Serum Retinol Level in Patients with Chronic Liver Disease

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Abstract: Chronic hepatitis C virus (HCV) infection is a major risk factor for hepatocellular carcinoma (HCC). The rate of HCC development among HCV-infected persons ranges between 1% and 3% after 30 years. Retinoids are known to possess an anti-tumor role by suppressing tumor promotion and progression. Retinoid depletion is often observed during pre-malignant status and cancer development. Loss of retinoid activity or responsiveness is closely linked to carcinogenesis in several organs. This work aimed to investigate serum retinol levels in patients with chronic hepatitis C, liver cirrhosis and HCC and to assess its importance as a risk factor for the development of HCC. A total of 90 subjects were included in the study and were assigned to the following 4 groups. Group I included 15 healthy subjects as a control group. Group II included 15 patients presented with chronic hepatitis C infection. Group ΠI included 45 patients with liver cirrhosis, it was further categorized according to Child Pugh classification into three groups; Group ΠI a: It included 15 Child grade A patients. Group ΠI b: It included 15 Child grade B patients. Group ΠI c: It included 15 Child grade C patients. Finally Group IV included 15 patients with hepatocellular carcinoma. Controls had significantly higher serum retinol level than HCV and HCC patients. Higher level of serum retinol was found in Group I, while the lower levels were found in Group IV and Group III c. Serum retinol mean value was significantly higher in patients with HCC on top of apparent normal liver than those on top of cirrhotic liver. Serum retinol was inversely correlated to age. No significant correlation was found between duration of interferon therapy, gender and serum retinol level. A positive correlation was found between serum retinol level and Hb, platelets count and albumin level, while negative correlation was found between serum retinol level and other liver function test parameters & serum creatinine. High significant difference was found between different histopathological grades in liver biopsy and serum retinol level. The highest serum retinol level was associated with normal liver and the lowest was associated with shrunken cirrhotic liver with multifocal lesion. Patients receive interferon therapy had a higher serum retinol level than patients do not receive interferon therapy. We concluded that serum retinol levels are lower in patients with chronic liver disease and is directly related to the severity of liver disease. Serum retinol levels are significantly lower in patients with HCC superimposed on liver cirrhosis compared with patients who have cirrhosis alone. Further studies are needed to elucidate role of retinol levels in the development of HCC and if retinol supplementation will be of help.

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1. Introduction:

Chronic hepatitis C is defined as an infection with the hepatitis C virus persisting for more than six months. Hepatitis C has infected nearly 200 million people worldwide, and infects 3-4 million more people per year **(Shepard et al., 2005)**.

Egypt has the highest seroprevalence for HCV, up to 20% in some areas. There is a hypothesis that the high prevalence is linked to a now-discontinued mass-treatment campaign for Schistosomiasis (**Frank et al., 2000)**.

Cirrhosis is a gradually developing chronic disease of the liver which always involves the organ as a whole. It is irreversible consequence and final stage of various chronic liver diseases of different etiology, or the result of long term exposure to various noxae (**Kuntz and Kuntz 2008)**.

Hepatocellular carcinoma (HCC) is one of the most common malignant primary liver tumors worldwide, it ranks fifth in frequency in men and eighth in women. Between 500000 and one million new cases are reported each year. The annual mortality rate is virtually the same as its annual incidence. Its geographical distribution varies greatly and correlates almost 100% with regional incidence of HCV and HBV infection. In Africa we have high incidence with 20-150 per 100000 inhabitants per year (**Bosch et al., 2004)**.

Retinol is the animal form of vitamin A and it is a fat-soluble vitamin, which is important in vision and bone growth. Retinol affects the differentiation and growth of many tissues and has antitumor properties. Retinol is among the most usable forms of vitamin A **(Eray et al., 2007)**.

Retinol has antioxidant activity and promotes cell differentiation, so it may protect against the development of hepatocellular carcinoma (HCC) by controlling hepatocellular differentiation and

reducing inflammatory responses (**Donato et al., 1998)**.

Only few small randomized controlled trials have demonstrated a possible role of retinol in chemoprevention as well as amelioration of symptoms in HCC; however the results of these trials need to be confirmed (**Newsome et al., 2000)**.

This study **aimed** to investigate serum retinol levels in patients with chronic liver disease (CLD) and to assess its importance as a possible risk factor for the HCC development in cirrhotic patients.

2. Subjects and methods

The present study was carried out in the departments of internal medicine and biochemistry, faculty of medicine, Zagazig University and El-Ahrar Hospitals, during the period from October 2009 to April 2010.

A - Subjects

This study included 75 patients with chronic liver diseases. Patients of Group II and Group III a were taken from department of internal medicine of El AHRAR Hospital and the other groups of patients were taken from department of internal medicine Faculty of Medicine, Zagazig University. Their ages ranged from 22 to 67years with a mean age ± SD of 45.56 ± 10.39. They were 49 males and 26 females.

The studied subjects were divided into the following groups:

Group I: it included 15 healthy volunteers as a control group. Their ages ranged from 19 to 61 years with a mean age ± SD of 37.13 ± 12.2. They were 9 males and 6 females.

Group II: it included 15 patients with chronic hepatitis C. Their ages ranged from 32 to 48 years with a mean age ± SD of 39.2 ± 5.65 .They were 11 males and 4 females.

» 14 patients were on interferon therapy for a duration ranging from 1 month to 12 months with a mean duration ± SD of 7.2 ± 0.9. They were 10 males and 4 females. Their ages ranged from 33 to 48 years with a mean age ± SD of 39.7 ± 5.61.

» Male patient was not on interferon therapy. He was 32 years old.

Group III: it included 45 patients with liver cirrhosis. They were further categorized according to Child Pugh classification into three groups:

1. Group IIIa: it included 15 Child grade A patients. Their ages ranged from 26 to 64 years with a mean age ± SD of 47.8 ± 10.96. They were 8 males and 7 females.

» 3 patients were on interferon therapy for a duration ranging from 2 months to 5 months, with a mean duration ± SD of 3.6 ±0.88. They were 2 females and one male. Their ages ranged from 26 to 33 years with a mean age ± SD of 29 ± 1.26.

» 12 patients were not on interferon therapy. They were 7 males and 5 females. Their ages ranged from 44 to 64 years with a mean age ± SD of 52.5 ± 7.37.

2. Group IIIb: it included 15 Child grade B patients. Their ages ranged from 22 to 50 years; with a mean age ± SD of 37.53 ± 8.65. They were 10 males and 5 females.

» 3 patients were presented with encephalopathy. They were 2 males and one female. Their ages ranged from 35 to 50 years with a mean age ± SD of 41.3 ± 7.76; with a disease duration ranged from 3.5 m to 10 m; with a mean duration ± SD of 5.83 ±2.08.

» 3 male patients were presented with jaundice. Their ages ranged from 22 to 44 years with a mean age ± SD of 36.33 ± 12.42; with a disease duration ranged from 8 m to 18 m, with a mean duration ± SD of 12.66 ± 2.9.

» 2 female patients were presented with hematemesis & melena (H&M). Their ages were 28 and 45 years with a mean age ± SD of 36.5 ± 12.02; with a disease duration ranged from 5 m to 6 m, with a mean duration ± SD of 5.5 ±0.5.

» Male patient presented with bleeding tendency, he was 33 years old with a disease duration about 7 m.

» 3 male patients were presented with combined encephalopathy and bleeding tendency. Their ages were between 31 and 46 years with a mean age ± SD of 37.66 ± 7.63; with a disease duration ranged from 9 m to 24 m, with a mean duration ± SD of 15 ±4.58.

» female patient presented with spontaneous bacterial peritonitis (SBP). She was 37 years old, with disease duration about 3 m.

» 2 patients presented with combined encephalopathy and H&M. They were one male and one female. Their ages were 25 and 49 years with a mean age ± SD of 37 ± 16.37, with a disease duration ranged from 4 m to 11 m with a mean duration ± SD of 7.5 ±3.5.

3. Group IIIc: it included 15 Child grade C patients. Their ages ranged from 34 to 67 years with a mean age ± SD of 51.66 ± 8.5. They were 9 males and 6 females.

» 4 patients were presented with combined H&M and encephalopathy. They were 2 males and 2 females. Their ages ranged from 41 to 57 years with a mean age ± SD of 48.75 ± 7.5; with a disease duration ranged from 3.5m to 13 m with a mean duration ± SD of 8.5 ±1.65.

» Male patient presented with combined bleeding tendency and encephalopathy. He was 54 years old with disease duration about 11m.

» Male patient presented with combined H&M , bleeding tendency and encephalopathy, he was 49 years old with a disease duration about 5m.
» 4 patients were presented with combined H&M, jaundice and encephalopathy. They were 3 males and one female. Their ages ranged from 49 to 67 years with a mean age ± SD of 58.75 ± 7.67; with a disease duration ranged from 9 m to 17 m with a mean duration ± SD of 12.5 ± 1.84.
» 2 patients were presented with combined SBP and encephalopathy. They were one male and one female. Their ages were 34 and 47 years with a mean age ± SD of 40.5 ± 9.19; with a disease duration ranged from 2 m to 4 m, with a mean duration ± SD of 3 ± 1.
» female patient presented with encephalopathy. She was 47 years old, with disease duration about 3 m.
» female patient presented with combined jaundice and encephalopathy. She was 55 years old with disease duration about 6 m.
» male patient presented with combined encephalopathy, jaundice and SBP. He was 59 years old, with disease duration about 11 m.

Group IV: it included 15 patients with hepatocellular carcinoma. Ten patients have cirrhotic liver and 5 patients with normally apparent liver. Their ages ranged from 34 to 66 years, with a mean age ± SD of 52.46 ± 8.5. They were 9 males and 6 females.
» 1 male patient was presented with combined encephalopathy and bleeding tendency. He was 66 years old with disease duration about 6 m.
» 2 male patients were presented with SBP. Their ages were 51 and 57 years with a mean age ± SD of 54 ± 2.24; with a disease duration ranged from 2 m to 12 m with a mean duration ± SD of 7 ± 5.
» 3 patients were presented with encephalopathy. They were 1 male and 2 females. Their ages were 49 and 61 years with a mean age ± SD of 53 ± 6.92 with a disease duration ranged from 2 m to 4 m with a mean duration ± SD of 3 ±0.54.
» 2 patients presented with jaundice, they were 1 male and one female, their ages were 34 and 54 years with a mean age ± SD of 44 ± 14.14 with a disease duration ranged from 1 m to 7 m with a mean duration ± SD of 4 ± 3.
» female patient was discovered accidentally. She was 53 years old.
» 6 patients presented with H&M. They were 4 males and 2 females. Their ages ranged from 37 to 60 years with a mean age ± SD of 52.16 ± 8.56, with a disease duration ranged from 3 m to 24 m with a mean duration ± SD of 12.5 ±3.43.

Exclusion criteria:

All conditions that affect retinol homeostasis are excluded from the study:
1. Advanced renal diseases.
2. Malignancies other than HCC.
3. Severe malnutrition.
4. Heavy parasitic infestation.
5. Drugs which interfere with retinol.

No one of the selected patients was excluded.

B -Methods:

All Subjects were subjected to the following:

I- Full medical history and detailed physical examination.

II- Ultrasonography and Computed tomography scanning of the abdomen:

Abdominal ultrasonographic examination and Computed Tomography scanning was done in fasting state evaluating:

* **The liver** for size, echo pattern and presence or absence of focal lesions (detected masses were evaluated as regards site, size, echo pattern).
* **The spleen** for size.
* **Portal vein diameter** (normally regarded as 9-12 mm) and presence or absence of thrombosis.
* **Presence or absence of hepatic periportal fibrosis.**
* **Abdominal lymph node enlargement** (para-aortic & porta-hepatic).
* Presence of ascites.

III- Laboratory investigations:
1. Complete blood picture (CBC).
2. Prothrombin time (PT).
3. Liver function test.
4. Kidney function tests.
5. Alpha fetoprotein (AFP) determination (Using AFPmab ELISA Kit by EQUIPAK).
6. Hepatitis virus markers: HBsAg, HBcAb (IgM) and HCV-Ab.
7. Liver biopsy

IV- Determination of Serum retinol level using thin layer chromatography in accordance to **MacCrehan and Schonberger (1987).**

Preparation and Processing of Measured Standard Samples

Weighed quantity of the standard was dissolved in chloroform. Carefully measured aliquots was then spotted separately on specially prepared silica gel plates and developed as outlined in the third section below. After development, the spots were visualized by UV light, scraped off the plate separately; and measured spectrophotometrically as described below.

An internal standard was prepared by adding 20µg quantities of retinal dissolved in 50µ l of ethanol to 3 ml of human serum. This prepared

standard and a control sample of serum was then carried through the extraction, fractionation, and quantification procedures described below.

Extraction of Retinol from Human Serum

First 20 ml of venous blood were drawn from each of the control and patient subjects in the fasting state. The blood was allowed to clot at room temperature for 20 min and the serum separated by centrifuging for 20 min at 2700 rpm. In order to obtain a nonsaponified sample, the retinol was extracted by a modification of the procedure of **Blankenhorn (1957)**. Then 3 ml of 95% ethanol were pipetted into each of two 15-ml stoppered centrifuge tubes, and 1.5 ml of serum was added dropwise through the ethanol into each tube. Finally, 3.0 ml of petroleum ether was added to each of the tubes, which were then stoppered tightly. The tubes were shaken vigorously by hand for 10 mm in order to liberate retinol from the carrier proteins and then centrifuged for 10 mm at 1500 rpm. The petroleum ether (upper phase) of the tubes, containing the retinol compounds, was carefully pipetted off and pooled into a single stoppered tube. The aqueous ethanol (lower phase), containing phospholipids and neutral lipids and the precipitated protein, was discarded. The pooled extract was taken to dryness under a stream of nitrogen and dissolved in 0.5 ml of n-hexane just prior to chromatography.

Development

Development is usually performed in rectangular glass tanks lined with filter paper. A convenient volume of solvent for 21*21*6 Cm tank is 100 mL. After equilibration period of 20 min, the plate is placed on the tank and allowed to stand until the desired developing distance is obtained.

Fractionation by Thin-Layer Chromatography

First, 20 X 20 X 0.5 cm glass chromatography plates were washed with Haemosol detergent, rinsed thoroughly with distilled water, and dried with acetone. The plates were coated to a thickness of 0.5 mm with a mixture of silica gel G and distilled water, 1:2 (w/v).

The coated Silica plates can be activated by heating at 120oC for 1 h in an attempt to enhance resolution, while spraying the plates with a solution of an antioxidant has been reported to prevent degradation of the compounds on the plates.

The chromatographic procedures were performed in a dark room illuminated only with a red light. A line was drawn on the plate 13 cm from the origin, and all silica gel above this line was scraped off with a razor blade. Then 40µ l of standard was spotted with a Hamilton microsyringe on the left quarter of the plate. The entire 0.5 ml of the n-hexane extract of serum was spotted horizontally across the remaining three-fourths of the plate. The plates were placed immediately into developing tanks containing a solvent system of benzene and ethyl ether, 90:10 (v/v), and developed to 13 cm in a nitrogen atmosphere for periods of 25-30 mm.

Quantitative Measurement of Fractions

Immediately after the plates were removed from the developing tank, the standard spots were visualized by UV light. The concentration of retinol was detected by using GAMAG TLC Scanner 3 apparatus.

Statistical analysis:

Data were entered, checked and analyzed using SPSS for windows version 10.

3. Results

Table 1 shows demographic data of patients and controls.

Table 2 shows demographic, clinical and laboratory data of different groups of patients. There were high significant differences between the studied groups of patients as regards, age, Hb level, platelet count, total bilirubin, albumin level, αFP, GGT, PT and serum creatinine ($p<0.001$), and significant differences between the studied groups of patients as regards, SGPT and SGOT ($p<0.01$).While no significant differences between the studied groups of patients were found as regards other studied parameters ($p >0.05$).

Table (3) shows the comparison of mean value ± SD of serum retinol level (µg/dl) of cases and controls. A high significant difference of serum Retinol level ($P < 0.001$) was found between cases and controls. Controls had a higher serum retinol level than cases.

Table (4) shows serum retinol level (µg/dl) among studied groups. A progressive decrease in serum retinol level was observed in the three groups of patients as compared to the control group & the lower most level was observed in group IV ($P<0.001$).

Table (5) shows LSD for serum retinol level (µg/dl) among studied groups of patients. A highly significant difference was obtained in comparing any of the studied groups of patients ($P < 0.001$) except for IV Group and III b, III c groups, also Group III c and Group III b.

Table (6) shows serum retinol level (µg/dl) in the two groups of HCC patients. Among patients with HCC (group IV) serum retinol level was significantly lower in HCC patients with liver cirrhosis than those with apparently normal liver ($P < 0.001$).

Table (7) shows correlation between serum retinol level (µg/dl) and different laboratory parameters. There was a positive highly significant correlation between serum retinol level and the following parameters: HB, platelets count and albumin level (P < 0.001).While an inverse high correlation was found between serum retinol level and other liver function test parameters, serum creatinine (P < 0.001) . No significant correlation was found between ALP, rrandom blood sugar and serum retinol level (P >0.05).

Table (8) shows correlation between serum retinol level (µg/dl) and age, blood pressure, gender and duration of interferon therapy. There was an inverse correlation between serum retinol level and age (P < 0.001), while a high positive correlation was found between serum retinol level and BL.P (P < 0.001). No significant correlation was found between duration of interferon therapy, gender and serum retinol level (P >0.05).

Table (9) shows mean serum retinol level (µg/dl) ± SD and liver biopsy findings in Child A and HCV groups. A high significant difference was found between the different grades of histopathological changes in liver biopsy findings as regard serum retinol level (P < 0.001).

Table (10) shows different abdominal ultrasound findings as regard serum retinol level (µg/dl). A significant relation was found between different findings of abdominal US and serum retinol level (P < 0.001). The highest serum retinol level was associated with normal liver and the lowest was associated with shrunken cirrhotic liver with multifocal lesion.

Table (11) shows relation between serum retinol level (µg/dl) and interferon therapy in HCV and Child A groups of patients. A significant difference of serum retinol level (p < 0.05) was found between patients receive interferon therapy and patients do not receive interferon therapy. Patients receive interferon therapy had a higher serum retinol level than patients do not receive interferon therapy.

Table (1) Demographic data of patients and controls

	Control	Cases	T p
Age (years) Mean ± (SD) Range	 37.1 ± 12.2 19 - 61	 45.73 ± 10.48 22 - 67	* 2.55 <0. 01 Sig
Sex			
	No %	No %	
Male	9 60	49 65.3	X2 p
Female	6 40	26 34.7	0.16 0.6

Table (2) Demographic, clinical and laboratory data of different groups of patients

	HCV		Child A		Child B		Child C		HCC		F	P
Age (years) Mean ± (SD) Range	39.2±5.6 32 - 48		47.8 ± 10.96 26 - 64		37.53 ± 8.65 22 - 50		51.66 ± 8.5 34 - 67		52.46 ± 8.5 34 - 66		** 9.8 <0.001 Sig	
Sex	No	%	No	%	No	%	No	%	No	%	X^2	P
Male	11	73.3	8	53.3	10	66.7	9	60	9	60		
Female	4	26.7	7	46.7	5	33.7	6	40	6	40	1.48	0.82
Symptoms	No	%	No	%	No	%	No	%	No	%	X^2	P
Encephalopathy	0	0	0	0	0	0	15	100	4	26	5.91	0.11
H&M	0	0	0	0	0	0	9	60	6	40	6.05	0.09
SBP	0	0	0	0	0	0	3	20	2	13	4.32	0.1
Jaundice	0	0	0	0	0	0	6	40	2	13	6.11	0.08
Bleeding tendency	0	0	0	0	0	0	2	13.5	1	8	4.59	0.19
HB	11.4 ±1.4		12.1 ±1		11.4 ±1.4		10.7 ±2.6		9.4 ±1.5 *		** 6.8 < 0.001	
Platelets count	242.7 ±70.7		248.9 ±55.7		242.7 ±70		170.1 ±110		144 ±58 *		** 11.1 < 0.001	
SGPT	25.7 ±16		21 ±8.8		41.3 ±20		23 ±20		31 ±16		4.38 0.01*	
SGOT	24.7 ±16		15.9 ±5.4		24.7 ±16		22 ±18.9		32.8 ±16.9		3.37 0.01*	
Bilirubin	0.77 ±0.2		1.3 ±0.35		2.5 ±0.6		3 ±1.3		2.9 ±1.1		28.08 0.001**	

Albumin	4 ±0.15	3.1 ±0.3	3 ±0.54	2.4 ±0.6	4 ±0.4	46.9 0.001**
αFP	46 ±16	48.6 ±15.8	46.5 ±16	117 ±27	930 ±394*	71.3 0.001**
GGT	19.2 ±6	20.8 ±8.5	19.3 ±6	41 ±16.1	29.6 ±13	10.68 0.001
ALP	48.9 ±16	59.9 ±18.8	48.8 ±16	66.5 ±34	61.7 ±21.1	1.49 0.11 NS
PT	11.7 ±1.1	15.3 ±0.8	15.2 ±1	18 ±2.4	16.7 ±2.7	37.08 0.001**
S. creatinine	0.58 ±0.2	0.6 ±0.2	0.58 ±0.2	1.38 ±0.5	1.26 ±0.6	17.8 0.001**
RBS	113 ± 55	106.7 ±14.6	113 ± 55	104.9 ±13.7	102.1 ±13.1	0.5 0.76 NS

Table (3) comparison of mean value ± SD of serum retinol level (µg/dl) of cases and controls

		Serum retinol level χ ± SD (Range)	T	P	Sig
cases	75	14.67 ± 3.48 (9 – 22)	15.78	< 0.001	HS
control	15	29.4 ± 2 (27 – 33)			

Table (4) serum Retinol level (µg/dl) among studied groups

	$\bar{\chi}$ ± SD (Range)	Median
Group I	29.4 ± 2 (27 – 33)	29.2
Group II	20.1 ± 1.2 (18.2 – 22)	20.2
Group III a	15.98 ± 0.3 (15.5 – 16.5)	16
Group III b	13.97 ± 0.3 (13.57 – 14.7)	13.99
Group III c	11.86 ± 0.6 (11 – 13)	11.79
Group IV	11.4 ± 2.65 (9 – 15.7)	10.18

K = 79.77 P < 0.001

Table (5) LSD for serum retinol level (µg/dl) among studied groups of patients

	Group I	Group II	Group III a	Group III b	Group III c
Group IV	< 0.001	< 0.001	< 0.001	NS	NS
Group III c	< 0.001	< 0.001	< 0.001	NS	
Group III b	< 0.001	< 0.001	< 0.001		
Group III a	< 0.001	< 0.001			
Group II	< 0.001				

Figure (1) Mean serum retinol level among studied groups

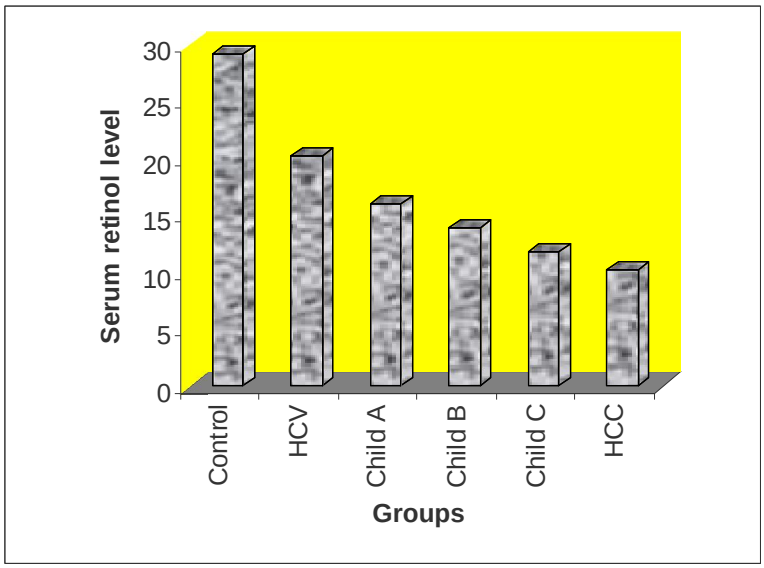

Table (6) Serum retinol level (µg/dl) in the two groups of HCC patients

	No	Retinol χ- ±SD (range)	T	p
Cirrhotic liver	10	9.6 ±0.81 (9 – 11)	12.4	** < 0.001
Apparently normal liver	5	14.85 ±0.64 (14.07 – 15.79)		

Table (7) Correlation between serum retinol level (µg/dl) and different laboratory parameters

	r	p	Sig
Haemogloubin	0.42	< 0.001	HS
platelets	0.52	< 0.001	HS
SGPT	-0.28	< 0.001	HS
SGOT	-0.31	< 0.001	HS
Bilirubin	-0.71	< 0.001	HS
Albumin	0.5	< 0.001	HS
αFB	-0.4	< 0.001	HS
GGT	-0.38	< 0.001	HS
ALP	-0.08	> 0.05	NS
PT	-0.72	< 0.001	HS
RBS	-0.09	> 0.05	NS
S.Creatinine	-0.45	< 0.001	HS
PCR (quantitative)	-0.66	< 0.001	HS

Table (8) Correlation between serum retinol level (µg/dl) and age, blood pressure, gender and duration of interferon therapy

	r	p	Sig
Age	-0.43	< 0.001 *	HS
Blood pressure	0.48	< 0.001 *	HS
Gender	0.31	. > 0.05	NS
Duration of interferon therapy	0.21	> 0.05	NS

Table (9) Mean serum retinol level (µg/dl) ± SD and liver biopsy findings in Child A and HCV groups

	Retinol χ- ±SD (range)
1/6	20.1 ± 1.35 (18.4 – 21.7)
2/6	19.9 ± 1.1 (18.2 – 21.5)
3/6	20.57 ± 1.6 (18.7 – 22)
4/6	15.9 ±0.3 (15.5 – 16.4)
5/6	16 ±0.38 (15.5 – 16.5)

F = 36.69 P < 0.001

Table (10) Different abdominal ultrasound findings as regard serum retinol level (µg/dl)

	Retinol χ- ±SD
Normal liver	20.98 ± 7.8
Fatty liver	18.76 ± 2.3
Periportal fibrosis	17.6 ± 2.6
Bright liver	15.75 ± 0.83
Early cirrhotic liver	13.9 ± 0.74
Shrunken cirrhotic liver	12.2 ± 1.1
Shrunken cirrhotic liver + focal lesion	10.2 ± 0.7
Shrunken cirrhotic liver + multifocal lesion	9 ± 0.01

Focal lesion + normally apparent liver	14.8 ± 0.6

F = 14.01 P < 0.001

Table (11) Relation between serum retinol level (µg/dl) and interferon therapy in HCV and Child A groups of patients

	No	Mean ± SD	Range
Patients on interferon therapy	17	19.39±2.02	15.53 - 22
Patients not on interferon therapy	13	16.35±1.21	15.5 – 20.24

T = 2.34 p < 0.05

4. Discussion

There has been a debate as to whether the measurement of serum retinol levels provides an accurate representation of tissue (hepatic) levels. Whilst the relationship is not linear in its entirety, it is accepted that there is a direct correlation between serum and hepatic levels during vitamin A deficiency. After a certain point, increasing hepatic retinol levels do not produce a proportional increase in serum retinol levels. More importantly, patients with cirrhosis fall towards the lower part of this curve, where a direct relationship exists, thus validate the usage of serum levels as a guide to hepatic levels (**Olson 1984)**.

In our study, a statistically significantly ($p<0.001$) lower serum level of retinol was observed among patients with chronic liver disease when compared with control group. The decrease in serum level of retinol found in our patients with chronic liver disease can be explained by the reduced hepatic vitamin storage **(Janczewska et al., 1995)**, reduced enzymatic conversion of ß-carotene into retinol **(Moreno et al., 2002),** and/or diminished release of binding proteins by the liver **(Janczewska et al., 1995)**.

Chronic inflammation and infection, which are part of clinical manifestations of chronic liver disease, are also responsible for the reduction of the serum level of retinol. This can be attributed to the reduction in the synthesis and release of retinol binding protein, during acute phase response (**Stephensen and Gildengorin 2000)**.

The inadequate consumption of vitamin A leads to the depletion of its organic reserves. In patients with cirrhosis, the inadequate intake of this vitamin, mainly the vitamin A from animal sources, which is highly available, can exacerbate an expected reduction in the serum levels of vitamin A. In addition, the reduction in protein ingestion, by unfounded patient's belief or inadequate recommendations in clinical practice, as prophylaxis for the hepatic encephalopathy contributes for the reduction of vitamin A from animal sources daily ingestion and the decreasing of bioconversion of provitamin A carotenoids **(Sklan et al., 1989)**. Moreover, the liver disease can course with intestinal alterations that can compromise the bioavailability and the bioconversion of carotenoids in vitamin A **(Albillos and Hera 2002)**.

Our findings were consistent with **Newsome et al. (2000) and Yuan et al. (2006)**. They had demonstrated that patients with chronic liver diseases have reduced levels of retinol in the blood and in the liver.

Our study not only confirmed that serum retinol levels were lower in patients with chronic liver disease; but also shown that this decrease was related to the severity of liver disease. Severity of liver disease can be assessed either clinically according to child and Pugh classification **(Pugh et al., 1973)**, according to histopathological grading **(Sun et al., 2009)**, or by default according to abdominal ultrasound findings **(Newaz Khan et al. 2000)**.

Patients with advanced liver cirrhosis (Child C group) had lower retinol levels when compared with other patients with less severe liver disease as chronic hepatitis C infection or Child A and B groups of cirrhosis.

High significant difference ($p<0.001$) was observed between serum retinol level and the different histopathological changes in liver biopsy. Significant relation ($p<0.001$) was found between different findings of abdominal ultrasound and serum retinol level. The highest serum retinol level was associated with normal liver and the lowest was associated with shrunken cirrhotic liver associated with focal lesion.

Hepatic stellate cells which are rich in retinol are activated to become myofibroblast while the liver undergoes a fibrotic transformation during cirrhosis. Myofibroblast lose retinol content **(Blomhoff and Wake 1991)**. The increase rate of conversion of hepatic stellate cells into myofibroblast is associated with loss of hepatic ability for retinol storage **(Knittel et al., 1999)**. **Newsome et al. (2000) and Yuan et al. (2006)** demonstrated that the synthesis of retinol binding protein by the liver is impaired with the progression of the liver disease.

Hepatocellular carcinoma is a common cause of morbidity and mortality in patients with liver cirrhosis, leading to more than a million deaths per year worldwide. Treatment options in such patients are limited **(Fried1998)**. A complementary approach to the management of HCC is to decrease its

incidence in at-risk populations. Retinoids have long been the focus of investigators searching for putative anticancer agents **(Goodman 1984)**. Many of the synthetic retinoids, which act as tumor differentiating agents, have demonstrated a potential for cancer prevention in animal models **(Parker 1996)**. The relationship between retinols and neoplasia has become the focus of much attention over the past 20 years. Recognition that its antioxidant properties, as determined in vitro, would be of benefit in the treatment of malignant conditions has led to its usage in clinical trials **(Issing et al., 1997)**.

In our study, the lower levels of serum retinol were found in HCC group, reduced serum levels of retinol could be the cause or consequence of HCC **(Yu et al., 1994)**. The reduction of hepatic stellate cells is a major cause of such retinoid depletion in the tumors. In addition, a rapid metabolism of retinoid into an inactive metabolite may participate in such depletion **(Okuno et al., 2001)**.

Several antioxidants, including β -carotene, vitamin E, and selenium, have been shown to inhibit chemically induced liver cancer in rodents **(Moreno et al., 2002)**.

Retinol has been shown to inhibit the formation of aflatoxin B1 (AFB 1) – DNA adducts in hepatocytes **(Yu et al., 1994)**. A crucial step in aflatoxin-induced hepatocarcinogenesis, and to inhibit the development of pre-neoplastic lesions in the liver of rats treated with chemical carcinogens **(Berberian et al. 1995)**. Retinoids also have been shown to inhibit the transformation (activation) of hepatic stellate cells **(Chi 2003)** and to suppress the proliferation of human hepatoma cells **(Piao et al., 2003)**.

Persistent inflammation caused by chronic infection with HBV and/or HCV is believed to be one mechanism of hepatocarcinogenesis and is probably mediated by inflammatory cytokines **(Nieters et al., 2005)**.

Retinoids inhibit the production of proinflamatory cytokines in cultured macrophages and reduce inflammatory reactions **(Brinckerhoff et al., 1983)**. Reactive oxygen species induced by chronic inflammation can cause damage to DNA, proteins, and lipids **(Rama and Balraj 2004)**. Reactive oxygen species – induced DNA damage is believed to be directly involved in hepatocarcino-genesis **(Loguercio and Federico 2003)**. Malfunction of retinoid nuclear receptor in hepatoma cells may contribute to hepatocarcinogenesis **(Okuno et al., 2001)**. Antioxidants, such as retinol and carotenoids, can neutralize reactive oxygen species and can possibly prevent the development of HCC **(Moreno et al., 2002)**.

Newsome et al. (2000) found that high serum retinol mean value in patients presented with HCC on top of apparent normal liver when compared with those on top of cirrhotic liver. We found same results with high significant difference between both ($P < 0.001$), this can be explained due to reduced number of hepatic stellate cells, prevalence of myofibroblast and deterioration of liver functions in cirrhotic patients **(Burt and Oakley Lecture 1993).**

Plasma concentration of albumin is a marker of synthetic ability of the liver **(Keith et al., 1999)**.While liver enzymes (ALT&AST) are markers of hepatocellular damage **(Kale et al., 2001)**, also plasma bilirubin concentration is a marker of hepatic excretory function of the liver **(Mayne 1994)**. With liver impairment the plasma level of albumin is decreased, while liver enzymes and bilirubin levels are increased (**Kuntz and Kuntz 2008)**. **Eray et al. (2007)** and **Zheng1 et al. (2008)** share us the high positive correlation ($P < 0.001$) between serum retinol level and albumin level, and also the inverse correlation ($P < 0.001$) between serum retinol level and liver enzymes and bilirubin.

Given that prothrombin is one of the hepatic dependant clotting factors. With impairment of hepatic functions, the plasma concentration of prothrombin is decreased, so subsequent increase in prothrombin time **Pinnas and Meinke (1992)**. A high significant inverse correlation ($P < 0.001$) between serum retinol level and prothrombin time was found. **Eray et al. (2007)** and **Zheng1 et al. (2008)** had found that Serum retinol level inversely correlated with the prothrombin time.

A highly significant inverse correlation ($p<0.001$) between serum retinol level and AFP was found, this can be explained as following high levels of AFP is considered as a biomarker for diagnosis of HCC **Bruix and Sherman (2005)**.

An inverse correlation ($P < 0.001$) between serum retinol level and age was found. **Ballew et al. (2009)** had found that Serum retinol level positively correlate with the patient´s age, while **Eray et al. (2007) and Newsome et al. (2000)** found that serum retinol level not associated with the patient´s age. Our finding can be explained as Egypt is considered one of the developing countries with low socioeconomic standards, absence of programs of geriatric care, bad nutritional habits of elderly**. Sommer (2008)** had announced that vitamin A deficiency is common in developing countries and the major cause of deficiency is diet which contains few animal sources.

A positive correlation ($P < 0.001$) was found between serum retinol level and blood pressure. This was in agreement to **Takebayashi et al. (2006)** who reported that serum retinol level correlated positively

with systolic blood pressure. **Connolly et al. (2007)** had reported that a negative correlation was found between serum retinol level and blood pressure. The possible mechanism of blood pressure lowering effect of retinoids has not yet been clarified. It may reflect alleviation of renal damage by retinoids, allowing the kidney to normalize blood pressure. Additionally, however, retinoids lower the expression of the angiotensin receptor in vitro and in vivo, so block the effects of angiotensin II **(Sabine et al. (2004)**.

In our study No statistically significant difference (p>0.05) between gender and serum retinol level was found. This was in agreement to **Eray et al. (2007)**.

No significant correlation (p>0.05) between ALP and serum retinol was found. This was in agreement to **Newsome et al. (2000).** No significant correlation (p>0.05) between rrandom blood sugar and serum retinol was found. This was in agreement to **Eray et al. (2007)**. While **Iwasa et al. (2009)** had found that Serum retinol level positively correlated with the rrandom blood sugar.

A positive high correlation (P < 0.001) between serum retinol level and hemoglobin was observed. In countries where vitamin A and iron deficiency are endemic in children; serum retinol concentration is positively correlated with hemoglobin concentration and biochemical iron tests **(Mejia and Chew 1988)**. The mechanisms explaining the relationship between vitamin A and iron status are not known, although it has been speculated that vitamin A is necessary for the release of iron from stores to maintain adequate levels of plasma iron to bone marrow and that vitamin A may enhance absorption of iron, perhaps by preventing the inhibitory effects of phytates and polyphenols on iron absorption **(Garcia-Casal et al., 1998)**.

A positive correlation (P < 0.001) between retinol and platelet count was observed. This was in agreement to **Zheng1 et al. (2008)** and **Iwasa et al. (2009).** Although the mechanism is unclear, But there are some evidence that vitamin A benefits hematopoiesis and hence platelets production **(Noreen and Gray-Donaldb 2002):**. Also platelet count in CLD is taken as an indicator of portal hypertension, which indicates the degree of cirrhosis. Platelet count progressively decreases with progressive decrease in liver functions cirrhotic patients (**Kuntz and Kuntz 2008)**.

A highly significant inverse correlation (P < 0.001) between quantitative PCR and serum retinol level was found. This was in agreement to **Kohge et al. (2009).** Retinol increases the antiviral effect of interferon α-2b plus ribavirin when retinol is taken during course of interferon therapy **(Kohge et al 2009)**.

No significant correlation (P >0.05) was found between duration of interferon therapy and serum retinol level. A significant difference (p < 0.05) of serum retinol level was found between patients receive interferon therapy and patients do not receive interferon therapy, but these results may be misleading because in our work we had taken only one blood sample from the patients who were on interferon therapy, we should take multiple blood sample in multiple setting (before, during and after treatment) from every patient on interferon therapy to compare effect of interferon on serum retinol level. Multiple further studies are needed for further evaluation of interferon therapy and serum retinol level.

This study demonstrated that serum retinol levels are lower in patients with chronic liver disease and have also shown that this decrease is directly related to the severity of liver disease. Low serum retinol levels may have a role in the development of HCC suggesting that supplementation may be of benefit. Serum retinol levels are significantly lower in patients with HCC superimposed on liver cirrhosis compared with patients who have cirrhosis alone.

Further studies are encouraged to determine serum retinol role in primary and secondary prophylaxis of HCC in cirrhotic patients, and to identify a serum retinol level below which the risk of HCC would substantially increase and hence provide a threshold level for retinol supplementation.

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