BIOCHEMICAL PATTERN FOR HEPATITIS C AND ACUTE LYMPHOBLASTIC LEUKEMIA IN HUMAN SERA

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Abstract: Present experimental work aimed to show role of the molecular biology in diagnosis of hepatitis C liver disease (HCV) and acute lymphoblastic leukemia (ALL) which occur as a result of the disturbances of protein and enzymes fractions at the molecular level. The study carried out using vertical slab gel electrophoresis for detection of the protein pattern, catalase and peroxidase. Protein fractionation of the control samples produced 13 bands with Rf ranged between 0.17 and 0.96 and (amount, 3.14 - 7.24). Comparing hepatitis C with control one out of these 13 bands are completely disappeared at Rf 0.86 (amount 9.34). Ten bands appeared to be common bands in all HCV samples except one sample only nine common bands were produced while the band number ten was disappeared. The data showed that 5 characteristic bands were produced. One from these five bands determined at Rf 0.7 in all HCV sera samples except the first sample. Comparing leukemia samples with control only two were considered as common bands. These bands completely appeared in all sera samples. On the other side one band was completely disappeared in all leukemia samples. The rest bands distributed between different leukemia samples. 15 bands produced as characteristic bands. Electrophoresis pattern for catalase mentioned that six bands were produced in control samples. When hepatitis C compared with the control showed that two out these six bands were completely disappeared and other all HCV four bands considered as common bands. The amount of catalase enzyme completely decreased in all bands. In leukemia five common bands were produced with the appearance of one characteristic band, from the other side one band was disappeared. A documentation of peroxidase pattern data showed that tow common bands were appeared with Rf 0.1 and 0.33, the amount of these two bands were decreased when the amount of HCV compared with control in the same rows. In leukemia there is only one common band was produced with appearance of a three characteristic bands. [Journal of American Science. 2010;6(11):203-216]. (ISSN: 1545-1003).

Keywords: HCV, Acute lymphoblastic leukemia, Protein electrophoresis, catalase, peroxidase

1. Introduction

Hepatitis C virus (HCV) is a major human blood-borne pathogen that has infected almost 170 million people worldwide (Liu et al., 2010). Hepatitis C virus is a single positive stranded RNA virus, classified as family Flaviviridae, genus Hepacivirus. This virus can be differentiated by RNA sequence analysis in to at least 6 major genotypes and more than 100 subtypes Richter, (2002) and Ismail et al., (2004).

Hoofnegle, 1997 and Bièche et al., 2005 reviewed that the infection with HCV is often asymptomatic, but once established, chronic infection can cause inflammation of the liver (chronic hepatitis). This condition can progress to scarring (fibrosis), and advanced scarring (cirrhosis). In some cases, those with cirrhosis will go on to develop liver failure or other complications of cirrhosis, including liver cancer.

Egypt has the highest countrywide prevalence of hepatitis C virus in the world, with an estimated 8–10 million among a population of 68 million having been exposed to the virus and 5–7 million active infections and the prevalence of antibodies to HCV is approximately 10-fold greater than in the United States and Europe (Ebeid and El-Bakry ., 2009). Frank et al., 2000 reported that HCV in Egypt associated with a high morbidity and mortality from chronic liver disease, cirrhosis, and hepatocellular carcinoma; the authors mentioned that Parenteral antischistosomal therapy had a major role in the spread of HCV throughout Egypt.

Leukemia originates from hematopoietic stem cells that lose their ability to differentiate normally for production of the mature blood cells (Battisti et al., 2008). The experimental studies reported that leukemia caused as a result of genetic changes include point mutations, gene deletions and rearrangements. This was able to cause disturbances in the gene expression without changing the DNA sequence (Melki and clark, 2002). There were several types of leukemia. The acute lymphblastic leukemia

(ALL) is a disease characterized by uncontrolled arrest in proliferation and maturation of lymphoid progenitor cells in bone marrow. This resulted in production of an excess of malignant cells (Battisti et al., 2008). The authors mentioned that the lymphoblasts replace the normal marrow elements, resulting in a marked decrease in the production of normal blood cells. Acute lymphoblastic leukemia (ALL) is the most common cancer found in the pediatric population and it accounts for more than 50% of the hematopoietic malignancies (Downing and Shannon, 2002 and Gaynon, 2005).

The present experimental work aimed to show role of the molecular biology in diagnosis of hepatitis C liver disease (HCV) and acute lymphoblastic leukemia (ALL) which occur as a result of the disturbances of protein and enzymes fractions at the molecular level. The study carried out using vertical slab gel electrophoresis for detection of protein, catalase and peroxidase patterns.

2. Material and Methods

This study was conducted in the nuclear research center, atomic energy authority, Inshas. Blood samples were taken as part of the experimental research. The study carried out on 29 cases divided into three groups. 10 patients diagnosed to have hepatitis C infection representing the HCV group, 9 diagnosed to have acute lymphoblastic leukemia representing the leukemia group and 10 were chosen from the same population and taken as control.

Hepatitis C patients

Ten patients; (males) mean age 30 ± 6 years, with hepatitis C infection. patients diagnosis depended completely on the historical review of each patient represented in elevation of serum transaminases, presence of anti-HCV antibodies The patients with other autoimmune liver diseases were excluded from the study.

Leukemia patients

Nine patients (males), mean age 28 ± 12 years infected with acute lymphplastic leukemia, The disease diagnosis depended completely on the historical review of each patient including clinical examination, complete blood picture, bone morrow aspiration . The patients with other diseases were excluded from the study.

Control group

Ten healthy individuals (male), mean age 27 ± 7 years. They were selected on the basis of general physical examination. They have no any disease of HCV, HBV and HIV. There was no pervious history of hepatitis and/or blood related cancers in any individual. They obey all the biochemical tests including liver functions (Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Gamma Glutamyl Transferase (GGT), complete blood count and bone marrow examination, protein profile (Total protein and albumin) showing no evidence of any abnormal disturbances.

Sample collection and assay:

Five milliliter blood samples were collected. Samples were collected on heparin (anti coagulant) in a sterile micro centrifuge tube and then centrifuged at 400 x g for five minutes. The relatively clear supernatant were divided into aliquots and frozen at -40oC till the assay time.

Total protein was determined according to Bradford, 1976, then Protein electrophoresis methods and procedures were taken from the book Gel electrophoresis of proteins (Hames, 1990). Resolving Gel (8%) solution were prepared for Native Poly acrylamide gel electrophoresis (PAGE) by mixing 14.4 ml distilled water, 8.1 ml of Acrylamide/Bis (30% T, 2.67% C) stock solution, and 7.5 ml Tris (1.5M, pH8.8). The total volume of the solution was 30 ml. To this solution 150µl of 10% APS, freshly prepared, and 30 µl of TEMED were added prior to pouring into the gel plate assembly. Then proper comb was inserted into the assembly to from wells in which samples were loaded. Electric current of 50 mA was applied on Serum protein samples under cooling conditions at 4 oC for about 4 hours. At the end of the run, electrical current was stopped and the gels were stained overnight then photographed after destaining.

Native protein gel was stained for catalase pattern depending on Gregory and Fridovich, (1974); Siciliano and Shaw, (1976) and Baker and Manwell, (1977). Peroxidase pattern was determined as Native protein gel then it was stained for using certain stain prepared according to Siciliano and Shaw, (1976) ; Misra and Fridovich, (1977) ; Shimoni, M. (1994) and Rescigno et al., (1997).

Data analysis

Gel plate was photographed, scanned and then analyzed using a gel pro Analyzer (Version 3.1 Media Cybernetics USA) for the analysis of tested samples. This program is a comprehensive computer software application designed to determine the relative fragmentation, the molecular weights and the amounts of protein as well as scanned graphical presentation of the fractionated bands of each lane.

The similarity index (S.I.) compares patterns within, as well as, between Control, hepatitis C and leukemia samples using the formula: S.I. = (2 Nab/Na + Nb) (Nei and Li, 1979). Where, Na and Nb are the

number of bands in individuals a and b and Nab is the number of shared bands between a and b. The similarity values were converted into genetic distance (D) using the formula: D = 1 – S.

3. Results

I -Protein electrophoresis of control and hepatitis C liver disease:

The protein pattern in sera of the control and HCV patients was revealed in table (1) and graphically illustrated in Fig. (1). protein fractionation of the control samples produced 13 bands with Rf ranged between 0.17 and 0.96 and (amount, 3.14 - 7.24). Comparing hepatitis C with control one out of these 13 bands are completely disappeared at row r18 with Rf 0.86 (amount 6.39) while bands in row r19 were disappeared in all HCV sera samples except first one; appeared with low amount (4.63) when compared with control at Rf 0.95. A documentation of protein in HCV sera showed that ten bands appeared to be common bands at rows r1, r3, r5, r6, r7, r8, r10, r11, r12, r13 and r17 in all HCV samples except HCV7 9 common bands were produced while the band number ten was disappeared at r8. The data showed that 5 characteristic bands were produced., HCV4 at r4 with Rf 0.26 (amount 3.39), HCV9 at r15 with Rf 0.68 (amount 4.24), HCV 10 at r2 and r14 at Rf 0.19 and 0.64 with amount 7.7 and 3.01 respectively, the five one appeared in r16 with Rf 0.7 and amount range (6.1 - 12.7) in all HCV sera samples except the first sample

The similarity indices between the control samples & hepatitis C samples (Table: 2) showed high value, S.I range (0.81- 0.96). from the other side, by comparing the infected sera samples with each other, the similarity index again recorded high values (range: 0.85-1).

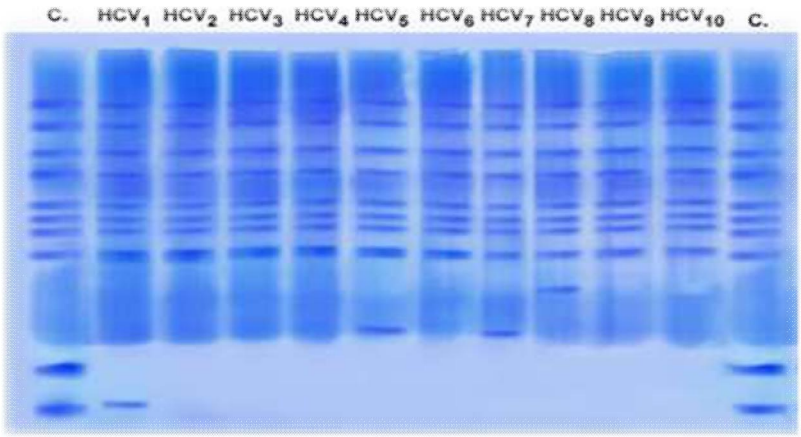

Fig. (1): The protein pattern detected in sera of control and different HCV patients.

Table (1): protein pattern in human sera of Control and HCV samples

Lanes	Control Lane 1		HCV_1 Lane 2		HCV_2 Lane3		HCV_3 Lane4		HCV_4 Lane 5	
Rows	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount%	(Rf.)	Amount %	(Rf.)	Amount %
R_1	0.17	9.2% (6.32)	0.17	13.1% (7.59)	0.17	14.03% (9.21)	0.17	14.8% (9.71)	0.18	14.5% (10.4)
R_2										
R_3	0.24	10.4% (7.12)	0.24	7.9% (4.59)	0.24	10.45% (6.86)	0.24	9.7% (6.34)	0.24	8.5% (6.1)
R_4									0.26	4.7% (3.39)

R_5	0.3	6.7% (4.59)	0.3	8.2% (4.75)	0.3	6.40% (4.2)	0.3	6.8% (4.46)	0.3	6.2% (4.46))
R_6	0.36	7.6% (5.19)	0.36	6.6% (3.84)	0.36	6.43% (4.22)	0.36	7.4% (4.87)	0.36	7.5% (5.33)
R_7	0.41	9.1 (6.2)	0.41	6.7% (3.89)	0.41	6.20% (4.07)	0.41	5.4% (3.51)	0.41	5.3% (3.81)
R_8	0.45	6.2% (4.27)	0.45	8.3% (4.84)	0.45	6.26% (4.11)	0.45	7.9% (5.15)	0.45	6.9% (4.95)
R_9										
R_{10}	0.48	4.8% (3.3)	0.48	5.4% (3.16)	0.48	5.94% (3.9)	0.48	5.9% (3.9)	0.48	5.3% (3.76)
R_{11}	0.51	4.6% (3.14)	0.51	4.5% (2.61)	0.51	5.03% (3.3)	0.51	5.4% (3.5)	0.51	4.3% (3.09)
R_{12}	0.54	5.6 (3.86)	0.54	6.3% (3.65)	0.54	5.54% (3.64)	0.54	6.3% (4.09)	0.54	5.3% (3.8)
R_{13}	0.6	5.2% (3.52)	0.6	12.6% (7.32)	0.6	9.40% (6.17)	0.6	9.4% (6.13)	0.6	9.5% (6.81)
R_{14}										
R_{15}										
R_{16}					0.7	13.37% (8.78)	0.7	10.2% (6.66)	0.7	11.6% (8.31)
R_{17}	0.79	10.6 (7.24)	0.79	12.4% (7.22)	0.79	10.95% (7.19)	0.79	10.8% (7.09)	0.79	10.2% (7.28)
R_{18}	0.86	9.4% (6.39)								
R_{19}	0.95	10.5% (7.2)	0.95	8.0% (4.63)						

Note: number between practices expressed the real mass of protein

Table (1): continued

HCV_5 Lane 6		HCV_6 Lane 7		HCV_7 Lane 8		HCV_8 Lane 9		HCV_9 Lane 10		HCV_{10} Lane 11		Control Lane 12	
(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %
0.17	15.2% (10.4)	0.17	15.7% (10.7)	0.17	14.4% (9.71)	0.17	16.9% (11)	0.17	14.2% (10.98)	0.17	13.9% (9.01)	0.17	9.2% (6.32)
								0.19	9.9% (7.7)				
0.24	10.0% (6.67)	0.24	8.8% (5.96)	0.24	9.7% (6.56)	0.24	9.0% (5.86)	0.24	8.3% (6.42)	0.24	9.4% (6.09)	0.24	10.4% (7.12)
0.3	6.9% (4.57)	0.3	7.2% (4.93)	0.3	6.5% (4.42)	0.3	6.6% (4.31)	0.3	6.3% (4.92)	0.3	6.9% (4.49)	0.3	6.7% (4.59)
0.36	8.2% (5.43)	0.36	6.6% (5.5)	0.36	8.3% (5.58)	0.36	7.3% (4.74)	0.36	5.3% (4.1)	0.35	7.3% (4.76)	0.36	7.6% (5.19)
0.41	5.3% (3.54)	0.41	6.8% (4.6)	0.41	6.1% (4.14)	0.41	8.4% (5.44)	0.41	5.5% (4.03)	0.41	6.2% (4)	0.41	9.1% (6.2)

0.45	6.5% (4.32)	0.45	7.0% (4.74)			0.45	7.0% (4.53)	0.45	6.0% (4.65)	0.45	7.2% (4.69)	0.45	6.2% (4.27)
0.48	5.8% (3.4)	0.48	4.7% (3.18)	0.48	4.9% (3.3)	0.48	5.6% (3.66)	0.48	3.9% (3.04)	0.48	5.8% (3.74)	0.48	4.8% (3.3)
0.51	5.9% (3.9)	0.51	4.7% (3.22)	0.51	5.2% (3.54)	0.51	4.6% (3.01)	0.51	4.5% (3.5)	0.51	4.8% (3.11)	0.51	4.6% (3.14)
0.54	4.8% (3.2)	0.54	5.6% (3.82)	0.54	6.4% (4.35)	0.54	5.1% (3.29)	0.54	4.8% (3.75)	0.54	6.4% (4.12)	0.54	5.6% (3.86)
0.6	8.1% (5.36)	0.6	9.8% (6.66)	0.6	8.9% (6)	0.6	9.1% (5.9)	0.6	8.1% (6.3)	0.6	9.3% (6.04)	0.6	5.2% (3.52)
								0.64	3.9% (3.01)				
						0.68	6.5% (4.24)						
0.7	12.5% (8.32)	0.7	12.0% (8.2)	0.7	18.8% (12.7)	0.7	9.3% (6.1)	0.7	8.9% (6.88)	0.7	10.4% (6.76)		
0.79	10.9% (7.23)	0.79	11.1% (7.55)	0.79	10.8% (7.29)	0.79	13.8% (8.98)	0.79	10.3% (7.97)	0.79	12.3% (8)	0.79	10.6% (7.24)
												0.86	9.4% (6.39)
												0.95	10.5% (7.2)

Rf: rate of flow
Note: number between practices expressed the real mass of protein

Table (2): Protein pattern similarity index (SI) and genetic distance (Gd) in sera of control and different HCV samples.

	S.I											
G.d	Lane	C.	HCV_1	HCV_2	HCV_3	HCV_4	HCV_5	HCV_6	HCV_7	HCV_8	HCV_9	HCV_{10}
	C.	.. ..	0.96	0.88	0.88	0.85	0.88	0.88	0.83	0.85	0.81	0.88
	HCV_1	0.04	.. ..	0.92	0.92	0.88	0.92	0.92	0.87	0.88	0.85	0.92
	HCV_2	0.12	0.08	.. ..	1	0.96	1	1	0.96	0.96	0.92	1
	HCV_3	0.12	0.08	0	.. ..	0.96	1	1	0.96	0.96	0.92	1
	HCV_4	0.15	0.12	0.04	0.04	.. ..	0.96	0.96	0.92	0.92	0.89	0.96
	HCV_5	0.12	0.08	0	0	0.04	.. ..	1	0.96	0.96	0.92	1
	HCV_6	0.12	0.08	0	0	0.04	0	.. ..	0.96	0.96	0.92	1
	HCV_7	0.17	0.13	0.04	0.04	0.08	0.04	0.04	.. ..	0.92	0.92	0.96
	HCV_8	0.15	0.12	0.04	0.04	0.08	0.04	0.04	0.08	.. ..	0.89	0.96
	HCV_9	0.19	0.15	0.08	0.08	0.11	0.08	0.08	0.08	0.11	.. ..	0.92
	HCV_{10}	0.12	0.08	0	0	0.04	0	0	0.04	0.04	0.08	.. ..

II. Protein electrophoresis of control and leukemia

Protein pattern in sera of the control and leukemia patients was revealed in table (3) and graphically illustrated in Fig. (2). Protein fractionation of the control samples produced 12 bands with Rf ranged between 0.08 and 0.73 and (amount, 3.46 - 15.2). Comparing leukemia samples with control only two of these 12 bands were considered as common bands these bands completely appeared in all sera samples. On the other side one band was completely disappeared in all leukemia samples at row r3 with Rf 0.14 (amount, 8.59). The rest nine bands distributed between different leukemia samples. 15 bands produced as characteristic bands ; two characteristic bands were

produced at row r4 with Rf 0.18 (amount, 8.2 and 7.27) for samples number five and seven respectively, at row r7 only one was detected in the four sample of leukemia with Rf 0.25 (amount,7.42), the same occurred at row r11 one band was determined with Rf 0.45 (amount, 10.1) for sample number 6, another two were appeared at row r12 with Rf 0.5 (amount, 8.74 and 8.3) in samples number five and seven respectively, while in the row r16 five characteristic bands were documented at Rf 0.7 (amount range: 5.2 and 7.82) for sera samples for patients number one, two, five, seven and nine. The rest four bands were fractionated at row r17 with Rf 0.78 and the bands mostly had the same amount (amount range: 5.71 And 6.27) in the first four samples.

The similarity indices between the control & acute lymphoblastic leukemia samples showed low value, S.I range (0.47-0.82) the data indicating to the severe effect of leukemia on the protein fractions (Table:4). From the other side, by comparing the infected sera samples of leukemic patients with each other, the similarity index again recorded (range: 0.33-0.95).

Fig. (2): Graphic illustration of protein pattern of Control and different leukemia patients.

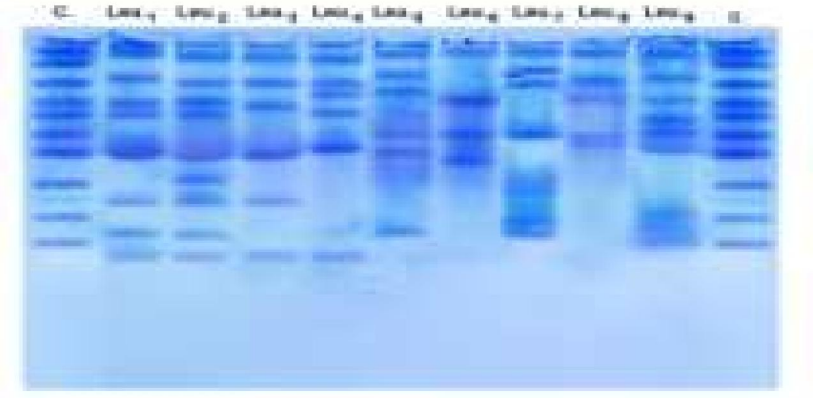

Table (3): Protein pattern of Control and acute lymphoblastic leukemia sera samples

Lanes:		**Control Lane 1**		**Leukemia$_1$ Lane 2**		**Leukemia$_2$ Lane 3**		**Leukemia$_3$ Lane 4**		**Leukemia$_4$ Lane 5**
Rows	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %	(Rf.)	Amount %
R_1	0.08	4.2% (3.96)	0.08	4.6% (3.34)	0.08	5.2% (3.85)	0.08	7.1% (3.87)	0.08	6.3% (3.3)
R_2	0.12	7.9% (7.54)	0.12	8.8% (6.41)	0.12	10.0% (7.36)	0.12	16.4% (8.91)	0.12	16.5% (8.6)
R_3	0.14	9.0% (8.59)								
R_4										
R_5	0.23	8.5% (8.14)	0.23	11.9% (8.62)	0.23	9.7% (7.09)	0.23	13.4% (7.26)	0.23	14.2% (7.4)
R_6									0.25	13.6% (7.42)
R_7	0.28	7.6% (7.27)	0.28	10.8% (7.87)	0.28	11.8% (8.7)	0.28	16.7% (9.1)		
R_8	0.33	15.9% (15.2)	0.33	20.7% (15)	0.33	12.4% (9.13)			0.33	17.5% (9.17)
R_9	0.37	12.7% (12.1)								
R_{10}	0.43	11.0% (10.5)	0.43	17.8% (12.9)	0.43	13.8% (10.1)	0.43	24.7% (13.4)	0.43	19.9% (10.4)
R_{11}										
R_{12}										
R_{13}	0.54	6.8% (6.48)			0.54	11.2% (8.26)				
R_{14}	0.59	3.6% (3.46)	0.59	8.1% (5.91)	0.59	10.3% (7.57)	0.59	10.9% (5.94)		
R_{15}	0.65	6.5%								

		(6.16)								
R_{16}			0.7	9.4% (6.81)	0.7	7.5% (5.49)				
R_{17}	0.73	6.3% (5.98)								
R_{18}			0.78	7.9% (5.71)	0.78	8.0% (5.89)	0.78	10.8% (5.85)	0.78	12.0% (6.27)

R_f: rate of flow

Table (3): continued

Leukemia$_5$ Lane 6		Leukemia$_6$ Lane 7		Leukemia$_7$ Lane 8		Leukemia$_8$ Lane 9		Leukemia$_9$ Lane 10		Control Lane 11	
Rf.	amount%	Rf.	Amount%	Rf.	Amount%	Rf.	Amount%	Rf.	Amount%	Rf.	Amount%
0.08	5.4% (3.7)	0.08	7.10% (3.05)	0.08	4.30% (3.24)	0.08	7.96% (3.58)	0.08	4.2% (3.04)	0.08	4.2% (3.96)
0.12	9.7% (6.59)	0.12	18.22% (7.83)	0.12	10.50% (7.92)	0.12	15.99% (7.19)	0.12	10.5% (7.7)	0.2	7.9% (7.54)
										0.14	9.0% (8.59)
0.18	12.1% (8.2)			0.18	10.96% (7.27)						
0.23	11.6% (7.9)			0.23	13.92% (10.5)	0.23	19.35% (7.7)	0.23	10.0% (7.34)	0.23	8.5% (8.14)
		0.28	24.43% (10.5)			0.28	19.75% (8.88)	0.28	10.3% (7.53)	0.28	7.6% (7.27)
0.33	11.6% (7.86)							0.33	12.3% (8.99)	0.33	15.9% (15.2)
0.37	10.7% (7.25)	0.37	26.76% (11.5)	0.37	13.79% (10.4)	0.37	19.64% (8.83)	0.37	15.2% (11.1)	0.37	12.7% (12.1)
0.43	14.5% (9.87)					0.43	17.30% (7.78)	0.43	13.1% (9.56)	0.43	11.0% (10.5)
		0.45	23.50% (10.1)								
0.5	12.9% (8.74)			0.5	11.00% (8.3)						
				0.54	8.33% (6.28)					0.54	6.8% (6.48)
				0.59	9.32% (7.03)					0.59	3.6% (3.46)
				0.65	10.34% (7.08)			0.65	10.5% (7.66)	0.65	6.5% (6.16)
0.7	11.5% (7.82)			0.7	7.54% (5.69)			0.7	6.9% (5.02)		
								0.73	7.1% (5.21)	0.73	6.3% (5.98)

R_f: rate of flow

Note: number between practices expressed the real mass of protein

Table (4): The protein pattern similarity index (SI) and genetic distance (Gd) in sera of control and Acute lymphoblastic leukemia sera samples.

	S.I										
G.d	lanes	control	$leuk_1$	$leuk_2$	$leuk_3$	$leuk_4$	$leuk_5$	$leuk_6$	$leuk_7$	$leuk_8$	$leuk_9$
	control	….	0.67	0.73	0.63	0.53	0.57	0.47	0.64	0.67	0.82
	$leuk_1$	0.33	….	0.95	0.88	0.75	0.67	0.43	0.53	0.67	0.74
	$leuk_2$	0.27	0.05	….	0.82	0.71	0.63	0.40	0.60	0.63	0.70
	$leuk_3$	0.37	0.13	0.18	….	0.71	0.50	0.50	0.47	0.77	0.59
	$leuk_4$	0.47	0.25	0.29	0.29	….	0.63	0.33	0.35	0.62	0.59
	$leuk_5$	0.43	0.33	0.37	0.50	0.38	….	0.43	0.74	0.67	0.74
	$leuk_6$	0.53	0.57	0.60	0.50	0.67	0.57	….	0.40	0.73	0.53
	$leuk_7$	0.36	0.47	0.40	0.53	0.65	0.26	0.60	….	0.50	0.60
	$leuk_8$	0.33	0.33	0.38	0.23	0.38	0.33	0.27	0.50	….	0.75
	$leuk_9$	0.18	0.26	0.30	0.41	0.41	0.26	0.47	0.40	0.25	….

Catalase electrophoresis:

Catalase pattern of control serum, hepatitis C and leukemia were shown in Table (5) and Figures (3) Electrophoresis pattern for catalase mentioned that six bands were produced in control samples with Rf range (0.05, 0.58), (amount range: 6.88-25.2). When hepatitis C compared with the control showed that two out these six bands were completely disappeared in r3 and r6 and other all HCV four bands considered as common bands. The amount of catalase enzyme completely decreased in all bands with the respect of control ones.

In leukemia five common bands were produced at rows r1, r2, r3, r6 and r7 with the appearance of one characteristic band at r5 with Rf 0.33 (amount, 10.7), from the other side one band was disappeared completely at r4 when compared with control. Similar to hepatitis C all bands of acute lymphoblastic leukemia was decreased in its amount.

The similarity indices between the control samples & hepatitis C samples showed high value (SI=0.8) and control samples with leukemia showed the highest value (SI=0.83) indicating that low obvious effect of hepatitis C virus & leukemia in the catalase activity of human body.

Fi

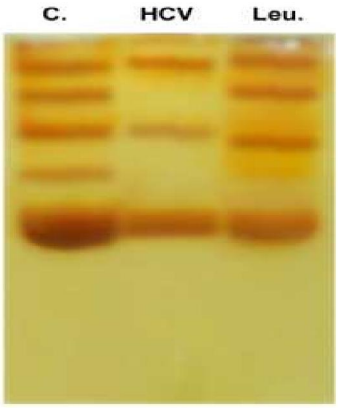

Fig (3): Catalase bands detected in serum of control, hepatitis C & Leukemia respectively

Table (5): Catalase pattern of serum of control, hepatitis C and leukemia

Lanes:	Control, Lane 1		HCV, Lane 2		Leukemia, Lane 3	
Rows	(Rf.)	amount %	(Rf.)	amount%	(Rf.)	amount%
R_1	0.05	9.7% (6.88)	0.05	8.4% (3.35)	0.05	6.2% (3.53)
R_2	0.097	16.3% (11.6)	0.097	21.1% (8.42)	0.097	16.4% (9.35)
R_3	0.19	11.6% (8.24)			0.19	15.1% (8.61)
R_4	0.3	15.6% (11.1)	0.3	23.3% (9.3)		
R_5					0.33	18.5% (10.6)
R_6	0.43	11.5% (8.19)			0.43	12% (6.89)
R_7	0.58	35.3% (25.2)	0.58	47.2% (18.8)	0.58	31.8% (18.2)

Rf: rate of flow; **Note:** number between practices expressed the real mass of protein

Table (6): Catalase similarity index (S.I) and genetic distance (G.d) between control, hepatitis C and leukemia samples.

	S.I			
	Lanes	Control	HCV	Leukemia
	Control	_____	0.8	0.83
G.d	HCV	0.2	_____	0.6
	Leukemia	0.17	0.4	_____

Peroxidase pattern

The peroxidase pattern of the serum isolated from control, hepatitis C and leukemia were shown in Table (7) and in Fig. (4). A documentation of peroxidase pattern data showed that the peroxidase fractionation in control appeared as three bands with Rf 0.1, 0.25 & 0.33 with amount (10.5, 12.8 & 11.1).When hepatitis C compared with the control, it was observed that two common bands were appeared at Rows (r2, & r5), with Rf 0.1 and 0.33 (amount, 4.37 and 6.2) respectively the amount of these two bands were decreased when the amount compared with control in the same rows.

When leukemia samples compared with the control, data showed there is only one common band was produced at r7 with Rf 0.33 (amount 13.9), with appearance of a three characteristic bands in the rows r1 at Rf 0.07 (amount, 8.06), r3 at Rf 0.13 (amount 9.98) and r4 at Rf 0.22 (amount, 12.4). On the other hand, the amount of the leukemia common band was higher than that of control.

The similarity index between the control, hepatitis C and leukemia recorded in the table (8). By comparing hepatitis C with control, the similarity index recorded values (SI=0.57). Also, comparing between the control and leukemia the similarity index recorded values (SI=0.3).indicating the effect of hepatitis C virus & leukemia on the peroxidase fractions and failure in overcomes effect of this diseases.

Fig(4): Peroxidase bands detected in serum of control, hepatitis C & Leukemia respectively

Table (7): Peroxidase pattern of serum of control, hepatitis C and leukemia

	Control		HCV		Leukemia	
Lanes:	**Lane 1**		**Lane 2**		**Lane 3**	
Rows	**(Rf.)**	**(amount)**	**(Rf.)**	**(amount)**	**(Rf.)**	**(amount)**
R_1			**0.07**	**11.4% (3.43)**	**0.070**	**18.2% (8.06)**
R_2	**0.1**	**30.53% (10.5)**	**0.1**	**15.7% (4.73)**		
R_3					**0.13**	**22.5% (9.98)**
R_4					**0.22**	**27.96% (12.4)**
R_5	**0.25**	**37.2% (12.8)**	**0.25**	**20.6% (6.2)**		
R_6			**0.29**	**51.3% (15.8)**		
R_7	**0.33**	**32.27% (11.1)**			**0.33**	**31.3% (13.9)**

Rf: rate of flow**.**
Note: number between practices expressed the real mass of protein**.**

Table (8): Peroxidase similarity index (S.I) and genetic distance (G.d) between control, hepatitis C and leukemia samples**.**

		S.I		
G.d	Lanes	**Control**	**HCV**	**Leu**
	Control	**_____**	**0.57**	**0.3**
	HCV	**0.43**	**_____**	**0.25**
	Leu	**0.7**	**0.75**	**_____**

3. Result

The proteome is the total complement of proteins expressed within a cell, a tissue or an organism. Proteins rather than genes or mRNAs represent the key players in the cell. Proteomics is the study of proteins, including their expression level, post-translational modification and interaction with other proteins, on a large scale. Expression levels of proteins determine the cellular phenotype and its plasticity in response to external signals. Since not all proteins are expressed at all times, but are dependent on physiological and environmental factors, proteomics can provide an excellent global view of disease processes at the protein level (Hütter et al., 2009 and Oie 2009).

I. Protein electrophoresis of control, HCV and leukemia patients

Data in the present study indicated that the specific protein bands of Hepatitis C and leukemia samples differed (through disappearance in some protein bands or appearance of new ones) after comparing with the control (there was a grate difference in the effect of the leukemic disease among patients' sera samples themselves. While in HCV the data indicating that the viral infection had low mutagenic effect on the protein pattern and mostly no-significant differences appeared of the different samples among themselves. The disappearance in certain protein bands of Hepatitis C & leukemia sera may be attributed to the effects of oxidative stress (translational modification and interaction with other proteins) which inhibit the synthesis and expression process of these deleted proteins (qualitative effect). In addition, even the bands remained it usually differs in the amount of protein and this may be explained as translational modification could not inhibit the synthesis of this protein type, but it may be affected only on the quantitative level. Protein is an essential nutrient made up of building- block chemicals called amino acids. Protein provides energy and is needed for the body to make new cells, to maintain and rebuild muscles, to carry other nutrients, to act as messengers in the body, and to support the immune system. Low levels may be seen in severe malnutrition and with conditions that cause malabsorption. Changes in total protein levels may be seen with chronic inflammation or infections such as viral hepatitis. They may be caused by bone marrow disorders such as different types of leukemia and multiple myeloma (Dhinaa, and Palanisamy, 2010).

Choi and James, 2006 reported that oxidative stress has emerged as a key player in the development and the progression of many pathological conditions,

including HCV-induced pathogenesis of liver. Oltra et al., 2001 and Al-Gayyar et al., 2007 mentioned that there was a relationship between leukemia and oxidative stress. Leukemic cells produce higher amounts oxidative stress (ROS) than non-leukemic cells.

Hawkins et al., 2009 and Rahmanto et al., 2010 discraped protein to be a major quantitative target for oxidative stress as a result of their abundance in cells (proteins compose ca. 70% of the dry mass of cells), plasma, and most tissues and their rapid rates of reaction with many oxidants.

Omar et al., 1995 founded that serum protein electrophoresis showed a significant decrease in albumin and increase in alpha-1, beta and gamma globulin levels in HCV infected group as compared to seronegative and anti-HCV positive groups, a finding that may reflect an increased burden on the liver. The significant increase in gamma globulins in the anti-HCV positive as well as the combined anti-HCV and HBsAg positive roups may be due to the increase in one or more of the immunoglobulins, necessitating immunoglobulin typing, an observation being currently investigated.

According to Battisti et al, 2008 Protein oxidation, determined by protein carbonyl content in serum of ALL patients observed that there was a significant difference between the patients and the controls. The protein carbonyl content was increased in the just diagnosed patients, when compared to the controls. These in the serum of patients with chronic leukemia and acute lymphoblastic leukemia and of bone marrow transplant recipients.

In acute lymphoblastic leukemia (ALL) show elevated levels of ox datively modified DNA lesions. Supportive of the finding those oxidative events are largely responsible for spontaneous mutagenesis, and strongly implicating such damage in the etiology of cancer. Oxidative mechanisms have been shown to have a potential role in a cell becomes malignant by DNA mutation, activation of proto-oncogenes and inactivation or loss of tumor suppressor genes (Evans et al., 2004 and Kong et al., 2009).

II. Isozymes electrophoretic pattern of control, HCV and leukemia.

II.I Catalases group

Catalase is an enzyme present in the cells of plants, animals and aerobic (oxygen requiring) bacteria. Catalase is located in a cell organelle called the peroxisome. The enzyme very efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen. Catalase has one of the highest turnover rates for all enzymes: one molecule of catalase can convert ≈6 million molecules of hydrogen peroxide to water and oxygen each minute.The significantly decreased capacity of a variety of tumours for detoxifying hydrogen peroxide is linked to a decreased level of catalase (Valko, et al., 2006).

Data in present study indicating that two bands were completely disappeared while, the amount of the enzyme completely decreased in all bands. In leukemia five common bands were produced with the appearance of one characteristic band, on the other hand one band was completely disappeared. In leukemia sera catalase amount appeared to be low when compared with control. Inhibition the synthesis and expression process of these deleted bands may refer to qualitative change. In addition, differs in the amount of the enzymes may be explained as translational modification could not inhibit the synthesis of this catalase type, but it may be affected only on the quantitative level.

There was a possible link between decreased catalase activity and increased levels of cellular alterations. The oxidative damage supported the idea that there was a persistence of oxidative stress in acute lymphoblastic leukemia (Battisti et al., 2008). Patients with chronic HCV infection are under the influence of oxidative stress associated with lower levels of antioxidant enzymes. Ebeid and El-Bakry., 2009 mentioned that the level of catalase was decreased in group of children with chronic hepatitis C, in comparison to the healthy children.

CAT activity in total blood of ALL patients just diagnosed was reduced when compared to controls. Battisti, et al 2008 suggested that oxidative damage accumulates in biological molecules during aging and that oxidative stress is relevant to the aging process. However, the antioxidant capacity of tissues decreases during aging. This phenomenon indicates a disturbance of the protective role of these enzymes against free radicals in ALL. These findings are in accordance with earlier studies of Oltra et al., 2001 confirmed decreased CAT activities in the lymphocytes of lymphocytic leukemia patients. The results are also in agreement with the reports of Sentuërker et al., 1997, who demonstrated reduced CAT activities in the lymphocytes of ALL patients, and Madej et al. 1988 who found a decreased activity of these enzymes during the development of the leukemic process in mice.

ii.ii. Peroxidase group

Glutathione peroxidase (GSH-Px) enzymes are the most important hydrogen peroxide (H2O2)-removing enzymes in mammalian cells (Nagwa, et al., 2010). Valko et al., 2006 reviwed that GSH-Px acts in conjunction with the tripeptide glutathione (GSH), which is present in cells in high (micromolar) concentrations. The substrate for the catalytic reaction

of GSH-Px is H2O2, or organic peroxide ROOH. GSH-Px decomposes peroxides to water (or alcohol).

Data in present study indicating that oxidative stress seemed to have effect on peroxidase enzyme activity. This effect appeared on HCV and leukemia samples when compared with control. The data show disappearance in some bands in HCV samples and even the bands remained it usually differs in the amount and this may be explained as oxidative stress may be inhabit or decreases preoxidase enzyme activity. On the other hand appearance of some bands of peroxidase enzymes in leukemia samples when compared with control. The data may explain as the role of peroxidase enzymes in the protection of cell from oxidative damage.

In hepatitis C virus GSHPx depleted when compared to the control. The viral activity is enhanced by redox imbalance and peroxidation with virus expression in chronically infected cells. The increase in peroxidation appears to contribute to disease progression with reduction of antioxidant activity that favors further viral replication and potentiates carcinogenesis (Stehbens., 2004). The author founded that Antioxidants suppress viral infections. Therefore, large doses of primary antioxidants should be the initial therapy to restore and thereafter to maintain serum and tissue concentrations at high normal values. Optimal plasma and tissue levels of all antioxidants require review, because when under severe stress, are too low to prevent pathological cellular changes.

Levent, et al., 2006 reviewed that the reduction in the amount of superoxide dismutases (SOD), and GSH-Px reflects both a decrease in the synthesize capacity of liver, and the antioxidant defense power of the patients. It can be argued that increased lipid peroxidation is caused by the inflammation related to viral infection and decreased the antioxidant levels may be an early marker of the oxidative stress. Lipid peroxides formed can be chemotactic for the neutrophils causing increased inflammation, which further drives oxidant-mediated injury in the liver.

GSH-Px activities were significantly increased in ALL when compared to the controls. Devi et al., 2000 reported that antioxidant enzyme activities showed significant increased red cell GSH-PX activity in leukemia patients. These scavenging enzymes play an important role in the protection of cell from oxidative damage. The authors mentioned that GSH-Px is known to detoxify lipid peroxides and thereby inhibit lipid peroxidation, thus although the generation of superoxide anion was increased the observed normal malonaldehyde (MDA) levels in patients could be due to the increased protective response of antioxidant enzymes. Alternatively, increased GSH-PX activity could also maintain normal MDA levels through utilization of lipid peroxide as substrates and produce alcohols as by products.

Conclusions

Proteins and enzymes were involved in cell proliferation, invasion, angiogenesis, metastasis, inflammation, synthesis, energy and metabolism. We recommended using common and characteristic bands as an investigation diagnostic biomarker as these bands reflects the physiological and pathological state where many proteins and enzymes in the resistant cell lines were found to have increased or decreased abundances, reflecting changes in both gene expression/regulation and protein degradation for identifying more selective targets for therapeutic intervention. Also more studies in different etiologies and a larger number of subjects should be considered.

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