment, also, we proposed that Zn and Se increased antiviral effect and in turns response to nutrition therapy in cases of hepatitis. The copper as trace element has a great role in biosynthesis of collagen and is considered as a co-factor against hepatic fibrosis in chronic hepatitis [21, 24-27].

IV. Conclusion

This study evaluated the effect of trace elements formulation as dietary supplements composed of Viusid, Pharma Nord, and SiliciumOrganiqe G5 (3 times per day on patients of hepatitis. Trace elements abnormalities distributions in particular, zinc, selenium, iron and copper, may reflect status of dysfunction in the liver. Also, they may be a way to predict the pathological process as well as the extent of the response to the treatment; we recommend further

studies to confirm the effectiveness of diet therapy. On the other hand, it would be important to evaluate whether the Viusid effects on the clinical outcomes are directly related to the significant reduction in portal pressure in viral hepatitis patients. Trace elements can serve as biomarkers for increases survival and reduces disease progression.

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