Assessment of serum iron markers and Hepcidin in patients with non-alcoholic fatty liver diseases

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Abstract: Objective: The aim of this study was to evaluate serum hepcidin and iron markers levels as biomarkers for inflammatory activity in patients with NAFLD. **Background:** Non alcoholic fatty liver disease (NAFLD) refers to a spectrum of hepatic disorders, ranging from simple or bland fatty liver to a non-alcoholic steato-hepatitis (NASH), which is characterized by an inflammatory reaction with hepatocytic injury, The role of hepcidin in non-alcoholic liver disease and its utility as a biomarker for non-alcoholic steatohepatitis (NASH) or non-alcoholic fatty liver disease (NAFLD) histological severity has generated much interest. **Patients and Methods:** This study was conducted on 20 patients with non alcoholic fatty liver with normal liver enzymes (Group A), 20 patients with non-alcoholic steato-hepatitis with elevated liver enzymes (Group B) and 15 healthy persons (control group). These patients were subjected to full history taking, complete clinical examination, CBC, Liver function tests, FBS, Lipid profile, HCV Ab, HBS Ag, abdominal ultrasound, measurement of serum iron markers and serum hepcidin level. **Results:** There was a significant statistical difference between three groups regarding Iron and Hepcidin. **Conclusion:** serum hepcidin may be a good predictor and a non-invasive marker for diagnosis of NAFLD.

[Tarek Elmahdy Korah, Mohamed Hamdy Badr, Eman Abd Elfatah Badr, Mohamed Saad Hashim and Mai Kamal Abd-El Mageed Bass**. Assessment of serum iron markers and Hepcidin in patients with non-alcoholic fatty liver diseases.** *Stem Cell* 2017;8(3):51-61]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 8. doi:10.7537/marsscj080317.08.

Key words: non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Lipid profile, iron markers, hepcidin.

1. Introduction:

Non alcoholic fatty liver disease (NAFLD) refers to a spectrum of hepatic disorders, ranging from simple or bland fatty liver (NAFL, non-alcoholic fatty liver) in which no inflammatory changes are seen except for macrovesicular or microvesicular steatosis to non-alcoholic steato-hepatitis (NASH), which is characterized by an inflammatory reaction with hepatocytic injury, such as balloonic degeneration and necroapoptosis with or without fibrosis [(1)].

Hepcidin, is a hormone which is exclusively synthesized in the liver, is low-molecular weight 25 amino acid (cysteine-rich) hepatic peptide that plays an important role in iron metabolism [(2)].

Dietary iron enters intestinal cells via specific transports. The iron is used by the cell (incorporated into enzymes), stored as ferritin (excreted in the feces when the intestinal cells sloughs) or is transferred to the plasma [(3)].

Production of hepcidin is regulated by iron and additionally by the erythropoietic requirement for iron [(4)].

The overall findings from that hepcidin deficiency is associated with iron overload and that the over expression of hepcidin is associated with a severe and often fatal iron-deficiency, is consistent with the hypothesis that hepcidin is a negative regulator of body iron [(2)].

This role has been confirmed in a number of studies which focused mainly on hepcidin acting as a systemic iron-regulatory hormone also in inhibiting intestinal absorption, macrophage release and in the placental passage of iron [(5)].

The role of hepcidin in non-alcoholic liver disease and its utility as a biomarker for non-alcoholic steatohepatitis (NASH) or non-alcoholic fatty liver disease (NAFLD) histological severity has generated much interest due to lack of any established biomarker. Since NASH is associated with both oxidative stress and proinflammatory cytokines; there has been a great interest to explore the biomarker potential of hepcidin as non-invasive marker for the presence of NASH [(6)].

Aim of the work:

The aim of this study was to evaluate serum hepcidin and iron markers levels as biomarkers for inflammatory activity in patients with NAFLD.

2. Patients and Methods:

This study was carried out at the Internal Medicine and Medical Biochemistry Departments, Faculty of Medicine, Menoufia University. It involved fifty five (55) individuals; Patients were selected from outpatient clinic and inpatients of Internal Medicine Department, Menoufia University Hospital in the period from October 2016 to January 2017. The selected subjects gave consent for participation in the study before they were exposed to examination and investigations and the study was approved by the ethics committee of Menoufia University hospital. The studied subjects included 3 groups:

- Group A: (20) Patients have non alcoholic fatty liver (NAFL) with normal liver enzymes.
- Group B: (20) Patients have non-alcoholic steato-hepatitis (NASH) with elevated liver enzymes.
- Group (control) 15 subjects served as healthy control.

All patients and controls were subjected to thorough history taking, complete clinical examination, laboratory investigations including (CBC, Liver function tests, FBS, Lipid profile, HCV Ab, HBV Ag.), imagining study as abdominal and pelvic ultra sound and measurement of serum iron markers and serum hepcidin level by ELISA method.

Blood sampling:

8 milliliters (ml) of blood samples were taken from each subject after overnight fasting and divided into: three portion, one for complete blood count (CBC) tubes, one for PT and INR in citrated tube, while the other portion was put in a plain tube, left to clot for 30 minutes at room temperature, then subjected to centrifugation for 10 minutes at 4000 rotations per minute (RPM) and the serum obtained was put in aliquots, stored at -80◦C until the time of assay of ALT, AST, FBS, Lipid profile, hepatitis markers, serum iron markers and serum hepcidin.

Assay methods

Complete blood picture was measured with Pentra-80 automated blood counter (ABX– France – Rue du Caducee- Paris Euromedecine-BP-7290.34184 Montpellier-Cedex 4.)

Liver function tests, FBS and lipid profile were analyzed on auto-analyzer (SYNCHRON CX5) from Beckman (Beckman, instrument Inc., Scientific Instrument Division, Fullerton, CA92634 - 3100).

Iron was measure by quantitative colorimetric method. Serum transferrin, ferritin and hepcidin were determined using commercial ELISA kits (Immunodiagnostic Systems Limited, Bolden, UK) and EIAab® Human Hepcidin prohormone ELISA kit, China respectively.

Results were collected, tabulated and analyzed by SPSS (statistical package for social science) version 17.0 on IBM compatible computer (SPSS Inc., Chicago, IL, USA) [(7)].

3. Results:

There was a significant statistical difference between three groups regarding BMI, DM and hypertension. While a non significant statistical difference regarding other parameters **(table 1).**

There was a significant statistical difference between control group & group B (NASH) and group A (NAFL) & group B (NASH) regarding WBC count, ALT and AST **(table 2)**.

There was a significant statistical difference between three groups regarding Cholestrol, TG, LDL, HDL and FBS **(table 3)**.

Table (1): Demographic characteristics of the studies groups of patients and control

Sociodemographic characteristics	Studied groups						Test of sig.	P. value
	Control (n=15)		Group A (n=20) (NAFL)		Group B (n=20) (NASH)			
	No	%	No	%	No	%		
Age (year) mean±SD Range	35.73±5.12 20-37		32.60±14.73 20-45		38.00±10.89 30-45		F.test 11.04	0.062(NS) P1=0.08 P2=0.092 P3=0.061
BMI mean±SD Range	21.50±1.18 20-24		30.90±2.79 27-36		34.75±2.65 30-40		133.6	**0.00**(S) P1=**0.00** P2=**0.00** P3=**0.00**
Gender Male Female	8 7	53.3 46.7	13 7	65 35	11 9	55 45	X^2 1.9	0.358(NS)
Diabetes mellitus	0	0	3	15	12	60	17.94	**0.000(S)**
Hypertension	0	0	1	5	8	40	12.98	**0.002(S)**

P1: Control group & Group A.; P2: Control group & Group B. P3: Group A & Group B.; BMI: Body Mass Index.; No: Number.; SD: Standard deviation.; F test: Anova test.; X^2: Chi- squared test.; NS: non significant (P-value>0.05).; S: significant. (P-value ≤0.05).; **%**: percentage.

Table (2): Statistical comparison between the studied groups of patients and controls as regards complete blood picture and liver function.

	Studied groups			F.test	P.value
	Control (n=15)	**Group A (n=20) (NAFL)**	**Group B (n=20) (NASH)**		
Hb% (gm/dl) mean±SD Range	13.96±0.80 13-15	13.29±0.88 12-14	13.43±0.96 12-14	4.35	0.058(NS) P1= 0.51 P2= 0.37 P3= 0.56
Platelets count ($\times10^3$) mean±SD Range	251.93±57.16 170-350	248.65±65.66 152-363	261.70±43.97 190-320	0.346	0.71(NS) P1= 0.864 P2= 0.575 P3=0.428
WBC count ($\times10^3$) mean±SD Range	6.99±1.45 4.9-10	7.75±1.74 5.3-11	9.24±2.24 5.5-14.5	6.9	**0.002**(S) P1= 0.238 P2= **0.001** P3= **0.014**
INR mean±SD Range	1.10±0.00 1.1-1.1	1.095±0.06 1-1.2	1.10±0.00 1.1-1.1	18.04	0.08(NS) P1= 0.52 P2= 1 P3=0.52
ALT (IU/L) mean±SD Range	22.53±5.93 16-36	23.75±8.63 11-40	119.80±45.41 79-239	74.2	**0.00**(S) P1= 0.9 P2= **0.00** P3=**0.00**
AST (IU/L) mean±SD Range	24.93±6.54 17-39	25.65±8.11 15-40	139.05±83.78 82-377	30.98	**0.00(S)** P1= 0.967 P2= **0.000** P3=**0.000**
Total bilirubin (gm/dl) mean±SD Range	0.65±0.32 0.2-1	0.69±0.28 0.2-1	0.65±0.29 0.2-1	0.093	0.911(NS) P1= 0.671 P2= 0.843 P3= 0.802
Direct bilirubin (gm/dl) mean±SD Range	0.11±0.07 0-0.2	0.14±0.07 0-0.2	0.13±0.07 0-0.2	0.383	0.684(NS) P1= 0.391 P2= 0.542 P3= 0.78
Serum albumin (gm/dl) mean±SD Range	4.71±0.76 3.5-5.5	4.60±0.73 3.5-5.5	4.4±0.48 3.5-5.0	4.8	0.52(NS) P1= 0.621 P2= 0.37 P3=0.47

Hb: Hemoglobin; WBC: White blood cell; INR: International Normalized Ratio; ALT: Alanine Aminotransferese; AST: Aspartate Aminotransferase. P1: Control group & Group A.; P2: Control group & Group B.; P3: Group A & Group B.; No: Number.; SD: Standard deviation. F test: Anova test. NS: non significant (P-value>0.05). S: significant. (P-value≤0.05).

There was a significant statistical difference between three groups regarding Iron and Hepcidin. and there is a significant statistical difference between control group & group B (NASH) and group A (NAFL) & group B (NASH) regarding Ferritin and Transferrin. While there is a non significant statistical difference between Control group & Group A regarding Ferritin and Transferrin **(table 4)**.

There was a significant statistical difference between three groups regarding Liver span **(table 5)**.

There was a postive significant statistical correlation between level of serum hepcidin with lipid profile (Cholestrol , TG and LDL) in group A. and also postive significant statistical correlation between level of iron markers and hepcidin with lipid profile (Cholestrol ,LDL and TG) and liver enzymes (ALT and AST) in group B **(table 8 & 9)**.

There was highly significant positive correlation between serum level of iron, ferritin, transferrin and hepcidin with each others among the patients group A & B **(table 10 & 11)**.

Table (3): Statistical comparison between the studied groups of patients and controls as regards lipid profile and fasting blood sugar.

	Studied groups			F.test	p.value
	Control (n=15)	Group A (n=20) (NAFL)	Group B (n=20) (NASH)		
Cholestrol (gm/dl) mean±SD Range	142.87±19.92 120-178	208.30±31.26 150-270	357.45±44.77 290-400	190.1	**0.00**(S) P1= **0.00** P2= **0.00** P3= **0.023**
TG (mg/dl) mean±SD Range	103.47±12.33 80-130	137.95±29.40 80-180	177.00±22.68 140-215	45.3	**0.00**(S) P1= **0.00** P2= **0.00** P3= **0.00**
LDL (mg/dl) mean±SD Range	76.00±12.39 55-93	105.80±12.79 83-125	123.10±14.62 100-150	55.86	**0.00**(S) P1= **0.00** P2= **0.00** P3= **0.00**
HDL (mg/dl) mean±SD Range	50.73±5.66 42-59	42.15±11.75 25-60	29.15±5.12 22-38	31.07	**0.00**(S) P1= **0.004** P2=**0.00** P3=**0.00**
FBS (mg/dl) mean±SD Range	78.87±10.74 70-105	100.70±28.48 75-180	115.95±20.55 80-146	7.27	**0.002**(S) P1= **0.018** P2= **0.00** P3=**0.024**

TG: Triglyceride.; LDL: Low denisty lipoprotein.; HDL: High denisty lipoprotien.; FBS: Fasting blood sugar.; P1: Control group & Group A.; P2: Control group & Group B.; P3: Group A & Group B.; No: Number.; SD: Standard deviation.; F test: Anova test. S: significant. (P-value≤0.05).

Table (4): Statistical comparison between the studied groups of patients and controls as regards serum iron, ferritin, transferin and hepcidin.

	Studied groups			F.test	p.value
	Control (n=15)	Group A (n=20) (NAFL)	Group B (n=20) (NASH)		
Iron mean±SD Range	44.98±9.56 26.48-61.49	57.21±6.77 45-94-69.79	91.40±22.77 69.35-153.5	48.17	**0.00**(S) P1= **0.021** P2= **0.00** P3= **0.00**
Ferritin mean±SD Range	24.33±5.59 17-35	30.90±8.89 17-48	72.65±17.78 49-125	88.55	**0.00**(S) P1= 0.122 P2= **0.00** P3=**0.00**
Transferrin mean±SD Range	1.45±0.29 1-2	1.64±0.25 1.2-2	2.65±0.34 2-3.2	93.21	**0.00**(S) P1=0.067 P2= **0.00** P3=**0.00**
Hepcidin mean±SD Range	79.53±14.39 50-95	132.75±23.20 95-170	195.00±16.70 170-225	171.5	**0.00**(S) P1= **0.00** P2= **0.00** P3=**0.00**

P1: Control group & Group A.; P2: Control group & Group B.; P3: Group A & Group B.; No: Number.; SD: Standard deviation.; F test: Anova test. S: significant. (P-value≤0.05).

Table (5): Statistical comparison between the studied groups of patients and controls as regards US data of portal vein diameter, liver and spleen span.

	Studied groups			F.test	p.value
	Control (n=15)	Group A (n=20) (NAFL)	Group B (n=20) (NASH)		
P.V diameter mean±SD Range	11.41±1.02 10.5-13	10.95±0.70 10-12	11.05±0.63 10-11.9	10.24	0.08(NS) P1= 0.2 P2= 0.12 P3= 0.13
Liver span mean±SD Range	14.41±0.32 14-14.9	18.20±0.79 17-19.5	19.19±0.85 18-21	209.7	**0.00**(S) P1= **0.00** P2= **0.00** P3= **0.00**
Spleen span mean±SD Range	10.89±0.97 9-12.4	10.44±0.87 9-12	10.28±0.63 9.5-11.1	2.56	0.087(NS) P1= 0.106 P2= **0.031** P3=0.548

P.V: Portal vein.; P1: Control group & Group A.; P2: Control group & Group B.; P3: Group A & Group B.; No: Number.; SD: Standard deviation.; F test: Anova test. NS: non significant (P-value>0.05). S: significant. (P-value≤0.05).

Table (6): Correlation between serum level of iron, ferritin, transferrin and hepcidin and demographic, US data and CBC among the patients Group A (n=20)

	Serum Iron		Serum Ferritin		Serum Transferrin		Serum hepcidin	
	r	p.value	R	p.value	r	p.value	R	p.value
Age (years)	0.324	0.164	0.213	0.368	0.159	0.502	-0.033	0.89
BMI	0.214	0.364	0.155	0.514	0.165	0.487	0.075	0.753
P.V diameter	0.088	0.711	0.07	0.77	0.222	0.348	0.249	0.29
Liver span	0.071	0.765	0.057	0.812	-0.096	0.687	-0.145	0.541
Spleen span	-0.099	0.679	-0.124	0.604	-0.121	0.612	0.03	0.9
Hb% (mg/dl)	-0.358	0.121	0.164	0.491	0.023	0.924	0.12	0.615
Platelets count ($\times10^3$)	-0.310	0.184	0.143	0.548	0.167	0.481	0.286	0.222
WBC count ($\times10^3$)	-0.051	0.832	-.051	0.832	-0.126	0.597	-0.145	0.541

BMI: Body Mass Index.; P. V: Portal vein. Hb: Hemoglobin; WBC: White blood cell; r: Spearman correlation coefficient

Table (7): Correlation between serum level of Iron, Ferritin, Transferrin and Hepcidin and demographic, US date and CBC among the patients Group B (n=20)

	Serum Iron		Serum Ferritin		Serum Transferrin		Serum hepcidin	
	r	p.value	R	p.value	r	p.value	R	p.value
Age (years)	-0.285	0.21	-0.210	0.361	-0.188	0.415	-0.132	0.57
BMI	-0.088	0.703	-0.024	0.917	0.034	0.883	-0.034	0.882
P.V diameter	-0.156	0.5	-0.111	0.631	-0.258	0.258	-0.221	0.336
Liver span	-0.097	0.675	-0.049	0.833	-0.029	0.9	-0.002	0.994
Spleen span	-0.006	0.979	0.204	0.376	0.234	0.307	0.038	0.872
Hb% (mg/dl)	-0.083	0.72	-0.167	0.47	-0.254	0.267	-0.153	0.508
Platelets count ($\times10^3$)	0.114	0.621	0.068	0.768	-0.024	0.919	0.025	0.914
WBC count ($\times10^3$)	0.232	0.311	0.282	0.215	0.34	0.132	0.422	0.057

BMI: Body Mass Index.; P. V: Portal vein. Hb: Hemoglobin; WBC: White blood cell; r: Spearman correlation coefficient

Table (8): Correlation between serum level of iron, ferritin, transferrin and hepcidin and liver function, lipid profile and fasting blood sugar among the patients group A (n=20).

	Serum Iron		Serum Ferritin		Serum Transferrin		Serum hepcidin	
	R	p.value	R	p.value	R	p.value	R	p.value
ALT (IU/L)	0.194	0.413	0.473	0.051	0.413	0.071	0.365	0.114
AST (IU/L)	0.111	0.642	0.507	0.052	0.436	0.054	0.492	0.08
Total bilirubin (mg/dl)	-0.069	0.771	0.066	0.783	0.144	0.545	0.129	0.588
Direct bilirubin (mg/dl)	0.26	0.269	0.225	0.34	0.339	0.143	0.322	0.166
Serum albumin (mg/dl)	0.036	0.88	0.084	0.723	0.186	0.433	0.225	0.34
Cholestrol (gm/dl)	0.249	0.289	0.116	0.627	0.154	0.518	0.095	**0.019***
TG (mg/dl)	0.94	0.694	0.124	0.603	0.093	0.695	0.142	**0.04***
LDL (mg/dl)	0.073	0.76	0.025	0.918	0.041	0.864	0.003	**0.01***
HDL (mg/dl)	-0.129	0.588	-0.219	0.354	-0.249	0.289	-0.248	0.291
FBS (mg/dl)	0.319	0.17	0.376	0.102	0.271	0.247	0.179	0.45

INR: International Normalized Ratio; ALT: Alanine Aminotransferese; AST: Aspartate Aminotransferase.; TG: Triglyceride.; LDL: Low denisty lipoprotein.; HDL: High denisty lipoprotien.; FBS: Fasting blood sugar.; r: Spearman correlation coefficient. ; * significant correlation.

Table (9):Correlation between serum level of iron, ferritin, transferrin and hepcidin and liver function, lipid profile and fasting blood sugar among the patients group B (n=20).

	Serum Iron		Serum Ferritin		Serum Transferrin		Serum hepcidin	
	r	p.value	R	p.value	R	p.value	R	p.value
ALT (IU/L)	0.047	**0.041***	0.095	**0.036***	0.025	**0.043***	0.118	**0.011***
AST (IU/L)	0.170	**0.024***	0.168	**0.046***	0.138	**0.049***	0.009	**0.001***
Total bilirubin (mg/dl)	0.025	0.915	0.124	0.592	0.039	0.865	-0.084	0.719
Direct bilirubin (mg/dl)	-0.140	0.545	-0.095	0.683	-0.235	0.306	-0.214	0.351
Serum albumin (mg/dl)	0.288	0.205	0.348	0.122	0.460	0.036	0.443	0.045
Cholestrol (gm/dl)	0.244	**0.028***	0.363	**0.015***	0.147	0.524	0.170	**0.043***
TG (mg/dl)	0.263	**0.024***	0.237	**0.03***	0.354	**0.015***	0.274	**0.022***
LDL (mg/dl)	0.052	**0.023***	0.013	**0.046***	0.068	0.77	0.039	**0.017***
HDL (mg/dl)	0.520-	0.06	0.592-	0.08	0.574-	0.07	-0.522	0.07
FBS (mg/dl)	0.189	0.412	0.086	0.709	0.149	0.519	0.286	0.208

INR: International Normalized Ratio; ALT: Alanine Aminotransferese; AST: Aspartate Aminotransferase. TG: Triglyceride.; LDL: Low denisty lipoprotein.; HDL: High denisty lipoprotien.; FBS: Fasting blood sugar. r: Spearman correlation coefficient. ;* significant correlation.

Table (10): Correlation between serum level of iron, ferritin, transferrin and hepcidin with each others among the patients group A (n=20)

	Serum Iron		Serum Ferritin		Serum Transferrin		Serum hepcidin	
	r	p.value	R	p.value	R	p.value	R	p.value
Iron	---	---	0.907	**0.000****	0.874	**0.000****	0.854	**0.000****
Ferritin	0.907	**0.000****	---	---	0.900	**0.000****	0.938	**0.000****
Transferrin	0.874	**0.000****	0.900	**0.000****	---	---	0.907	**0.000****
Hepcidin	0.854	**0.000****	0.938	**0.000****	0.907	**0.000****	---	---

** Highly significant correlation

Table (11): Correlation between serum level of iron, ferritin, transferrin and hepcidin with each others among the patients group B (n=20)

	Serum Iron		Serum Ferritin		Serum Transferrin		Serum hepcidin	
	r	p.value	R	p.value	R	p.value	R	p.value
Iron	---	---	0.934	**0.001****	0.811	0.001**	0.918	**0.001****
Ferritin	0.934	**0.001****	---	---	0.885	0.001**	0.887	**0.001****
Transferrin	0.811	**0.001****	0.885	**0.001****	---	---	0.886	**0.001****
Hepeidin	0.918	**0.001****	0.887	**0.001****	0.886	0.001**	---	---

** Highly significant correlation

4. Discussion:

In the present study, statistical analysis revealed no significant difference between the three studied groups regarding gender as they were sex matched but NAFLD was more common in men (65%) in group NAFL and (55%) in group NASH in agreement with **Ruhl et al** [8]. who reported that NAFLD was more prevalent in men than in women finding essentially explained by the higher waist – to – hip circumference (WHR) ratio in men. WHR is correlated with visceral adipose tissue (VAT) and visceral adiposity is associated with both peripheral and hepatic IR.

Also in **Amor et al.** [9]. NAFLD is more common in male (56%) in agreement with our study. In another study using cohort size, **Clark et al.** [10]. also reported that men have higher prevalence of NAFLD than women in agreement with our result.

Wang et al. [11]. also reported that NAFLD is more common in males than females. We also found that there was non statistical significant difference between the three studied groups according to age as they were age matched from the start of this study with range from 20-45 in the two patients groups. Also, **Carulli et al.** [12] Stated that NAFLD tends to increase from younger to middle-aged groups of individuals and the prevalence of disease begins to decline at the age of 50 or 60.

This study also found that there was statistical significant difference between the three studied groups according to BMI, DM and hypertension also, **Rocha et al. (2005)** [13]. stated that NAFLD is strongly associated with high BMI due to presence of at least one element of metabolic syndrome. Also **Janssen et al.** [14]. stated that NAFLD showed strong association with increase BMI (obesity) as the presence of intra-abdominal fat has been proposed as the major determinant of insulin resistance which is the key mechanism in the pathogenesis of NASH/NAFLD. Also **Amor et al.** [9]. Stated that there was statistical significant difference with obesity, D.M, hypertension and patients with dyslipedimia with control group especially in NASH group and this study agree with us. Also **Wang et al.** [11] stated that the prevalence of males, hypertension, hyperglycaemia, smoking and regular exercise were significantly different between the incident NAFLD and non-NAFLD groups.

Additionally, and more importantly, a relationship between NAFLD and metabolic syndrome has been proposed in many studies which revealed that components of metabolic syndrome such as obesity, hypertension, dyslipidaemia and hyperglycaemia were independently associated with NAFLD. **(Fan and Farrell)** [15]. **(Hu et al.,)** [16]. **(Angulo)** [17]**.**

The present study also revealed mild or moderate elevation of serum levels of asparatate aminotransferase (AST), alanine aminotransferase (ALT) or both in group B which are the most common laboratory abnormality found in patients with NASH in agreement with **Agarwal et al.** [18]. who stated that there was a statistical significant difference in AST, ALT values is a reasonable observation considering the fact that elevation of enzymes as well as sonographic qualities of the liver was the most important considerations.

Our study revealed mild elevation of white blood cells in NASH group result in a significant statistical difference between control group and NASH also between group A (NAFL) and group B (NASH) in agreement with **Wang et al.** [11]. who proved that an elevated WBC level is related to NAFLD incidence, a finding which provides novel and powerful evidence for a significant relationship between WBC level and NAFLD and WBC in subjects with only NAFLD are different from that in those with NAFLD, cirrhosis and non-alcoholic steatohepatitis (NASH). This study suggests that inflammation plays an important role in the occurrence of NAFLD, especially, hepatic steatosis as a result of systemic inflammation.

Also, NAFLD may be the liver manifestation of MS, as a relationship between WBC count and MS components has been documented in previous studies **Chao et al.,** [19]. **and Gharipour et al.,** [20].. which lead relationship between WBC count and NAFLD. We also found that there was no statistically significant difference between the three studies groups regarding albumin level as the cases have normal liver function and not cirrhotic.

The study by **Nakahara et al.** [21]. reported that hyperlipidemia is associated with NAFLD and hyper-LDL. Cholesterolemia was present in 37.5% of patients with NAFLD in whom liver biopsy was performed and hypertriglyceridemia is most prevalent among patients with NAFLD which goes with our study by showing that there were statistically significant difference the three studies groups regarding triglyceride, LDL, HDL and cholesterol levels. So it seems that inappropriate food hapits and physical inactivity are the reasons for this dyslipidemia, which could be improved or treated by changing lifestyle and diet among high risk people, especially in early stages.

Also, **Song et al.,** [22]. **and Wang et al.,** [11]. stated that NAFLD is associated with dyslipidemia and there was statistically significant difference between NAFLD group and non NAFLD group regarding to TG, LDL HDL and cholesterol this agree with our study.

Also, **Amor et al.** [9]. agree with our study as indicate that there was statistically significant difference between NAFLD group and non NAFLD group regarding to TG, LDL HDL and cholesterol and increase TG, LDL and cholesterol and decrease HDL especially in NASH. In our study, there was a statistical significant difference between the three studied groups regardind to liver span and hepatic echogenisty so in NASH is large and more echogenic than NAFL and control group in agreement with **Kirovski et al** [23]**.**

Current study showed that transferrin was higher in NASH group than NAFL and control groups and also ferrtin may be related to (dysmetabolic iorn overload syndrome, DIOS) in agreement with **Turlin et al.** [24]. who noticed that iron deficiency anaemia and an excessive accumulation of iorn (DIOS) may develop in patients with NAFLD, DIOS is a term defining the typical stiution in patients who have mild to moderate iron overload and increased serum ferritin levels, transferrin saturation is also seen at the upper limit of the normal range as in NAFLD.

Kowdley et al,. [25]. demonstrated in the large NASH Clinical Research Network (CRN) cohort of 628 patients that a serum ferritin concentration greater than 1.5 times the upper limit of normal was independently associated with advanced fibrosis and increased NAFLD activity score. **Sumida et al,.** [26]., have demonstrated the utility of incorporating serum ferritin into a clinical scoring system to predict steatohepatitis in Japanese patients with NAFLD. these studies agree with the present study.

However, other studies have not found such a clear association **Chandok et al.** [27]. and **Valenti et al.** [28].

Current study showed that a statistical significant difference between the three studied groups according to iron in agreement with **Zhang and Rovin.** [29]. who reported that the increase in ferritin levels in NAFLD with metabolic syndrome and obesity as they associated with chronic inflammation due to increased adipokine levels that increased inflammation could induce the release of hepcidin.

Bugianesi et al. [30]. **and Moon et al.** [31] declared few contradictory reports on the role of the iron burden in patients with NAFLD as it is not clear whether the serum ferritin is a consequence of systemic inflammation or a marker of iron overload in patients with NAFLD so further studies to confirm the role iron in NAFLD are needed.

Studies by **Fargion et al.** [32]. and **Manousou et al.** [33]. found that there is a correlation between hepatic iron overload with chronic liver disease with accumulation evidence suggests a link between altered iron metabolism and NAFLD and its progression to NASH as even mild iron overload might aggravate insulin resistance, atherosclerosis, colonic neoplasia, and NAFLD, moreover, iron depletion therapy, such as with a phlebotomy, improves the metabolic complications and elevated liver enzymes in patients with NAFLD.

We detected an increase in hepcidin level in patients with NAFLD and NASH groups in comparison with the control group with a statistical significant difference between them. In agreement with **Senantes et al.** [6] on patients with biopsy-proven NAFLD which demonstrated increased hepcidin levels compared to healthy subjects and hepcidin is thought to play an important role in iron bioavailability in NAFLD patients.

Aigner et al [34] indicated increase hepcidin formation in iron-overloaded NAFLD patients. indicate that the cause in increase hepicidin due to iron overload in agreement with our study. This study was done on three groups iron overloaded NAFLD, NAFLD patients without iron overloaded and healthy control group. Also with agreement with **Demircioglu et al.** [35]. who reported that hepcidin values of NAFLD patients were significantly higher than healthy subjects but **Penkova et al.** [36] showed a decrease in serum hepcidin levels in patients with NAFLD and in chronic liver disease with increase iorn markers. This can be explained as in the early phase of chronic liver disease, hepcidin may be prominently suppressed by NAFLD and chronic liver disease, but as iron accumulates the negative influence of these factors may be masked by the positive stimulation of iron.

Barisani et al. [37]. have previously shown that hepcidin mRNA expression in patients with dysmetabolic hepatic iron overload significantly

correlated with indices of lipid metabolism namely total cholesterol, LDL-C, and triglycerides. In addition, **Fernandez-Real et al.** [38]. have previously shown an association prohepcidin with LDL-C, and triglycerides levels thereby confirming the existence of subtle interactions between hepcidin production and abnormalities of lipid metabolism. This agrees with our study as there is positive correlation between hepcidin and lipid profile.

In this study, we observed a positive correlation between elevated iorn stores, measured by serum ferritin levels, iron serum and the prevalence of the metabolic syndrome positively correlated with serum triglycerides, an individual component of the metabolic syndrome, as well as marker of insulin resistance which goes with **Piperno et al.** [39] and **Williams et al.** [40]. as they had previously examined the association between iron stores and individual cardiovascular risk factors, including hypertension, dyslipidemia, elevated fasting blood glucose and insulin, and central adiposity. This agrees with our study.

In our study, there was a highly significant positive correlation between serum levels of iron, ferritin, transferrin and hepcidin indicate that hepicidin is a hormone regulator of iron. Hepcidin production is increased by plasma and liver iron as a feedback mechanism to maintain stable body iron levels this agree. **Elgari et al** [41]. also stated a highly significant positive correlation between serum level of Iron, ferritin, transferrin and hepcidin in a study on iron deficiency anemia patients. Also **Cherian et al.** [42], **Sanad M and Gharib AF.** [43]. and **Müller et al.** [44] agree with our study. Also **Osman et al.** [45] stated that a significant positive correlation between serum level of iron and serum hepicidin.

In our study, there was a highly significant positive correlation between Hepcidin and iron markers with liver enzymes: Alanine Aminotransferese (ALT) and Aspartat Aminotransferase(AST) . In agreement with **An et al**[46]. who stated that alanine transaminase (ALT) was found to be significantly associated with serum hepcidin also increased serum transaminase levels were also associated with elevated serum ferritin indcating that When hepatocytes are damaged, liver enzymes leak into the circulation and can be detected in the serum. both ALT and AST are sensitive markers for detecting liver injury. and associated with elevated serum hepcidin and ferritin. These result from liver injury.

Conclusion:

From the previously mentioned results we can conclude that there was increase of serum hepcidin levels in patients with nonalcoholic fatty liver disease especially with staetohepatitis patients with prompt increase of the levels of iron and lipid profile in comparison with control group, so serum hepcidin may be a good predictor and a non-invasive marker for diagnosis of NAFLD.

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5/30/2017