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RESEARCH ARTICLE

Physiological and hematological changes in patients with chronic renal failure undergoing hemodialysis

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Abstract

In this study , 79 patients (54 males and 25 females) suffering from chronic renal failure who were obtained from AL-Hakeem Hospital, AN Najaf, Iraq, and 79 healthy individuals were included as control group . The sera were separated from samples of patients blood and subjected to physiological investigation. We found that renal failure was more predominant among the patients ages group ranging from 19 – 60 years old. The results shows a significant increase in iron, ferritin and TIBC levels in CRF patients in both males ($P<0.05$) and females ($P<0.05$) but UIBC levels a significant increase in both males ($P<0.05$) and females ($P<0.05$) in (CRF) patients as compared with their control groups, furthermore no significant changing in (TS%) levels in both males ($P>0.05$) and females ($P>0.05$) in (CRF) patients as compared with their corresponding control groups , in addition to that a significant change in transferring levels in both males and females ($P<0.05$) (CRF) patients as compared with their parallel control. Then we subjected this sample to hematological studies and we found a significant decrease in (Hemoglobin) level s and (PCV) in both males and females ($P<0.05$) in (CRF) patients as compared with their corresponding control groups.

INTRODUCTION

The kidneys are responsible for filtering and excreting wastes from the blood without proper functioning, toxic waste products will be accumulated and the patient will died. Therefore, kidneys are vital to maintain life. Kidney diseases were classified into acute kidney disease (AKD) and chronic kidney disease (CKD).

Hepcidin is a peptide produced primarily in the liver. Its mature form consists of 25 amino acids with a calculated mass of 2.8 kDa. Hepcidin has a key role in iron homeostasis by interacting with ferroportin.(Weiss and Goodnough, 2005) Hepcidin is the regulator of iron homeostasis in humans and other mammals. In humans, HAMP is the gene that encodes for hepcidin (Ganz, 2003). 25-amino acid peptide was first identified in human urine and plasma. This peptide contains four disulfide bonds (Krause et al., 2000). It is synthesized, processed and secreted primarily by hepatocytes. In vitro, human hepcidin has anti-bacterial and antifungal activities (Park et al.,2001).

Iron is an essential element for nearly all living organisms (Aisen et al., 2001). Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. The amount of ferritin stored reflects the amount of iron stored. The protein is produced by almost all living organisms, including algae, bacteria and higher plants, and animals. In humans, it acts as a buffer against iron deficiency and iron overload (Theil, 1987) .

Hepcidin inhibits iron transport by binding to the iron channel ferroportin, which is located on the basolateral surface of gut enterocytes and the plasma membrane of reticuloendothelial cells (macrophages). Inhibiting ferroportin shuts off the iron transport out of these cells, which store iron (Rossi, 2005). Hepcidin regulates iron

homeostasis by binding to and inducing the internalization and degradation of ferroportin, the sole cellular iron efflux channel in iron transporting cells (Nemeth et al., 2004)

Hemodialysis is a method that is used to achieve the extracorporeal removal of waste products such as creatinine and urea and free water from the blood by an artificial kidney machine when the kidneys are in a state of renal failure. The basic principle of the artificial kidney is to pass blood through minute blood channels bounded by a thin membrane. On the other side of the membrane is a dialyzing fluid into which unwanted substances in the blood pass by diffusion (Guyton and Hall, 2011).

MATERIALS AND METHODS

Sample collection and patients

Samples (79 patients and 79 healthy) were collected from Artificial Kidney Unit, Al-Hakeem General Hospital, AN Najaf, Iraq. Further, 79 patients included in this study were 54 males and 25 females and their ages ranged from 19 to 60 years. The patients were diagnosed as Chronic renal failure for both sexes based on the history, clinical examination.

ELIZA

A Hepcidin level were assessed using ELISA reader M6 and Hepcidin Kit according to manufactured instructions (Biolabo, France).Briefly, serum samples were thawed, centrifuged at 3000 rpm for 5 minutes, and incubated with micro beads for 2 hours. After a wash step, the beads were incubated with the detection antibody for 2 hours. After an additional wash step, the beads were incubated with Streptavidin-HRP conjugate for 30 minutes. After wash the beads were incubated with substrate for 11minutes. After adding stop solution the samples were placed in the array reader at absorbance was measured at 450 nm for determination of the respective cytokine concentration.

Serum Iron

Serum iron was measured by using a colorimetric Cromatest® kit supplied by Linear Chemicals, Spain according to manufacture instructions.

Serum Ferritin

Ferritin patient's serum were assessed using Enzyme-linked Immunosorbent Assay (ELISA) as described previously.

Statistical analysis

Data were analyzed using the software packages Graphpad prism for Windows (5.04, Graphpad software Inc. USA). Data are presented as the mean±standard Devation (SD). The comparison between the patients and healthy groups were analyzed by one-way T-test [p-value less than 0.05was consider significant].

Measurment of TIBC, UIBC and TS

Total Iron Binding Capacity (TIBC) calculated by the formula supplied in iron kit and according to manufactured instruction as mentioned below:

TIBC = Iron conc. in supernatant (µmol/L) x 3 (Dilution Factor)

Unsaturated Iron binding capacity (UIBC) calculated by the formula UIBC= TIBC - Serum iron concentration (Tietz, 1995). Transferrin saturation percentage is calculated from the following equation: TS% =Iron×100/TIBC (Christine et al., 2001)

RESULTS:

In the present study, the Hepcidin , iron, ferritin , TIBC and UIBC levels increased in CRF patients in both males (P<0.05) and females (P<0.05) as compared with their control groups, furthermore no significant changing in (TS%) levels in both males (P>0.05) and females (P>0.05) in (CRF) patients as compared with their corresponding control groups , in addition to that a significant change in transferring levels in both males and females (P<0.05) (CRF) patients as compared with their parallel control groups as shown in **Figures 1 &2.**

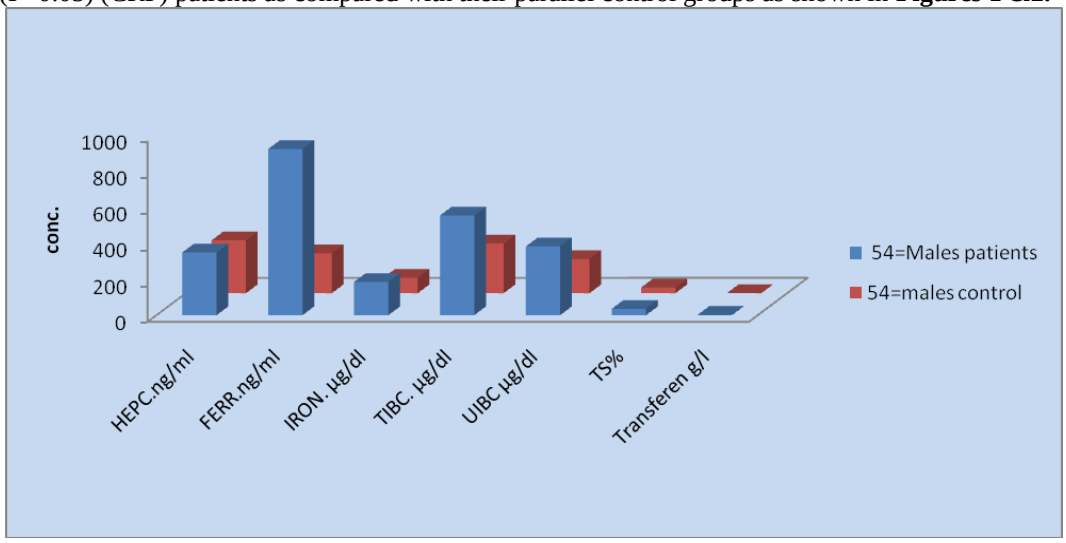

Figure.1. Variability of distribution of Hepcidin and iron status in CRF males Patients.

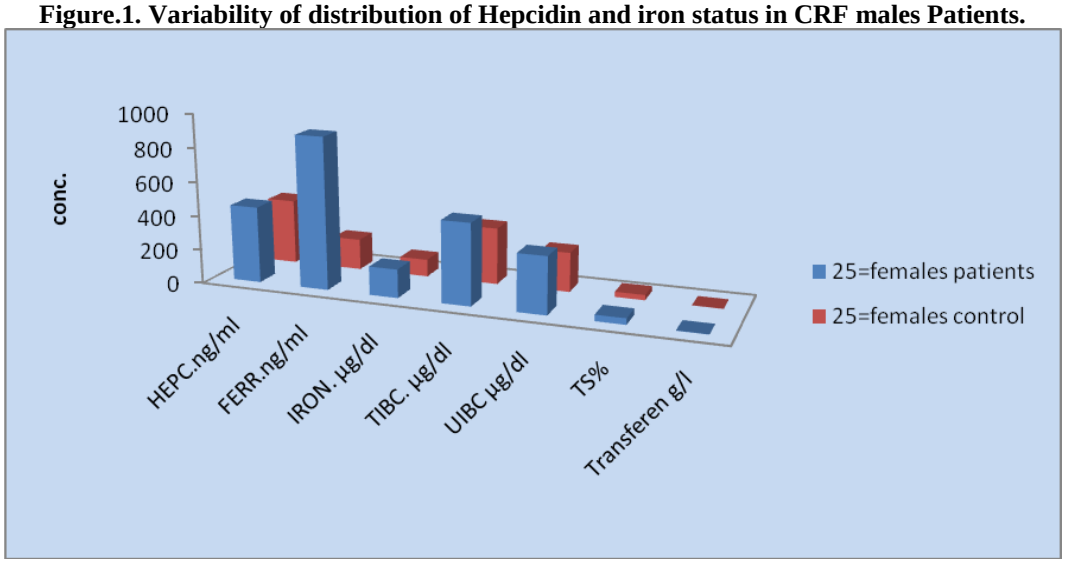

Figure.2. Variability of distribution of Hepcidin and iron status in CRF females Patients.

Clinical data of the Hematological investigations are presented in (Figures 3&4) that shows a significant decrease in (Hemoglobin) levels and (PCV) in both males and females (P<0.05) in (CRF) patients as compared with their corresponding control groups.

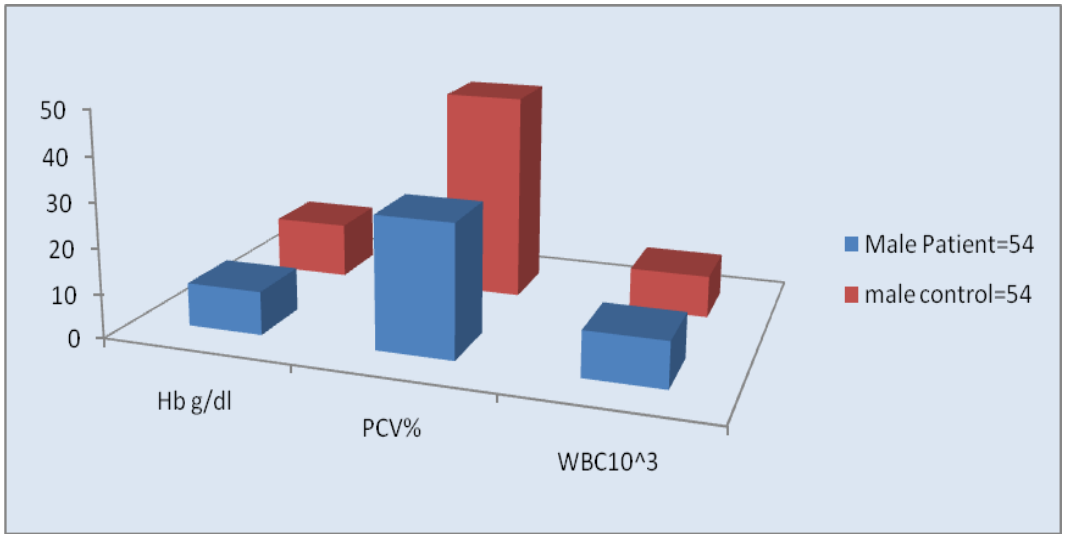

Figure.3. Variability of distribution of Hematological Parameter in CRF males Patients.

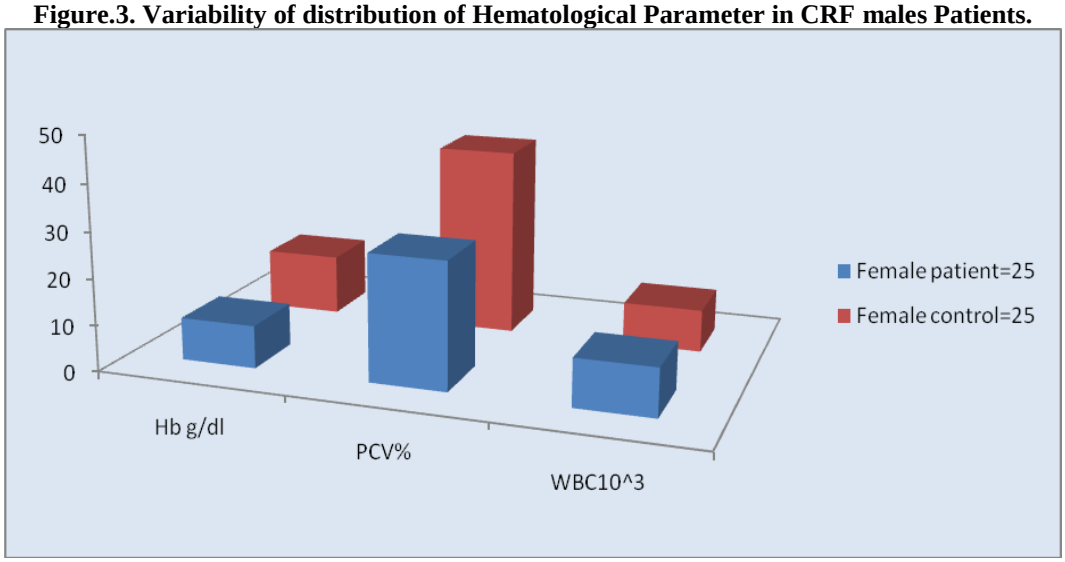

Figure.4. Variability of distribution of Hematological parameter in CRF females Patients.

DICUSSION

Renal failure refers to a condition where the kidneys lose their normal functionality, which may be due to various factors including infections, auto immune diseases, diabetes and other endocrine disorders, cancer, and toxic chemicals (Meyer and Hostetter, 2007).The results in **Figures 1 &2** show a significant increase in hepcidin levels in both males and females in patients with chronic renal failure as compared with their parallel control groups. Its levels are increased in the blood by infection, or any type of chronic inflammation, and these conditions may convert what would otherwise be a low level of ferritin from lack of iron, into a value in the normal range. For this reason, low ferritin levels carry more information than those in the normal range, low ferritin may also indicate hypothyroidism, vitamin C deficiency or celiac disease (Guyaton et al; 1990). The synthesis of hepcidin is greatly stimulated by inflammation or by iron overload. Hepcidin is the predominant negative regulator of iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages (Tomas, 2003). In CRF patients suffering with drop all of the humeral and cellular immune and getting infection (Amore and Coppo, 2002). In this study, the CKD patients showed significant elevation of serum levels of hepcidin as compared to controls of interest. Another important findings were that this high levels of hepcidin is associated with significant elevation of serum ferritin but not other serum iron status parameters, early epidem-icologic investigations that related body iron stores to CKD were criticized because of the use of nonspecific markers, such as serum iron and/or transferrin (Liao et al., 1994). If ferritin is high, there is iron in excess or else there is an acute inflammatory reaction in which ferritin

is mobilized without iron excess. For example, ferritins may be high in infection without signalling body iron overload, Ferritin is also used as a marker for iron overload disorders, such as hemochromatosis, hemosiderosis. Adultonset Still's disease, porphyria and Hemophagocytic lymphohistiocytosis are diseases in which the ferritin level may be abnormally raised, As ferritin is also an acute-phase reactant, it is often elevated in the course of disease. A normal C-reactive protein can be used to exclude elevated ferritin caused by acute phase reactions, Ferritin can be elevated during periods of acute malnourishment (Kennedy et al; 2004). Studies that used serum ferritin have been considered more informative because ferritin is strongly correlated with body iron stores in healthy individuals. In the current study patients were associated with normal iron and TIBC levels which make the possibility of the regulatory role of iron level as a cause of the recorded hepcidin elevation less likely. Moreover, a positive correlation was found between serum ferritin, and hepcidin which is now recognized as a very reliable marker of inflammation and between hepcidin on one hand and ferritin on the other hand (Ridker, 2001).

The study shows a statistically significant decrease in both PCV and Hb concentration in CRF patients compare to the control groups in Figures 3&4 .The result explained that anemia first appears when the GFR falls below 40 ml/minute, and is present in most patients with ESRD because in renal failure, erythropoietin production it is usually is insufficient to stimulate adequate red blood cell production by the bone marrow (Jones et al., 2005).

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