AIM OF THE WORK

The aim of this work was to evaluate the utility of serum hyaluronic acid (HA) as a simple, non invasive marker which can assess the extent of liver fibrosis and can predict risk of serious liver events in patients with chronic HCV infection and in patients with HCV/HIV co- infection.

And to evaluate changes of serum hyaluronic acid (HA) level before and after Interferon therapy in patients with chronic HCV infection to identify the relationship between the level of this marker and the effectiveness of Interferon therapy.

SUBJECTS

This study was carried out on eighty four patients divided as follows:

Group I: A twenty-four patients with chronic Hepatitis C Virus infection (HCV) fit for Interferon therapy recruited from EL-Kabary Hospital, twelve patients before the beginning of Interferon therapy (group Ia) and twelve patients (were age and sex matched with the pretreatment group) had completed 48 doses of Interferon therapy course (group Ib).

Group II: Included twenty-four patients of chronic hepatitis C recruited from Alexandria Fever Hospital, twelve patients with compensated liver insufficiency (group IIa) and twelve patients with decompensated liver disease (group IIb).

Group III: Enrolled thirty six patients recruited from Alexandria Fever Hospital divided into three sub groups:-

- Twelve patients with Hepatitis C Virus/Human Immunodeficiency Virus co infection with liver affection (group IIIa).
- Twelve patients with Hepatitis C Virus/Human Immunodeficiency Virus co infection with no liver affection (group IIIb).
- Twelve patients with Human Immunodeficiency Virus monoinfection as a control group (group IIIc).

Exclusion criteria:

- Patients who had liver fibrosis due to any other cause were excluded.
- Patients with diseases and conditions that may affect level of hyaluronic acid (HA) giving false positive results as seen in rheumatoid arthritis, active osteoarthritis, sclerosis, systemic lupus erythematosus, scleroderma, malignant tumours and pregnancy.
- Patients with severe systemic illness.

METHODS

All patients were subjected to:-

1. Informed consent was obtained from all patients before sampling
2. Thorough history taking including risks for acquiring infection, any treatment received before, investigations done before and any associated medical diseases.
3. Clinical evaluation including :
   a. Symptoms :dyspepsia, diarrhea, abdominal pain, distension, lower limb edema, hemetemesis and fever.
   b. Signs: Pallor, jaundice, hepatomegaly, splenomegaly, signs of portal hypertension and liver decompensation.
4. Laboratory investigations including :
   a. Routine investigations:
      i. Complete blood picture.
      ii. Fasting blood glucose level
      iii. Serum urea and creatinine.
   b. Liver function tests and liver enzymes including serum alanine transaminase (ALT), serum aspartate transaminase (AST), serum albumin, serum bilirubin (total, direct), prothrombin activity, alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) .
   c. Specific investigations:
      - Viral markers:
        - Hepatitis C Virus anti-bodies (HCV Abs).[62]
        - Hepatitis B surface antigen (HBsAg).
        - For patients with HCV infection HCV RNA quantitative polymerase chain reaction (PCR) was done.[66]
      - For diagnosis of Human Immunodeficiency Virus (HIV):[5]
        - Enzyme linked immunosorbent assay (ELISA).
        - Western blot.
      - For patients who received interferon therapy ,the following investigations were done:
        - Thyroid Stimulating Hormone (TSH).

- Anti- shistosomal antibodies.
- Anti -nuclear antibodies (ANA).
- α-fetoprotein.
- Ultrasound guided liver biopsy.

- **Per-cutaneous ultrasound guided liver biopsy:** was done to group I patients just before the initiation of PEG INF+ RBV therapy after exclusion of all contraindications for liver biopsy and obtaining patient's consent using 14- gauge sterilized Trucut needle,the liver samples were subjected to histopathological examination where they were processed as paraffin blocks . H&E and masson Trichrome sections were prepared. Grading and staging of hepatitis was done according to the modified histological activity index (HAI).[79]

5. Medical imaging:

- Abdominal ultrasonography

The US score was determined from the right and left lobes and the average score for each parameter was calculated as follows: (1) liver edge : score 0 for sharp; score 1 for mildly blunted; score 2 for blunted; (2) liver surface : score 0 for smooth; score 1 for mildly irregular; score 2 for irregular; score 3 for highly irregular; and (3) liver parenchymal texture : score 0 for fine; score 1 for mildly coarse; score 2 for coarse; score 3 for highly coarse. These were counted and given a score of nine and then classified into normal (zero), mild (one to three), moderate (four to seven) and severe (eight to nine).[187]

6. Serum Hyaluronic acid by enzyme-linked immunosorbant assay (ELISA) using specific ELISA kit (produced by USCN Life Science Inc.) which is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of HA in serum, plasma and other biological fluids.

Figure (11): Serum hyaluronic acid ELSA kit.

Specimen Collection And Preparation

Blood samples were collected in serum separator tubes, centrifuged separating serum samples which were frozen at - 20°C .

Test Principle

This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to HA has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled HA and unlabeled HA (Standards or samples) with the pre-coated antibody specific to HA. After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of HA in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of HA in the sample.

Assay Procedure

1. 50µL of each of dilutions of standard, blank and samples was put into the appropriate wells and then 50 µL of detection reagent A was added to each well immediately then incubated for 1 hour at 37°C.
2. The wells were washed 3 times with a wash solution.
3. 100µL of Detection Reagent B working solution was added to each well and incubated for 30 minutes at 37°C.
4. The wells were washed for 3 times.
5. 90µL of Substrate Solution was added to each well and incubated for 15- 25 minutes at 37°C.
6. 50µL of stop solution was added to each well.
7. The results were read at 450 nm.

Calculation of Results

This assay employed the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between HA concentration in the sample and the assay signal intensity. A standard curve was created on log-log or semi-log graph paper, with the log of HA concentration on the y-axis and absorbance on the x-axis.

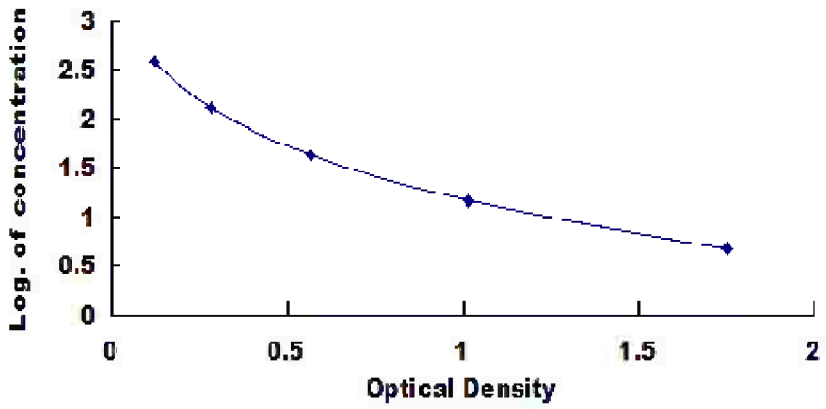

Figure (12): Typical Standard Curve for HA ELISA.

Normal Range

HA normal range 0 – 75 ng/mL.

Statistical Analysis of Results

Statistics of the results were carried out according to the following formulae:

1. Arithmetic mean ($\overline{X}$):

Was calculated as follows:

$$\overline{X} = \frac{\sum x}{n}$$

Where: $\overline{X}$ = arithmetic mean

Σx = Sum of observations

n = number of observations

2. Standard deviation (SD):

Was calculated as follows:

$$SD = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Where: Σx^2 = sum of squared observations.

$(\Sigma X)^2$ = square of the sum of observations.

n = number of observations.

3. Chi-square (X^2):

For comparison between distributions of patients according to different items of study and use this formula for calculation:

$$X^2 = \sum \frac{(O-E)^2}{E}$$

Where: O = Observed results

$$E = \text{Expected results} = \frac{\text{Total row X total column}}{\text{Grand total}}$$

$(O-E)^2$ = Difference squared

4. Analysis of Mann Whitney test:

Used as a non-parametric statistical test to compare between tow numerical groups.

$$U = n_1 n_{2+} \frac{n_2 (n_2 + 1)}{2} - \sum_{i=n_1+1}^{n_2} R_i$$

Where:

n_1 and n_2 are the two sample sizes

R_i is the ranks of the samples.

The test assumes the samples are random.

5. Analysis of T-test:

Used as a parametric statistical test to compare between tow numerical groups.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

Where:

$\bar{X}_1$= Mean of first set of values

$\bar{X}_2$= Mean of second set of values

S_1= Standard deviation of first set of values

S_2= Standard deviation of second set of values

n_1= Total number of values in first set

n_2= Total number of values in second set.

6. Analysis of Kruskal-Wallis test:

Used as a non-parametric statistical test to compare between three or more numerical groups.

$$U = (N-1)\frac{\sum_{i=1}^{g} ni(\bar{r}_i - \bar{r})^2}{\sum_{i=1}^{g}\sum_{j=1}^{ni}(r_{ij} - \bar{r})^2}$$

Where:

n_i = is the number of observations in group *i*

r_{ij} = is the rank (among all obserbations) of observation *j* from group *i*

N = is the total number of observations across all groups

$$\bar{r}_i = \frac{\sum_{j=1}^{ni} r_{ij}}{n_i}$$

$\bar{r} = \frac{1}{2}(N+1)$ is the average of all the r_{ij}

7. Analysis of ANOVA:

Used as a parametric statistical test to compare between three or more numerical groups.

$$F = \frac{\frac{\sum n(x-\bar{x})^2}{P-1}}{\frac{\sum(n-1)S^2}{N-P}}$$

Where:

P = total number of populations

n = total number of samples in a population

S = Standard deviation of the sample

N = total number of observations

RESULTS

The present study was conducted on eighty four subjects classified into three groups as the following:

- **Group (I):** 24 patients with chronic Hepatitis C Virus infection (HCV) fit for Interferon therapy divided into two groups:
  - **Group (Ia):** 12 patients before the beginning of Interferon therapy
  - **Group (Ib):** 12 patients had completed 48 doses of Interferon therapy course
- **Group (II):** 24 patients of chronic hepatitis C divided into two groups:
  - **Group (IIa):** 12 patients with compensated liver insufficiency.
  - **Group (IIb):** 12 patients with decompensated liver disease.
- **Group (III):** 36 patients divided into three groups:
  - **Group (IIIa):** 12 patients with Hepatitis C Virus/Human Immunodeficiency Virus co infection had liver disease
  - **Group (IIIb):** 12 patients with Hepatitis C Virus/Human Immunodeficiency Virus co infection had no liver disease.
  - **Group (IIIc):** 12 patients with Human Immunodeficiency Virus infection as a control group

Age and Sex Distribution: (Tables 8,9 –Figures 13,14)

Group (Ia) consisted of 8 (66.7%) males and 4 (33.3%) females, their ages were between 25-42 years with mean± S.D. 34.75±5.578 years while group Ib subjects consisted of 8 (66.7%) males and 4 (33.3%) females, their ages were between 27-46 years with mean± S.D. 35.25±6.032 years. In group IIa 10 (83.3%) out of the patients were females and 2 (16.7%) out of the patients were males, their ages ranged between 29-56 years with mean± S.D. 43.5±7.914 years while patients of group IIb consisted of 10 (83.3%) males and 2 (16.7%) females, their ages ranged between 44-73 years with mean± S.D. 56.33±6.985 years. Regarding group IIIa, all paients (12,100%) were males with ages ranged from 31 to 44 years with mean± S.D. 36.92±4.274 years, while group IIIb consisted of 11 (91.7%) males and 1 (8.3%) female, their ages ranged between 19-42 years with mean± S.D. 31±6.523 years and in group IIIc 10 (83.3%) out of the patients were male, only 2 (16.7%) were females with age ranged between 22-39 years with mean± S.D. 30.08± 5.401 years. There was statistically significant differences between group II groups and group III subgroups where P=0.000, 0.009 respectively. (significant level at P less than 0.05)

Table (8): Comparison between groups regarding to patient's age

Age	Group I		Group II		Group III		
	Ia	Ib	IIa	IIb	IIIa	IIIb	IIIc
Min.	25	27	29	44	31	19	22
Max.	42	46	56	73	44	42	39
Mean	34.75	35.25	43.50	56.33	36.92	31.00	30.08
S.D.	5.578	6.032	7.914	6.985	4.274	6.523	5.401
P Value	0.835		0.000*		0.009*		

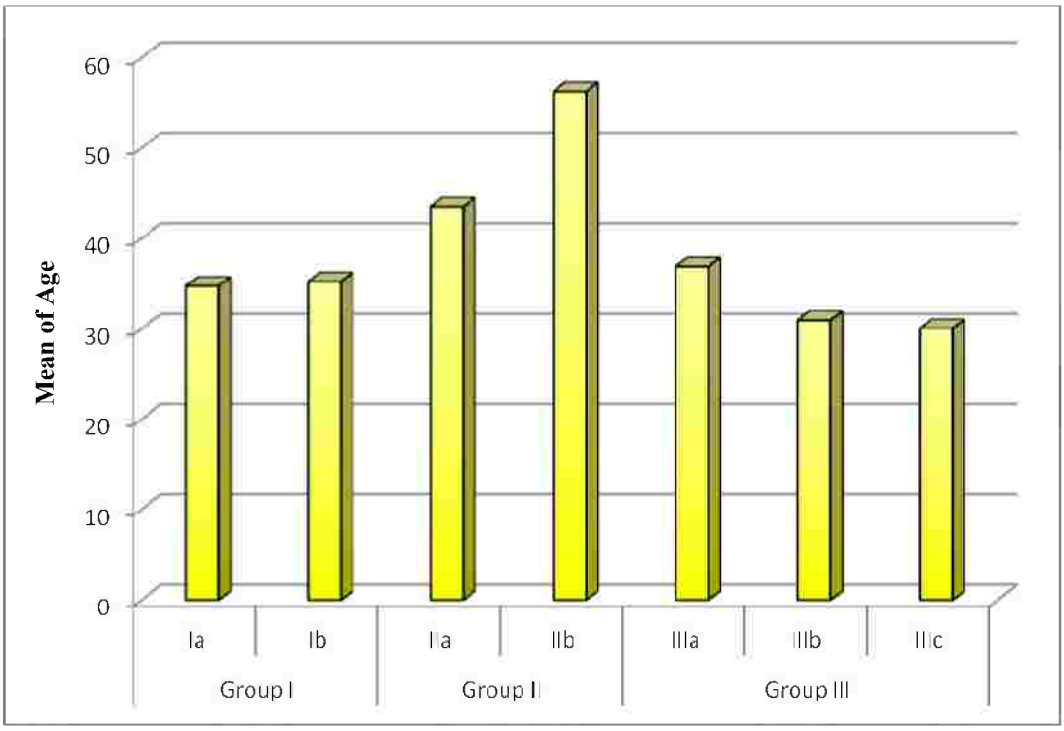

Figure (13): Comparison between groups regarding to patient's age

Table (9): Comparison between patients groups regarding to gender

Gender	Group I				Group II				Group III					
	Ia		Ib		IIa		IIb		IIIa		IIIb		IIIc	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Female	4	33.3	4	33.3	10	83.3	2	16.7	0	0	1	8.3	2	16.7
Male	8	66.7	8	66.7	2	16.7	10	83.3	12	100	11	91.7	10	83.3
Total	12	100	12	100	12	100	12	100	12	100	12	100	12	100
P Value	1.000				0.003*				0.336					

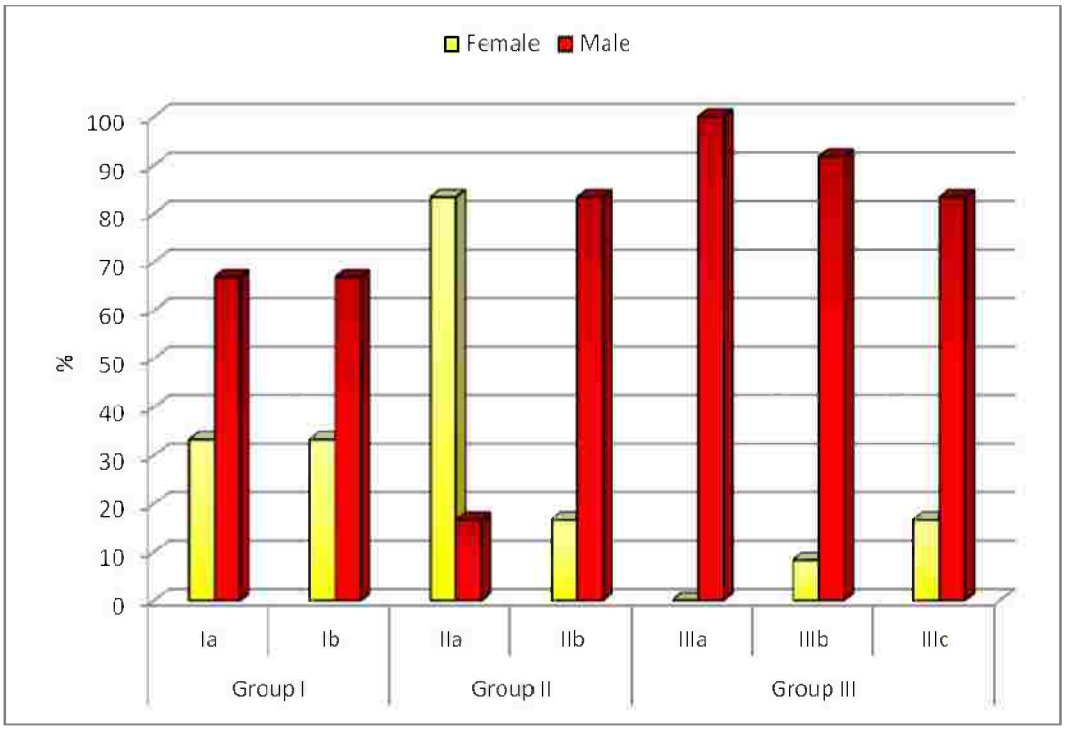

Figure (14): Comparison between groups regarding patients gender

Risk analysis: (Tables 10, 11 and Figures 15,16)

Regarding risk of acquiring infection, in group Ia 9 (75%) out of the patients had no risk of acquiring infection while in group Ib 11(91.7%) out of the patients had no risk of acquiring infection. Similar to these results, in group IIa 10(83.3%) out of the patients had no risk of acquiring infection and all patients (100%) of group IIb had no risk of acquiring infection. On the contradictory, presence of risk of acquiring infection was frequent in group III in 100%,91.7% and 100% in groups IIIa, IIIb and IIIc successively. There was no statistically significant differences between any group subgroups where p value for group I was 0.590, for group II was 0.478 and 0.358 for group III. (significant level at P less than 0.05)

By analysis of type of risk, it was found that **Blood transfusion** was found in 8.3% of patients of group Ia and patients of group IIa, **Cesarean section** represented risk in 8.3% of patients of group Ia, **Hemophilia** was a risk in 8.3% of patients of group IIIb, **IV Drug addiction** was a risk in 100% of group IIIa, 75% of group IIIb and 83.3% of group IIIc, **Previous surgery** was a risk in 8.3 of patients of groups Ia, Ib and IIa while **Sexually transmitted** infection was the risk in 8.3% of group IIIb and 16.7% of group IIIc patients.

Table (10): Comparison between groups regarding risk of acquiring infection

Risk of acquiring infection	Group I				Group II				Group III					
	Ia		Ib		IIa		IIb		IIIa		IIIb		IIIc	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
No	9	75	11	91.7	10	83.3	12	100	0	0	1	8.3	0	0
Yes	3	25	1	8.3	2	16.7	0	0	12	100	11	91.7	12	100
Total	12	100	12	100	12	100	12	100	12	100	12	100	12	100
P Value	0.590				0.478				0.358					

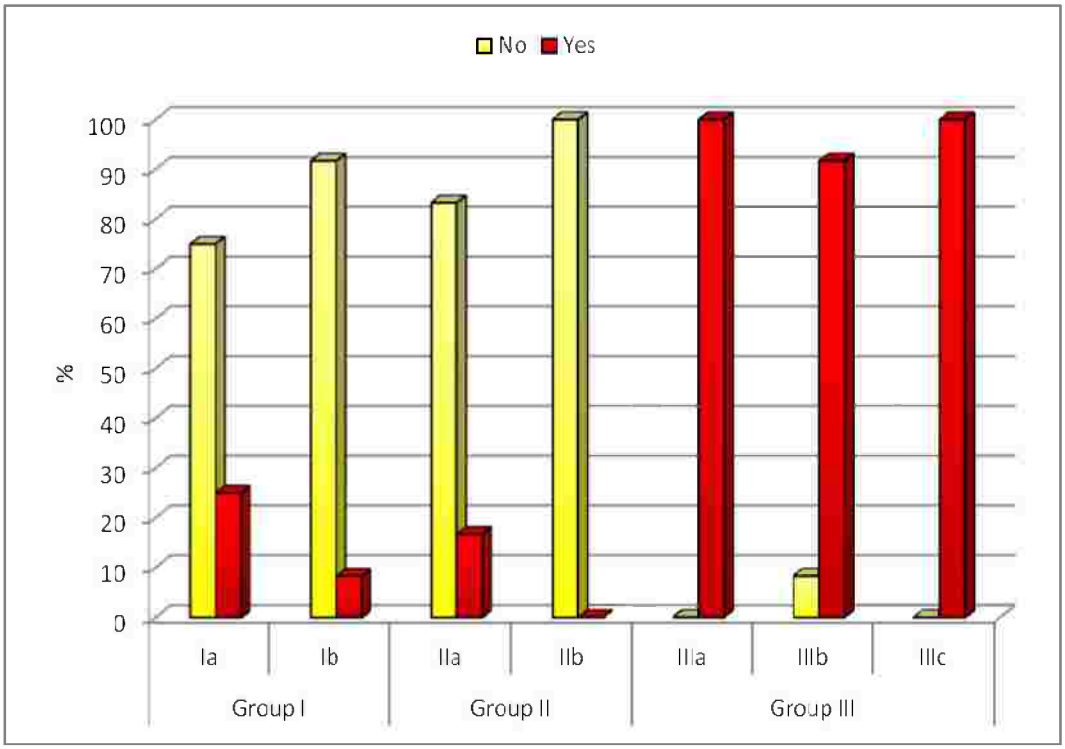

Figure (15): Comparison between groups regarding risk of acquiring infection

Table (11): Comparison between groups regarding to type of risk

Type of risk	Group I				Group II				Group III					
	Ia		Ib		IIa		IIb		IIIa		IIIb		IIIc	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Blood transfusion	1	8.3	0	0	1	8.3	0	0	0	0	0	0	0	0
Cesarean section	1	8.3	0	0	0	0	0	0	0	0	0	0	0	0
Hemophilia	0	0	0	0	0	0	0	0	0	0	1	8.3	0	0
IV Drug addiction	0	0	0	0	0	0	0	0	12	100	9	75	10	83.3
Previous surgery	1	8.3	1	8.3	1	8.3	0	0	0	0	0	0	0	0
Sexually transmitted	0	0	0	0	0	0	0	0	0	0	1	8.3	2	16.7
P Value	0.532				0.336				0.375					

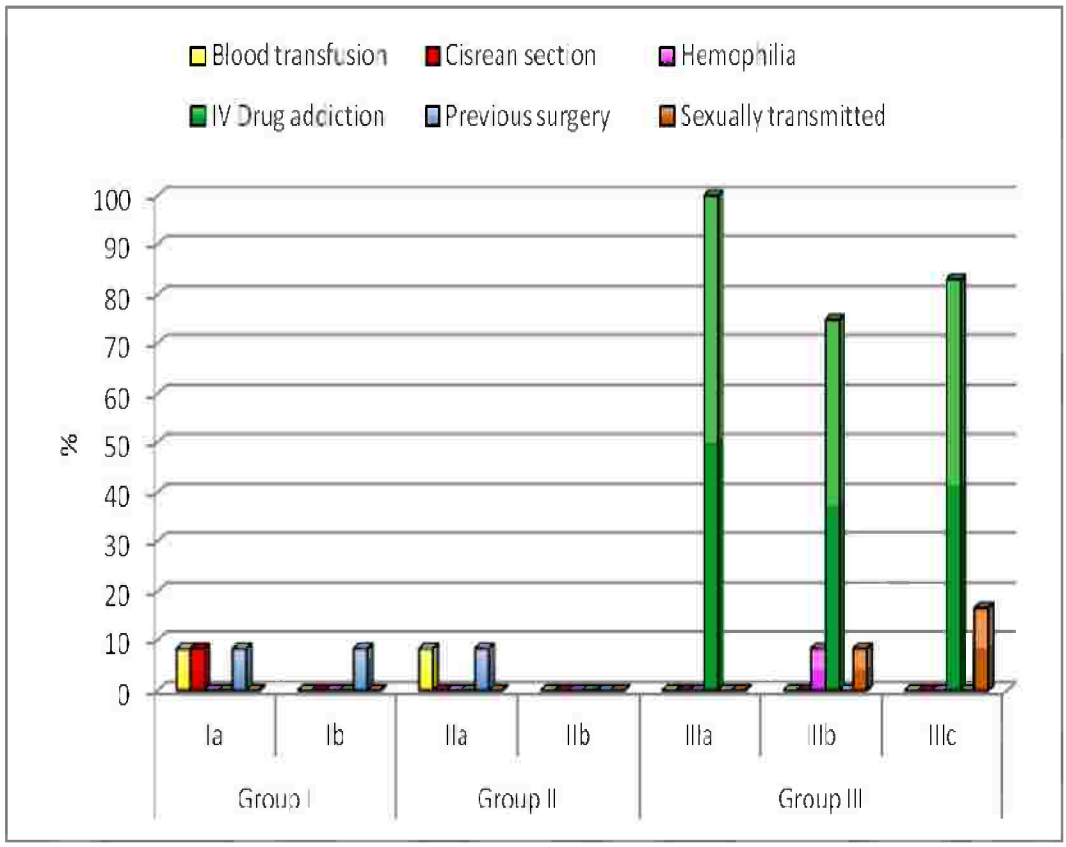

Figure (16): Comparison between groups regarding to type of risk

Clinical Findings: (Tables 12,13 –Figures 17,18)

Regarding to symptoms, in group (Ia) the most frequent complaints were dyspepsia (41.7%), abdominal pain (25%) and fever (8.3%) while in group (Ib) the most frequent complaints were abdominal pain (58.3%), dyspepsia (33.3%) and fever (8.3%). In group (IIa) the most frequent complaints were dyspepsia (75%), abdominal pain (75%), abdominal distension (58.3%), heamatemesis (33.3%) and diarrhea (8.3%) while in group (IIb) the most frequent complaints were dyspepsia (100%), abdominal pain (100%), abdominal distension (100%), heamatemesis (75%), diarrhea (58.3%) and fever (33.3%). In group (IIIa) the most frequent complaints were fever (100%), diarrhea (83.3%), abdominal pain (75%), dyspepsia (66.7%), heamatemesis (33.3%) and abdominal distension (16.7%), in group (IIIb) the most frequent complaints were fever (100%), diarrhea (75%), abdominal pain (41.7%) and dyspepsia (33.3%) and in group (IIIc) the most frequent complaints were fever (100%), diarrhea (91.7%), abdominal pain (83.3%) and dyspepsia (41.7%).

Table (12): Comparison between groups as regard to patient's symptoms

Symptoms	Group I				Group II				Group III					
	Ia		Ib		IIa		IIb		IIIa		IIIb		IIIc	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Dyspepsia	5	41.7	4	33.3	9	75	12	100	8	66.7	4	33.3	5	41.7
Diarrhea	0	0	0	0	1	8.3	7	58.3	10	83.3	9	75.0	11	91.7
Abdominal pain	3	25	7	58.3	9	75	12	100	9	75	5	41.7	10	83.3
Abdominal distension	0	0	0	0	7	58.3	12	100	2	16.7	0	0	0	0
Heamatemesis	0	0	0	0	4	33.3	9	75	4	33.3	0	0	0	0
Fever	1	8.3	1	8.3	0	0	4	33.3	12	100	12	100	12	100

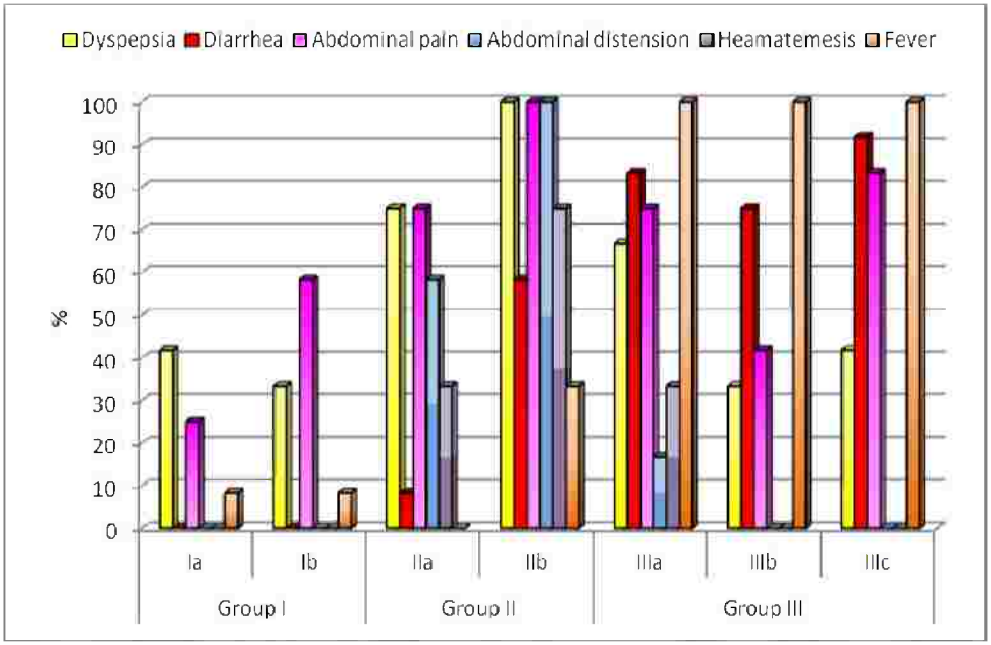

Figure (17): Comparison between groups as regard to patient's symptoms

As regard to clinical examination, in group (Ia) hepatomegaly was present in 58.3% of patients while 41.7% had splenomegaly and in group (Ib) hepatomegaly was present in 58.3% of patients while 41.7% had splenomegaly in group (IIa) 100% of patients presented with hepatomegaly, 91.7% with splenomegaly, 66.7 with jaundice and 33.3% with lower limb edema while in group (IIb) 100% of patients presented with jaundice, 100% with splenomegaly, 100% with ascites, 100% with lower limb edema, 66.7 with hepatomegaly and 41.7% with hepatic encephalopathy and in group (IIIa) the most frequent signs were jaundice (100%), hepatomegaly (83.3%), splenomegaly (33.3%), lower limb edema (33.3%), ascites (8.3%) and hepatic encephalopathy (8.3%) in group (IIIb and IIIc) there was no specific clinical signs.

Table (13): Comparison between patients groups as regard to patient's clinical signs

Signs	Group I				Group II				Group III					
	Ia		Ib		IIa		IIb		IIIa		IIIb		IIIc	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Jaundice	0	0	0	0	8	66.7	12	100	12	100	0	0	0	0
Hepatomegaly	7	58.3	7	58.3	12	100	8	66.7	10	83.3	0	0	0	0
Splenomegaly	5	41.7	4	33.3	11	91.7	12	100	4	33.3	0	0	0	0
Ascites	0	0	0	0	0	0	12	100	1	8.3	0	0	0	0
Hepatic encephalopathy	0	0	0	0	0	0	5	41.7	1	8.3	0	0	0	0
Lower limb edema	0	0	0	0	4	33.3	12	100	4	33.3	0	0	0	0

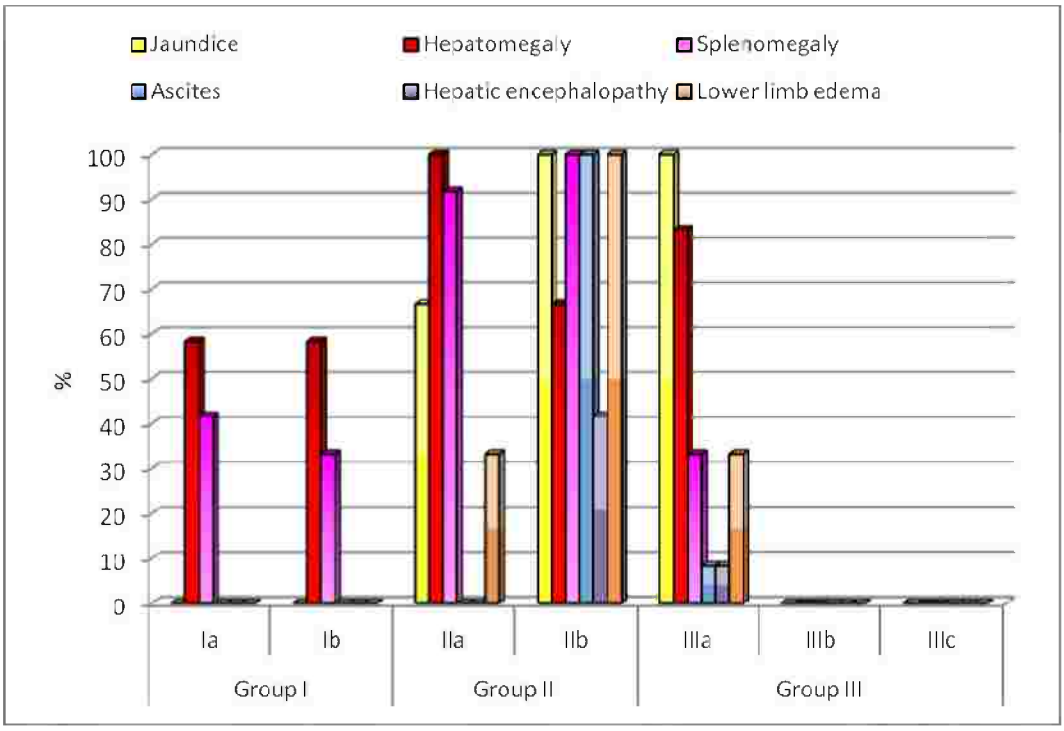

Figure (18): Comparison between groups as regard to patient's clinical signs

Laboratory findings

Hematological findings: (Table 14, Figure 19,20)

As regard to Hb, in group Ia it ranged between 11.5-13.4 gm/dl with mean of. 12.533 ± 0.6184 gm/dl while in group Ib it ranged between 11.5-13.5 gm/dl with mean of 12.442 ± 0.6415 gm/dl; in group IIa it ranged between 10.5-13 gm/dl with mean of 11.542 ± 0.8918 gm/dl while in group IIb it ranged between 8.5-12.2 gm/dl with a mean of. 10.492 ± 0.9558 gm/dl; in group IIIa it ranged between 7.2-11.2 gm/dl with a mean of 9.308 ± 0.9995 gm/dl, in group IIIb it ranged between 6-10.5 gm/dl with a mean of 8.525 ± 1.4423 gm/dl and in group IIIc it ranged between 7.8-11.5 gm/dl with a mean of 9.8±1.1394 gm/dl. There was statistically significant difference between group II subgroups and group III subgroups where P=0.011, 0.045 respectively. (P significant level at P less than 0.05)

As regard to Platelets count multiplied by 10^3 , in group Ia it ranged between 214 - 416 with a mean of 316.17 ± 61.261 while in group Ib it ranged between 190 - 310 with a mean of 231.58 ± 43.488; in group IIa it ranged between 165 - 215 with a mean of 199.17 ± 14.953 while in group IIb it ranged between 65-167 with a mean of 123 ± 34.314; in group IIIa it ranged between 87-190 with a mean of 151± 27.601, in group IIIb it ranged between 146-348 with a mean of 215.92 ± 64.545 and in group IIIc it ranged between 165-317 with a mean of 237.83 ± 57.55. There was statistically significant difference between group I, II and III subgroups where P=0.000 in all (P significant level at P less than 0.05).

Table (14): Comparison between groups as regard to patient's haematological findings

		Group I		Group II		Group III		
		Ia	Ib	IIa	IIb	IIIa	IIIb	IIIc
Hb (gm/dl)	Min.	11.5	11.5	10.5	8.5	7.2	6.0	7.8
	Max.	13.4	13.5	13.0	12.2	11.2	10.5	11.5
	Mean	12.533	12.442	11.542	10.492	9.308	8.525	9.800
	S.D.	0.6184	0.6815	0.8918	0.9558	0.9995	1.4423	1.1394
	P Value	0.733		0.011*		0.045*		
Platelets $x10^3$/ µL	Min.	214	190	165	65	87	146	165
	Max.	416	310	215	167	190	348	317
	Mean	316.17	231.58	199.17	123.00	151.00	215.92	237.83
	S.D.	61.261	43.488	14.953	34.314	27.601	64.545	57.550
	P Value	0.000*		0.000*		0.000*		

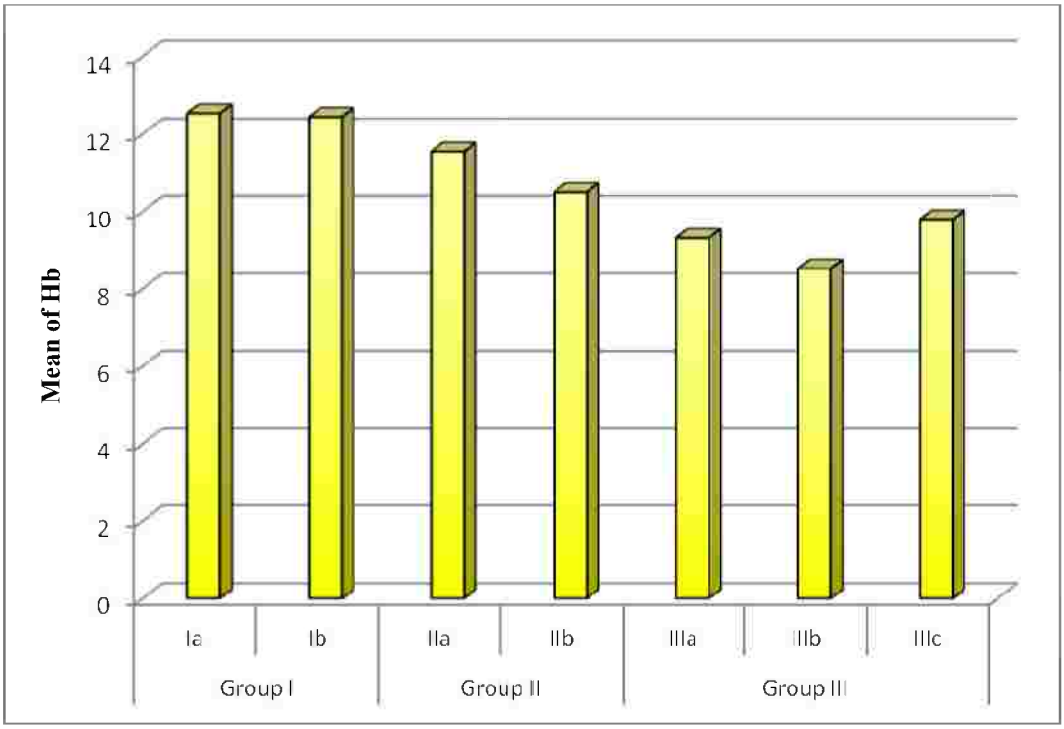

Figure (19): Comparison between groups as regard to patient's Hb

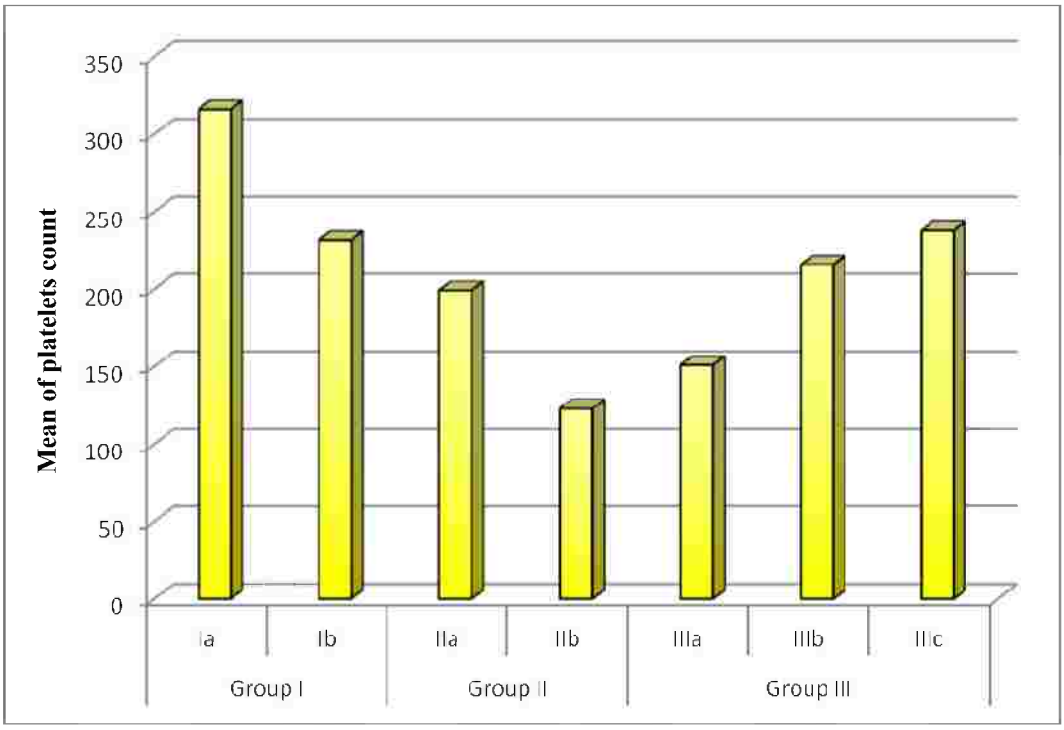

Figure (20): Comparison between groups as regard to patient's platelets count

Liver Function tests:

Regarding serum alanine transferase (ALT), in group Ia; it ranged between 38-118 u/l with a mean of 63.5 ± 23.181 u/l while in group Ib; it ranged between 24-98 u/l with a mean of 39.08 ± 22.326 u/l; in group IIa it ranged between 32-55 u/l with a mean of 41.67 ± 7.82 u/l while in group IIb it ranged between 31-46 u/l with a mean of 35.92 ± 5.632 u/l; in group IIIa; it ranged between 35-150 u/l with a mean of 58.67±32.469 u/l, in group IIIb it ranged between 18-32 u/l with a mean of 24.92±4.4 u/l and in group IIIc, it ranged between 16-28 u/l with a mean of 20.33±3.701 u/l. There were statistically significant differences between group I, II and III subgroups where P=0.001, 0.045 and 0.000 respectively (P significant level at P less than 0.05). (Table 15, Figure 21)

Regarding to serum aspartate transferase (AST), in group Ia it ranged between 30-96 u/l with a mean of 51.42±18.313 u/l while in group Ib, it ranged between 22-87 u/l with a mean of 33±20.701 u/l; in group IIa it ranged between 32-81 u/l with a mean of 50.50 ± 14.842 u/l while in group IIb it ranged between 33-70 u/l with a mean of 46.75±12.122 u/l; in group IIIa, it ranged between 36-106 u/l with a mean of 57.33 ± 21.402 u/l, in group IIIb, it ranged between 18-35 u/l with a mean of 27.58±4.166 u/l and in group (IIIc) it ranged between 22-32 u/l with a mean of 26.33±2.774 u/l. There were statistically significant differences between group I subgroups and group III subgroups where P=0.002 and 0.000 respectively (P significant level at P less than 0.05) (Table 15, Figure 22)

Regarding to serum alkaline phosphatase (ALP), in group Ia it ranged between 81-171 u/l with a mean of 129.21±30.007 u/l while in group Ib it ranged between 95-166 u/l with a mean of 124.5±24.04 u/l; in group IIa it ranged between 120-188 u/l with a mean of 161.25±20.191 u/l while in group IIb it ranged between 98-200 u/l with a mean of 146.42±38.116 u/l; in group IIIa it ranged between 85-135 u/l with a mean of 108.67±15.370 u/l, in group IIIb, it ranged between 43-120 u/l with a mean of 79.42 ± 22.387 u/l and in group IIIc, it ranged between 42-110 u/l with a mean of 66.67±19.901 u/l. There was statistically significant difference between group III subgroups where P= 0.000 (P significant level at P less than 0.05) (Table 15, Figure 23)

Regarding to Prothrombin activity, in group Ia, it ranged between 76-91% with a mean of 84±4.954 while in group Ib it ranged between 68-82% with a mean of 74.5±3.989%; in group IIa, it ranged between 68-74% with a mean of 70 ± 2% while in group IIb, it ranged between 20-60% with a mean of 38.99 ± 12.402%; in group IIIa it ranged between 44-78% with a mean of 67.33 ± 9.5%, in group IIIb, it ranged between 72-95% with a mean of 81.75±7.399% and in group IIIc, it ranged between 69-92% with a mean of 80.67±7.426%. There were statistically significant differences between group I, II and III subgroups where P=0.000 in all. (P significant level at P less than 0.05) (Table 15, Figure 24)

Regarding to serum total bilirubin levels, in group Ia it ranged between 0.6-1.1 mg/dl with a mean of 0.892±0.138 mg/dl while in group Ib, it ranged between 0.8-1.2 mg/dl with a mean of 0.967±0.137 mg/dl; in group IIa, it ranged between 0.8-5.2 mg/dl with a mean of 2.938±1.587 mg/dl while in group IIb, it ranged between 2.9-14.2 mg/dl with a mean of 6.592±3.793 mg/dl; in group IIIa, it ranged between 2.1-6.5 mg/dl with a mean of 4.117±1.185 mg/dl, in group IIIb, it ranged between 0.4-1.1 mg/dl with a mean of 0.783±0.204 mg/dl and in group IIIc, it ranged between 0.6-9.8 mg/dl with a mean of

1.592±2.591 mg/dl. There was statistically significant differences between group II subgroups and group III subgroups where P= 0.005, 0.000 respectively. (significant level at P less than 0.05). (Table 15, Figure 25)

Regarding to serum direct bilirubin levels, in group Ia it ranged between 0.2-0.4 mg/dl with a mean of 0.308±0.079 mg/dl while in group Ib, it ranged between 0.2-0.5 mg/dl with a mean of 0.375±0.087 mg/dl; in group IIa, it ranged between 0.3-3 mg/dl with a mean of 1.754±1.102 mg/dl while in group IIb, it ranged between 1.4-11.2 mg/dl with mean±S.D. 4.75±3.172 mg/dl; in group (IIIa) it ranged between 1.5-4 mg/dl with a mean of 2.85±0.748 mg/dl, in group IIIb, it ranged between 0.2-0.5 mg/dl with a mean of 0.317±0.094 mg/dl and in group IIIc, it ranged between 0.2-0.8 mg/dl with a mean of 0.408±0.156 mg/dl. There were statistically significant differences between group II subgroups and group III subgroups where P=0.004 and 0.000 respectively. (significant level at P less than 0.05) (Table 15, Figure 26)

Regarding to serum albumin levels, in group Ia, it ranged between 3.4-4.5 gm/dl with a mean of 3.925±0.317 gm/dl while in group Ib, it ranged between 3.3-4.2 gm/dl with a mean of 3.758±0.231 gm/dl; in group IIa it ranged between 3-3.8 gm/dl with a mean of 3.292±0.223 gm/dl while in group IIb, it ranged between 1.8-2.6 gm/dl with a mean of 2.205±0.308 gm/dl; in group IIIa, it ranged between 2.7-3.8 gm/dl with a mean of 3.342±0.294 gm/dl, in group IIIb, it ranged between 3.5-4.2 gm/dl with a mean of 3.825±0.242 gm/dl and in group IIIc, it ranged between 3.2-4.2 gm/dl with a mean of 3.783±0.307 gm/dl. There were statistically significant differences between group II subgroups and group III subgroups where P=0.000 in both (P significant level at P less than 0.05). (Table 15, Figure 27)

Table (15): Comparison between groups as regard to liver function tests

		Group I		Group II		Group III		
		Ia	Ib	IIa	IIb	IIIa	IIIb	IIIc
ALT (u/l)	Min.	38	24	32	31	35	18	16
	Max.	118	98	55	46	150	32	28
	Mean	63.50	39.08	41.67	35.92	58.67	24.92	20.33
	S.D.	23.181	22.326	7.820	5.632	32.469	4.400	3.701
	P	0.001*		0.045*		0.000*		
AST (u/l)	Min.	30	22	32	33	36	18	22
	Max.	96	87	81	70	106	35	32
	Mean	51.42	33.00	50.50	46.75	57.33	27.58	26.33
	S.D.	18.313	20.701	14.842	12.122	21.402	4.166	2.774
	P	0.002*		0.590		0.000*		
ALP (u/l)	Min.	81	95	120	98	85	43	42
	Max.	171	166	188	200	135	120	110
	Mean	129.21	124.50	161.25	146.42	108.67	79.42	66.67
	S.D.	30.007	24.040	20.191	38.116	15.370	22.387	19.901
	P	0.676		0.246		0.000*		
Prothrombin activity(%)	Min.	76	68	68	20	44	72	69
	Max.	91	82	74	60	78	95	92
	Mean	84.00	74.50	70.00	38.99	67.33	81.75	80.67
	S.D.	4.954	3.989	2.000	12.402	9.500	7.399	7.426
	P	0.000*		0.000*		0.000*		
Total bilirubin (mg/dl)	Min.	0.60	0.80	0.80	2.90	2.10	0.40	0.60
	Max.	1.10	1.20	5.20	14.20	6.50	1.10	1.20
	Mean	0.892	0.967	2.938	6.592	4.117	0.783	0.896
	S.D.	0.138	0.137	1.587	3.793	1.185	0.204	0.142
	P	0.195		0.005*		0.000*		
Direct bilirubin (mg/dl)	Min.	0.20	0.20	0.30	1.40	1.50	0.20	0.20
	Max.	0.40	0.50	3.00	11.20	4.00	0.50	0.80
	Mean	0.308	0.375	1.754	4.750	2.850	0.317	0.408
	S.D.	0.079	0.087	1.102	3.172	0.748	0.094	0.156
	P	0.089		0.004*		0.000*		
Serum albumin (gm/dl)	Min.	3.4	3.3	3.0	1.8	2.7	3.5	3.2
	Max.	4.5	4.2	3.8	2.6	3.8	4.2	4.2
	Mean	3.925	3.758	3.292	2.205	3.342	3.825	3.783
	S.D.	0.317	0.231	0.223	0.308	0.294	0.242	0.307
	P	0.155		0.000*		0.000*		

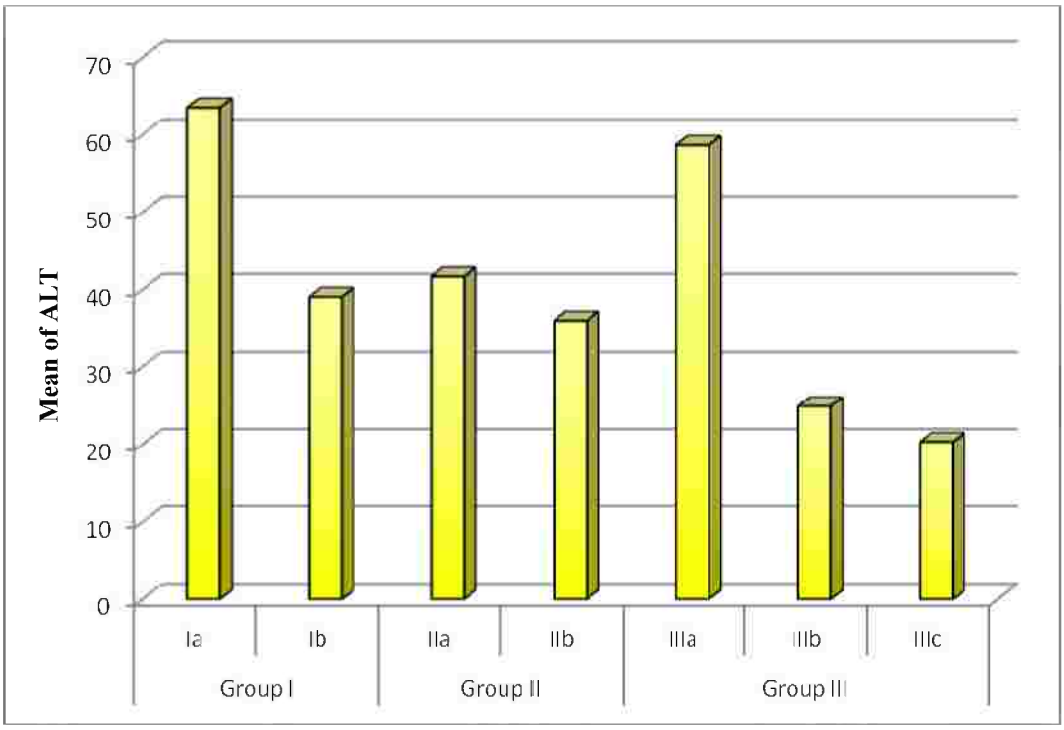

Figure (21): Comparison between groups as regard to ALT

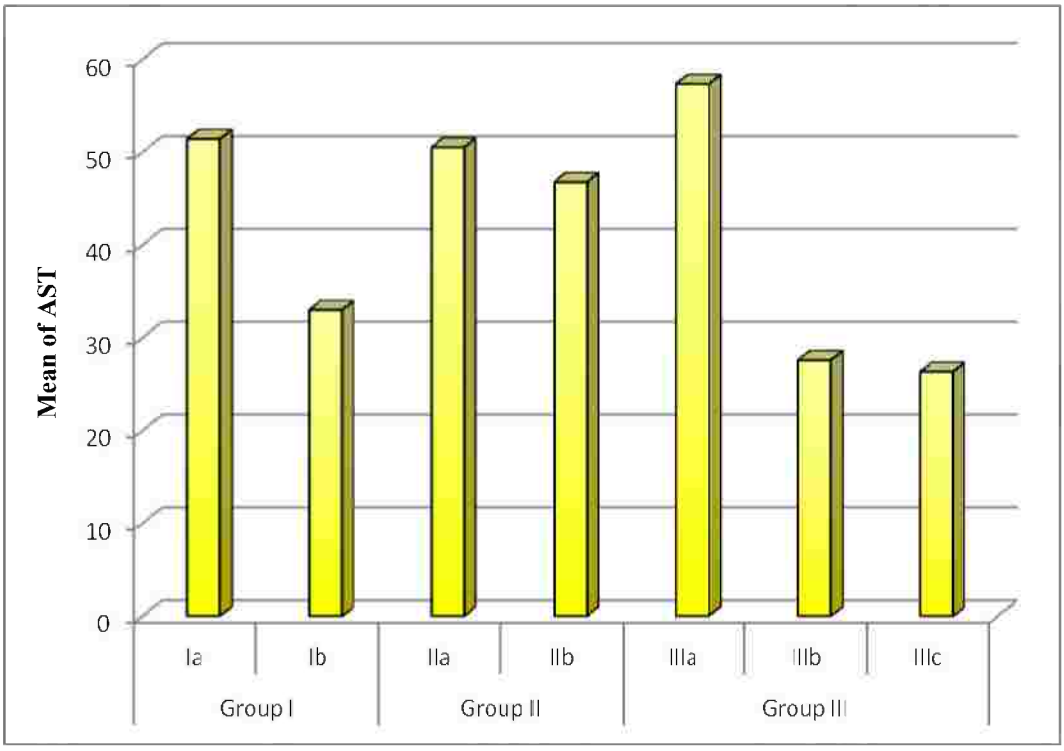

Figure (22): Comparison between groups as regard to AST

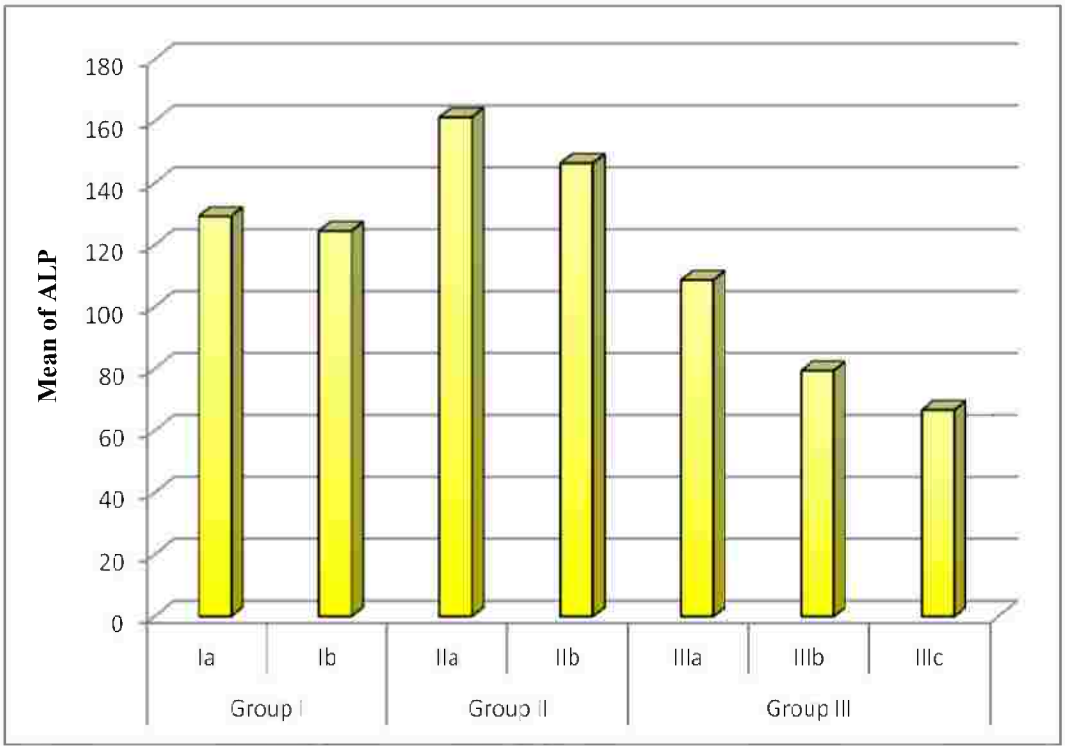

Figure (23): Comparison between groups as regard to ALP

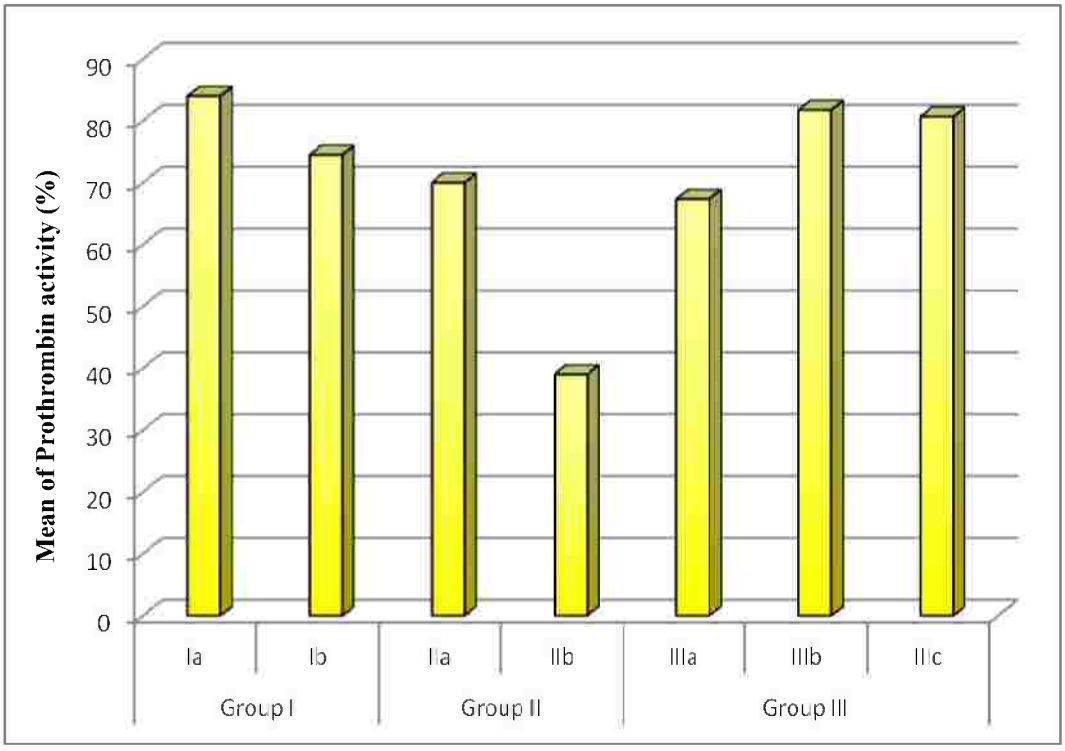

Figure (24): Comparison between groups as regard to Prothrombin activity(%)

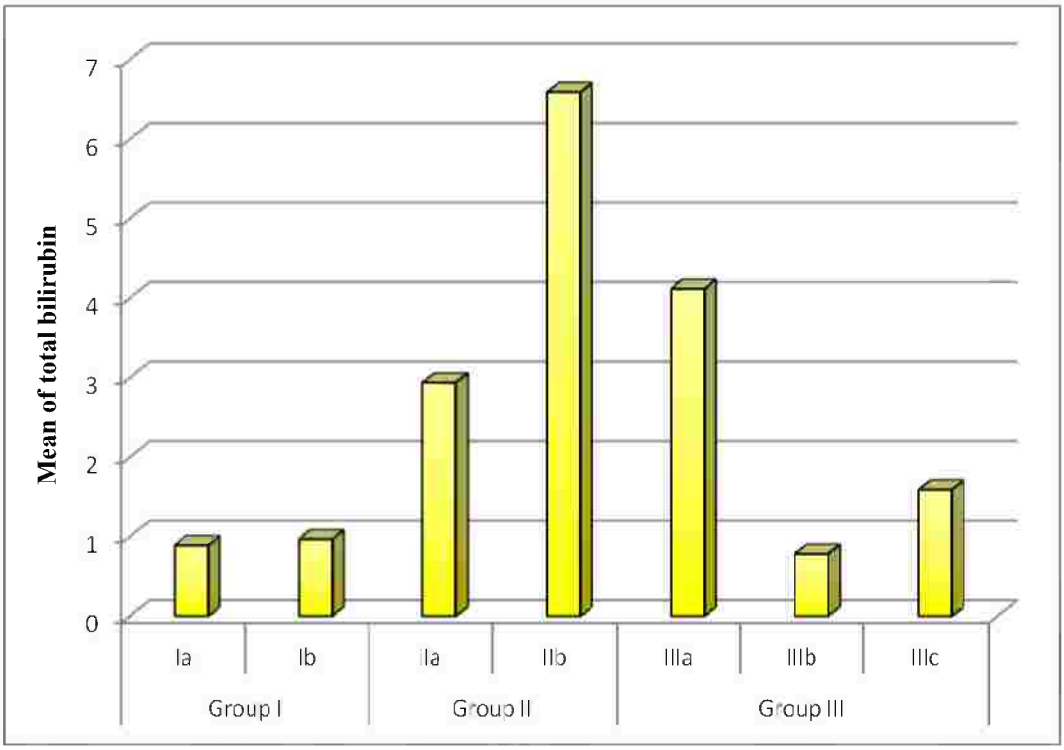

Figure (25): Comparison between groups as regard to total bilirubin

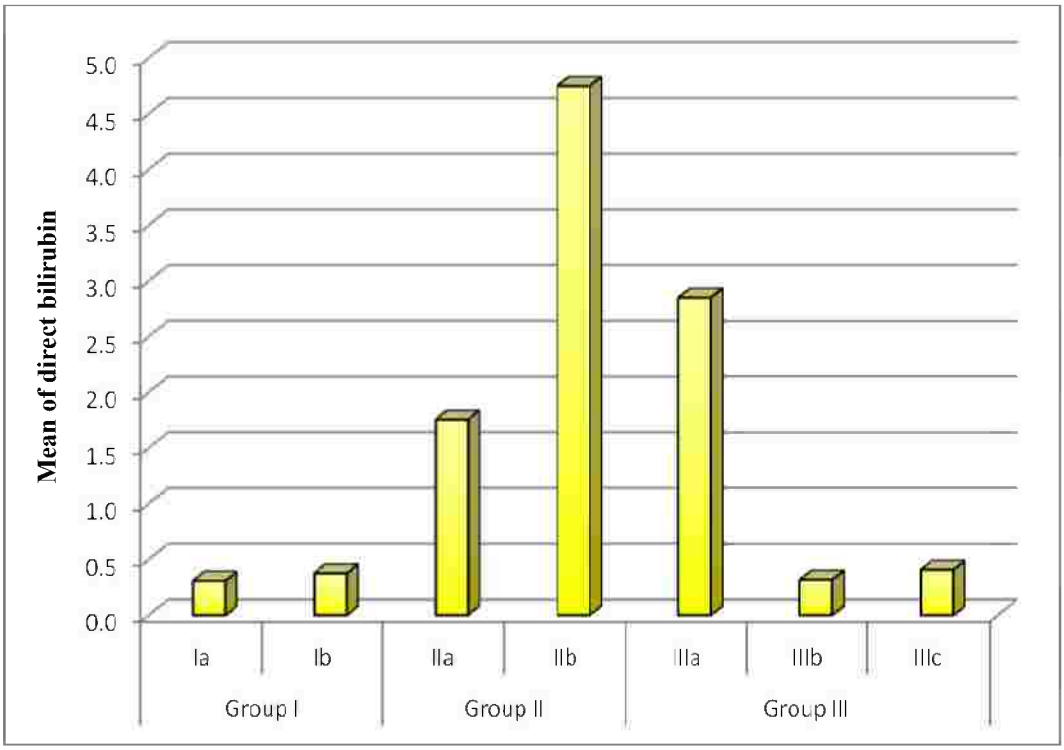

Figure (26): Comparison between groups as regard to direct bilirubin

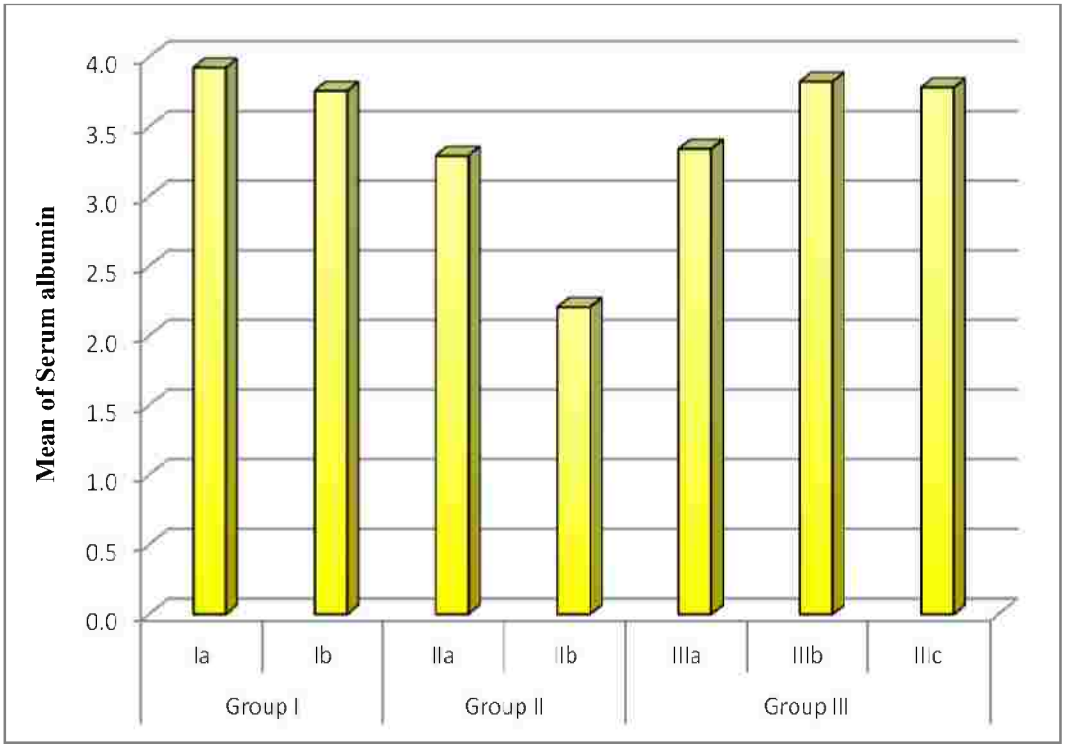

Figure (27): Comparison between groups as regard to Serum albumin

Liver Biopsy: (Table 16, Figure 28)

Liver biopsies were obtained from patients of group I and regarding to it, in group Ia, 2 (16.7%) out of the patients had fibrotic score 0, 2 (16.7%) out of the patients had fibrotic score 1 while 6 (50%) out of the patients had fibrotic score 2 and 2(16.7%) out of the patients were given fibrotic score 3. In group Ib, 6 (50%) out of the patients had fibrotic score 1, 4 (33.3%) out of the patients had fibrotic score 2 and 2 (16.7%) out of the patients had fibrotic score 3. There was no statistically significant differences between group Ia and group Ib regarding liver biopsy results where P=0.221 (P significant level at P less than 0.05).

Table (16): Comparison between group I patients as regard to liver biopsy

Stage of liver fibrosis	Group I			
	Ia		Ib	
	No.	%	No.	%
0	2	16.7	0	0
1	2	16.7	6	50
2	6	50	4	33.3
3	2	16.7	2	16.7
Total	12	100	12	100
P Value	0.221			

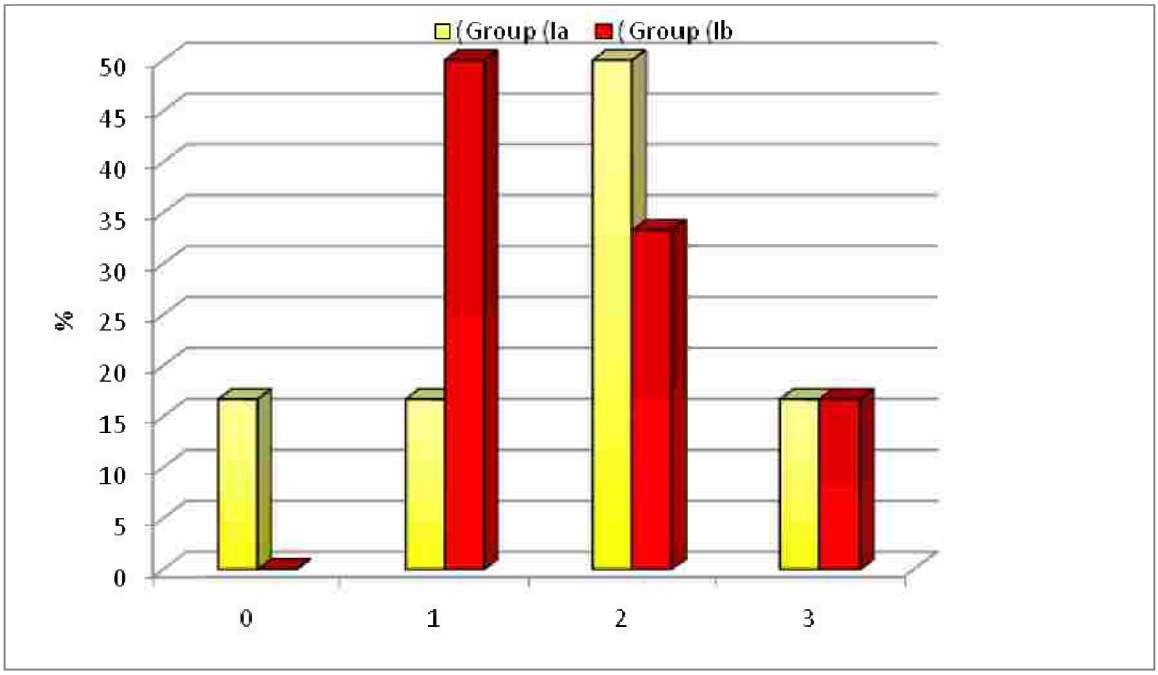

Figure (28): Comparison between group I patients as regard to liver biopsy

Serum Hyaluronic Acid: (Table 17, Figure 29)

As regard to serum hyaluronic acid (HA), in group Ia, it ranged between 15-65 ng/ml with a mean of 44.12±16.340 ng/ml while in group Ib, it ranged between 12-42 ng/ml with a mean of 22.73±9.686 ng/ml; in group IIa, it ranged between 36-85 ng/ml with a mean of 48.94±16.413 ng/ml while in group IIb, it ranged between 73-255 ng/ml with a mean of 148.33±66.496 ng/ml; in group IIIa it ranged between 30-152 ng/ml with a mean of 58.59±39.743 ng/ml, in group IIIb, it ranged between 9-26 ng/ml with a mean of 19.07±6.765 ng/ml and in group IIIc, it ranged between 12-26 ng/ml with a mean of 17.78±4.877 ng/ml. HA was significantly higher in group Ia than Ib (P=0.001), in group IIb than IIa (P=0.000) and in group IIIa than both IIIb and IIIc (P=0.000). (P significant level at P less than 0.05)

Table (17): Comparison between groups as regard to serum hyaluronic acid (HA)

HA	Group I		Group II		Group III		
	Ia	Ib	IIa	IIb	IIIa	IIIb	IIIc
Min.	15	12	36	73	30	9	12
Max.	65	42	85	255	152	26	26
Mean	44.12	22.73	48.94	148.33	58.59	19.07	17.78
S.D.	16.340	9.686	16.413	66.496	39.743	6.765	4.877
P Value	0.001*		0.000*		0.000*		

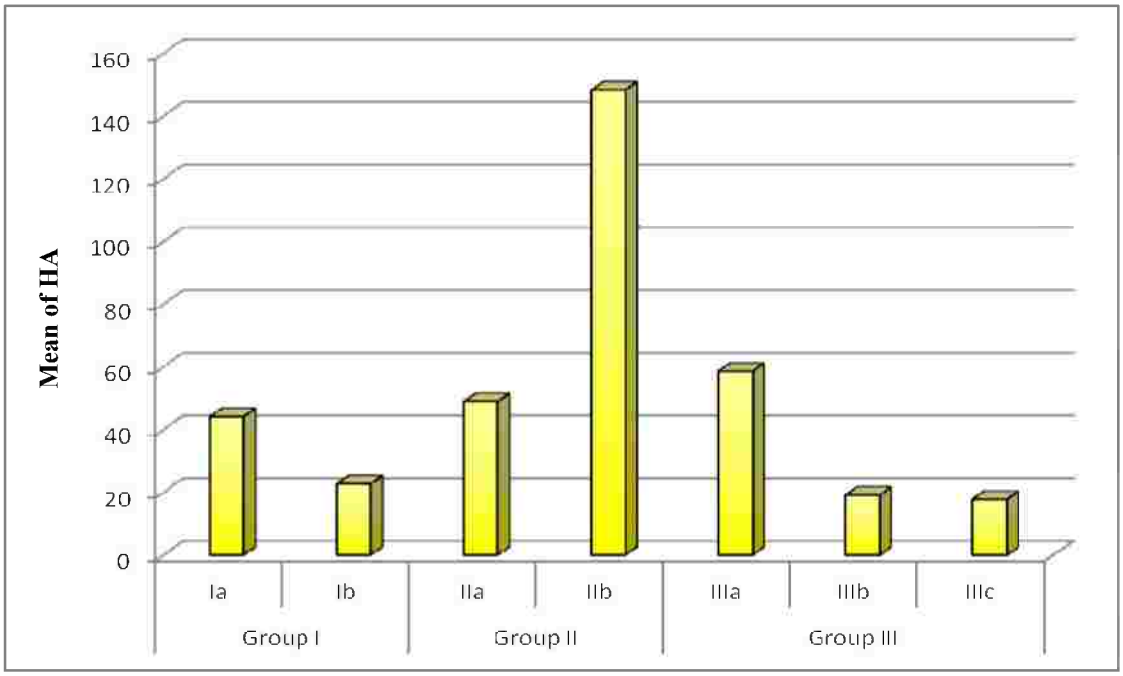

Figure (29): Comparison between groups as regard to serum hyaluronic acid (HA)

Ultrasound findings: (Table18, Figure 30)

As regard to estimation of the severity of liver fibrosis using US, in group Ia, 3(25%) out of the patients had normal liver texture while 7(58.3%) out of the patients had mild fibrosis and 2(16.7%) out of the patients had moderate fibrosis and the same is in group Ib, 3(25%) out of the patients had normal liver texture while 7(58.3%) out of the patients had mild fibrosis and 2(16.7%) out of the patients had moderate fibrosis; in group IIa, 3(25%) out of the patients had mild fibrosis while 6(50%) out of the patients had moderate fibrosis and 3(25%) out of the patients were classified as severe fibrosis while in group IIb all patients in this group were classified as severe fibrosis, in group IIIa, 5(41.7%) out of the patients had mild fibrosis while 5(41.7%) out of the patients had moderate fibrosis and 2(16.7%) out of the patients were classified as severe fibrosis while in groups IIIb, and IIIc all patients had normal texture liver. There was statistically significant differences between group II and III subgroups where P=0.001, 0.000 respectively (P significant level at P less than 0.05).

Table (18): Comparison between groups as regard to Ultrasound finding

US	Group I				Group II				Group III					
	Ia		Ib		IIa		IIb		IIIa		IIIb		IIIc	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Normal	3	25	3	25	0	0	0	0	0	0	12	100	12	100
Mild fibrosis	7	58.3	7	58.3	3	25	0	0	5	41.7	0	0	0	0
Moderate	2	16.7	2	16.7	6	50	0	0	5	41.7	0	0	0	0
Severe	0	0	0	0	3	25	12	100	2	16.7	0	0	0	0
Total	12	100	12	100	12	100	12	100	12	100	12	100	12	100
P Value	1.000				0.001*				0.000*					

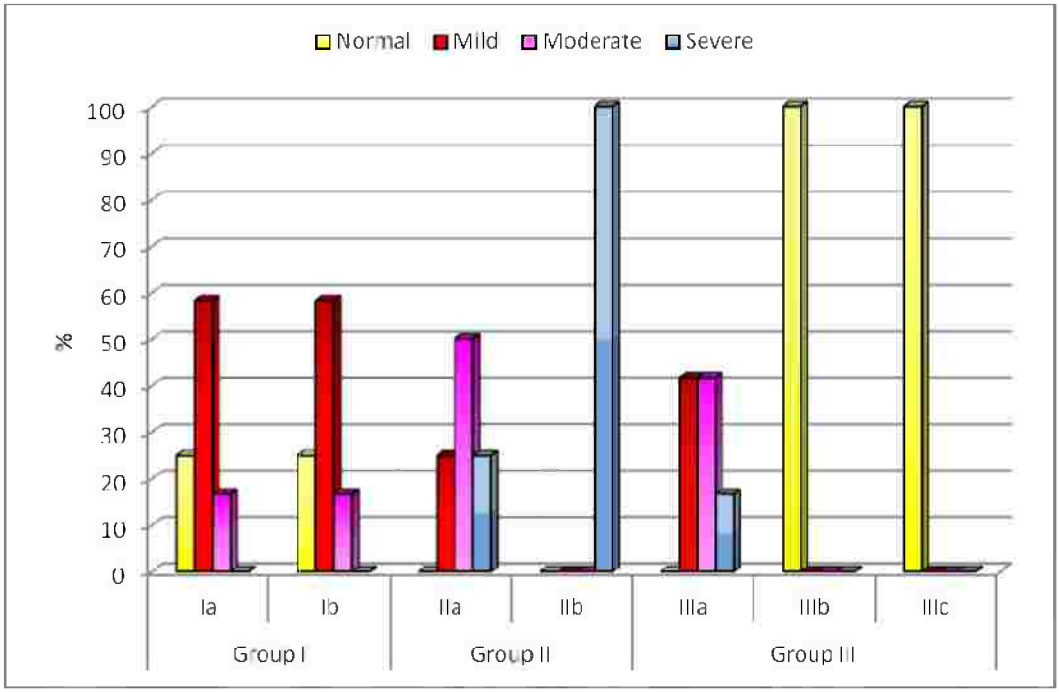

Figure (30): Comparison between groups as regard to Ultrasound finding

When comparing ultrasound findings and liver biopsy results in group I , the next table showed that normal US finding had fibrosis score ranged between 0-1 while the mild US finding had fibrosis score ranged between 1-2 and the moderate US finding had fibrosis score 3. This indicates that US can be used to assess degree of liver fibrosis.(Table 19)

Table (19): Comparison between ultrasound finding and fibrosis in group I

Fibrosis		Ultra sound Finding		
		Normal	Mild	Moderate
Group Ia	Min.	0	1	3
	Max.	1	2	3
	Mean	0.333	1.857	3.000
	S.D.	0.577	0.378	0.000
Group Ib	Min.	1	1	3
	Max.	1	2	3
	Mean	1.000	1.571	3.000
	S.D.	0.000	0.535	0.000
Group I	Min.	0	1	3
	Max.	1	2	3
	Mean	0.667	1.714	3.000
	S.D.	0.516	0.469	0.000

Correlation between HA and liver function tests: (Table 20, Figures 31-36)

There was positive but not significant correlation between HA level and ALT while there was high positive significant correlation between HA and each of AST, Total bilirubin and direct bilirubin and there was high negative significant correlation between HA level and each of serum albumin and prothrombin activity.

Table (20): Correlation between serum hyaluronic acid (HA) and liver function tests

		Serum hyaluronic acid level (ng/ml)
ALT	**R**	0.048
	P	0.667
AST	**R**	0.243
	P	0.026*
Albumin	**R**	-0.718
	P	0.000*
Prothrombin activity(%)	**R**	-0.673
	P	0.000*
Total bilirubin	**R**	0.484
	P	0.000*
Direct bilirubin	**R**	0.515
	P	0.000*

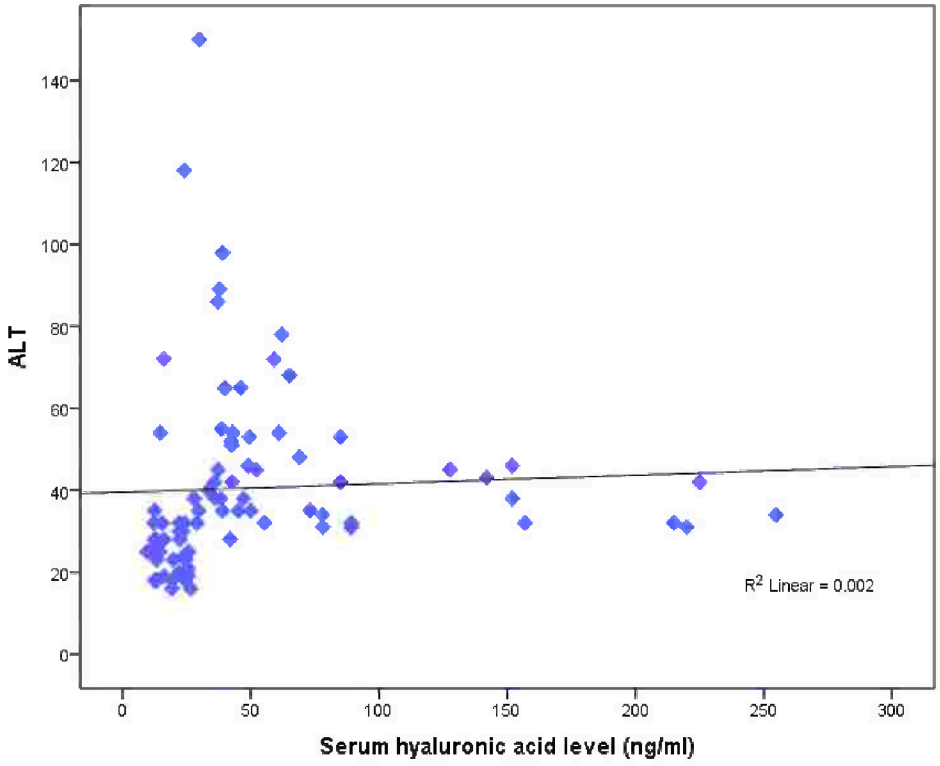

Figure (31): Correlation between serum hyaluronic acid (HA) and ALT

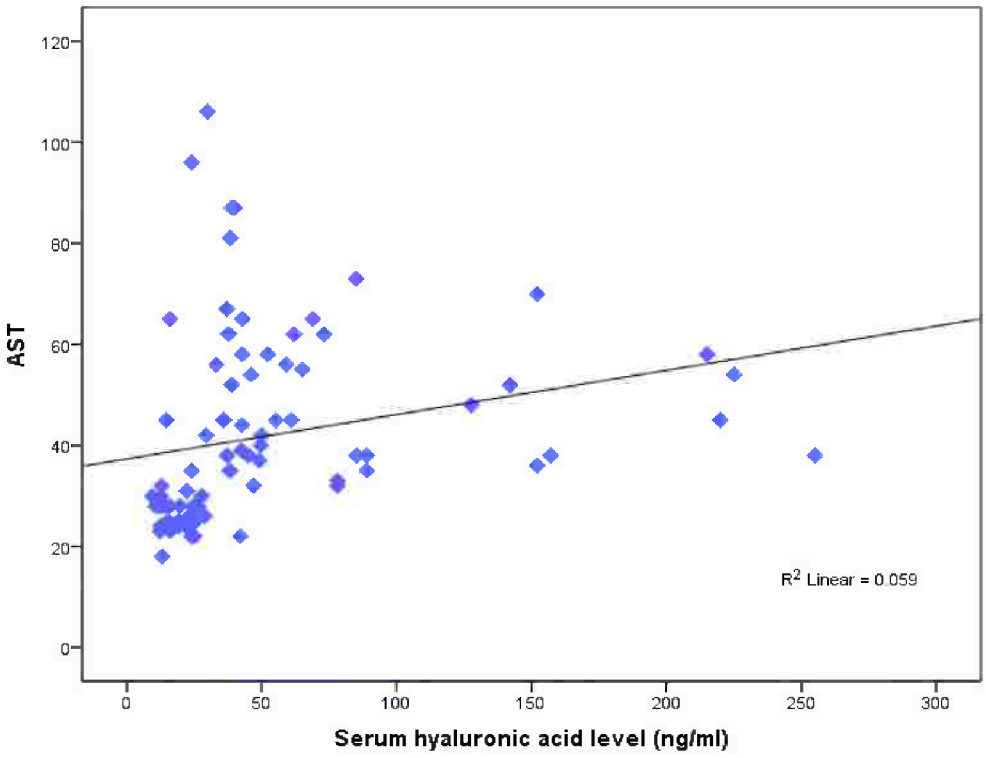

Figure (32): Correlation between serum hyaluronic acid (HA) and AST

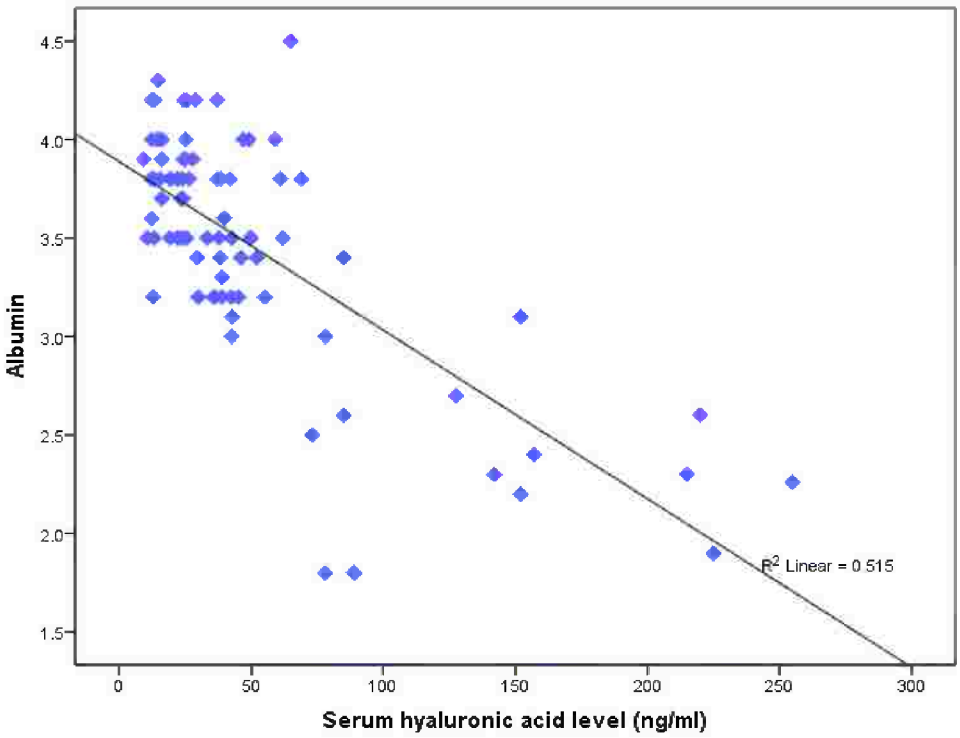

Figure (33): Correlation between serum hyaluronic acid (HA) and Albumin

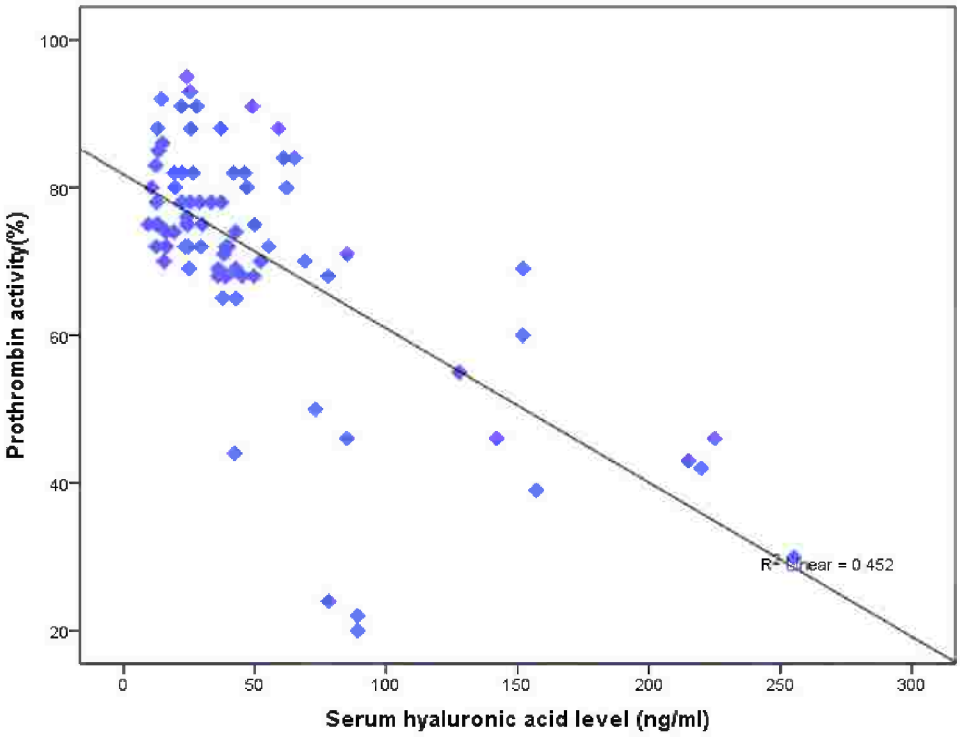

Figure (34): Correlation between serum hyaluronic acid (HA) and prothrombin activity

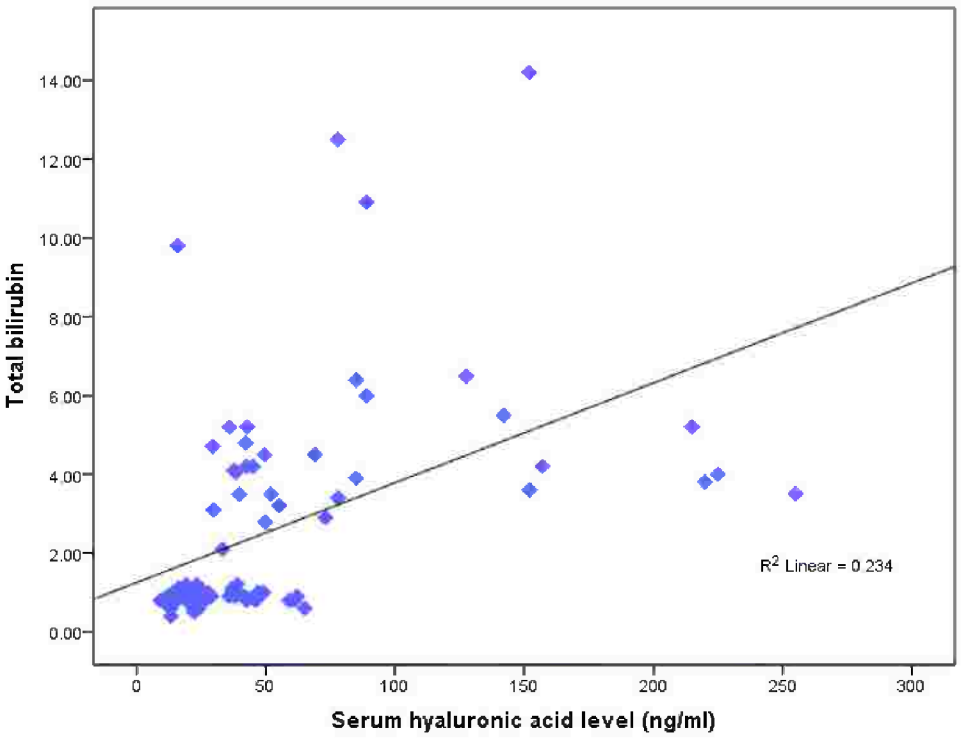

Figure (35): Correlation between serum hyaluronic acid (HA) and total bilirubin

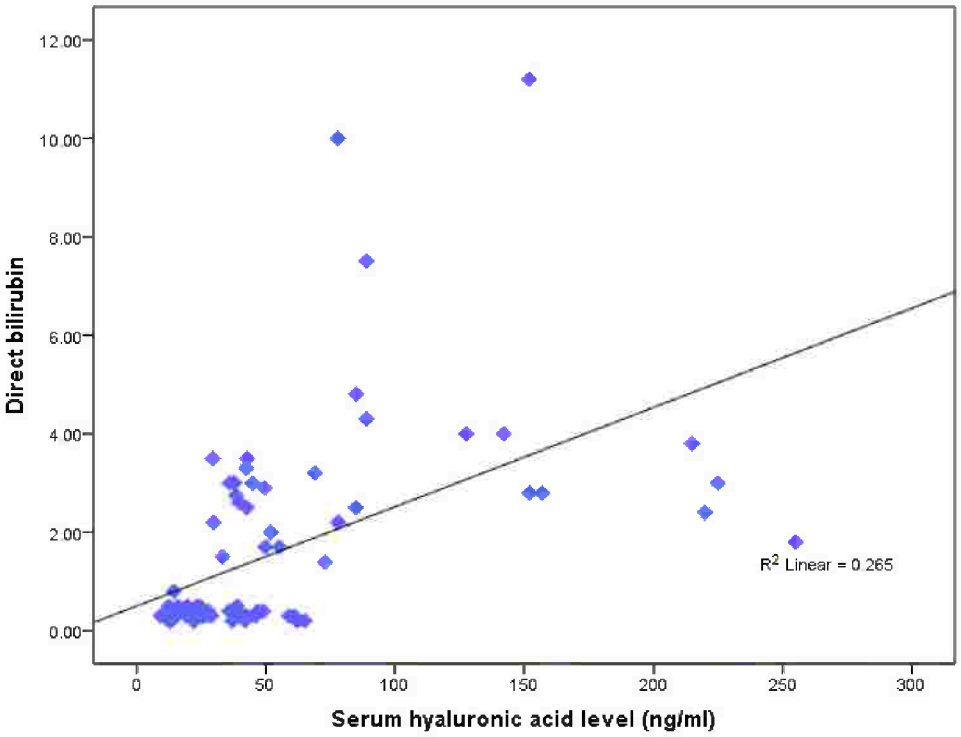

Figure (36): Correlation between serum hyaluronic acid (HA) and direct bilirubin

Correlation between HA and fibrosis score in group I: (Table 21, Figure 37-39)

There was strong positive correlation between HA and liver fibrosis score by liver biopsy in group I.

Table (21): Correlation between serum hyaluronic acid (HA) and fibrosis

		Serum hyaluronic acid level (ng/ml)
Group (Ia)	**R**	0.856
	P	0.000*
Group (Ib)	**R**	0.755
	P	0.007*
Group (I)	**R**	0.619
	P	0.002*

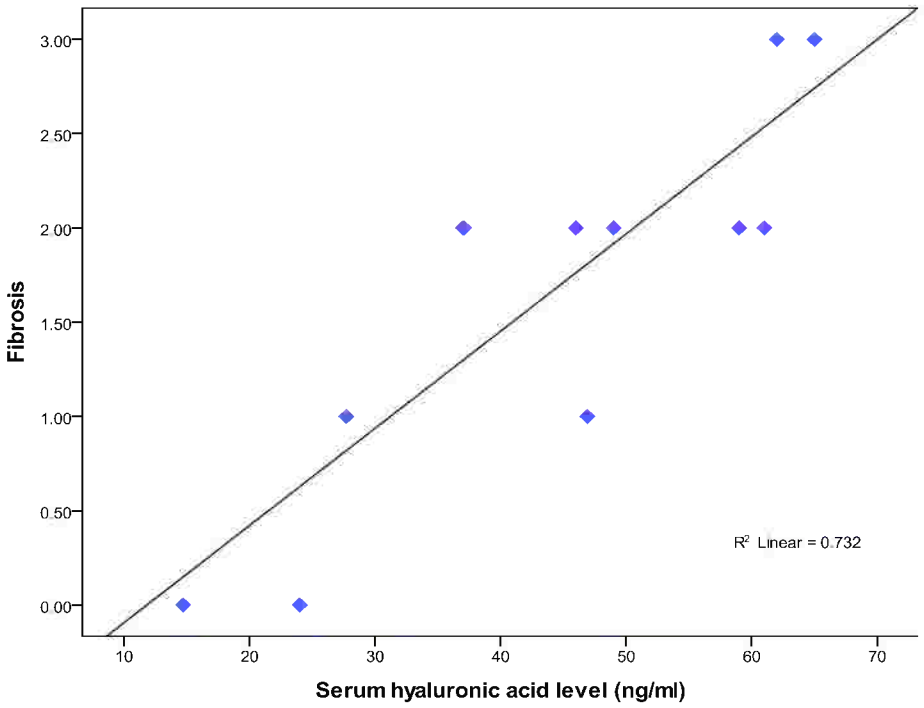

Figure (37): Correlation between serum hyaluronic acid (HA) and fibrosis in group Ia

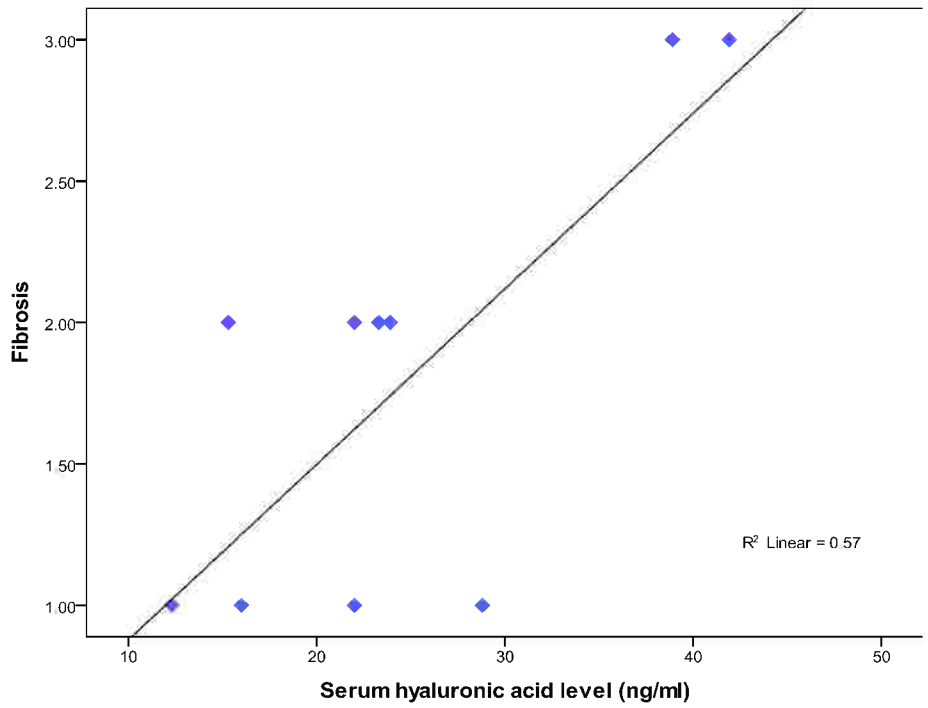

Figure (38): Correlation between serum hyaluronic acid (HA) and fibrosis in group (Ib)

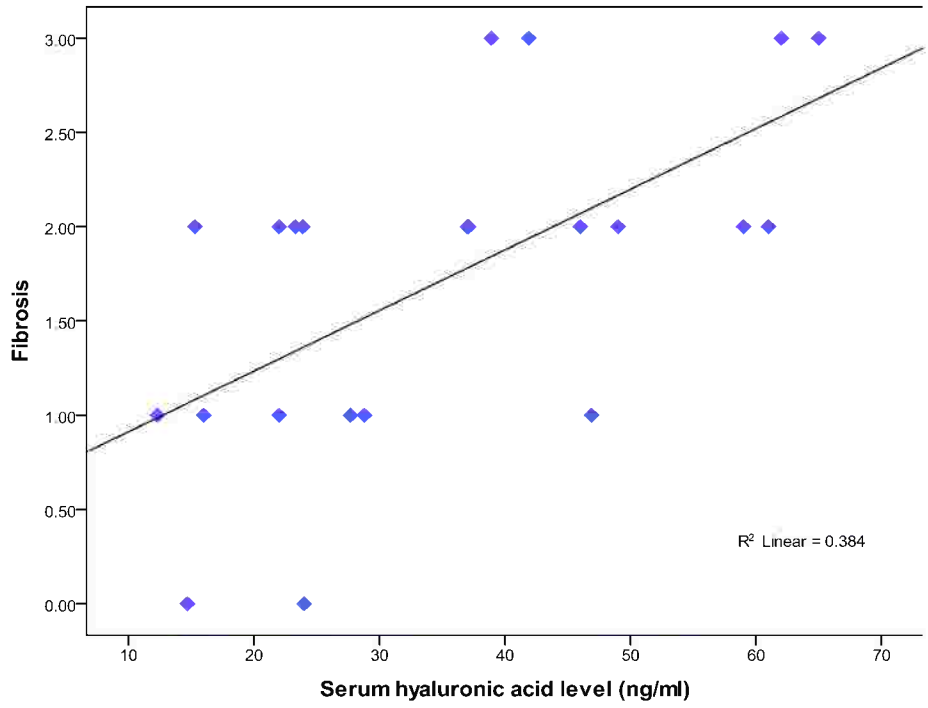

Figure (39): Correlation between serum hyaluronic acid (HA) and fibrosis in group (I)

Correlation between HA and Ultrasound findings: (Table 22, Figure 40)

There was a high positive significant correlation between HA and Ultra sound findings of all groups where R=0.861 and P=0.000.

Table (22): Correlation between serum hyaluronic acid (HA) and Ultra sound findings

		Serum hyaluronic acid level (ng/ml)
Ultra sound findings	**R**	0.861
	P	0.000*

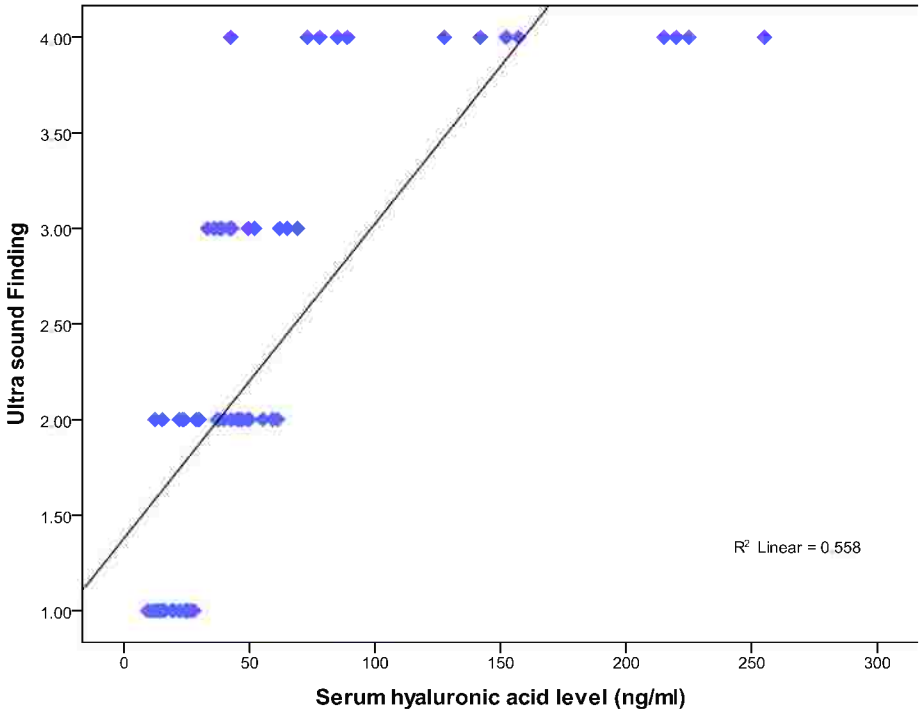

Figure (40): Correlation between serum hyaluronic acid (HA) and Ultra sound findings