

**Sudan University of Science and Technology**  
**College of Graduate Studies**

**The Effect of Hemodialysis on Serum Levels of Iron and  
C-Reactive Protein in Patients with Renal Failure**

تأثير الغسيل الدموي على معدلات الحديد و بروتين سي التفاعلي لدى مرضى الفشل الكلوي

**Dissertation submitted in partial fulfillment for master degree  
(M.Sc) in clinical chemistry**

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*تأثير الغسيل الدموي على معدلات الحديد وبروتين سي ال إنفلافين لدى*

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وهي منتج فكري أصيل . وباختياري أعطى حقوق طبع ونشر هذا العمل لكلية الدراسات العليا - جامعة السودان للعلوم والتكنولوجيا، عليه يحق للجامعة نشر هذا العمل للأغراض العلمية .

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قال تعالى:

{ الرَّحْمَنُ (1) عَلَّمَ الْقُرْآنَ (2) خَلَقَ الْإِنْسَانَ (3) عَلَّمَهُ الْبَيَانَ (4) الشَّمْسُ وَالْقَمَرُ بِحُسْبَانٍ (5) }.

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إهداء

أهدي ثمرة هذا الجهد المتواضع إلى:

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إلى أساتذتي الأجلاء وكل من ساعدني في هذا العمل

وإلى كل من ساعدني في هذه الحياة

صفاء

# **Dedication**

**To my family for their love**

**To my teachers for their  
patience**

**To my friends for their  
help**

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All love to my beautiful daughter (Malak) and my great husband (Haisam) .



## **Abstract**

This study was carried out to assess the effect of hemodialysis on iron and C-reactive protein (CRP) in patients with renal failure. Sixty samples were collected from patients in period between April to May 2015, chosen randomly from Association specialized hospital in Khartoum states, and thirty apparently healthy individuals as controls.

Measure plasma iron and CRP by using Hitachi/cobas C311 system, and results were analyzed using statistical of package social science (SPSS), computer program.

The results showed that, when the control were compared with the samples the serum level of iron was significantly decreased ( $17.5 \pm 7.527$  versus  $13.8 \pm 6.955$   $\mu\text{mol/L}$ , P-value=0.024) and the plasma level of CRP was significantly increased ( $0.651 \pm 0.608$  versus  $45.4 \pm 110.791$  mg/L, P-value=0.030) in the Sudanese patients under hemodialysis.

Also the findings in this study showed that, there were insignificant differences (increase or decrease) between the levels of iron and CRP according to gender.

The result as follow males versus female.

Iron: ( $14.19 \pm 7.53$  versus  $13.04 \pm 5.72$   $\mu\text{mol/L}$  p=0.550).

CRP: ( $50.18 \pm 118.8$  versus  $35.93 \pm 94.88$  mg/L  $p=0.643$ ).

Person correlation showed that, there were insignificant correlation between duration of dialysis and the levels of iron and CRP.

Iron: ( $r= -0.130$ ,  $p$ -value  $0.324$ ). CRP: ( $r=0.045$ ,  $p$ -value  $0.733$ ).

There was significant week positive correlation between the dose of treatment (Erythropoiesis stimulating agents -ESAs) and the level of iron. ( $r=0.267$ ,  $p$ -value  $0.039$ ).

It is concluded that; the serum level of iron was significantly decrease in the Sudanese patients with renal failure under hemodialysis. And the serum level of CRP was significantly increased in the Sudanese patients with renal failure under hemodialysis. There was no correlation between duration of dialysis and the levels of iron and CRP, while there was significant week positive correlation between the dose of treatment (Erythropoiesis stimulating agents -ESAs) and the level of iron.

## مستخلص البحث

أجريت هذه الدراسة لقياس مدى تأثير الغسيل الدموي على مستويات الحديد و بروتين سي التفاعلي في مرضى الفشل الكلوي. ستون عينة أخذت من هؤلاء المرضى في الفترة مابين شهر ابريل وحتى مايو 2015 تم إختيارهم بطريقة عشوائية من مستشفى الجمعيه التخصصي, مع ثلاثين عينة من الأصحاء كمجموعة ضابطه.

تم قياس المستويات بواسطة إستخدام جهاز الكيمياء السريري هيتاشي\كوباس سي311, وتم تحليل البيانات بواسطة برنامج الحزمة الإحصائية للعلوم الإجتماعية .

توصلت النتائج عند مقارنة مجموعة التحكم مع المرضى في هذه الدراسة إلى ان هناك إنخفاض ملحوظ في مستويات الحديد في المرضى الذين يخضعون للغسيل الدموي ( $7.527 \pm 17.5$  مقابل  $6.955 \pm 13.8$  مايكرو مول/لتر للحديد وكان الإحتمال الإحصائي للمقارنة =0.024) و أن هناك إرتفاع ملحوظ في مستوى بروتين سي التفاعلي ( $0.608 \pm 0.651$  مقابل  $\pm 45.4$  110.79 مايكرو جرام/لتر لبروتين سي التفاعلي وكان الإحتمال الإحصائي للمقارنة =0.030).

وجد أيضا في هذه الدراسة أنه لا يوجد تغيير ملحوظ في مستويات الحديد و بروتين سي التفاعلي تبعاً للجنس وكانت النتائج كالآتي:-

"المتوسط + الانحراف المعياري عند الذكور مقارنة بالاناث"

( $7.53 \pm 14.19$  مقابل  $5.72 \pm 13.4$  مايكرو مول/لتر , الإحتمال الإحصائي للمقارنة = 0.55 للحديد).

( $118.8 \pm 50.18$  مقابل  $94.88 \pm 35.93$  مايكرو جرام/لتر , الإحتمال الإحصائي للمقارنة = 0.643 لبروتين سي التفاعلي) .

معامل بيرسون للإرتباط أوجد أنه ليس هناك علاقه ملحوظه بين الفترة الزمنية للغسيل وبين مستويات الحديد و بروتين سي التفاعلي.

(معامل بيرسون للإرتباط = 0,130 , مستوى المعنويه = 0,324) للحديد.

(معامل بيرسون للإرتباط = 0,045 , مستوى المعنويه = 0,733) لبروتين سي التفاعلي.

وجد أيضا أن هناك علاقه ذات دلالة إحصائية بين كمية الجرعة الدوائية وبين مستويات الحديد حيث كان معامل بيرسون للإرتباط (0,267) و مستوى المعنويه = (0,039).

خلصت هذه الدراسة إلى أن مستويات الحديد يحدث بها نقصان ملحوظ في مرضى الفشل الكلوي الذين يخضعون للغسيل الدموي كما أن مستويات بروتين سي التفاعلي يحدث بها زيادة ملحوظه في مرضى الفشل الكلوي الذين يخضعون للغسيل الدموي. كما وجد أنه ليس هناك علاقه ملحوظه بين الفترة الزمنية للغسيل وبين مستويات الحديد و بروتين سي التفاعلي بينما هناك علاقه ذات دلالة إحصائية بين كمية الجرعة الدوائية وبين مستويات الحديد.

## List of contents

<b>No</b>	<b>Titles</b>	<b>Pages</b>
<b>1</b>	<b>A verse from the Quran</b>	<b>I</b>
<b>2</b>	<b>Dedication</b>	<b>II</b>
<b>3</b>	<b>Acknowledgment</b>	<b>III</b>
<b>4</b>	<b>English abstract</b>	<b>IV</b>
<b>5</b>	<b>Arabic abstract</b>	<b>VI</b>
<b>6</b>	<b>contents</b>	<b>VIII</b>
<b>7</b>	<b>List of Tables</b>	<b>XII</b>
<b>8</b>	<b>List of Figures</b>	<b>XIII</b>
<b>9</b>	<b>Abbreviation</b>	<b>XIV</b>
<b>Chapter One</b>		
<b>1.1</b>	<b>Introduction</b>	<b>1</b>
<b>1.2</b>	<b>Rational</b>	<b>3</b>
<b>1.3</b>	<b>Objective</b>	<b>4</b>
<b>Chapter Two</b>		
<b>2</b>	<b>Literature Review</b>	<b>5</b>
<b>2.1</b>	<b>The Renal</b>	<b>5</b>
<b>2.1.1</b>	<b>Renal Anatomy</b>	<b>5</b>
<b>2.1.2</b>	<b>Renal functions</b>	<b>7</b>

<b>2.1.3</b>	<b>Renal failure</b>	<b>7</b>
--------------	----------------------	----------

<b>2.1.3.1</b>	<b>Acute Renal Failure</b>	<b>7</b>
<b>2.1.3.2</b>	<b>Chronic Renal Failure</b>	<b>8</b>
<b>2.1.3.3</b>	<b>Causes of renal failure</b>	<b>8</b>
<b>2.1.4</b>	<b>Renal function test</b>	<b>9</b>
<b>2.1.4.1</b>	<b>Glomerular Filtration Tests</b>	<b>9</b>
<b>2.1.4.2</b>	<b>Clearance Tests</b>	<b>9</b>
<b>2.1.4.3</b>	<b>Clinical Significance</b>	<b>9</b>
<b>2.1.5</b>	<b>Dialysis</b>	<b>10</b>
<b>2.1.5.1</b>	<b>Hemodialysis (HD)</b>	<b>11</b>
<b>2.1.5.2</b>	<b>Pretoneal dialysis (PD)</b>	<b>11</b>
<b>2.2</b>	<b>Iron</b>	<b>12</b>
<b>2.2.1</b>	<b>Iron component</b>	<b>12</b>
<b>2.2.2</b>	<b>Storage Component</b>	<b>12</b>
<b>2.2.3</b>	<b>Diatary Iron</b>	<b>13</b>
<b>2.2.4</b>	<b>Iron Absorbtion</b>	<b>13</b>
<b>2.2.5</b>	<b>Iron Excretion</b>	<b>14</b>
<b>2.2.6</b>	<b>Relationship between iron and renal diseases</b>	<b>15</b>
<b>2.2.7</b>	<b>Treatment of nemia in people on dialysis</b>	<b>16</b>
<b>2.3</b>	<b>C-reactive protein</b>	<b>16</b>
<b>2.3.1</b>	<b>Relationship between CRP and renal diseases</b>	<b>18</b>

<b>2.3.2</b>	<b>Relationship between CRP and EPO</b>	<b>18</b>
--------------	---	-----------

<b>Chapter three</b>		
<b>3</b>	<b>Materials and Methods</b>	<b>19</b>
<b>3.1</b>	<b>Materials</b>	<b>19</b>
<b>3.1.1</b>	<b>Study design</b>	<b>19</b>
<b>3.1.2</b>	<b>Study area</b>	<b>19</b>
<b>3.1.3</b>	<b>Study population</b>	<b>19</b>
<b>3.1.4</b>	<b>Samples collection and processing</b>	<b>19</b>
<b>3.1.5</b>	<b>Ethical consideration</b>	<b>19</b>
<b>3.1.6</b>	<b>Requirement</b>	<b>20</b>
<b>3.1.7</b>	<b>Reagent and stability</b>	<b>20</b>
<b>3.1.8</b>	<b>Data analysis</b>	<b>20</b>
<b>3.1.9</b>	<b>Data collection</b>	<b>20</b>
<b>3.2</b>	<b>Methods</b>	<b>21</b>
<b>3.2.1</b>	<b>Estimation of iron</b>	<b>21</b>
<b>3.2.2</b>	<b>Estimation of iron</b>	<b>21</b>
<b>3.3</b>	<b>Quality control</b>	<b>21</b>
<b>Chapter four</b>		
<b>4</b>	<b>Results</b>	<b>22</b>
<b>Chapter five</b>		
<b>5</b>	<b>Discussion, Conclusion and Recommendation</b>	<b>28</b>
<b>5.1</b>	<b>Discussion</b>	<b>28</b>

<b>5.2</b>	<b>Conclusion</b>	<b>32</b>
<b>5.3</b>	<b>Recommendation</b>	<b>33</b>

	<b>References</b>	<b>34</b>
	<b>Appendix I</b>	<b>38</b>
	<b>Appendix II</b>	<b>39</b>
	<b>Appendix III</b>	<b>42</b>

### **List of tables:**

<b>No</b>	<b>Description</b>	<b>page</b>
<b>2.1</b>	Stages of chronic kidney disease	<b>10</b>
<b>4-1</b>	Age and gender of patients with renal failure disease	<b>24</b>
<b>4-2</b>	The mean of plasma iron and CRP in both study groups	<b>24</b>
<b>4-3</b>	Comparison of the mean of plasma iron and CRP concentration in male and female	<b>24</b>



## **List of figures:**

<b>No</b>	<b>Description</b>	<b>Page</b>
<b>2.1</b>	The kidney	<b>6</b>
<b>2.2</b>	Dietary iron	<b>15</b>
<b>4-1</b>	Scatter plot shows the correlation between iron level and duration of dialysis	<b>25</b>
<b>4-2</b>	Scatter plot shows the correlation between CRP level and duration of dialysis	<b>26</b>
<b>4-3</b>	Scatter plot shows the correlation between iron level and the dose of Erythropoietin	<b>27</b>

## Abbreviations

<b>Abbreviation</b>	<b>Full term</b>
<b>ARF</b>	Acute renal failure
<b>CKD</b>	chronic kidney disease
<b>CRP</b>	C-reactive protein
<b>CRF</b>	Chronic renal failure
<b>EPO</b>	Erythropoietin
<b>ESAs</b>	Erythropoiesis stimulating agents
<b>Fe</b>	Iron
<b>GFR</b>	Glomerular filtration rate
<b>HD</b>	Hemodialysis
<b>PD</b>	Peritoneal dialysis

# **Chapter 1**

## **Introduction**

## **1.1-Introduction**

The kidneys are two bean-shaped organs, each about the size of fist. They are located just below the rib cage, one on each side of the spine. Every day, the two kidneys filter about 120-150 quarts of blood to produce about 1-2 quarts of urine, composed of wastes and extra fluid. (Rockville et al, 2009).

Renal failure exists in both acute and chronic forms. The progression to end-stage renal disease is characterized by a marked decrease in the glomerular filtration rate patients may present with many different symptoms relating to the particular disorder involved; oliguria, edema, and azotemia. However, supportive renal dialysis may be required to maintain patients until the inflammation subsides. (Susan et al, 2008).

Iron is a mineral that our bodies need for many functions. For example, iron is part of hemoglobin, a protein which carries oxygen from our lungs throughout our bodies. It helps our muscles store and use oxygen. Iron is also part of many other proteins and enzymes.

The body needs the right amount of iron. If the body has too little iron, they may develop iron deficiency anemia. Causes of low iron levels include blood loss, poor diet, or an inability to absorb enough iron from foods. People at higher risk of having too little iron are young children and women who are pregnant or have periods.

Too much iron also can damage the body. Taking too many iron supplements can cause iron poisoning. Some people have an inherited

disease called hemochromatosis. It causes too much iron to build up in the body. ( Rockville, 2015).

Anemia commonly occurs in people with chronic kidney disease (CKD)—the permanent, partial loss of kidney functions. Anemia might begin to develop in the early stages of CKD, when someone has 20 to 50 percent of normal kidney function. (Brugnara et al, 2011).

Acute phase protein molecules that are normally present in serum. The serum concentration of a number of these proteins increases rapidly during infection and they are therefore called acute phase proteins. One example of an acute phase protein is C reactive protein (CRP), so-called because of its ability to bind to the C protein of pneumococci. This promotes the uptake of pneumococci by phagocytes, a process known as opsonization. (David et al, 2007).

CRP appears to be a risk marker for renal function loss. The mechanism of this relationship remains to be clarified. Elevated CRP was positively associated with diminished filtration. (Kannel, 2000).

## **1.2-Rationale:**

Renal failure is a devastating medical, social and economic problem in Sudan and it is fatal unless treated properly. Recent studies were done in Sudan to determine the mortality. According to the latest WHO data published in April 2013 Kidney diseases deaths in Sudan reached 8,782 or 2.38% of total deaths. The age adjusted death rate 42.39 per 100.000 of population ranks Sudan in 49<sup>th</sup> in the world. (Sudan health profile, 2015).

Low iron levels are referred to as “iron deficiency” and can lead to anemia in people with chronic kidney disease (CKD). Some of the common causes of low iron levels in dialysis patients are:

- Blood loss because of bleeding from the access, surgery, frequent blood tests or remaining blood in blood lines.
- Poor absorption of iron from food in the intestinal tract.
- Not eating enough high iron foods because of poor appetite. (Allen et al, 1999).

There is a strong relationship between renal failure and anemia and high rate of C-reactive protein (CRP). Some researchers have assumed that there is a correlation between the iron and CRP levels and the severity of renal failure caused by loss of function and diminished filtration. From this points many studies around the world were done and showed that serum iron levels are lower and serum CRP are higher under hemodialysis and returned that to many causes but the mechanism of this relationship remains to be clarified. (Abraham et al, 2015).

This study was conducted to highlight the effect of hemodialysis on iron and CRP in Sudanese patients with renal failure under hemodialysis.

### **1.3-Objectives:**

#### **-General objectives:**

To study the effect of hemodialysis on iron and CRP in plasma of Sudanese patients with renal failure.

#### **-Specific objectives:**

1-To assess the level of iron and CRP in plasma of Sudanese patients with renal failure under hemodialysis compared to control group.

2-To correlate between the duration of dialysis and iron and CRP in Sudanese patients with renal failure under hemodialysis.

3-To study the correlation between the dose of treatment (2 units, 3 units or 4 units) and iron in Sudanese patients with renal failure under hemodialysis.

# **Chapter2**

## **Literature Review**



## **2-Literature Review**

### **2.1-The renal:**

The kidneys are vital organs that perform a variety of important functions. The most prominent functions are removal of unwanted substances from plasma, homeostasis of the body's water, electrolyte and acid-base status, and participation in hormonal regulation. In the clinical laboratory, kidney function tests are used in assessment of renal disease, Water balance, acid-base disorders and in situations of trauma, head injury, surgery and infectious disease. (kara et al, 2010).

#### **2.1.1-Renal Anatomy:**

The kidneys are paired, bean-shaped organs located retroperitoneally on either side of the spinal column. Macroscopically, a fibrous capsule of connective tissue encloses each kidney. When dissected longitudinally, two regions can be clearly discerned—an outer region called the cortex and an inner region called the medulla. The pelvis can also be seen. It is a basinlike cavity at the upper end of the ureter into which newly formed urine passes. The bilateral ureters are thick-walled canals, connecting the kidneys to the urinary bladder. Urine is temporarily stored in the bladder until voided from the body by way of the urethra. Nephrons are the functional units of the kidney that can only be seen microscopically. Each kidney contains approximately 1 million nephrons. Each nephron is a complex apparatus comprised of five basic parts. (Kara et al, 2010).

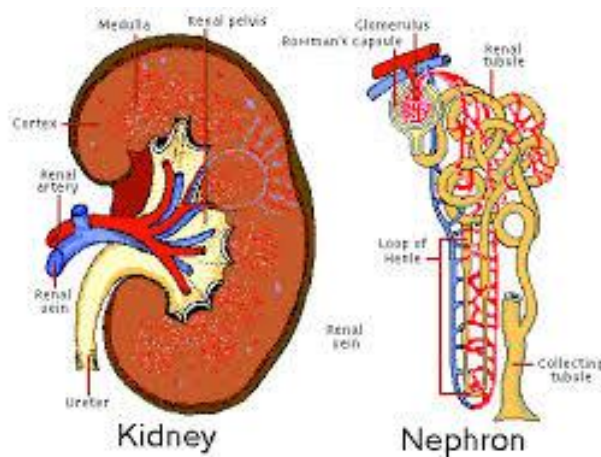


Fig (2.1): The kidney.

- The glomerulus—a capillary tuft surrounded by the expanded end of a renal tubule known as Bowman's capsule. Each glomerulus is supplied by an afferent arteriole carrying the blood in and an efferent arteriole carrying the blood out. The efferent arteriole branches into peritubular capillaries that supply the tubule.
- The proximal convoluted tubule—located in the cortex.
- The long loop of Henle—composed of the thin descending limb, which spans the medulla, and the ascending limb, which is located in both the medulla and the cortex, composed of a region that is thin and then thick.
- The distal convoluted tubule—located in the cortex.
- The collecting duct—formed by two or more distal convoluted tubules as they pass back down through the cortex and the medulla to collect the urine that drains from each nephron. Collecting ducts eventually merge and empty their contents into the renal pelvis.

There are three basic renal processes:

1. Glomerular filtration
2. Tubular reabsorption
3. Tubular secretion. (Kara et al, 2010).

### **2.1.2- Renal functions:**

The basic functioning unit of the kidney is called the nephron. The kidneys together comprise greater than 2 million nephrons, and each is capable of forming urine. The nephron's function is to clean the blood of unwanted substances as it flows past. The nephron is composed of the glomeruli, through which the blood is filtered, and then the tubules, which receive and process the filtered fluid. Kidney function is estimated using the glomerular filtration rate or GFR. This is the amount of filtrate formed in all nephrons. (Marguerite et al, 2014).

It has an endocrine function playing a part in the production of vitamin D and erythropoietin and as part of the renin/angiotensin/aldosterone axis. Healthy kidneys produce a hormone called erythropoietin (EPO). A hormone is a chemical produced by the body and released into the blood to help trigger or regulate particular body functions. EPO prompts the bone marrow to make red blood cells, which then carry oxygen throughout the body. (Cheung et al, 2009).

### **2.1.3-Renal failure:**

#### **2.1.3.1-Acute Renal Failure:**

Acute renal failure is a sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys, defined as occurring when the glomerular filtration rate (GFR) is reduced to less than 10 mL/minute. This syndrome is subdivided into three types, depending on the location of the precipitating defect. (Kara et al, 2010).

- **Prerenal failure:** The defect lies in the blood supply before it reaches the kidney. Causes can include cardiovascular system failure and consequent hypovolemia.

- **Primary renal failure:** The defect involves the kidney. The most common cause is acute tubular necrosis; other causes include vascular obstructions/inflammations and glomerulonephritis.
- **Postrenal failure:** The defect lies in the urinary tract after it exits the kidney. Generally, acute renal failure occurs as a consequence of lower urinary tract obstruction or rupture of the urinary bladder. (Kara et al, 2010).

#### **2.1.3.2-Chronic Renal Failure:**

Chronic renal failure describes abnormal kidney function and /or structure. There is evidence that treatment can prevent or delay the prognosis of chronic kidney disease, reduce or prevent the development of complications, and reduce the risk of cardiovascular disease (CVD).

The chronic renal failure is based on the presence of the kidney damage (i.e. albuminuria) or decreased kidney function (i.e. glomerular filtration rate - GFR) for three months or more, irrespective of clinical diagnosis. (Levey et al, 2012).

#### **2.1.3.3-Causes of renal failure:**

- Poorly controlled diabetes.
- Poorly controlled high blood pressure.
- Chronic glomerulonephritis.
- polycystic kidney disease, reflux nephropathy (damage caused by urine backflow from the bladder into the ureters and kidney) ,Nephrotic syndrome ,kidney stone (Benjamin, 2015)

## **2.1.4-Renal function test:**

### **2.1.4.1-Glomerular Filtration Tests:**

The standard test used to measure the filtering capacity of the glomeruli is the clearance test. As its name implies, a clearance test measures the rate at which the kidneys are able to remove (to clear) a filterable substance from the blood. (Susan et al, 2008).

### **2.1.4.2-Clearance Tests:**

The earliest glomerular filtration tests measured urea because of its presence in all urine specimens and the existence of routinely used methods of chemical analysis. Because approximately 40% of the filtered urea is reabsorbed, normal values were adjusted to reflect the reabsorption, and patients were hydrated to produce a urine flow of 2 mL/min to ensure that no more than 40% of the urea was reabsorbed. At present, the use of urea as a test substance for glomerular filtration has been replaced by the measurement of other substances including *creatinine*, *inulin*, *beta2 microglobulin*, *cystatin C*, or radioisotopes. (Susan et al, 2008).

Currently, routine laboratory measurements of GFR employ creatinine as the test substance. Creatinine, a waste product of muscle metabolism that is normally found at a relatively constant level in the blood, provides the laboratory with an endogenous procedure for evaluating glomerular function. (Susan et al, 2008).

### **2.1.4.3-Clinical Significance:**

When interpreting the results of a creatinine clearance test, the GFR is determined not only by the number of functioning nephrons but also by the functional capacity of these nephrons. In other words, even though

half of the available nephrons may be nonfunctional, a change in the GFR will not occur if the remaining nephrons double their filtering capacity. This is evidenced by persons who lead normal lives with only one kidney. Therefore, although the creatinine clearance is a frequently requested laboratory procedure, its value does not lie in the detection of early renal disease. Instead, it is used to determine the extent of nephron damage in known cases of renal disease, to monitor the effectiveness of treatment designed to prevent further nephron damage, and to determine the feasibility of administering medications, which can build up to dangerous blood levels if the GFR is markedly reduced. (Susan et al, 2008).

**Table 1. Stages of Chronic Kidney Disease**

Stage	Description	GFR (mL/min/1.73 m <sup>2</sup> )
1	Kidney damage with normal or ↑ GFR	≥90
2	Kidney damage with mild ↓ GFR	60-89
3	Moderate ↓ GFR	30-59
4	Severe ↓ GFR	15-29
5	Kidney failure	<15 (or dialysis)

Chronic kidney disease is defined as either kidney damage or GFR <60 mL/min/1.73 m<sup>2</sup> for ≥3 months. Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies.

### 2.1.5-Dialysis:

Dialysis is the process of separating macromolecules from ions and low molecular weight compounds in solution by the difference in their rates of diffusion through a semi permeable membrane. Crystalloids pass readily through this membrane, but colloids pass very slowly or not at all. Two distinct physical processes are involved diffusion and convection. (Michael et al, 2008).

#### **2.1.5.1-Hemodialysis (HD):**

Hemodialysis is the most common method used to treat advanced and permanent kidney failure. Operationally, it involves connecting the patient to a hemodialyzer into which their blood flows, after filtration to remove the wastes and extra fluids, the cleansed blood is returned to the patient. It is a complicated and inconvenient therapy requiring a coordinated effort from a healthcare team. (Michael et al, 2008).

Surgeons can build a fistula, connection a large artery and vein in the body, usually in the arm, that allows a large amount of blood flow into the vein. This makes the vein swell or dilate, and its walls become thicker so that it can tolerate repeated needle sticks to attach tubing from the body to the machine. Since it takes many weeks or months for a fistula to mature enough to be used. Significant planning is required if hemodialysis is to be considered as an option. (Benjamin, 2015).

#### **2.1.5.2-Peritoneal dialysis (PD):**

Peritoneal dialysis is a type of dialysis in which dialysate is instilled into the patient peritoneal cavity with the peritoneum then employed as the dialysis membrane. Continuous ambulatory peritoneal dialysis (CAPD) is now available that is performed in ambulatory patients during normal activities. (Michael et al, 2008).

Peritoneal dialysis differs from hemodialysis, a more commonly used blood-filtering procedure. With peritoneal dialysis, patient can give himself treatments at home, at work or while traveling. Patients may be able to use fewer medications and eat a less restrictive diet than hemodialysis. Peritoneal dialysis isn't an option for everyone with kidney failure. Patients need manual dexterity and the ability to care for himself at home or a reliable caregiver. Peritoneal dialysis may be done to manage kidney failure until a kidney transplant is possible. Kidney failure

itself usually results from a long-term (chronic) disease that causes kidney damage over a number of years. (Mayo Clinic, 2013).

## **2.2-Iron:**

### **2.2.1-Iron component:**

Iron is a mineral that our bodies need for many functions. For example, iron is part of hemoglobin, a protein which carries oxygen from our lungs throughout our bodies. It helps our muscles store and use oxygen. Iron is also part of many other proteins and enzymes. (Rockville et al, 2015).

Iron is a part of heme, which is the active site of electron transport in cytochromes and cytochrome oxygenase, essential coenzymes in the Krebs cycle. Heme is also the site of O<sub>2</sub> uptake by myoglobin and hemoglobin, providing the means of O<sub>2</sub> transport to tissues. Hemoglobin approximately 2 g of body iron of men and 1.5 g in women is in hemoglobin, which is 0.34 percent iron by weight. One mL of packed erythrocytes contains approximately 1 mg of iron. (Anderson et al, 1987).

### **2.2.2-Storage Component:**

Iron is stored either as ferritin or as hemosiderin. The former is water-soluble; the latter is water-insoluble. Ferritin is composed of a core ferrihydrite crystal ( $\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ ) within an apoferritin shell. Ferritin occurs in virtually all cells of the body and also in tissue fluids. In blood plasma ferritin is present in minute concentration. It is largely composed of H monomers. The plasma (serum) ferritin concentration usually correlates with total-body iron stores, which makes this measurement important in the diagnosis of disorders of iron metabolism. Hemosiderin occurs predominantly in macrophages of the monocyte-macrophage system (marrow, liver, and spleen). It can be seen microscopically in



unstained tissue sections or marrow films as clumps or granules of golden refractile pigment. Hemosiderin contains approximately 25 to 30 percent iron by weight. Under pathologic conditions, it may accumulate in large quantities in almost every tissue of the body. Hemosiderin consists of aggregates of ferrihydrite core crystals largely devoid of apoferritin. (Ernest et al, 2000).

### **2.2.3-Dietary Iron:**

The iron content of the diet is variable. An average American male ingests 10 to 20 mg of iron daily. The amount of iron absorbed by a normal adult male need only balance the small amount that is excreted, mostly in the stool, approximately 1 mg per day. A higher iron requirement exists during growth periods or when there is blood loss. In women, iron absorbed must be sufficient to replace that lost through menstruation or diverted to the fetus during pregnancy. Gastric juice stabilizes dietary ferric iron, preventing its precipitation as insoluble ferric hydroxide. (Ernest et al, 2000).

The iron gained by food during cooking or other food processing is in the form of simple inorganic salts or iron-amino acid complexes. Heme, as from hemoglobin and myoglobin, normally comprises about one-third of dietary iron. (Ernest et al, 2000).

### **2.2.4-Iron Absorption:**

Iron is absorbed at the brush border of epithelial cells of the intestinal villi, particularly in the duodenum and upper jejunum. It is absorbed in the form of heme, or as ferric or ferrous ions. In humans little of the heme absorbed by mucosal cells passes directly into plasma. In microsomes, heme oxygenase converts heme to biliverdin, CO, and Fe<sup>+++</sup>. Iron may

also be trapped in ferritin within the epithelial cells of the gastrointestinal tract, thereby preventing its absorption when body iron stores are high.

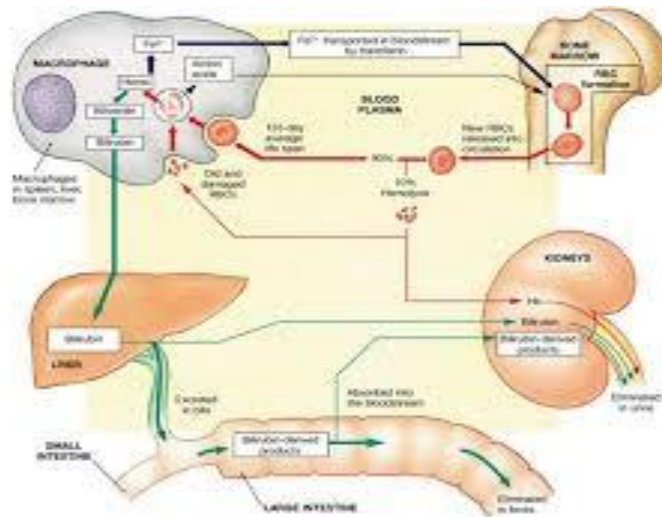
With the passage of time the mucosal cell advances to the tip of the villus, is sloughed and lost in the feces, together with its retained iron. (Ernest et al, 2000).

The absorption of iron is modulated to meet the body's needs: Iron absorption is enhanced when there is chronic liver disease. Bile may facilitate iron absorption among the factors operating outside the alimentary tract to increase iron absorption are hypoxia, anemia, depletion of iron stores, and increased erythropoiesis. Each of these factors appears to exert an independent effect, but it is not known how they “instruct” the bowel to absorb more iron. The degree of transferrin saturation, the plasma iron concentration, the rate of plasma iron clearance, and the plasma erythropoietin concentration have each been considered as humoral messengers. The fine control of the rate of iron absorption may depend on more than one humoral mechanism. (Ernest et al, 2000).

#### **2.2.5-Iron Excretion:**

The body conserves iron with remarkable efficiency: less than a thousandth of it is lost each day, an amount easily replaced if dietary sources are adequate. Almost all this iron loss occurs by way of the feces, and it normally amounts to about 1 mg per day. Exfoliation of skin and dermal appendages results in a much smaller loss, as does perspiration. Even in tropical climates, the loss of iron in sweat is minimal. Iron is excreted also in urine, but in very small amounts. In humans, lactation may cause excretion of about 1 mg iron daily, thus doubling the overall rate of iron excretion. Blood loss by normal menstruation contributes to negative iron balance. (Ernest et al, 2000).

While total daily iron excretion is normally about 1 mg for males and about 2 mg for menstruating women, persons with marked iron overload, as in hemochromatosis, may lose as much as 4 mg of iron daily by these mechanisms, a quantity insufficient to prevent the accumulation of storage iron. (Ernest et al, 2000).



**Fig (2.2): Dietary iron.**

### 2.2.6-Relationship between iron and renal diseases:

When kidneys are diseased or damaged, they do not make enough EPO. As a result, the bone marrow makes fewer red blood cells, causing anemia. When blood has fewer red blood cells, it deprives the body of the oxygen it needs. Other common causes of anemia in people with kidney disease include blood loss from hemodialysis and low levels of the following nutrients found in food:

- iron
- vitamin B12
- folic acid

These nutrients are necessary for red blood cells to make hemoglobin, the main oxygen-carrying protein in the red blood cells.

If treatments for kidney-related anemia do not help, the health care provider will look for other causes of anemia, including:

- Other problems with bone marrow
- Inflammatory problems—such as arthritis, lupus, or inflammatory bowel disease—in which the body's immune system attacks the body's own cells and organs.
- Chronic infections such as diabetic ulcers.
- Malnutrition. (National Kidney Foundation, 2015).

#### **2.2.7-Treatment of anemia in people on dialysis:**

In people on dialysis, anemia is treated with:

- Drugs called erythropoiesis stimulating agents (ESAs). ESAs replace the EPO that is low in people with kidney failure, so they can make red blood cells.
- Extra iron. Diet alone cannot supply enough iron to meet body needs. Extra iron will likely be necessary. In fact, once patients start taking ESAs, he will make more red blood cells and his iron supply will be used up faster.
- The best way to get extra iron is IV through the dialysis machine. (National Kidney Foundation, 2015).

#### **2.3-C-Reactive Protein:**

C-reactive protein (CRP) is synthesized in the liver and is one of the first acute-phase proteins to rise in response to inflammatory disease. CRP rises sharply whenever there is tissue necrosis, whether the damage originates from a pneumococcal infection or some other source. CRP bound to bacteria and fungi promotes the binding of complement, which facilitates their uptake by phagocytes. This protein-coating process to

enhance phagocytosis is known as opsonization. Furthermore, interventions such as weight loss, diet, exercise, and smoking cessation and administration of pharmacologic agents such as statins all lead to reduced CRP levels. Most studies to date have focused on heart disease, but new research shows that having CRP in the high-normal range may also be associated with other diseases such as colon cancer, complications of diabetes, obesity, and the risk of developing type 2 diabetes. C reactive protein content of serum also is mildly elevated in older individuals without an apparent inflammatory process. (Lynda et al, 2010).

Individuals with CRP levels greater than 3 mg/L have a risk of the development of diabetes 4 to 6 times higher than that of individuals with lower levels of CRP. Part of the link between heart disease and diabetes is due to inflammation. CRP is not specific but does have value as a general indicator. Normally, there are minimal levels of CRP in blood. A high or increasing amount of CRP suggests an acute infection or inflammation. Although a result above 1 mg/L is usually considered high for CRP, most infections and inflammations result in CRP levels above 10 mg/L. In cases of inflammatory rheumatic diseases, such as rheumatoid arthritis and SLE, the CRP test is used to assess the effectiveness of a specific arthritis treatment and monitor periods of disease eruption. However, even in known cases of inflammatory disease, a low CRP level is possible and is not indicative of absence of inflammation. (Lynda et al, 2010).

It is significantly elevated in acute rheumatic fever, bacterial infections, myocardial infarctions, rheumatoid arthritis, carcinomatosis, gout, and viral infections. CRP is generally measured by immunologic methods, including nephelometry and EIA. The traditional methods have a sensitivity of approximately 3-5 mg/L. (Lynda et al, 2010).

### **2.3.1-Relationship between CRP and renal diseases:**

CRP is a significant predictor of death in chronic dialysis patients, independent of serum albumin and other possible confounders. Dialysis patients with high CRP levels should be carefully evaluated and monitored regardless of serum albumin concentrations in the normal range. (European Renal Association, 2015).

### **2.3.2- Relationship between CRP and Erythropoietine:**

Inflammation is one of the major causes of resistance to erythropoietin (EPO) treatment. In the present study, the relationship between serum C-reactive protein (s-CRP) and the dose of recombinant human EPO required to maintain hemoglobin levels at approximately 12 g/dL was analyzed in 30 hemodialysis patients. The weekly EPO dose in patients with s-CRP  $\geq 20$  mg/L was, on average, 80% higher than in patients with s-CRP less than 20 mg/L. The EPO doses and s-CRP were both inversely correlated to the levels of serum albumin and serum iron, suggesting that the principal mechanism by which inflammatory cytokines inhibit erythropoiesis is coupled to iron metabolism, ie, functional iron deficiency. Our results demonstrate the usefulness of s-CRP as a predictor of resistance to EPO treatment. (peter et al, 1999).

# **Chapter 3**

## **MATERIALS AND METHODS**

### **3-Materials and methods**

#### **3.1-Materials:**

**3.1.1-Study design:** This is a case control study.

**3.1.2-Study area:** This study was conducted in Association specialized hospital in Khartoum/states, Bahri/local.

#### **3.1.3-Study population;**

This study included 60 Sudanese patients with renal failure in Khartoum state under hemodialysis, during the period from April to May 2015.

**Inclusion criteria:** Sudanese patients with renal failure under hemodialysis and apparently healthy volunteers were included.

**Exclusion criteria:** Patients with hepatitis positive were excluded.

#### **3.1.4-Samples collection and processing:**

Blood sample from all participants were taken by vein puncture using disposable syringes from each specimen 3ml. The blood sample transferred in to anticoagulant container (lithium heparin). Plasma was separated from blood cells after centrifugation for 5 minutes at 5000 r.p.m, at room temperature.

#### **3.1.5-Ethical consideration:**

Consent was taken regarding acceptance to participate in the study and reassurance of confidentiality. Before the specimen was collected, the donor knew that this sample was collected for research purpose.



### **3.1.6-Requirement:**

- Sterile needle.
- 70% alcohol.
- Cotton.
- Plan and heparinize container.
- Constant temperature
- Cuvette.
- Desktop centrifuge.
- Cobas system (Spectrophotometer).

### **3.1.7-Reagent and stability:**

**Iron reagent and stability:** The reagent and standard are provide ready to use and are stable up to 6 weeks if store at 2-8 °C .

**CRP reagent and stability:** The reagent and standard are provide ready to use and are stable up to 12 weeks if store at 2-8 °C.

### **3.1.8-Data analysis:**

Differences in means were tested with an independent T test. The SPSS (Statistical Package for Social Sciences) were used for all statistical analysis and value 0.05 considered significant ( $p \text{ value} \leq 0.05$ ).

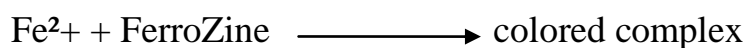
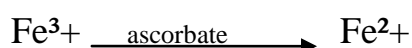
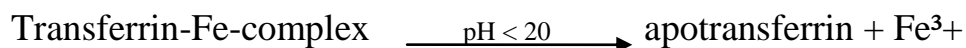
### **3.1.9-Data Collection:**

The clinical data were obtained from history; clinical examination and hospital follow up records and were recorded on a questionnaire sheet.

### 3.2-Method

#### 3.2.1- Estimation of iron: (Appendix II).

**Principle of the reaction:** Method used for determined iron is based on formation of color complex between iron in form of ( $\text{Fe}^{2+}$ ) and FerroZine under acidic conditions.



#### 3.2.2- Estimation of C-reactive protein: (Appendix III).

**Principle of the reaction:** Humane CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

#### 3.3-Quality control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it application for the measurement of test and control samples.

# **Chapter 4**

## **Results**

## 4- Results

The results of the biochemical parameters of plasma iron and CRP in patients with renal failure are given in Tables and Figures:

**Table (4-1)** Illustrates the sex, ages and family history of patients with renal failure. The result showed that the patients whose ages over Fifty Five years were more susceptible for renal failure with the percentage of (74.4%) compared to those with age below Fifty years (25.6%).

The number of male patients was 40 (66.6%), while the numbers of females was 20 (33.3%).

Patients whose have family history of disease constitute (34%) while that has no family history of disease constitute (66%).

**Table (4-2)** Represents the mean of the levels of plasma iron and CRP in both study groups.

The level of plasma iron was significant decrease in patients with renal failure compared to control group, (mean  $\pm$  SD:  $13.8 \pm 6.955 \mu\text{mol/L}$  versus  $17.5 \pm 7.527 \mu\text{mol/L}$ , P-value 0.024).

The level of plasma CRP was significant increase in patient with renal failure compared to control group, (mean  $\pm$  SD:  $45.4 \pm 110.791 \text{ mg/L}$  versus  $0.651 \pm 0.608 \text{ mg/L}$ , P-value 0.030 ).

**Table (4-3)** shows insignificant difference in the mean of the levels of the plasma iron and CRP in male patients with renal failure and the mean of the levels of the plasma iron and CRP in female patients with renal failure.

Iron: (mean  $\pm$  SD  $14.19 \pm 7.53$  versus  $13.04 \pm 5.72 \mu\text{mol/L}$  p=0.550).

CRP: (mean  $\pm$  SD  $50.18 \pm 118.8$  versus  $35.93 \pm 94.88 \text{ mg/L}$  p=0.643).

**Figure (4-1)** a scatter plot shows the correlation between duration of dialysis (months) and iron level. Showed no correlation between iron level and increase duration of dialysis ( $r = -0.130$ ,  $p$ -value 0.324).

**Figure (4-2)** a scatter plot shows the correlation between duration of dialysis (months) and CRP level. Showed no correlation between CRP and increase duration of dialysis ( $r = 0.045$ ,  $p$ -value 0.733).

**Figure (4-3)** a scatter plot shows the correlation between the dose of treatment and iron level. Showed significant correlation between iron and the dose of Erythropoiesis stimulating agents ( $r = 0.267$ ,  $p$ -value 0.039).

**Table (4-1)**

Age, gender and family history of patients with renal failure:

<b>Variable</b>	<b>Number</b>	<b>%</b>
Age 30-55 years	42	70
Age 56-80 years	18	30
Sex Male	40	66.6
Sex Female	20	33.3
Family history disease (Yes)	21	34
Family history disease (No)	39	66

**Table (4-2)**

The mean of plasma iron and CRP in both study groups:

<b>variables</b>	<b>Case Mean <math>\pm</math> SD</b>	<b>Control Mean <math>\pm</math> SD</b>	<b>P-value</b>
Iron ( $\mu\text{mol/L}$ )	13.8 $\pm$ 6.955	17.5 $\pm$ 7.527	0.024
CRP (mg/L)	45.4 $\pm$ 110.791	0.651 $\pm$ 0.608	0.030

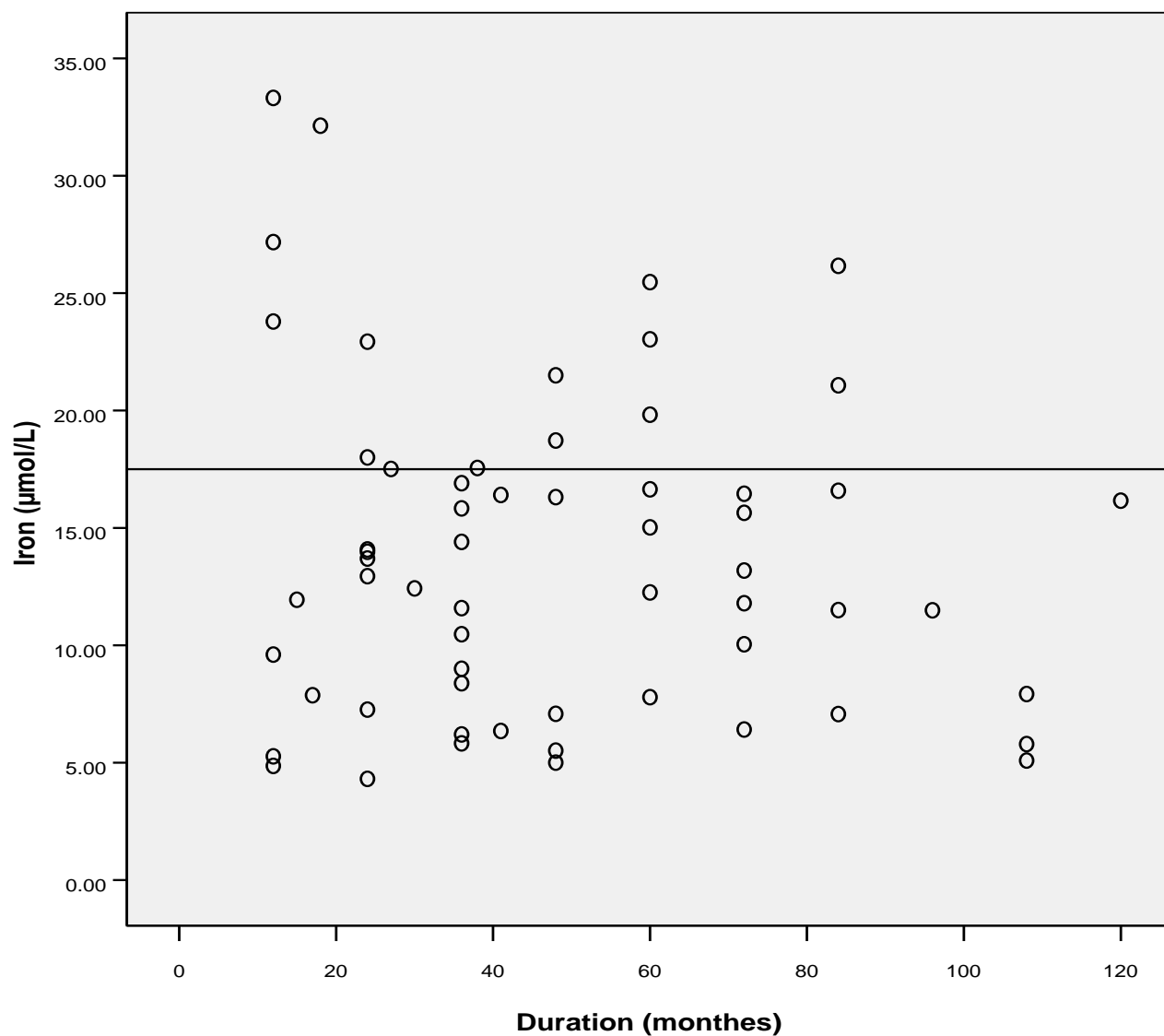
- Results given in mean  $\pm$  SD.
- P-value  $\leq$  0.05 consider significant.

**Table (4-3)**

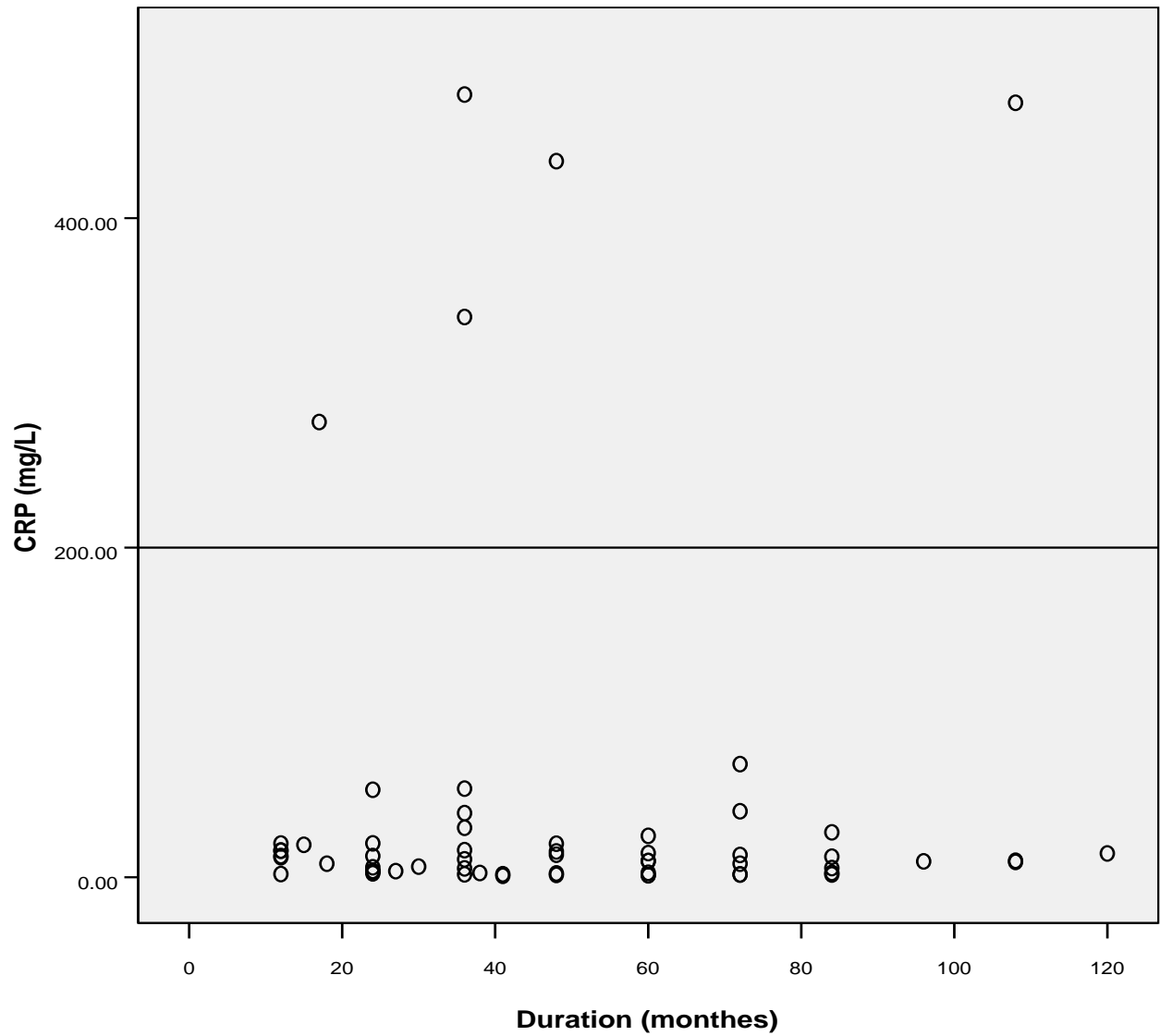
Comparison of the mean of plasma iron and CRP concentration in male and female:

<b>variables</b>	<b>Male Mean <math>\pm</math> SD</b>	<b>Female Mean <math>\pm</math> SD</b>	<b>P-value</b>
Iron ( $\mu\text{mol/L}$ )	14.19 $\pm$ 7.53	13.04 $\pm$ 5.72	0.550
CRP (mg/L)	50.18 $\pm$ 118.8	35.93 $\pm$ 94.88	0.643

- Results given in mean  $\pm$  SD.
- P-value  $\leq$  0.05 consider significant.

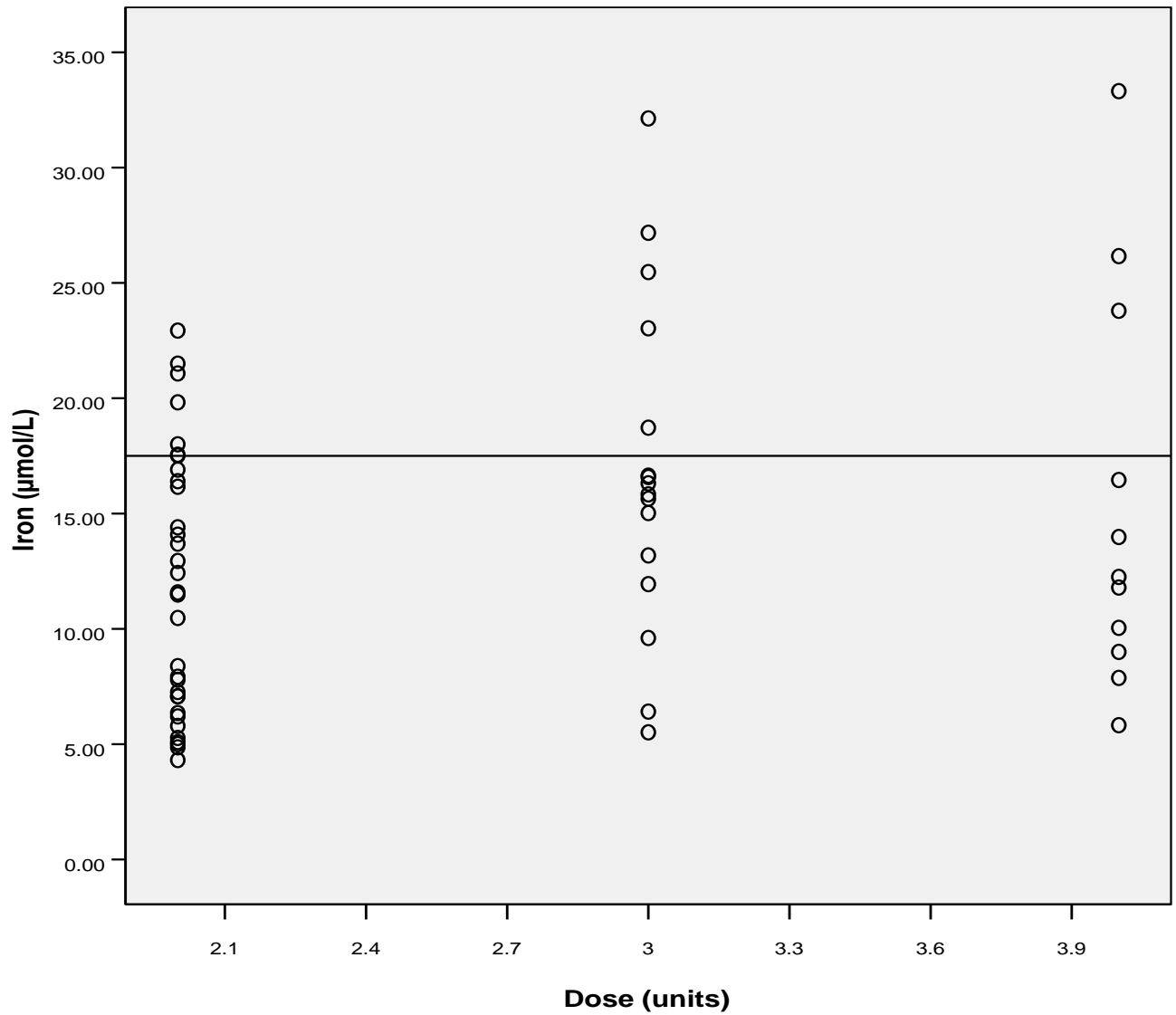


**Figure (4.1):** Scatter plot between serum iron level (μmol/L) and duration of dialysis (months).  $r = -0.130$ ,  $p\text{-value} = 0.324$



**Figure (4-2):** Scatter plot between serum CRP (mg/L) level and duration of dialysis (months).  $r=0.045$ ,  $p$ -value 0.733.





**Figure (4-3):** Scatter plot between serum iron level ( $\mu\text{mol/L}$ ) and the dose of treatment (ESAs) Erythropoiesis stimulating agents (2, 3, 4 units).  
 $r=0.267$ ,  $p\text{-value } 0.039$ .

# **Chapter 5**

## **Discussion, Conclusion and Recommendations**

## **5-Discussion, Conclusion and Recommendation:**

### **5.1-Discussion:**

Kidney failure is a condition in which the kidney fails to remove metabolic end product from the blood, so when kidney failure is reached the end stage dialysis is needed. (Benjamin, 2015).

Hemodialysis affects many substances in the body by increasing, decreasing or removing them. This study conducted to explore the effect of hemodialysis on levels of iron and CRP.

Preliminary investigation and findings obtained from specially designed questionnaire revealed that the majority of patients under dialysis participated in this study in the average ages of about 55 years. This agreed with previous published results of studeis cared by coresh, whose finding confirmed that after the age of 30 years, glumerular filtration rate (GFR) progressively declines at an average rate of 8 mL/min/173 m<sup>2</sup> per decade, and the risk of renal failure increased with age (coresh et al, 2003). This result also was reported by; (Christian, 2014), which showed that the average age of British person with renal failure is 77 years.

Sex distribution in patients under hemodialysis of this study revealed that 66.6% were males. This agreed with the previous study which documented in the field of nephrology, showed that women seem to be somewhat protected from developing end stage renal failure; the cumulative incidence of the disease remains low during the reproductive ages and begins to rise 10 years later. (Iseki, 1996).

Social clinical history index of patients under the study indicate that positive family history of renal failure of first degree relatives found to be in 34% of cases. This finding may indicate that hereditary play role in the pathogenesis of renal failure patients. This result agree with previous

study results showed that, there is a high prevalence of family history-end stage renal disease among US population, about 23%. (William et al, 2007).

Other study showed the same result also; family history of renal disease is one of the most important risk factors associated with development of nephropathy. (Scott et al, 2005).

In this study the comparison of level of iron between case and control participants in this study showed that significant decrease of level of iron in patients with renal failure under hemodialysis when compared with control. ( $13.8 \pm 6.955 \mu\text{mol/L}$  versus  $17.5 \pm 7.527 \mu\text{mol/L}$ , p-value 0.024). This result was agreed with result carried by many authors, whose finding confirmed that, there was significantly reduction in the level of iron in patients with renal failure compared to control group, (Stauffer et al, 2014). The National Health and Nutrition Examination Survey (NHANES) study showed that the prevalence of anemia increases as glomerular filtration rate falls. Data collected in 2007-2010 showed that anemia was twice as prevalent in people with Chronic Kidney Diseases (15.4%) as it was in the general population (7.6%). The prevalence increased with the stage of Chronic Kidney Diseases, from (8.4%) at stage 1 to (53.4%) at stage 5.

Also the result agreed with previous study which found that, the iron deficiency is also common in patients with chronic kidney disease (CKD). The iron deficiency may be absolute, often due to poor dietary intake or sometimes occult bleeding, or functional, when there is an imbalance between the iron requirements of the erythroid marrow and the actual iron supply. Iron deficiency leads to a reduction in formation of red cell haemoglobin, causing hypochromic microcytic anaemia. (Singh et al, 1999).

Also the result agreed with another result which confirmed that, the causes of anaemia in chronic kidney disease include the presence of uraemic inhibitors (eg, parathyroid hormone, inflammatory cytokines), reduced half-life of circulating blood cells and deficiencies of folate or vitamin B12. ( Singh et al, 1999).

Studies of patients with Chronic Kidney Disease have shown that the prevalence of anemia (defined as a haemoglobin level less than 12 g/dL in men and postmenopausal women and less than 11 g/dL in premenopausal women) is about (12%). (NICE Clinical Guideline, 2011).

The findings of this study showed that, there was significant increase in level of CRP in patients with renal failure compared with control group. ( $45.4 \pm 110.791$  mg/L versus  $0.651 \pm 0.608$  mg/L, P-value 0.030). This result was in agreement with finding done by (Edith et al; 2003).The confirmed that, CRP was significant increased in patients with renal failure. The official journal of the international society of nephrology result found that, there was significantly increased level of CRP in patients with renal failure.

In this study the results showed that there were insignificant differences between iron and CRP according to gender. The finding of this study showed that there was insignificant correlation between the duration of dialysis and Iron level, in figure (4-1), ( $r=-0.130$ , p-value- 0.324).

The result was disagreement with another research which showed that, the long-term hemodialysis patients there is a variety of factors make massive iron overload of various organs a likely occurrence, severe hepatosplenic siderosis may occur in marrow-iron-depleted patients, and serum ferritin levels in this setting may not always accurately reflect the status of marrow iron store.(JAMA, 1980).

Also in this study as appeared in figures (4-2), which showed insignificant correlation between duration of dialysis and CRP level. ( $r=0.045$ ,  $p$ -value 0.733).

This result was disagreed with another result carried by (peter et al, 1996), which found that there was correlation between duration of dialysis and CRP level.

The finding of this study showed that, there was significant correlation between the dose of treatment (EPO) and iron level, in figure (4-3), ( $r=-0.267$ ,  $p$ -value 0.039). This result is similar to another result which showed that, there was significant correlation between Erythropoietin (EPO) and iron level. (National kidney foundation, 2015).

## **5.2-Conclusion:**

From the results and findings of this study, it is concluded that:

- 1-Iron is significantly decreased in the blood of patients with renal failure.
- 2-CRP is significantly increased in the blood of patients with renal failure.
- 3-The duration of dialysis has an effect on the concentration of iron and CRP.
- 4-The dose of the treatment has an effect on the concentration of iron.

### **5.3-Recommendation**

It is recommended that:

- Patients with renal failure should receive iron supplement and Erythropoietin to avoid anemia.
- Uptake of adequate iron in rich nutrition.



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# Appendixes

## Appendix I

### Questioner

Topic: the effect of hemodialysis on the rate of iron and CRP in patients with renal failure.

Hospital:.....

-Name: .....

-Sex: .....

Age: .....

-Type of dialysis:   Heamodialysis       .....

                                  Pretonialdialysis       .....

-Family history of diseases    -Yes ..... -No.....

-Duration of dialysis: .....

-The dosage of treatment: .....

-Any other diseases or inflammation: .....

-Investigation:

-Iron ..... $\mu$ mol/L.

-CRP .....mg/L.

# CRPHS

Cardiac C-Reactive Protein (Latex) High Sensitive

cobas®

• Indicates cobas c systems on which reagents can be used

## Order information

Cardiac C-Reactive Protein (Latex) High Sensitive

300 tests	Cat. No. <b>04628918</b> 190
Calibrator f.a.s. Proteins (5 x 1 mL)	Cat. No. <b>11355279</b> 216
Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Cat. No. <b>11355279</b> 160
CRP T Control N (5 x 0.5 mL)	Cat. No. <b>20766321</b> 322
Precinorm Protein (3 x 1 mL)	Cat. No. <b>10557897</b> 122
Precinorm Protein (3 x 1 mL, for USA)	Cat. No. <b>10557897</b> 160
Diluent NaCl 9 % (50 mL)	Cat. No. <b>04489357</b> 190

System-ID 07 6866 9
Code 656
Code 656
Code 235
Code 302
Code 302
System-ID 07 6869 3

Roche/Hitachi cobas c systems

cobas c 311 cobas c 501/502

• •

## English

### System information

For cobas c 311/501 analyzers:

CRPHS: ACN 217

For cobas c 502 analyzer:

CRPHS: ACN 8217

### Intended use

In vitro test for the quantitative determination of C-reactive protein (CRP) in human serum and plasma on Roche/Hitachi cobas c systems. Measurement of CRP is of use for the detection and evaluation of inflammatory disorders and associated diseases, infection and tissue injury. Highly sensitive measurement of CRP may also be used as an aid in the assessment of the risk of future coronary heart disease. When used as an adjunct to other laboratory evaluation methods of acute coronary syndromes, it may also be an additional independent indicator of recurrent event prognosis in patients with stable coronary disease or acute coronary syndrome.

### Summary<sup>1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21</sup>

C-reactive protein is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains that form a five-member ring having a molecular weight of 105000 Daltons. CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. Complexed CRP activates the complement system beginning with C1q. CRP then initiates opsonization and phagocytosis of invading cells, but its main function is to bind and detoxify endogenous toxic substances produced as a result of tissue damage.

CRP assays are used to detect systemic inflammatory processes (apart from certain types of inflammation such as SLE and Colitis ulcerosa); to assess treatment of bacterial infections with antibiotics; to detect intrauterine infections with concomitant premature amniorrhexis; to differentiate between active and inactive forms of disease with concurrent infection, e.g. in patients suffering from SLE or Colitis ulcerosa; to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy; to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to distinguish between infection and bone marrow transplant rejection.

Sensitive CRP measurements have been used and discussed for early detection of infection in pediatrics and risk assessment of coronary heart disease. Several studies came to the conclusion that the highly sensitive measurement of CRP could be used as a marker to predict the risk of coronary heart disease in apparently healthy persons and as an indicator of recurrent event prognosis. Increases in CRP values are non-specific and should not be interpreted without a complete clinical history. The American Heart Association and the Centers for Disease Control and Prevention have made several recommendations concerning the use of high sensitivity C-Reactive Protein (hsCRP) in cardiovascular risk assessment.<sup>21</sup> Testing for any risk assessment should not be performed while there is an indication of infection, systemic inflammation or trauma. Patients with persistently unexplained hsCRP levels above 10 mg/L (95.2 nmol/L) should be evaluated for non-cardiovascular etiologies. When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values. Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Screening the entire adult population for hsCRP is not recommended, and hsCRP is not a substitute for traditional cardiovascular risk factors. Acute coronary

syndrome management should not depend solely on hsCRP measurements. Similarly, application of secondary prevention measures should be based on global risk assessment and not solely on hsCRP measurements. Serial measurements of hsCRP should not be used to monitor treatment.

Various assay methods are available for CRP determination, such as nephelometry and turbidimetry. The Roche CRP assay is based on the principle of particle-enhanced immunological agglutination.

### Test principle<sup>22,23</sup>

Particle enhanced immuno-turbidimetric assay.

Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

### Reagents - working solutions

**R1** TRIS buffer with bovine serum albumin and immunoglobulins (mouse); preservative; stabilizers

**R2** Latex particles coated with anti-CRP (mouse) in glycine buffer; preservative; stabilizers

### Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

### Reagent handling

Ready for use.

Mix cobas c pack well before placing on the analyzer.

### Storage and stability

#### CRPHS

Shelf life at 2-8 °C: See expiration date on cobas c pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

#### Diluent NaCl 9 %

Shelf life at 2-8 °C: See expiration date on cobas c pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

### Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin and K<sub>2</sub>-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability: <sup>24</sup>	11 days at 15-25 °C
	2 months at 2-8 °C
	3 years at (-15)-(-25) °C





# CRPHS

Cardiac C-Reactive Protein (Latex) High Sensitive

## Materials provided

See "Reagents - working solutions" section for reagents.

## Materials required (but not provided)

See "Order information" section.

General laboratory equipment

## Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum and plasma

### cobas c 311 test definition

Assay type	Rate A
Reaction time / Assay points	10/7-57
Wavelength (sub/main)	- /546 nm
Reaction direction	Increase
Units	mg/L (nmol/L, mg/dL)
Reagent pipetting	Diluent (H <sub>2</sub> O)
R1	82 µL 42 µL
R2	28 µL 20 µL
<b>Sample volumes</b>	<b>Sample</b> <b>Sample dilution</b>
	<b>Sample</b> <b>Diluent (NaCl)</b>
Normal	6 µL - -
Decreased	6 µL 10 µL 140 µL
Increased	12 µL - -

### cobas c 501/502 test definition

Assay type	Rate A
Reaction time / Assay points	10/12-70
Wavelength (sub/main)	- /546 nm
Reaction direction	Increase
Units	mg/L (nmol/L, mg/dL)
Reagent pipetting	Diluent (H <sub>2</sub> O)
R1	82 µL 42 µL
R2	28 µL 20 µL
<b>Sample volumes</b>	<b>Sample</b> <b>Sample dilution</b>
	<b>Sample</b> <b>Diluent (NaCl)</b>
Normal	6 µL - -
Decreased	6 µL 10 µL 140 µL
Increased	12 µL - -

## Calibration

Calibrators	S1: H <sub>2</sub> O S2: C.f.a.s. Proteins Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the six-point calibration curve: S2: 0.0125 S5: 0.100 S3: 0.0250 S6: 0.200 S4: 0.0500
Calibration mode	Line Graph
Calibration frequency	Full calibration - after reagent lot change - and as required following quality control procedures

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).<sup>25</sup>

cobas c systems

cobas®

## Quality control

For quality control, use control materials as listed in the "Order information" section.

Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors: mg/L x 9.52 = nmol/L  
mg/L x 0.1 = mg/dL

## Limitations - interference<sup>26</sup>

Criterion: Recovery within ± 10 % of initial values at CRP levels of 5.0 mg/L.

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L (1000 mg/dL)).

Lipemia (Intralipid): No significant interference up to an L index of 600. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 1200 IU/mL do not interfere.

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>27,28</sup>

High dose hook-effect: No false result occurs up to a CRP concentration of 1000 mg/L.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Although measures were taken to minimize interference caused by human anti-mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. The latest version of the Carry over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1 + 2/SCCS Method Sheets. For further instructions refer to the operator manual.

**cobas c 502 analyzer:** All special wash programming necessary for avoiding carry over is available via the cobas link, manual input is not required.

**Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.**

## Limits and ranges

### Measuring range

0.15-20.0 mg/L (1.43-190 nmol/L, 0.015-2.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:15 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 15.

### Lower limits of measurement

#### Lower detection limit of the test

0.15 mg/L (1.43 nmol/L, 0.015 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

### Functional sensitivity

0.3 mg/L (2.96 nmol/L, 0.03 mg/dL)

The functional sensitivity is the lowest CRP concentration that can be reproducibly measured with an inter-assay coefficient of variation of < 10 %.

2 / 4

2010-03, V 5 English

# CRPHS

Cardiac C-Reactive Protein (Latex) High Sensitive

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## Expected values

Consensus reference interval for adults:<sup>29</sup>

IFCC/CRM 470

mg/dL	mg/L	nmol/L
< 0.5	< 5.0	< 47.6

The CDC/AHA recommended the following hsCRP cut-off points (tertiles) for CVD risk assessment:<sup>21,30</sup>

hsCRP level (mg/L)	hsCRP level (nmol/L)	Relative risk
< 1.0	< 9.52	low
1.0-3.0	9.52-28.6	average
> 3.0	> 28.6	high

Patients with higher hsCRP concentrations are more likely to develop myocardial infarction and severe peripheral vascular disease.

5-95 % reference intervals of neonates and children:<sup>31</sup>

Neonates (0-3 weeks): 0.1-4.1 mg/L (0.95-39.0 nmol/L)

Children (2 months-15 years): 0.1-2.8 mg/L (0.95-26.7 nmol/L)

**It is important to monitor the CRP concentration during the acute phase of the illness.**

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges. Increases in CRP values are non-specific and should not be interpreted without a complete clinical history.

When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values. Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Measurements should be compared to previous values. When the results are being used for risk assessment, patients with persistently unexplained hsCRP levels of above 10 mg/L (95.2 nmol/L) should be evaluated for non-cardiovascular origins. Testing for any risk assessment should not be performed while there is indication of infection, systemic inflammation or trauma.<sup>21</sup>

## Specific performance data

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

## Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability\* (n = 21), intermediate precision\*\* (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability*	Mean	SD	CV
	mg/L (nmol/L, mg/dL)	mg/L (nmol/L, mg/dL)	%
Precinorm Protein	9.00 (85.7, 0.900)	0.10 (1.0, 0.010)	1.2
CRP T Control N	4.34 (41.3, 0.434)	0.04 (0.4, 0.004)	1.0
Human serum 1	15.9 (151, 1.59)	0.1 (1, 0.01)	0.4
Human serum 2	0.54 (5.14, 0.054)	0.01 (0.10, 0.001)	1.6
Intermediate precision**	Mean	SD	CV
	mg/L (nmol/L, mg/dL)	mg/L (nmol/L, mg/dL)	%
Precinorm Protein	9.06 (86.3, 0.906)	0.11 (1.1, 0.011)	1.3
CRP T Control N	4.28 (40.8, 0.428)	0.11 (1.1, 0.011)	2.6
Human serum 3	13.3 (126, 1.33)	0.3 (3, 0.03)	2.1
Human serum 4	0.53 (5.05, 0.053)	0.05 (0.48, 0.005)	8.4

\* repeatability = within-run precision

\*\* intermediate precision = total precision / between run precision / between day precision

## Method comparison

CRP values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared to those determined with the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 192

Passing/Bablok <sup>32</sup>	Linear regression
$y = 0.992x + 0.254 \text{ mg/L}$	$y = 0.946x + 0.514 \text{ mg/L}$
$r = 0.944$	$r = 0.996$

The sample concentrations were between 0.500 and 19.7 mg/L (4.76 and 188 nmol/L, 0.050 and 1.97 mg/dL).

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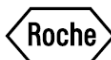
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# IRON2

Iron Gen.2

**cobas**<sup>®</sup>

• Indicates **cobas c** systems on which reagents can be used

## Order information

Iron Gen.2

200 tests

Calibrator f.a.s. (12 x 3 mL)

Calibrator f.a.s. (12 x 3 mL, for USA)

Precinorm U plus (10 x 3 mL)

Precinorm U plus (10 x 3 mL, for USA)

Precipath U plus (10 x 3 mL)

Precipath U plus (10 x 3 mL, for USA)

Precinorm U (20 x 5 mL)

Precipath U (20 x 5 mL)

Cat. No. **03183696** 122

Cat. No. **10759350** 190

Cat. No. **10759350** 360

Cat. No. **12149435** 122

Cat. No. **12149435** 160

Cat. No. **12149443** 122

Cat. No. **12149443** 160

Cat. No. **10171743** 122

Cat. No. **10171778** 122

System-ID 07 6596 1

Code 401

Code 401

Code 300

Code 300

Code 301

Code 301

Code 300

Code 301

Roche/Hitachi **cobas c** systems

**cobas c** 311 **cobas c** 501

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## English

## System information

IRON2: ACN 661

## Intended use

In vitro test for the quantitative determination of iron in human serum and plasma on Roche/Hitachi **cobas c** systems.

## Summary<sup>1,2,3,4,5</sup>

Ingested iron is mainly absorbed in the form of Fe<sup>2+</sup> in the duodenum and upper jejunum. The trivalent form and the heme-bound Fe<sup>3+</sup> component of iron in food has to be reduced by vitamin C. About 1 mg of iron is assimilated daily. Upon reaching the mucosal cells, Fe<sup>2+</sup> ions become bound to transport substances. Before passing into the plasma, these are oxidized by ceruloplasmin to Fe<sup>3+</sup> and bound to transferrin in this form. The transport of Fe ions in blood plasma takes place via transferrin-iron complexes. A maximum of 2 Fe<sup>3+</sup> ions per protein molecule can be transported. Serum iron is almost completely bound to transferrin.

Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissue of the two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease. Iron determinations are performed for the diagnosis and monitoring of microcytic anemia (e.g. due to iron metabolism disorders and hemoglobinopathy), macrocytic anemia (e.g. due to vitamin B<sub>12</sub> deficiency, folic acid deficiency and drug-induced metabolic disorders of unknown origin) as well as normocytic anemias such as renal anemia (erythropoietin deficiency), hemolytic anemia, hemoglobinopathy, bone marrow disease and toxic bone marrow damage.

Numerous photometric methods have been described for the determination of iron. All have the following in common:

- Liberation of Fe<sup>3+</sup> ions from the transferrin complex using acids or detergents.
- Reduction of Fe<sup>3+</sup> ions to Fe<sup>2+</sup> ions.
- Reaction of the Fe<sup>2+</sup> ions to give a colored complex.

The method described here is based on the FerroZine method without deproteinization.

## Test principle

Colorimetric assay.

Transferrin-Fe-complex  $\xrightarrow{\text{pH} < 2.0}$  apotransferrin + Fe<sup>3+</sup>

Fe<sup>3+</sup>  $\xrightarrow{\text{ascorbate}}$  Fe<sup>2+</sup>

Fe<sup>2+</sup> + FerroZine  $\longrightarrow$  colored complex

Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe<sup>3+</sup> ions to Fe<sup>2+</sup> ions which then react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically.

## Reagents - working solutions

**R1** Citric acid: 200 mmol/L; thiourea: 115 mmol/L; detergent

**R3** Sodium ascorbate: 150 mmol/L; FerroZine: 6 mmol/L; preservative

## Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Safety data sheet available for professional user on request.

Disposal of all waste material should be in accordance with local guidelines.

**Warning:** This reagent contains thiourea, a substance known to the State of California to cause cancer or reproductive harm. It may also cause skin reactions. In the event of contact, flush affected areas with copious amounts of running water. Get immediate medical attention for contact with the eyes or if ingested.

Contact phone: all countries: +49-621-7590,

USA: +1-800-428-2336

## Reagent handling

Ready for use.

## Storage and stability

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Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 6 weeks

When removing the **cobas c** pack from the instrument during use, please immediately store at 2-8 °C.

Do not shake the **cobas c** pack to avoid foaming.

Store protected from light.

## Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum (free from hemolysis).

Plasma (free from hemolysis): Li-heparin plasma.

Do not use EDTA or oxalate plasma.

Separate serum or plasma from the clot or cells within 1 hour.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay.

Stability:<sup>6,7</sup>

7 days at 15-25 °C

3 weeks at 2-8 °C

several years at (-15)-(-25) °C

## Materials provided

See "Reagents - working solutions" section for reagents.

# IRON2

Iron Gen.2

## Materials required (but not provided)

See "Order information" section.

Distilled water

General laboratory equipment

## Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum and plasma

### cobas c 311 test definition

Assay type	2 Point End
Reaction time / Assay points	10 / 23-27
Wavelength (sub/main)	700/570 nm
Reaction direction	Increase
Units	µmol/L (µg/dL, mg/L)

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (H <sub>2</sub> O)
Normal	8.5 µL	–	–
Decreased	4.0 µL	–	–
Increased	17.0 µL	–	–

### cobas c 501 test definition

Assay type	2 Point End
Reaction time / Assay points	10 / 34-42
Wavelength (sub/main)	700/570 nm
Reaction direction	Increase
Units	µmol/L (µg/dL, mg/L)

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (H <sub>2</sub> O)
Normal	8.5 µL	–	–
Decreased	4.0 µL	–	–
Increased	17.0 µL	–	–

## Calibration

Calibrators	S1: H <sub>2</sub> O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration <ul style="list-style-type: none"> <li>- after <b>cobas c</b> pack change</li> <li>- after 7 days on board</li> <li>- and as required following quality control procedures</li> </ul>

Traceability: This method has been standardized against a primary reference material (SRM 937).

## Quality control

For quality control, use control materials as listed in the "Order information" section.

Other suitable control material can be used in addition.

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The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation

The analyzer automatically calculates the analyte concentration of each sample.

Conversion factors:	µmol/L x 5.59 = µg/dL
	µmol/L x 0.0559 = mg/L
	µg/dL x 0.179 = µmol/L
	µg/dL x 0.010 = mg/L

## Limitations - interference<sup>8</sup>

Criterion: Recovery within ± 10 % of initial value at an iron concentration of 26.9 µmol/L (150 µg/dL).

Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L (60 mg/dL)).

Hemolysis: No significant interference up to an H index of 200 (approximate hemoglobin concentration: 125 µmol/L (200 mg/dL)). Higher hemoglobin concentrations lead to artificially increased values due to contamination of the sample with hemoglobin-bound iron.

Lipemia (Intralipid): No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>9,10</sup>

In patients treated with iron supplements or metal-binding drugs, the drug-bound iron may not properly react in the test, resulting in artificially low values.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.

**Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.**

## Limits and ranges

### Measuring range

0.90-179 µmol/L (5.00-1000 µg/dL, 0.05-10.0 mg/L)

Determine samples having higher concentrations via the rerun function. For samples with higher concentrations, the rerun function decreases the sample volume by a factor of 2.1. The results are automatically multiplied by this factor.

### Lower limits of measurement

#### Lower detection limit of the test

0.90 µmol/L (5.00 µg/dL, 0.05 mg/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

## Expected values<sup>11</sup>

Females	6.6-26 µmol/L	37-145 µg/dL	0.37-1.45 mg/L
Males	11-28 µmol/L	59-158 µg/dL	0.59-1.58 mg/L

Serum/plasma iron levels are dependent on diet and subject to circadian variation.<sup>12</sup>

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.



# IRON2

Iron Gen.2

## Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

### Precision

Precision was determined using human samples and controls in an internal protocol. Repeatability\* (n = 21), intermediate precision\*\* (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability*	Mean $\mu\text{mol/L}$ ( $\mu\text{g/dL}$ )	SD $\mu\text{mol/L}$ ( $\mu\text{g/dL}$ )	CV %
Precinorm U	19.8 (111)	0.1 (0.6)	0.6
Precipath U	32.8 (183)	0.2 (1.1)	0.6
Human serum 1	11.3 (63.2)	0.2 (0.6)	1.3
Human serum 2	54.5 (305)	0.5 (3)	0.8

Intermediate precision**	Mean $\mu\text{mol/L}$ ( $\mu\text{g/dL}$ )	SD $\mu\text{mol/L}$ ( $\mu\text{g/dL}$ )	CV %
Precinorm U	20.1 (112)	0.3 (2)	1.5
Precipath U	33.5 (187)	0.5 (3)	1.5
Human serum 1	11.8 (66.0)	0.2 (1.1)	1.8
Human serum 2	55.1 (308)	0.7 (4)	1.3

\* repeatability = within-run precision

\*\* intermediate precision = total precision / between run precision / between day precision

### Method comparison

Iron values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 85

Passing/Bablok <sup>13</sup>	Linear regression
$y = 1.003x + 0.29 \mu\text{mol/L}$	$y = 1.000x + 0.37 \mu\text{mol/L}$
$r = 0.986$	$r = 1.000$

The sample concentrations were between 3.5 and 162  $\mu\text{mol/L}$  (19.6 and 906  $\mu\text{g/dL}$ ).

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