# AIM OF THE WORK

The aim of this study is to evaluate the influence of continuous ampulatory peritoneal dialysis and on line -hemodiafiltration on anemia and serum hepcidin levels in chronic kidney disease patients

# SUBJECTS

Informed consents were taken from all subjects participated in the study. After the approval of the Ethical Committee of the Medical Research Institute, ten patients with end stage renal disease under maintenance online-HDF (for more than 6 months) and another ten patients under maintenance CAPD (for more than 6 months) and another ten patient as a control were included in this study.

All patients were of matched age and socioeconomic status and were hepatitis C (HCV) &B (HBV) negative. The patients were divided into three groups as follows:

- **Group I (control group):**

  It consisted of ten patients who are completely normal.

- **Group II:**

  It consisted of tern patients, who are under maintenance CAPD.

- **Group III:**

  It consisted of ten patients, who are under maintenance OL-HDF.

## Inclusion criteria:

1. Patients who received recombinant erythropoietin and iron supplements are enrolled provided that their doses had been stable for at least four weeks.
2. Parenteral iron supplements will be stopped for one week before measurement of Hepcidin.

## Exclusion criteria:

1. Patients with previously diagnosed non renal causes of anaemia.
2. Patients with chronic infection or inflammation.
3. Patients with evidence of active or occult bleeding.
4. Patients with history of blood transfusion in the past four months.
5. Patients with history of malignancy and end stage liver disease or chronic hypoxia.
6. Patients with recent hospitalization or infection that required antibiotics within the past four weeks.

# METHODS

To all subjects the following parameters were carried out:

1. Full clinical examination including detailed history taking with emphasis on cause and duration of dialysis and complete physical examination.

2. Laboratory Investigation:

## Sampling:

- Following an overnight fasting period and immediately before dialysis session, ten millimeters whole venous blood were withdrawn from each subject before heparinization of the line and before the patient being connected to the dialysis machine.
- 1ml of whole blood was mixed with 50 µl of 3.8% ethylene dinamine tetraacetic acid (EDTA) for determination of complete blood count (CBC).
- The rest of the blood was left to clot. After coagulation, samples were centrifuged, and serum was harvested and aliquoted.
- Serum iron, total iron binding capacity (TIBC), and ferritin were immediately determined.
- Transferrin saturation (TSAT) was calculated
- Two tubes were frozen at -20˚C for estimation of serum hepcidin and hs-CRP.

## Methods:

- **Complete Blood Count (CBC):** [180]

It was performed on mindray cell counter. The hemoglobin concentration, hematocrit, red and white cell counts as well as platelets were measured, along with automatic calculation of hematological indices. A blood smear was spread on glass slide, left to dry and stained with leishmann stain for determination of differential white cell count.

- **Evaluation of plasma Creatinine:** [181]

Creatinine was determined without deprotinization using jaffe reaction in a kinetic manner. After 20 seconds delay, the rate of increased absorbance (ΔA/min) due to complex formation between creatinine in the sample ($\Delta A_T$) and alkaline picrate reagent was monitored kinetically over a period of 1 minute at λ 500 nm and compared to a standard creatinine solution ($\Delta A_S$) OF Known concentration ($C_S$) similarly treated. Creatinine concentration ($C_T$) was determined as follows:

Mg/ dl creatinine ($C_T$) = $\Delta A_T / \Delta A_S$ XCs (mg/dl)

µmol/L=mg/dl X88.4

1. **Estimation of serum High sensitive C-reactive protein (hsCRP):** [182]

- **Principle of the Method**

The hsCRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the on the CRP molecule

This mouse monoclonal anti-CRP antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl changing the color to yellow. The concentration of CRP is directly proportional the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

**Reagents**

1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with mouse monoclonal anti-CRP.
2. *Reference Standard Set (1.0 ml/vial)*
   Contains 0, 0.005, 0.010, 0.025, 0.050 and 0.100 mg/l CRP in serum based buffer-BSA solution with preservatives.
3. *hsCRP Sample Diluent (50 ml/vial)*
   Contains phosphate buffer-BSA solution with preservatives.
4. *CRP Enzyme Conjugate Reagent (12 ml/vial)*
   Contains goat anti-CRP conjugated to horseradish peroxidase with preservatives.
5. *TMB Reagent (11 ml/bottle)*
   Contains one-step TMB solution.
6. *Stop Solution (1 bottle, 11 ml/bottle)*
   Contains diluted hydrochloric acid (1N HCl).

**Reagent Preparation**

1. All reagents were allowed to reach room temperature (18-25°C) before use.

***Patient serum were diluted 100 fold prior to use. Prepare a series of small tubes (i.e., 1.5 ml microcentrifuge tubes) and mix 5 µl of serum with 495 µl (0.495 ml) Sample Diluent.***

3. Samples with expected CRP concentrations over 10 mg/l were quantitated by further dilution (10 fold) of the 100-fold diluted solution with sample diluent (i.e., 10 µl of the 100-fold diluted sample to 90 µl sample diluent).

**Assay Procedure**

1. Patient serum and control serum were diluted 100 fold prior to use.
2. The desired number of coated wells were secured in the holder.
3. 10 µl of CRP standards, DILUTED specimens, and DILUTED controls were Dispensed into appropriate wells.
4. 100 µl of CRP Enzyme Conjugate Reagent were dispense into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at room temperature (18-25 °C) for 45 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with deionized or distilled water. DO NOT USE TAP WATER.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
13. ***Read absorbance at 450 nm with a microtiter well reader*** within 15 minutes.

**Calculation of Results**

1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in mg/l on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CRP (mg/l) from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The obtained values of the patient samples and control sera should be multiplied by the dilution factor of 100 to obtain CRP results in mg/l.
5. Patient samples with CRP concentrations greater than 10 mg/l should be further diluted 10-fold after the initial 100-fold dilution (total dilution 1:1,000), and the final CRP values should be multiplied by 1,000 to obtain CRP results in mg/l.
6. NOTE: Patient samples with CRP concentrations less than 0.1 mg/l should reported as "<0.1 mg/l CRP".

## Estimation of serum iron: [183]

In an acidic medium, transferring bound iron dissociates into free ferric ($Fe^{2+}$). The ferrous ions then react with (2,4,6,Tri –(2- pyridyl) -5-triazine) TPTZ to form a blue coloured complex which was measured bichromatically at 600/800nm.

The increase in absorption is directly proportional to amount of iron present in the sample. Results are expressed in mg/dl and was calculated as follows:

$$C_T\,(mg/dl) = A_T / A_S \times C_S\,(mg/dl)$$

## Determination of total iron binding capacity (TIBC): [183]

The serum total iron binding capacity (TIBC) is determined by addition of sufficient fe3+ to saturate iron binding sites on transferring. The excess fe3+ is moved by adsorption with light magnesium hydroxide carbonate powder, and the iron in the supernatant is than spectrophoto metrically measured.

**TIBC** = Iron concentration in supernatant X 3 (dilution)

- **Calculation of transferrin saturation (TSAT):**

Transferring saturation is calculated as follows:

Transferring saturation (%) = 100 X serum iron /TIBC

## Estimation of serum ferritin: [184]

**Principle:**

Ferritin was assayed as a solid-phase, two-site chemiluminescent immunometric assay on IMMULITE 1000 (Siemens, United Kingdom). Monoclonal anti-ferritin antibody was used for solid phase immobilization and an enzyme-linked goat anti-ferritin antibody was in the antibody solution. The test sample was allowed to react simultaneously with tow antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies

**Reagents:**

**Ferritin test units:**

Each bar-coded test unit contained one bead coated with monoclonal murine antiferritin. The kit contained 100 test units bags were left to come to room temperature before opening.

**Ferritin reagent wedge:**

Reagent with barcode consisted of 7.5ml alkaline phospahtase conjugated to polyclonal goat antiferritin in buffer, with preservative.

**Ferritin adjustors:**

Two vials (low and high), 2.5 ml each, of ferritin in a human protein-based matrix with preservative. Each vial was reconstituted with 4.0ml distilled water. It was mixed by gentle swirling until the lyophilized naterials were fully dissolved.

**Sample:**

10ml serum were aspirated form sample cup with dead volume 100ml.

**Performance data:**

Calibration range: up to 1500ng/ml, samples higher than this range were diluted by saline as follows (1 part serum + 4 parts saline)

Analytical sensitivity: 1.5 ng/ml

**Normal expected values:**

Males: 30-300 ng/ml

Females: 10-200ng/ml

- **Estimation of serum Hepcidin: [185]**

**Principle:**

The kit assay Human hepcidin level in the sample, use purified Humen Hepcidin antibody to coat microtiter plate wells, make solid-phase antibody. Then add Hepcidin to wells, combined Hepcidin which with enzyme labeled, become antibody-antigen-enzyme-antibody complex, after washing complerely, add substrate, substrate becomes blue coor at HRP enzyme- catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at wavelength of 450nm. The concentration of Hepcidin in the samples is then setermind by comparing the O.D. of the samples to the standard curve.

**Peagents:**

48 microtiterwells coated with monoclonal anti-Hepcidin antibodies. Standard (standard 0-5), 6 vials, each contained 0.5ml lyophilized synthetic hepcidin with concentrations: 0.0-3.85-11.5-23-70-180.0 ug/l.

Controls (low and high), 2 vials contained lyophilized synthetic hepcidin each 0.5ml. the low control mean concentration was 19.8ng/ml and range 9.1- 27.3 ng/ml and the high control mean concentration was 19.8 ng/ml and the range 9.1-27.3ng/ml and the high control mean concentration was 44.5ng/ml and the range 22.2-66.6

**Reagent preparation:**

All reagents and strips were brought to room temperature prior use. Standards: the lyophilized contents of the standard vials reconstituted with 0.5ml distilled water.

Control: the lyophilized contents of control vials were reconstituted with 0.5ml distilled water and let stand for 10 minutes and mixed several times before use.

Wash solution: the 30ml concentrated wash solution (40X) was diluted with 1170ml deionized water to a final volume of 1200ml.

**Assay procedure:**

1- Dilute and add sample to standard: set 10 standard wells on the microtiter plate coated, add standard 100ul the first and the second well. Then, add standard dilution 50ul to the first and

second well, mix; take out 100ul form the first and second well then add it to the third and forth well separately.

Then, add standard dilution 50ul to the third forth well, mix; then take out 50ul form the third and forth well separately again and them to the fifth and sixth well separately, then add standard dilution 50ul to the fifth and six well, then add standard dilution 50ul form the fifth and sixth well and add to the seventh and eighth well, then add standard dilution 50ul to the seventh and tenth well, add standard dilution 50ul to the ninth and tenth well, mix, take out 50ul form the ninth and tenth well discard. Keep 50ul in each well after diluting, (density: 120ug/L, 80/L,20mg/L).

2- Add sample set blank wells separately (blank comparison wells don not add sample and enzyme conjugate, the other step are same). Add sample dilution 40ul to sample well, then add sample 10ul (sample final dilution is 5-fold). Do not touch the wall and bottom of the well during adding samples. Then mix gently.
3- Incubate: after closing plate with closure plate membrane, incubate for 30 minutes at 37.
4- Prepare solution: 30-fold wash solution diluted 30-fold with distilled water and reserve.
5- Manual washing: remove incubation mixture by aspirating contents of the plate into a sink of proper waste container.

Using a squirt bottle, fill each well completely with wash solution then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for more times for a total of FIVE washes. After final wash, invert plate, and plot dry by hitting plate onto absorbed paper or paper towels until on moisurere appear. Note: hold the sites of the plate frame firmly then washing the plate to assure to assure all strips remains securely in frame.

6- Add enzymes: add 50ul enzyme conjugate reagent to each well, except blank well.
7- Incubate: operation with 3.
8- Washing operation with 5.
9- Colure at 50ul substrate A and substrate B to each wall, cover and incubate for 15 minutes at 37 C.
10- Stop the reaction: add 50ul stop solution to each well. Mix well.
11- As say: determine the optical density of each well within 15 minutes by a microplate reader.

**Calculation of the result:**

Take the standard density as the horizontal, the OD value for the vertical, draw standard curve on graph paper, find out the corresponding density according to the sample OD value by the sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with standard density and the OD value, with the sample OD value in the equation, calculate the sample density, multiplied by the dilution factor, the result is the sample actual density.

**Assay range:**

7.5ug/L-150ug/L

## Statistical analysis of the data [186]

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. [187] Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described as range (minimum and maximum), mean, median, and standard deviation. The distributions of quantitative variables were tested for normality using kolmogorov-Smirnov test, Shapiro-Will test and D'Agstino test. In addition, histogram and QQ plot were used for vision test. If it revealed normal data distribution, paramedic tests were applied. Statistical differences between the groups were identified using one-way analysis of variance (ANOVA) followed by post test. Coefficient was used to analyses correlation between any two normally distributed variables (in case of not normally distributed data, we used logarithmic measure). Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at 5% level.

# RESULTS

The study was conducted on 30 subjects divided into 3 groups:

**Group I:** 10 normal individuals of average age and sex served as a control.

**Group II:** 10 patients with end stage renal disease on Continuous Ambulatory Peritoneal Dialysis (CAPD) for more than 6 months.

**Group III:** 10 patients with end stage renal disease on on/line hemodiafiltration for more than 6 months**.**

## 1-Demographic data:

## a-Age:

- In group I, the age ranged from 43 to 57 years with a mean of 51.1±4.75 years.
- In group II, the age ranged from 45 to 63 years with a mean of 52.73± 5.93 years.
- In group III, the age ranged from 40 to 65 years with a mean of 55± 8.27 years.

There was no statistically significant difference observed between the two patients groups and the control group as regard the age

**Table (2): Comparison between the different studied groups regarding age (years).**

| | **Group I** | **Group II** | **Group III** |
|---|---|---|---|
| **Age** | | | |
| Min | 43 | 45 | 40 |
| Max | 57 | 63 | 65 |
| Mean | 51.10 | 52.73 | 55.00 |
| S.D. | 4.75 | 5.93 | 8.27 |
| Sig.bet.grps | $P_1$=0.227, $P_2$=0.075, $P_3$=0,118 | | |

P1 comparison between control and CAPD.
P2 comparison between control and ON-LINE HDF
P3 comparison between CAPD and ON-LINE HDF

## b-Sex:

All patients included in this study were males.

## 2-Laboratory data:

### a- Renal function tests (RFTs):

### 1-Blood urea and serum creatinine (Table 2):

- In group I, urea ranged between 23 and 44 mg/dl with a mean of 35.30± 6.57 mg/dl, serum creatinine level ranged between 0.7 and 1.20 mg/dl with a mean of 1.02± 0.24 mg/dl.
- In group II, urea ranged between 102.70 and 131.20 mg/dl with a mean of 118.88± 8.28 mg/dl, serum creatinine level ranged between 9.50 and 11.7 mg/dl with a mean of 10.29± 0.77 mg/dl.
- In group III, urea ranged between 85 and 189 mg/dl with a mean of 140.80± 35.12 mg/dl, serum creatinine level ranged between 6.0 and 12.0 mg/dl with a mean of 8.53±.194 mg/dl.

There was statistically significant difference between group I and II as regard the mean urea level (P=0.001), There was also statistically significant difference between group I and III (P=0.001). There was no statistically significant difference between group II and III (P=0.87).

There was statistically significant difference between both group I and II as regard the mean creatinine (P=0.001), There was also statistically significant difference between group I and III (P=0.001). There was astatistically significant difference between both group II and III (P=0.012).

**Table (3): Comparison between the studied groups according to renal function**

| | Control (n = 10) | CAPD (n = 10) | HDF (n = 10) | F | p |
|---|---|---|---|---|---|
| **Urea** | | | | | |
| Min. – Max. | 23.0 – 44.0 | 102.70 – 131.20 | 85.0 – 189.0 | | |
| Mean ± SD. | 35.30 ± 6.57 | 118.88 ± 8.28 | 140.80 ± 35.12 | 69.142* | <0.001* |
| Median | 35.50 | 119.05 | 144.0 | | |
| **Sig. bet. grps** | $p_1<0.001^*$, $p_2<0.001^*$, $p_3=0.087$ | | | | |
| **Creatinine** | | | | | |
| Min. – Max. | 0.70 – 1.20 | 9.50 – 11.70 | 6.0 – 12.0 | | |
| Mean ± SD. | 0.97 ± 0.15 | 10.29 ± 0.77 | 8.53 ± 1.94 | 168.505* | <0.001* |
| Median | 0.95 | 10.01 | 7.95 | | |
| **Sig. bet. grps** | $p_1<0.001^*$, $p_2<0.001^*$, $p_3=0.011^*$ | | | | |

F: F test (ANOVA)
$p_1$: p value for Post Hoc test (Scheffe) for comparing between control and CAPD
$p_2$: p value for Post Hoc test (Scheffe) for comparing between control and HDF
$p_3$: p value for Post Hoc test (Scheffe) for comparing between CAPD and HDF
*: Statistically significant at $p \leq 0.05$

## b - Hb (table 3):

-In group I, Hb ranged between 12.07 and 14.20 mg/dl with a mean of 13.04 ± 0.67 mg/dl.

- In group II, Hb ranged between 10.44 and 12.84 mg/dl with a mean of 11.80 ± 0.81 mg/dl.
- In group III, Hb ranged between 11.45 and 13.75 mg/dl with a mean of 12.56 ± 0.76 mg/dl.

There was statistically significant difference in both group I and II as regard the mean Hb (P=0.004*). There was no statistically significant difference between group I and III (P=0.377). There was also no statistically significant difference between group II and III (P=0. 096).

**Table (4): Comparison between the studied groups according to Hb**

| | **Control (n = 10)** | **CAPD (n = 10)** | **HDF (n = 10)** | **F** | **p** |
|---|---|---|---|---|---|
| **Hb** | | | | | |
| Min. – Max. | 12.07 – 14.20 | 10.44 – 12.84 | 11.45 – 13.75 | | |
| Mean ± SD. | 13.04 ± 0.67 | 11.80 ± 0.81 | 12.56 ± 0.76 | 6.905* | 0.004* |
| Median | 13.0 | 12.0 | 12.60 | | |
| **Sig. bet. grps** | $p_1 = 0.004^*$, $p_2 = 0.377$, $p_3 = 0.096$ | | | | |

F: F test (ANOVA)
$p_1$: p value for Post Hoc test (Scheffe) for comparing between control and CAPD
$p_2$: p value for Post Hoc test (Scheffe) for comparing between control and HDF
$p_3$: p value for Post Hoc test (Scheffe) for comparing between CAPD and HDF
*: Statistically significant at $p \leq 0.05$

## c-Iron profile (table 4 ):

### * serum iron:

- In group I, serum iron ranged between 64.90 and 101.90 mg/dl with a mean of 91.06 ± 10.96 mg/dl, in group II, serum iron ranged between 63.0 –and 114.0 mg/dl with a mean of 78.60 ± 15.02 mg/dl, in group III, serum iron ranged between 74.0 and 117.0 mg/dl with a mean of 89.90 ± 14.79 mg/dl.

There was no statistically significant difference between group I and II as regard the mean serum iron (P=0. 0.147). There was also no statistically significant difference between group II and III (P=0.202). There was no statistically significant difference between group I and III (P=0.982).

### * Serum ferritin:

- In group I, serum ferritin ranged between 85 and 148 mg/dl with a mean of 114.4± 23.7 mg/dl, in group II, serum ferritin ranged between 212.0 and 636.0 mg/dl with a mean of 434.30±147.42 mg/dl, in group III, serum ferritin ranged between 205.70 and 530.0 mg/dl with a mean of 319.76 ± 107.44 mg/dl.

There was statistically significant difference between group I and II as regard the mean serum ferritin (P<0.001*). There was also statistically significant difference between group I and III (P<0.001*). There was no statistically significant difference between group II and III (P=0.069).

### * TIBC :

- In group I, TIBC ranged between 185.0 and 308.0 mg/dl with a mean of 248.95 ± 38.11 mg/dl, in group II, TIBC ranged between 288.0 and 311.0 mg/dl with a mean of 298.0 ± 7.62 mg/dl, in group III, TIBC ranged between 200.0 and 315.0 mg/dl with a mean of 250.90 ± 51.26 mg/dl,

There was statistically significant difference between group I and II as regard the mean TIBC (P<0.001*). There was also statistically significant difference between group II and III (P=0.030*). There was no statistically significant difference between group I and III (P=0.993).

### * TSAT :

- In group I, TSAT ranged between 26.73% and 50.27% with a mean of 37.58 ± 8.55, in group II, TSAT ranged between 20.26% – 39.18 % with a mean of 26.42 ± 5.32% , in group III, TSAT ranged between 24.92 % – 48.10% with a mean of 37.01 ± 8.25% .

There was statistically significant difference between group I and II as regard the mean TSAT (P=0.003*). There was also statistically significant difference between group II and III (P=0.010*). There was no statistically significant difference between group I and III (P=0. 082).

**Table (5): Comparison between the studied groups according to iron profile**

| | Control (n = 10) | CAPD (n = 10) | HDF (n = 10) | Test of sig. | p |
|---|---|---|---|---|---|
| **Serum iron** | | | | | |
| Min. – Max. | 64.90 – 101.90 | 63.0 – 114.0 | 74.0 – 117.0 | | |
| Mean ± SD. | 91.06 ± 10.96 | 78.60 ± 15.02 | 89.90 ± 14.79 | F= 2.518 | 0.099 |
| Median | 94.05 | 78.0 | 86.0 | | |
| **Sig. bet. grps** | $^{Sch}p_1$= 0.147, $^{Sch}p_2$= 0.982, $^{Sch}p_3$= 0.202 | | | | |
| **Serum ferritin** | | | | | |
| Min. – Max. | 85.0 – 148.0 | 212.0 – 636.0 | 205.70 - 530.0 | | |
| Mean ± SD. | 114.40 ± 23.70 | 434.30±147.42 | 319.76 ± 107.44 | $^{KW}\chi^2$= 20.851* | <0.001* |
| Median | 104.0 | 450.0 | 284.15 | | |
| **Sig. bet. grps** | $^{MW}p_1$<0.001*, $^{MW}p_2$ <0.001*, $^{MW}p_3$=0.069 | | | | |
| **TIBC** | | | | | |
| Min. – Max. | 185.0 – 308.0 | 288.0 – 311.0 | 200.0 – 315.0 | | |
| Mean ± SD. | 248.95 ± 38.11 | 298.0 ± 7.62 | 250.90 ± 51.26 | F= 5.592* | <0.001* |
| Median | 243.75 | 296.50 | 262.0 | | |
| **Sig. bet. grps** | $^{Sch}p_1$= 0.023*, $^{Sch}p_2$ = 0.993, $^{Sch}p_3$= 0.030* | | | | |
| **TSAT** | | | | | |
| Min. – Max. | 26.22 – 50.27 | 20.26 – 39.18 | 24.92 – 48.10 | | |
| Mean ± SD. | 37.58 ± 8.55 | 26.42 ± 5.32 | 37.01 ± 8.25 | $^{KW}\chi^2$= 10.392* | 0.006* |
| Median | 37.81 | 25.80 | 37.98 | | |
| **Sig. bet. grps** | $^{MW}p_1$ = 0.003*, $^{MW}p_2$ = 0.821, $^{MW}p_3$ = 0.010* | | | | |

F: F test (ANOVA)
$^{KW}\chi^2$: Chi square for Kruskal Wallis test
Sch: Post Hoc Test (Scheffe)
MW: Mann Whitney test
$p_1$: p value for comparing between control and CAPD
$p_2$: p value for comparing between control and HDF
$p_3$: p value for comparing between CAPD and HDF
*: Statistically significant at $p \leq 0.05$

## d- High sensitive C-reactive protein (hs-CRP) (table 5 ):

- In group I, hs-CRP ranged between 2.4 and 4.5mg/dl with a mean of 3.42± 0.71 mg/dl.
- In group II, hs-CRP ranged between 3.50 and 6.20 mg/dl with a mean of 5.25± 0.84 mg/dl.
- In group III, hs-CRP ranged between 3.20 and 8.90 mg/dl with a mean of 5.51± 1.71 mg/dl.

There was statistically significant difference between group I and II as regard the mean hs-CRP (P=0.007). There was also statistically significant difference between group I and III (P=0.002). There was no statistically significant difference between group II and III (P=0.887).

**Table (6): Comparison between the studied groups according to CRP**

| | **Control (n = 10)** | **CAPD (n = 10)** | **HDF (n = 10)** | **F** | **p** |
|---|---|---|---|---|---|
| **CRP** | | | | | |
| Min. – Max. | 2.40 – 4.50 | 3.50 – 6.20 | 3.20 – 8.90 | | |
| Mean ± SD. | 3.42 ± 0.71 | 5.25 ± 0.84 | 5.51 ± 1.71 | 9.432* | 0.001* |
| Median | 3.15 | 5.41 | 5.15 | | |
| **Sig. bet. grps** | $p_1$=0.007*, $p_2$=0.002*, $p_3$=0.887 | | | | |

F: F test (ANOVA)
$p_1$: p value for Post Hoc test (Scheffe) for comparing between control and CAPD
$p_2$: p value for Post Hoc test (Scheffe) for comparing between control and HDF
$p_3$: p value for Post Hoc test (Scheffe) for comparing between CAPD and HDF
*: Statistically significant at $p \leq 0.05$

## e - Serum hepcidin (table 6 ):

- In group I, serum hepcidin ranged between 43.5 and 109.5 mg/dl with a mean of 61.40± 19.30 mg/dl.
- In group II, serum hepcidin ranged between 56.50 and 168 mg/dl with a mean of 125.80± 39.63 mg/dl.
- In group III, serum hepcidin ranged between 43.5 and 111.5 mg/dl with a mean of 69.15± 23.55 mg/dl.

There was statistically significant difference between group II and III as regard the mean serum hepcidin (P=0.010*). There was also statistically significant difference between group I and II (P=0.003*). There was no statistically significant difference between group I and III (P=0.405).

**Table (7): Comparison between the studied groups according to hepcidin**

| | **Control (n = 10)** | **CAPD (n = 10)** | **HDF (n = 10)** | **$^{KW}\chi^2$** | **p** |
|---|---|---|---|---|---|
| **Hepcidin** | | | | | |
| Min. – Max. | 43.50 – 109.50 | 56.50 – 168.0 | 43.50 – 111.50 | | |
| Mean ± SD. | 61.40 ± 19.30 | 125.80 ± 39.63 | 69.15 ± 23.55 | 10.804* | 0.005* |
| Median | 58.50 | 142.25 | 65.25 | | |
| **Sig. bet. grps** | $p_1$=0.003*, $p_2$=0.405, $p_3$=0.010* | | | | |

$^{KW}\chi^2$: Chi square for Kruskal Wallis test
$p_1$ : p value for Mann Whitney test for comparing between control and CAPD
$p_2$ : p value for Mann Whitney test for comparing between control and HDF
$p_3$ : p value for Mann Whitney test for comparing between CAPD and HDF
*: Statistically significant at $p \leq 0.05$

## Statistical Correlations

- **In group II,**There was a statistically negative correlation between Hb and hsCRP and serum hepcidin (P=0.015, <0.022 respectively).(Table 7).

There was a statistically positive correlation between hs-CRP and serum ferritine and hepcidin (P=0.018, =0.041 (respectively).(Table 7).

There was a statistically positive correlation between serum iron and TSAT and ferritine and hepcidin (P< 0.001, =0.002, =0,013 (respectively). (Table 7).

There was a statistically positive correlation between serum ferritine and hepcidin (P<0.001). (Table 7).

- **In group III,**

There was a statistically positive correlation between hsCRP and serum iron and serum ferritine and serum hepcidin (P=0.004, =0,035 =0.004 respectively).(Table 8).

There was a statistically positive correlation between serum ion and serum ferritine and serum hepcidin (P =0.003,<0.001 respectively)).(Table 8).

There was a There was a statistically positive correlation between serum ferritine and hepcidin (P<0.001) (Table 8).

**Table (8): Correlation between different studied parameters in CAPD group**

| | | Urea | Cr | CRP | S. Iron | TSAT | TIBC | S.Ferritin | Hepcidin |
|---|---|---|---|---|---|---|---|---|---|
| **Hb** | **r** | 0.059 | -0.145 | -0.736* | -0.443 | -0.387 | -0.250 | -0.599 | -0.709* |
| | **p** | 0.872 | 0.689 | 0.015 | 0.200 | 0.270 | 0.485 | 0.067 | 0.022 |
| **Urea** | **r** | | -0.563 | -0.517 | 0.251 | 0.217 | 0.282 | -0.001 | -0.275 |
| | **p** | | 0.090 | 0.126 | 0.484 | 0.546 | 0.430 | 0.997 | 0.441 |
| **Cr** | **r** | | | 0.325 | -0.249 | -0.261 | 0.131 | -0.047 | 0.089 |
| | **p** | | | 0.359 | 0.489 | 0.467 | 0.719 | 0.897 | 0.807 |
| **CRP** | **r** | | | | 0.183 | 0.159 | 0.016 | 0.539 | 0.631* |
| | **p** | | | | 0.612 | 0.660 | 0.965 | 0.108 | 0.041 |
| **S. Iron** | **r** | | | | | 0.994* | -0.275 | 0.846* | 0.748* |
| | **p** | | | | | <0.001 | 0.442 | 0.002 | 0.013 |
| **TSAT** | **r** | | | | | | -0.379 | 0.815* | 0.723* |
| | **p** | | | | | | 0.280 | 0.004 | 0.018 |
| **TIBC** | **r** | | | | | | | -0.071 | -0.097 |
| | **p** | | | | | | | 0.845 | 0.790 |
| **S. Ferritin** | **r** | | | | | | | | 0.893* |
| | **p** | | | | | | | | <0.001 |

r: Pearson coefficient
*: Statistically significant at $p \leq 0.05$

**Table (9): Correlation between different studied parameters in HDF group**

| | | Urea | Cr | CRP | S. Iron | TSAT | TIBC | S.Ferritin | Hepcidin |
|---|---|---|---|---|---|---|---|---|---|
| **Hb** | **r** | -0.079 | 0.181 | -0.355 | -0.537 | -0.597 | 0.276 | -0.225 | -0.450 |
| | **p** | 0.827 | 0.616 | 0.315 | 0.110 | 0.069 | 0.439 | 0.532 | 0.192 |
| **Urea** | **r** | | 0.383 | 0.032 | 0.006 | -0.118 | 0.074 | -0.410 | 0.052 |
| | **p** | | 0.274 | 0.931 | 0.988 | 0.745 | 0.839 | 0.239 | 0.886 |
| **Cr** | **r** | | | -0.168 | -0.192 | 0.089 | -0.196 | -0.522 | -0.323 |
| | **p** | | | 0.642 | 0.595 | 0.807 | 0.587 | 0.122 | 0.363 |
| **CRP** | **r** | | | | 0.817* | 0.277 | 0.288 | 0.669* | 0.814* |
| | **p** | | | | 0.004 | 0.439 | 0.419 | 0.035 | 0.004 |
| **S. Iron** | **r** | | | | | 0.455 | 0.194 | 0.837* | 0.972* |
| | **p** | | | | | 0.187 | 0.592 | 0.003 | <0.001 |
| **TSAT** | **r** | | | | | | -0.778* | 0.292 | 0.364 |
| | **p** | | | | | | 0.008 | 0.413 | 0.301 |
| **TIBC** | **r** | | | | | | | 0.259 | 0.260 |
| | **p** | | | | | | | 0.470 | 0.468 |
| **S. Ferritin** | **r** | | | | | | | | 0.863* |
| | **p** | | | | | | | | 0.001 |

r: Pearson coefficient
*: Statistically significant at $p \leq 0.05$

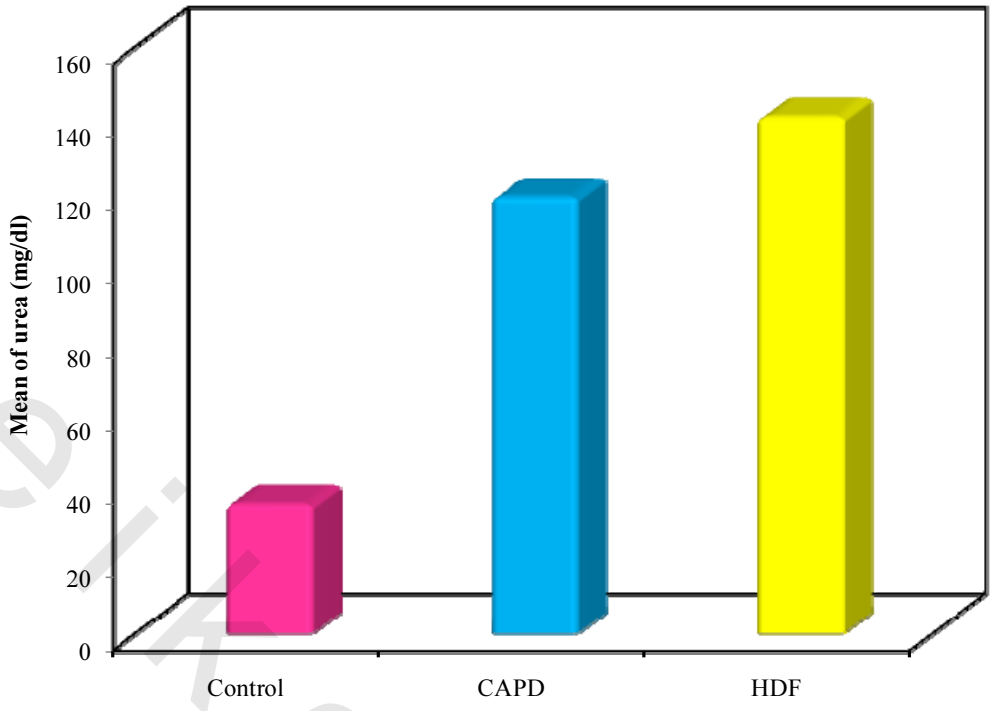


**Figure (9): Comparison between the studied groups according to urea**

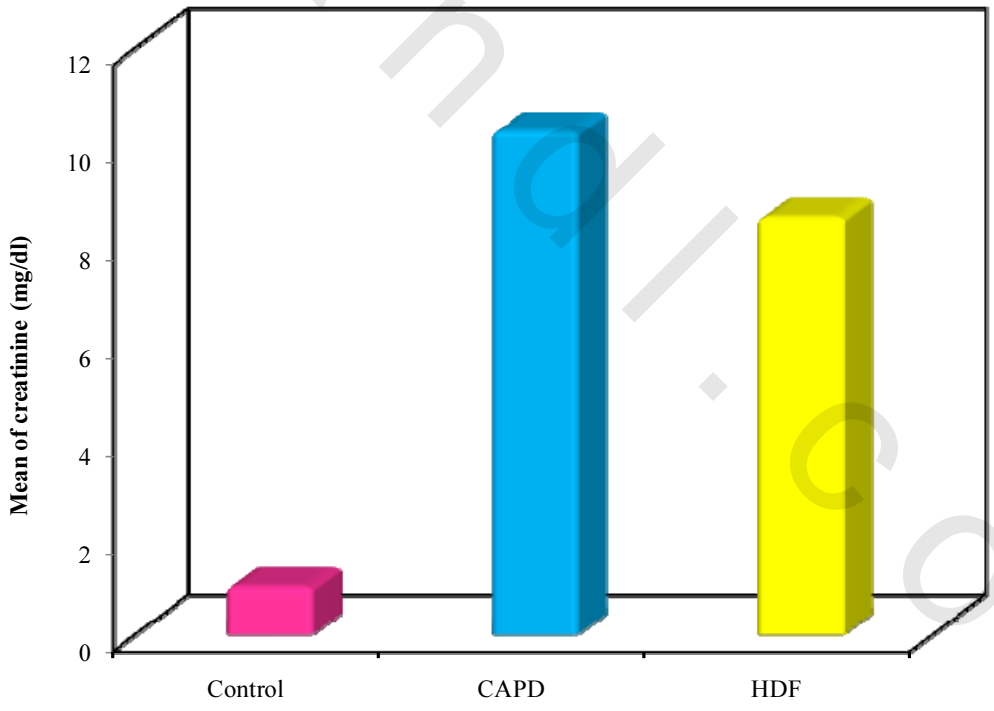


**Figure (10): Comparison between the studied groups according to creatinine**

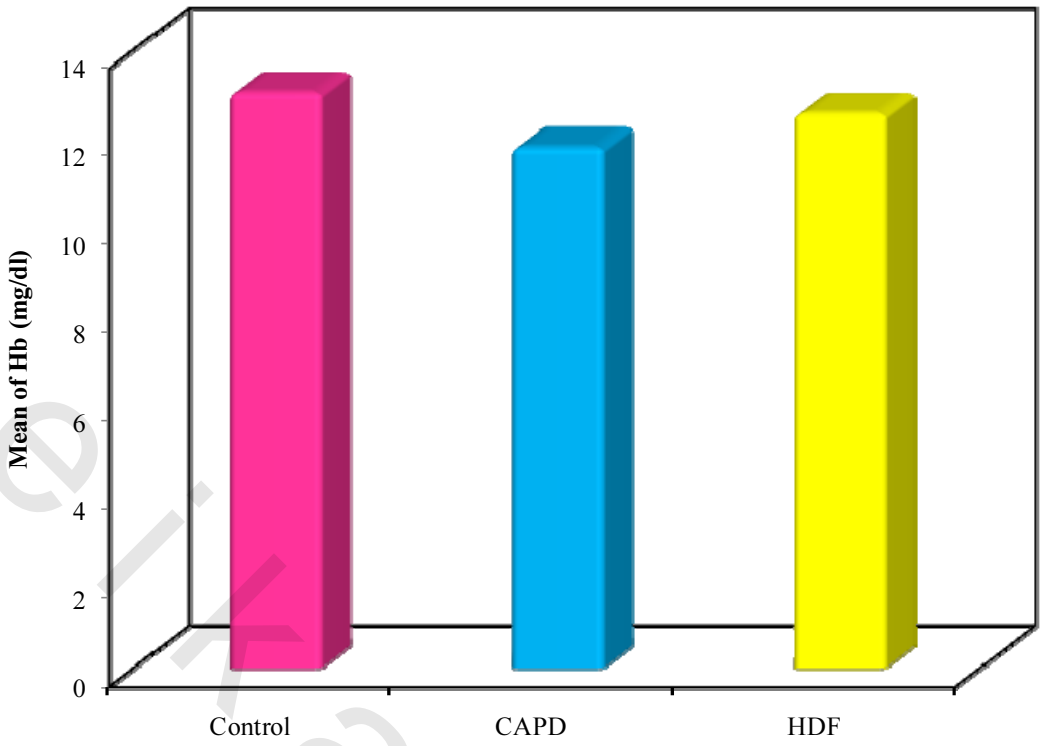


**Figure (11): Comparison between the studied groups according to Hb**

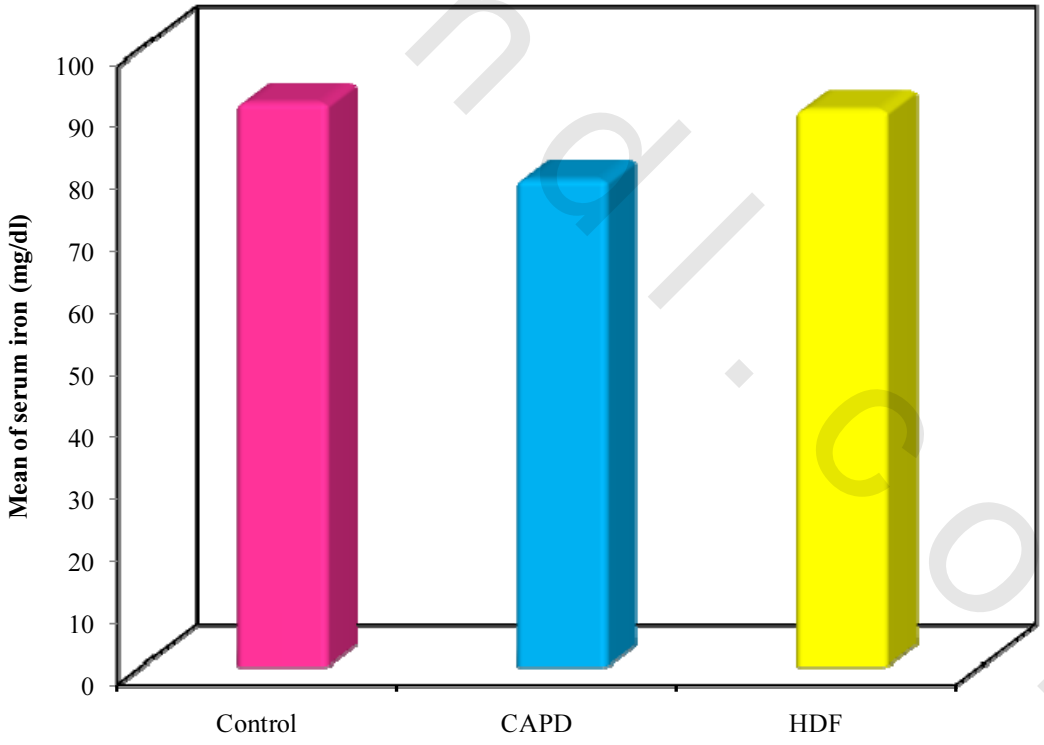


**Figure (12): Comparison between the studied groups according to serum iron**

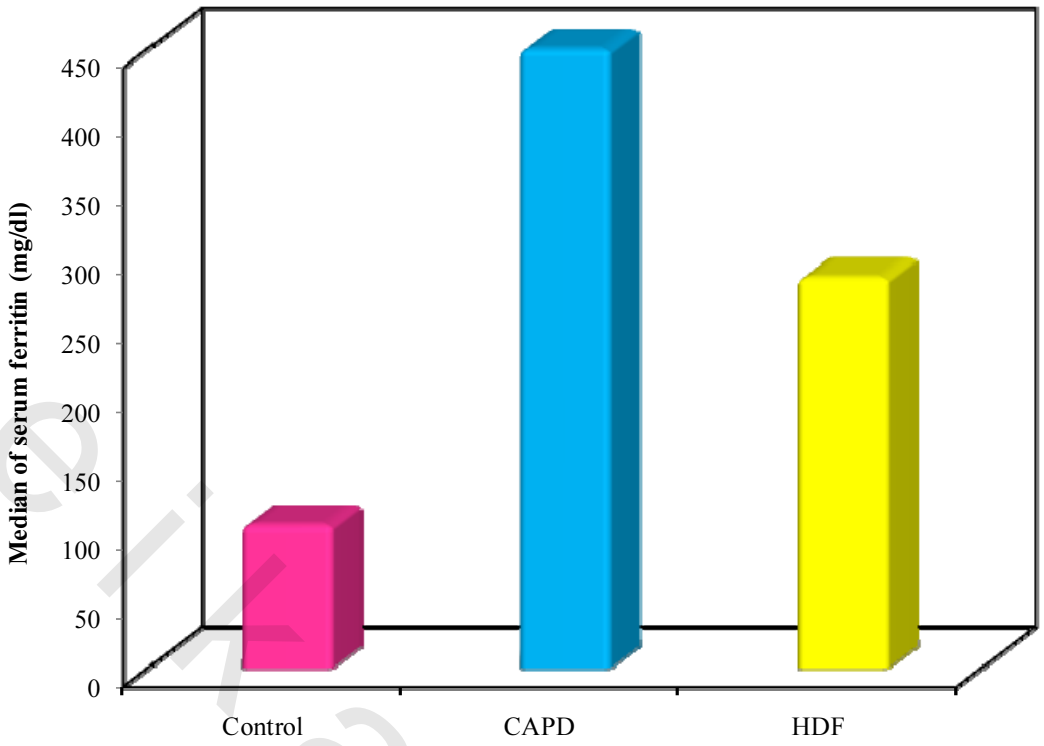


**Figure (13): Comparison between the studied groups according to serum ferretin**

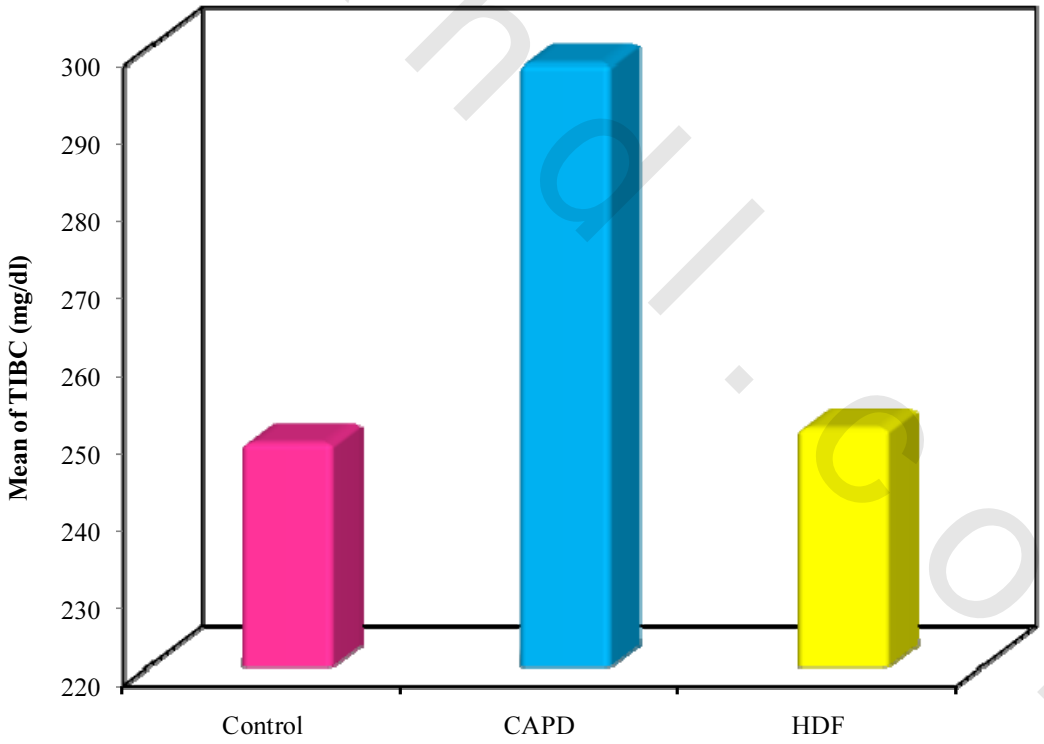


**Figure (14): Comparison between the studied groups according to TIBC**

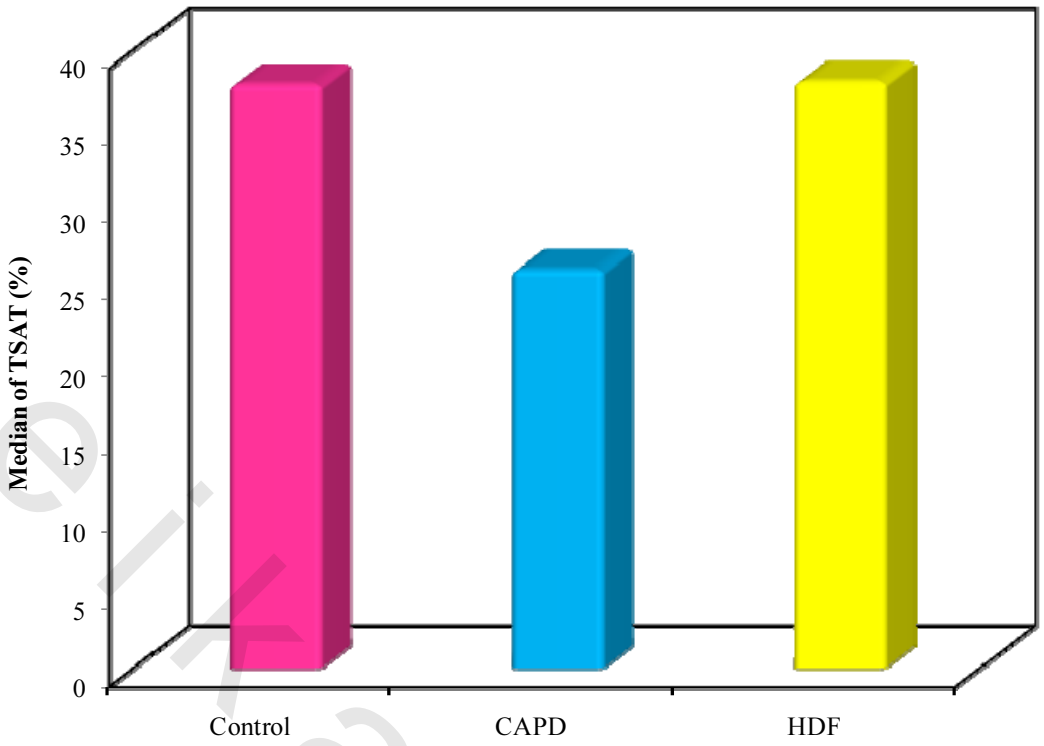


**Figure (15): Comparison between the studied groups according to TSAT**

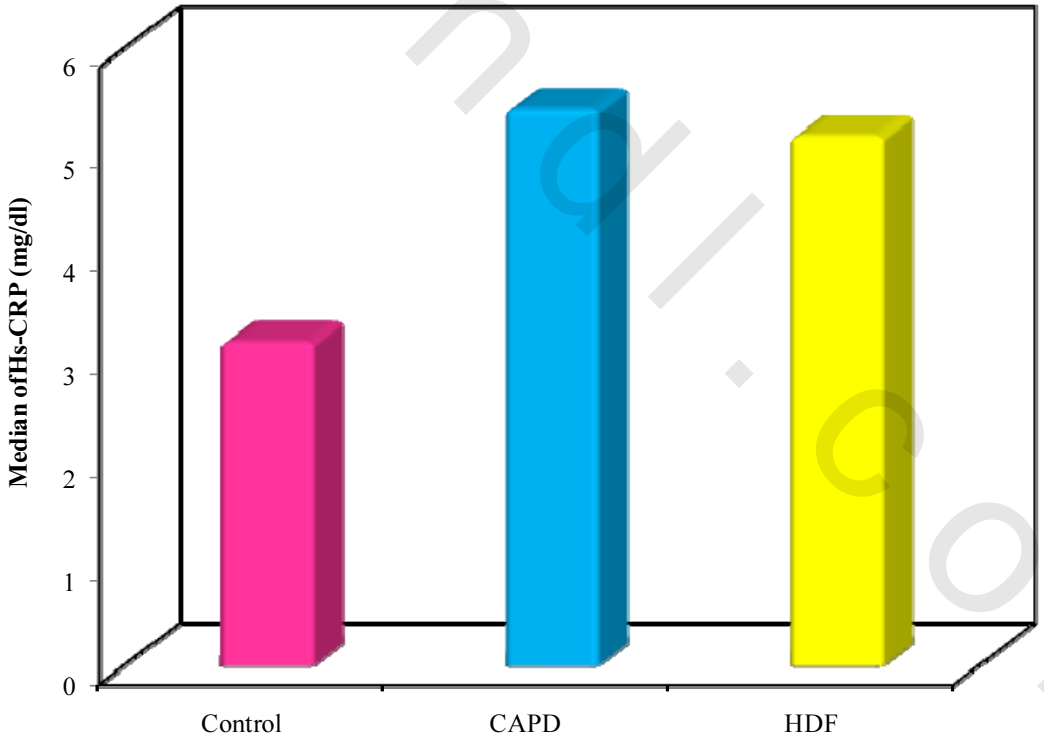


**Figure (16): Comparison between the studied groups according to CRP**

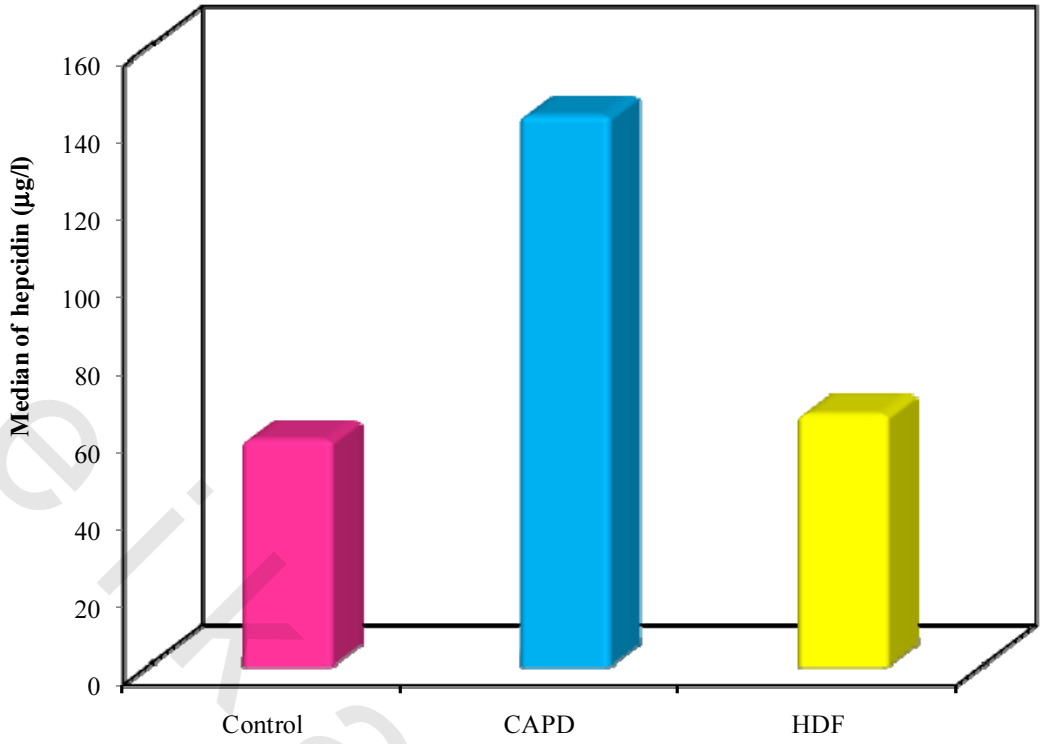


**Figure (17): Comparison between the studied groups according to hepcidin**

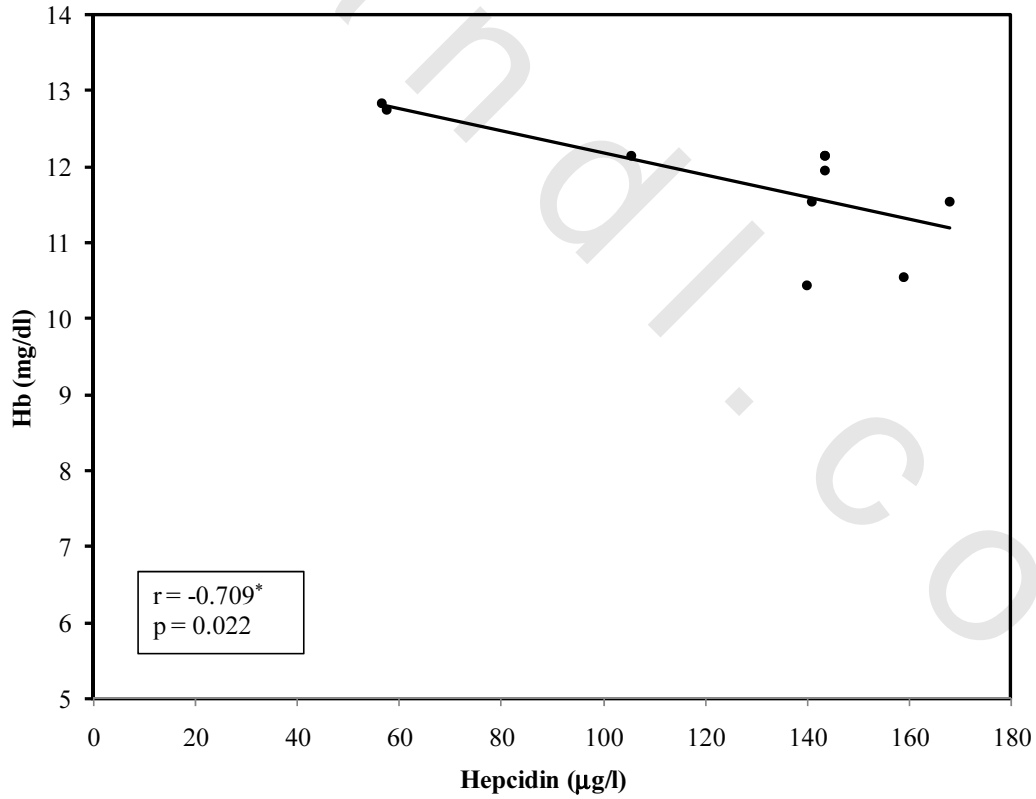


**Figure (18): Correlation between Hepcidin with Hb in CAPD group**

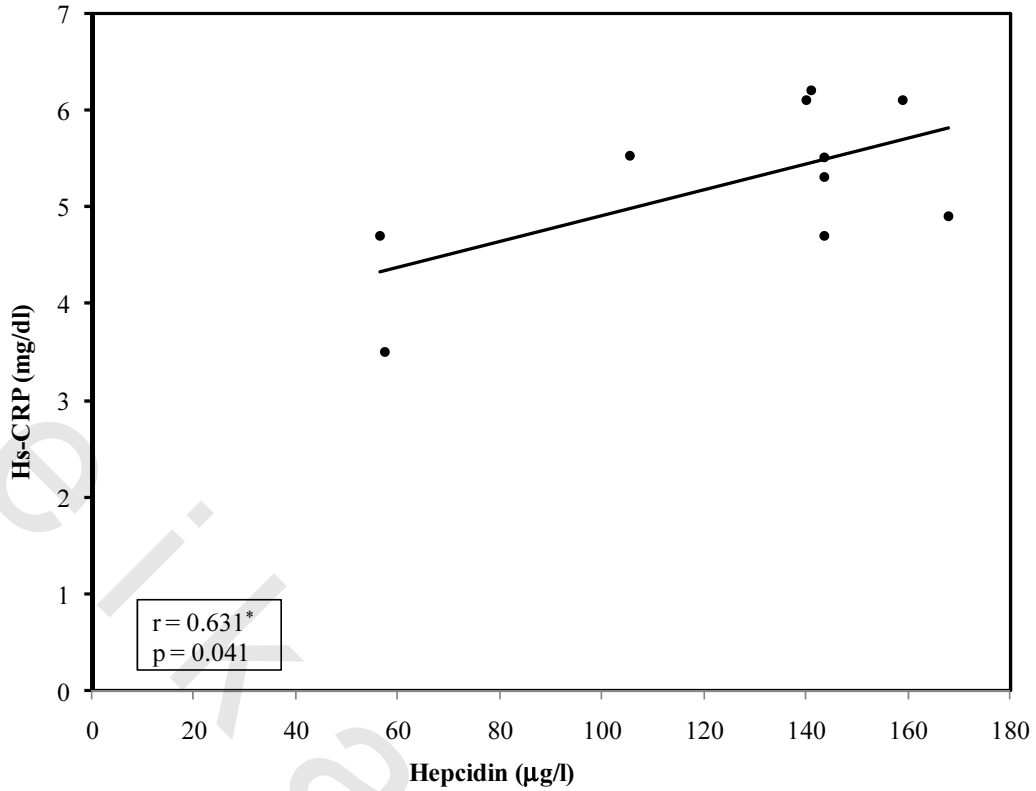


**Figure (19): Correlation between Hs-CRP with Hepcidin in CAPD group**

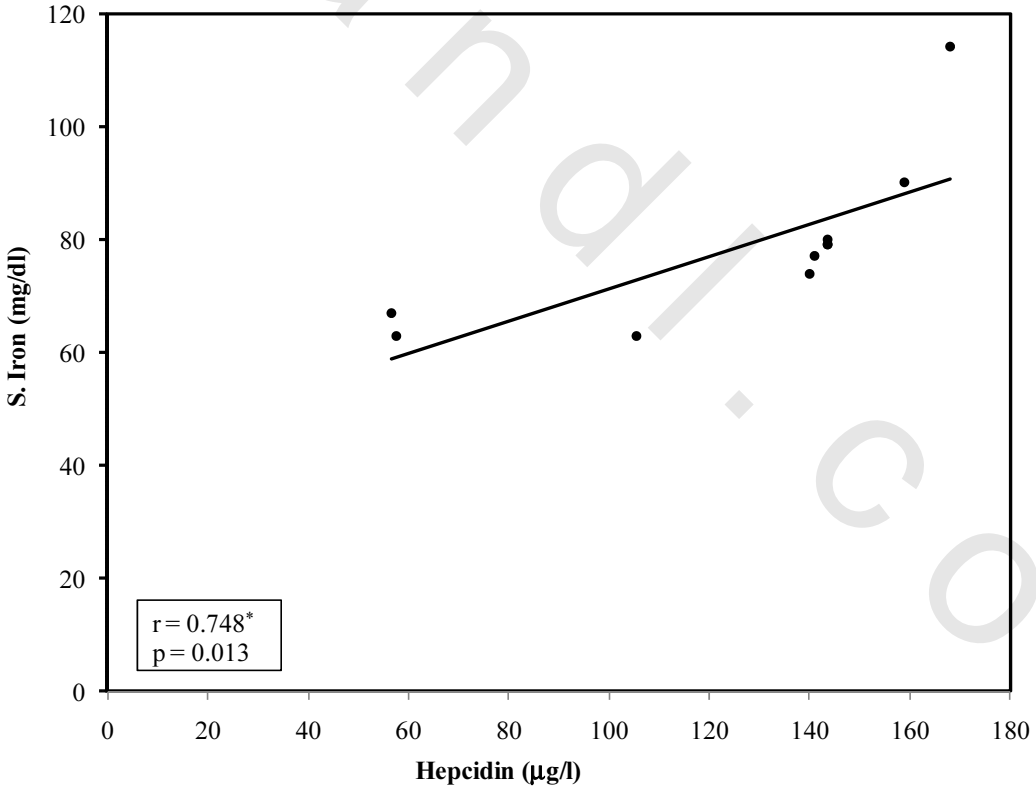


**Figure (20): Correlation between Hepcidin with S. Iron in CAPD group**

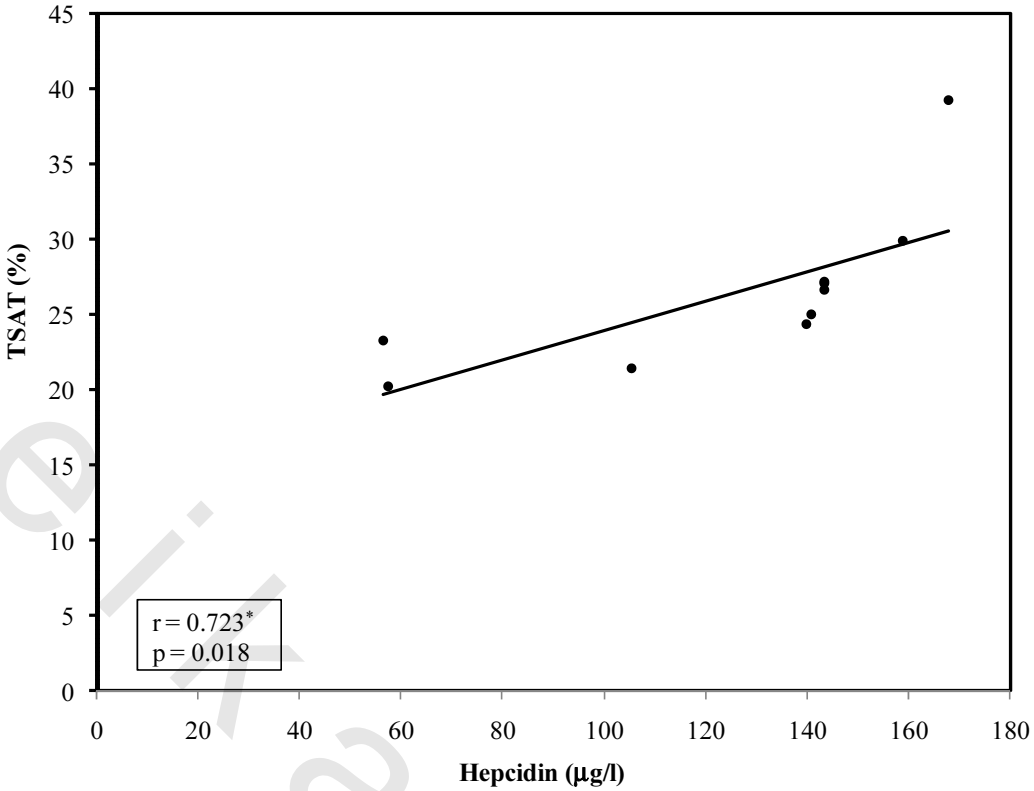


**Figure (21): Correlation between hepcidin with TSAT in CAPD group**

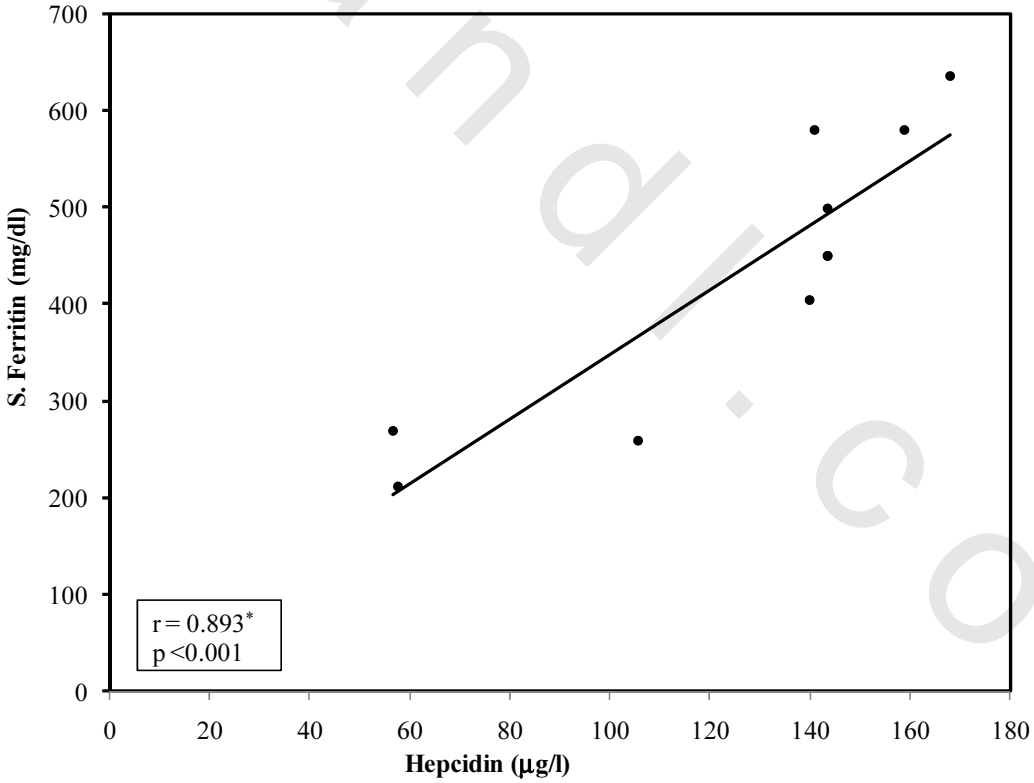


**Figure (22): Correlation between hepcidin with S. Ferritin in CAPD group**

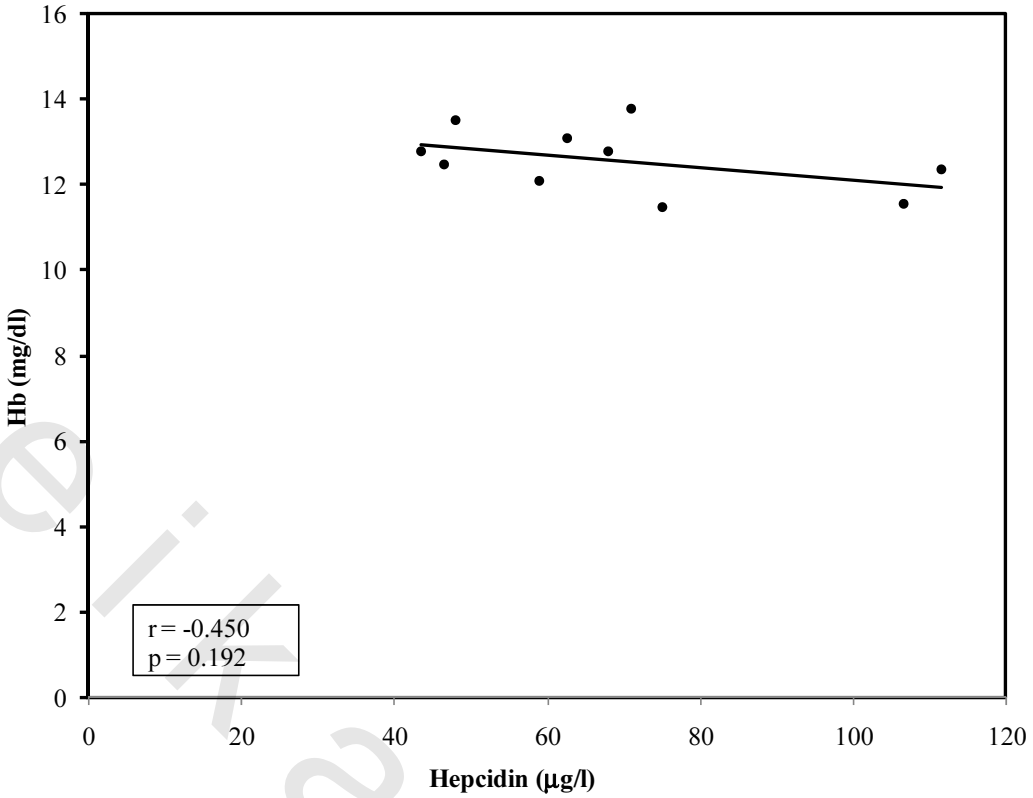


**Figure (32): Correlation between Hepcidin with Hb in HDF group**

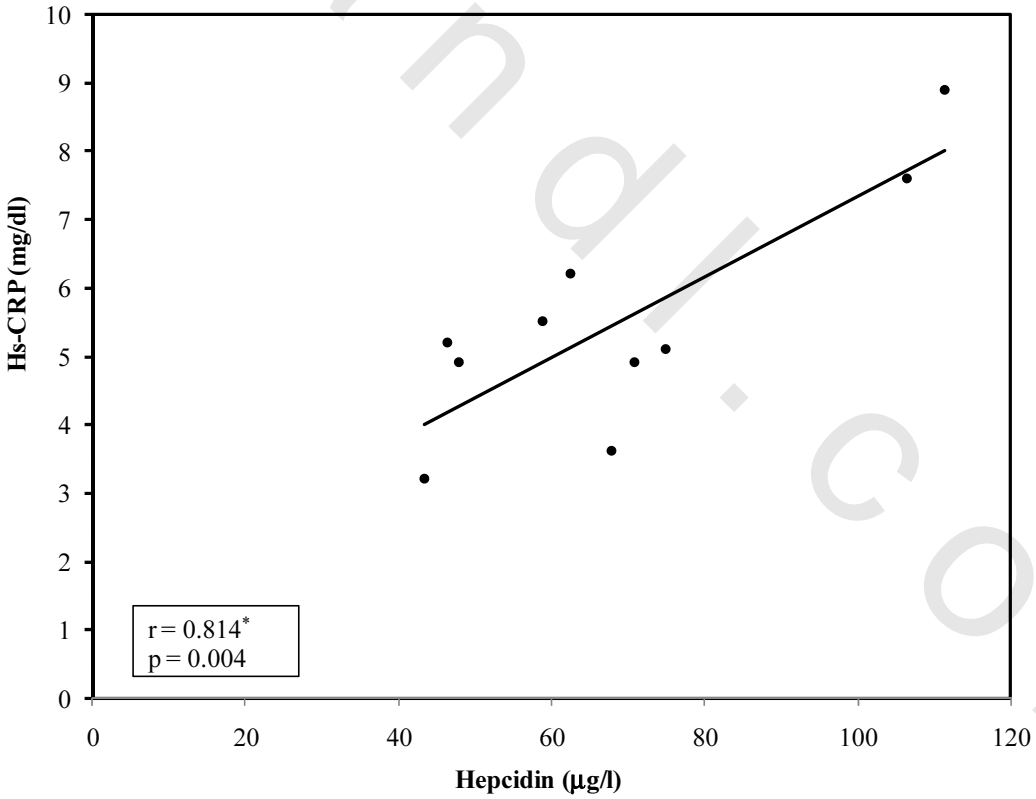


**Figure (24): Correlation between Hepcidin with Hs-CRP in HDF group**

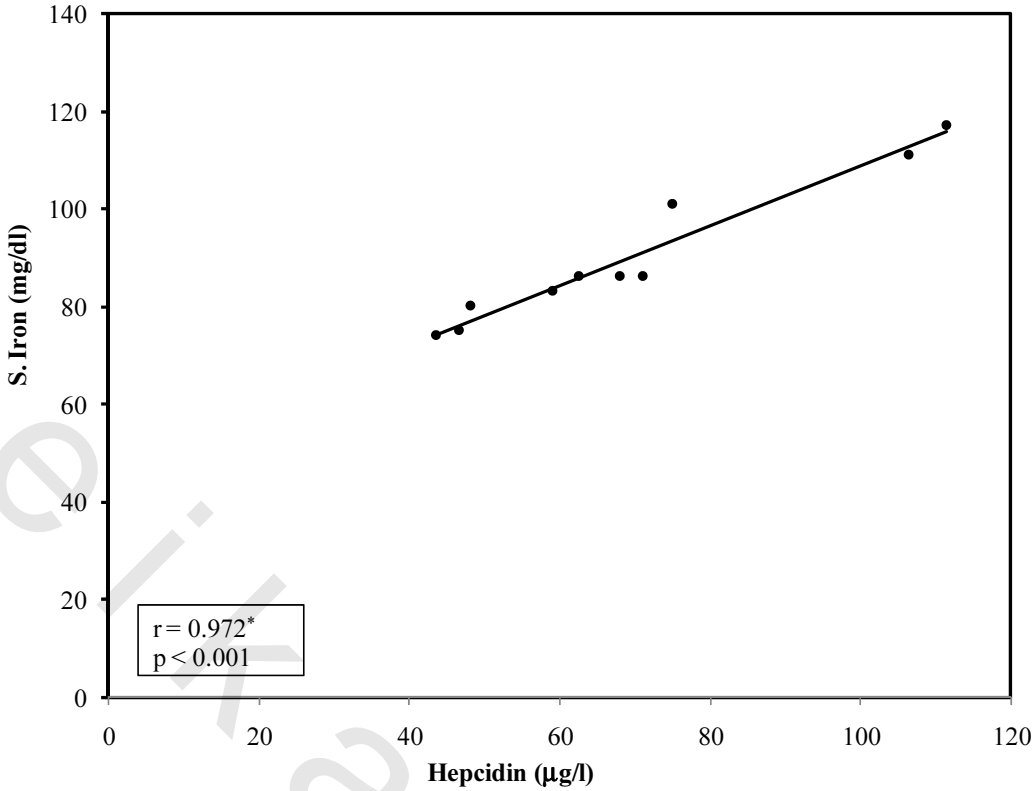


**Figure (25): Correlation between hepcidin with S.iron in HDF group**

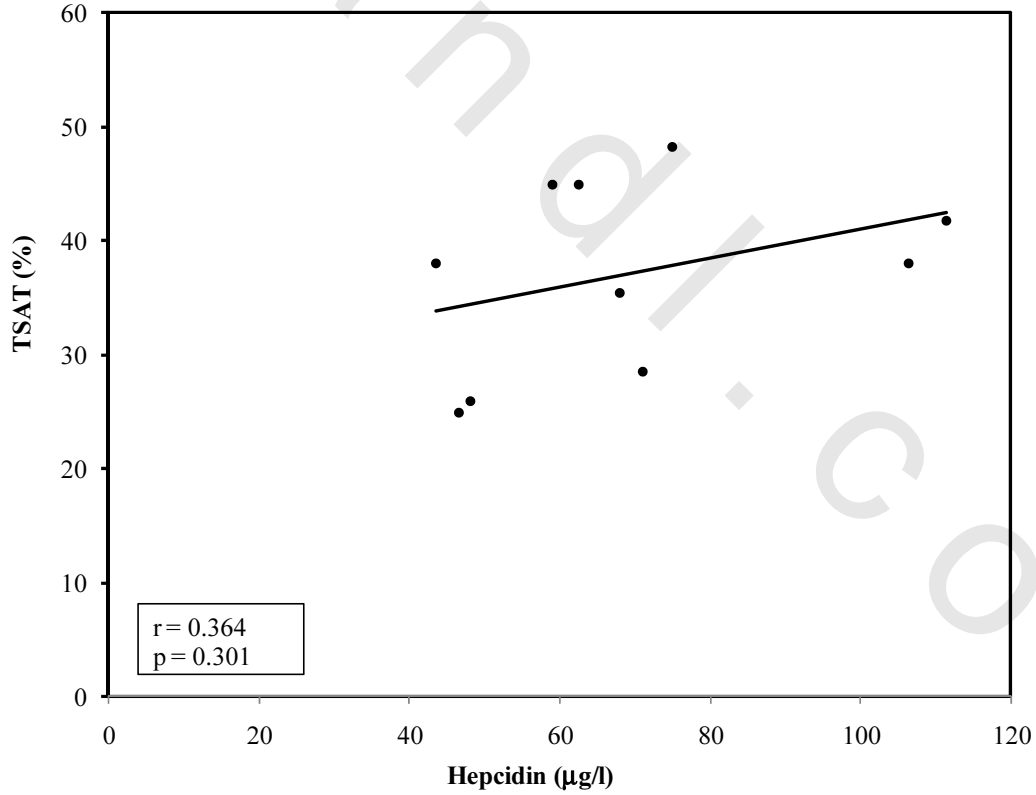


**Figure (26): Correlation between hepcidin with TSAT in HDF group**

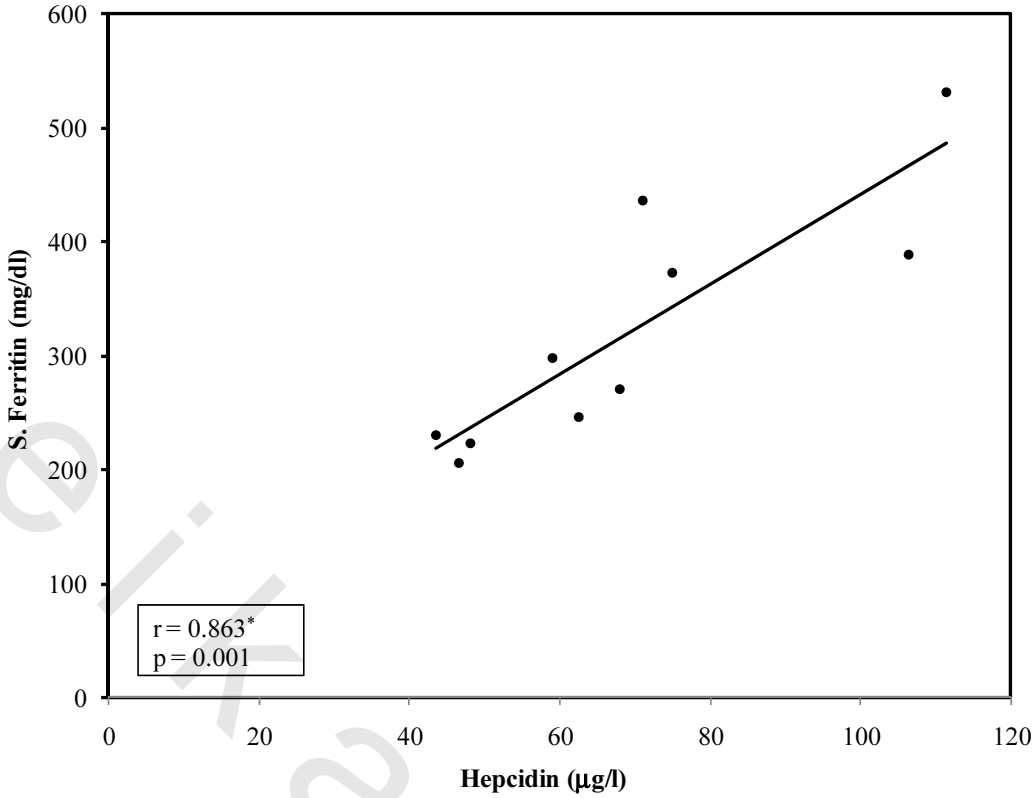


**Figure (27): Correlation between hepcidin with S.ferritin in HDF group**