بسم الله الرحمن الرحيم

Sudan University of Science & Technology

College of Graduate Studies

Assessment of IronProfile, Hemoglobin Level and Reticulocyte Count Among Sudanese Dialysis Patients Receiving Erythropoietin Injection, Khartoum State

تقييم نسبة الحديد والهيموقلوبين و عدد الخلايا الشبكية لدى مرضى غسيل الكلى الذين يتلقون حقن الإريثروبويتين, ولاية الخرطوم

A Dissertation Submitted in Partial Fulfillment of M.Sc Degree in Medical Laboratory Science (Haematology and Immunohaematology)

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الآيـــــة

قال تعالى:

وَلَقَدْ خَلَقْنَا الْإِنْسَانَ مِنْ سُلَالَةٍ مِنْ طِينٍ ﴿12﴾ ثُمَّ جَعَلْنَاهُ نُطْفَةً فِي قَرَارٍ مَكِينٍ ﴿13﴾ ثُمَّ خَلَقْنَا النُّطْفَةَ عَلَقَةً فَخَلَقْنَا الْعَلَقَةَ مُضْغَةً فَخَلَقْنَا الْمُضْغَةَ عِظَامًا فَكَسَوْنَا الْعِظَامَ لَحْمًا ثُمَّ أَنْشَأْنَاهُ خَلْقًا آخَرَ ۚ فَتَبَارَكَ اللَّهُ أَحْسَنُ الْخَالِقِينَ ﴿14﴾

صدق الله العظيم

سورة المؤمنون- الآية(12-14)

Dedication

First and always thanks to God Almighty.

To the light of my eyes and the love of my life my mother.

To that warrior who taught me everything and was behind every success in my life, my dear father.

To my dear brothers and sisters.

To every precious friend in my life.

To my supervisor, who was the reason for advice and guidance to me.

Finally to myself.

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First I wish to thank Allah for granting me the Confidence and Success to complete this study. I wish to thank my supervisor who was more than generous with their expertise and precious time. A special thanks to **Prof. Dr.Munsoor Mohammed,** my supervisor for his countless hours of reflecting, reading, encouraging, and most of all patience throughout the entire process.

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List of Abbreviations

CHr	reticulocyte hemoglobin content
CKD	Chronic kidney disease
EBPG	European Best Practice Guidelines
EPO	erythropoietin
ESAs	erythropoiesis-stimulating agents
ESRD	end-stage renal disease
HIF	hypoxiainducible factor
IV	intravenous
K/DOQI	Kidney Disease Outcomes Quality Initiative
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
NTBI	non-transferrin bound iron
PD	Permanent dialysis
RBCs	red blood cells
RHuEPO	recombinant human erythropoietin
STfR	soluble transferrin receptor
SC	Subcutaneous
TIBC	total serum iron binding capacity

Abstract

Background: This was retrospective analytical case control studyconducted in hemodialysis patients in Bashaier hospital during the period of April 2019 to January 2020, aimed to total of sixty subject (thirty dialysis patient and thirty healthy individual as control group)

Objective: the aim of this study was to asses of iron Profile& reticulocyte count among Sudanese dialysis patients.

Materials & Methods: in this case control study conducted in Bashaier hospital at Khartoum State – Sudan. Sixty subjects including: (thirty Dialysis patients at the study area who completed questionnaires and collecting data & thirty healthy individuals as control group). Serum total iron and TIBC concentrations were estimated by enzymatic method in fully Automated Biochemistry Analyzer (Mindray BS-380, china). Serum total ferritin concentrations were estimated by specific enzyme-linked immunosorbent assay (ELISA) kit method in fully Automated Biochemistry Analyzer (TOSOH AIA 2000, Japan). Hemoglobin level were estimated by automated hematology analyzer (sysmex KX-N21) and manual reticulocyte count by normal light microscope.

Results: In our study, Serum Iron Profile levels in Dialysis patients compared to control group. the mean serum level of total iron, TIBC, Transferrin saturation %, Ferritin, Hemoglobin g/dl and retic count in Dialysis patients respectively were (1.07±0.2, 1.33±0.4, 1.27±0.4, 1.80±0.4,1.40±0.4, 1.07±0.2), and in control group respectively were(1.47±0.5, 1.60±0.4, 1.03±0.1, 1.13±0.3,1.47±0.5, 1.33±0.4), and there was a significant decrease of serum level of total iron and Transferrin saturation in Dialysis patients compared to healthy individuals (p value=0.01, 0.00)and significant increase of TIBC, Ferritin and retic count in Dialysis patients compared to healthy individuals (p value=0.01, 0.00, 0.00)

Discussion: -Iron deficiency and Anemia are frequent complications in chronically hemodialyzed patients, rHuEPO considered an effective medication in the treatments of anemia of CKD but presence of other side elements will interfere such as blood transfusion and IV iron.

Conclusion: - Significant decrease of serum level of total iron and Transferrin saturationand significant increase of TIBC, Ferritin,Hemoglobin and retic count in Dialysis patients treated with EPO compared to healthy individuals

المستخلص

الخلفية: كانت هذه دراسة حالة تحليلية بأثر رجعي أجريت على مرضى غسيل الكلى في مستشفى بشائر خلال الفترة من أبريل 2019 إلى يناير 2020 ، واستهدفت ما مجموعه ستين موضوعًا (ثلاثون مريض غسيل كلى وثلاثون فردًا سليمًا كمجموعة ضابطة).

الهدف: كان الهدف من هذه الدراسة هو تقييم خصائص الحديد والهيموجلوبين وعدد الخلايا الشبكية بين مرضى غسيل الكلى السودانيين.

المواد والطرق: في هذه الحالة أجريت دراسة شواهد في مستشفى بشائر بولاية الخرطوم - السودان. ستون موضوعاً من ضمنهم: (ثلاثون مريضاً بغسيل الكلى بمنطقة الدراسة ممن أكملوا الاستبيانات وجمعوا البيانات وثلاثون من الأصحاء كمجموعة ضابطة). تم تقدير تركيزات الحديد الكلي و TIBC في المصل بالطريقة الأنزيمية في محلل الكيمياء الحيوية الآلي بالكامل (Mindray BS-380 ، الصين). تم تقدير تركيزات الفيريتين الكلية في المصل من خلال طريقة مجموعة مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) في محلل الكيمياء الحيوية المؤتمت بالكامل (TOSOH AIA 2000 ، اليابان). تم تقدير مستوى الهيموغلوبين بواسطة محلل الدم الآلي (sysmex KX-N21) وعدد الخلايا الشبكية اليدوي بواسطة المجهر الضوئي العادي.

النتائج: في دراستنا ، مستويات الحديد في مصل الدم لدى مرضى غسيل الكلى مقارنة بمجموعة التحكم. كان متوسط مستوى المصل للحديد الكلي ، TIBC ، ترانسفيرين ، نسبة التشبع ، الفيريتين ، الهيموجلوبين جم / ديسيلتر ، العد الشبكي في مرضى غسيل الكلى على التوالي (1.07 ± 0.2 ، 1.33 ± 0.4 ، 1.27 ± 0.4 ، 1.80 ± 0.4 ، 1.40 ± 0.4 ، 1.07) ± 0.2) ، وفي المجموعة الضابطة على التوالي كانت (1.47 ± 0.5 ، 1.60 ± 0.4 ، 1.03 ± 0.1 ، 1.13 ± 0.3 ، 1.47 ± 0.5 ، 1.33 ± 0.4) ، وكان هناك انخفاض معنوي في مستوى المصل من إجمالي الحديد وترانسفرين. التشبع في مرضى غسيل الكلى مقارنة بالأفراد الأصحاء (قيمة p = 0.01، 0.00) وزيادة معنوية في Ferritin،TIBC ، وعدد شبكي في مرضى غسيل الكلى مقارنة بالأفراد الأصحاء (قيمة p = 0.01، 0.00 ، 0.00)

المناقشة: - يعتبر نقص الحديد وفقر الدم من المضاعفات المتكررة في مرضى التحلل الدموي المزمن ، ويعتبر rHuEPO دواءً فعالاً في علاج فقر الدم المصاحب لمرض الكلى المزمن ولكن وجود عناصر جانبية أخرى سيتدخل مثل نقل الدم والحديد الوريدي.

الخلاصة: - انخفاض كبير في مستوى مصل الدم من إجمالي الحديد وتشبع الترانسفيرين وزيادة معنوية في TIBC و Ferritin و Hemoglobin وعدد شبكي في مرضى غسيل الكلى المعالجين بـ EPO مقارنة بالأفراد الأصحاء

CHAPTER I

INTRODUCTION

1. Introduction

1.1 Introduction

Chronic kidney disease (CKD) is considered a public health problem worldwide with high incidence and prevalence rates. In end-stage renal disease (ESRD), renal function must be replaced by dialysis or renal transplantation (Schoolwerth *et al*., 2006). In Sudan, the number of patients on dialysis has increased gradually over the years. Anemia is one of the most frequent early complications of CKD (Hsu *et al*., 2002). The main cause is erythropoietin (EPO) deficiency due to impaired kidney function. However, other causes should be considered when the severity of anemia is inconsistent with the decrease in renal function; when there is evidence of iron deficiency or matching decreases in hemoglobin, leukopenia and/or thrombocytopenia are also found **(**Mercadal *et al.*, 2012). The definition of anemia in CKD patients has changed with some guidelines being produced over the last few years. In 2004 the Revised European Best Practice Guidelines (EBPG) on Anemia defined low hemoglobin levels as values less than 11.5 g/dL in adult females and less than 13.5 g/dL in adult males (less than 12 g/dL in over 70-year olds**) (**Agarwal., 2006**).** Patients with CKD should maintain a hemoglobin level above 11 g/dL (hematocrit >33%). In addition, levels above 12 g/dL are not recommended for patients with severe cardiovascular disease(Barany *et al.*, 2007).In patients with normal kidney function, absolute iron deficiency is characterized by low serum ferritin concentration (less than 30 μg/l). The ferritin cut-off level for absolute iron deficiency in CKD patients is 100 μg/l by the experience that chronic inflammation increases serum ferritin levels approximately three-fold. The Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines recommend serum ferritin levels >200 μg/l for the adult hemodialysis patient population (Obrador*et al*., 2001).The treatment of anemia in CKD patients usually involves the use of recombinant human erythropoietin (rHuEPO). The main cause of rHuEPO treatment failure is the loss or low iron availability. The prevalence of iron deficiency is very common in CKD, affecting as many as 50% of patients (Nemoto *et al*., 2001). However, despite rHuEPO and intravenous iron in the majority of patients, the prevalence of anemia reaches 34% of those patients. This indicates the existence of other important factors related to rHuEPO resistance. Most of these patients also needs supplementation with regular iron injections to secure iron availability for proper erythropoiesis. Following intravenous iron injection, non-

transferrin bound iron (NTBI) can appear in the circulation, capable of inducing harmful oxidative reactions (Astor *et al.*, 2002).

CHAPTER II

LITERATURE REVIEW

RATIONALE & OBJACTIEVES

2. Literature Review

2.1 Patients on dialysis

Chronic kidney disease (CKD) is increasing in prevalence worldwide. Anemia is one of the most common complications of this disease. Luckily, it is also one of the most responsive to treatment. The use of iron and erythropoiesis-stimulating agents (ESAs) is the most effective means of treating the anemia of CKD (Egrie *et al., 2001*). Anemia in CKD is usually normocytic and normochromic. The characteristics of erythrocytes as determined by hematimetric indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) can characterize the etiology of anemia (Levin *et al.*, 1999). In addition to hematimetric indices, the laboratory investigation includes complete blood cell count, reticulocyte count, serum iron, determination of the transferrin saturation and serum ferritin, as well as occult blood in stools and the levels of folic acid and vitamin B12 (Foley, 2003).Iron deficiency is another common contributing factor to the anemia seen in CKD patients. Iron deficiency is also one of the major causes of resistance to rHuEPO treatment seen in clinical practice. It is crucial that the iron stores be replete prior to the start of rHuEPO treatment (*Macdougall et al., 2008*). The causes of iron deficiency in CKD include increased blood loss during dialysis and blood drawn for laboratory testing, decreased iron absorption, occult gastrointestinal bleeding and dietary restrictions *(Inriget al., 2011*). Increased erythropoiesis itself can induce a state of functional iron deficiency where total body iron stores are adequate, but iron is not made available to keep up with rHuEPO driven accelerated erythropoiesis (Khankin *et al.*, 2010). More recently, inflammation has emerged as an important cause of not only erythropoietin resistance but also impaired utilization of iron stored in the reticuloendothelial system (Obrador*et al*., 2001).The biochemical markers of iron deficiency (serum iron, ferritin, transferrin saturation and soluble transferrin receptor – sTfR) have limited value in functional iron deficiency as they are changed in several clinical conditions such as the ones that evolve with rHuEPO therapy. However, reticulocyte hemoglobin content (CHr or Ret-He) is a sensitive indirect marker of iron deficiency, which reflects recent changes in erythropoiesis. The measurement of CHr in peripheral blood samples is useful for assessing the amount of functional iron that was available in the bone marrow for new red blood cell production over the previous 3–4 days. CHr may be a more sensitive marker of

functional iron deficiency in patients receiving erythropoietin therapy. It may also be an early indicator of the effectiveness of iron replacement therapy (Obrador *et al*., 2001).

2.2 Iron metabolism

Iron is a critical body substance, transporting oxygen to tissues via hemoglobin and functioning as a cofactor in a number of enzyme systems. Iron is stored in reticuloendothelial cells of the liver, spleen, and bone marrow bound to ferritin and hemosiderin. This storage iron constitutes one third of the 3 to 4 g of total body iron. The remaining iron is present in erythropoietic tissue or red blood cells(RBCs) (Macdougall *et al*., 2002) .The majority of circulating iron is carried by transferrin, although at any one time, only 3 to 4 mg of iron are present on transferrin. Ingested iron varies from very small amounts up to 200 mg in end-stage renal disease (ESRD) patients given supplements. However, only 1% to 2% of this iron is absorbed (Nemeth *et al*., 2009). Internal iron exchange between the erythroid marrow, circulating red blood cells, and the reticuloendothelial system involves 20 mg or more of iron daily. In the absence of rHuEPO, patients with advanced renal disease have less iron exchanged from the erythroid marrow to RBCs and more stored in the reticuloendothelial system (Andrews, 2005).

2.3 Iron deficiency in dialysis patients

2.3.1 Pathogenesis

There are three important mechanisms that have been proposed to explain the high frequency of iron deficiency in dialysis patients, increasing demand for iron and consequent storage in RBCs when erythropoiesisis stimulated with rHuEPO. The second include abnormal iron absorption, and last external blood loss and functional iron deficiency. (Fishbane *et al*., 2001)

2.3.2 Iron absorption in dialysis patients

Absorption of iron from the gastrointestinal tract is modulated by the level of body iron stores, EPO, and the extent of erythropoiesis (Mast *et al*., 2008). Iron absorption takes place almost exclusively in the duodenum and proximal jejunum and is regulated depending on dietary intake, intra luminal factors, erythropoietic activity, the functional capacity of the intestinal mucosal cells, and the tissue-iron storage

level. Ingested iron is reduced to the ferrous form and is bound to high molecular weight chelators (Brugnara, 2003). Red meat contains primarily heme iron, which is more avidly absorbed. Restrictions in the ingestion of red meat, common in dialysis patients, may account, in part, for the small amount of iron that is absorbed in this population. It should be noted that the proportion of ingested iron absorbed in individuals with normal renal function is also low, with 1 mg of iron or less absorbed daily (Fishbane *et al*., 2001). On the other hand, iron absorption generally increases in the face of accelerated erythropoiesis or a decrease in body iron stores. Conflicting data have appeared in the literature regarding the balance of these factors in influencing iron absorption in dialysis patients (Hsu *et al*., 2002). Early studies suggested that iron absorption was normal in patients on dialysis, whereas more recent studies using ferrokinetic techniques found that uptake of iron by intestinal mucosal cells and iron retention was decreased significantly in dialysis patients. High ferritin levels, which can occur in dialysis patients despite low iron stores, may also impair the normal feedback that would increase absorption during deficiency states (Brugnara, 2003).

2.3.3 External blood loss

There are several factors that contribute blood loss in dialysis patients, including blood retained in the dialyzer and blood tubing at the end of each dialysis treatment, frequent blood testing, occult gastrointestinal the lial blood loss and blood lost from puncturing or removing needles from hemodialysis vascular accesses. It has been shown that 1 to 3 g of iron is lost annually from these causes. It should be kept in mind that the higher the hematocrit achieved with rHuEPO, the greater iron loss with each milliliter of lost blood, because of the higher percentage of RBCs containing iron that are lost at a higher hematocrit. Quality Initiative suggested that 25 to100 mg of iron would need to be replaced weekly in hemodialysis patients just to offset the iron lost because of the ongoing external blood losses described earlier here (Maconi *et al*., 2009). There are no data available on the external blood losses that occur in peritoneal dialysis patients. Clearly, the lack of blood loss in the dialyze rand tubing, the absence of needle sticks, and heparin use would lead one to predict a substantially smaller blood loss in this patient population. For those peritoneal dialysis patients receiving rHuEPO, however, the increased demand for iron, as erythropoiesis is stimulated by rHuEPO, will still need to be addressed (Astor *et al*., 2002).

2.4 Functional iron deficiency

A further complicating feature in the management or identification of iron deficiency in the dialysis population is the introduction of the concept of functional iron deficiency. Functional iron deficiency is present when the usual tests for iron deficiency in dialysis patients do not indicate absolute iron deficiency (ferritin of more than 100 ng/ml; Transferrrin saturation of more than 20%) (Macdougall *et al*., 2006), but patients respond to additional iron administration with a rise in hematocrit at a stable EPO dose or maintain a stable hematocrit with a lower EPO dose. Patients with functional iron deficiency therefore have apparently insufficient available iron to keep up with the demands of the stimulated erythropoiesis that occur when exogenous EPO is administered. In some such patients, the inability to mobilize iron rapidly relates to the presence of reticuloendothelial blockade. Dialysis patients may have coexisting occult infections or other conditions that may preclude an appropriate response to iron therapy or prevent the use of iron stores that would otherwise be sufficient in additional erythropoiesis. This effect may be caused by increased levels of circulating cytokines that are capable of inducing macrophages of the reticuloendothelial system to more avidly take up and hold on to iron. The effect of cytokines may involve a decrease in endogenous EPO production or a decrease in responsiveness of erythroid precursor cells to endogenous or exogenous EPO. In particular, interleukin-1b (possibly acting through interferon g) and tumor necrosis factor-have been shown to have both of these effects. In addition, interleukin-6 has recently been shown to decrease responsiveness to EPO as well. Such patients typically have high Transferrin saturation levels and elevated levels of serum ferritin (Thomas *et al*., 2002). Iron therapy in these patients, unlike other patients with functional iron deficiency, generally will not produce a favorable response. The only current method of separating the two groups is a therapeutic trial of exogenous iron. The rapeutictrials in such patients should therefore closely monitor subsequent ferritin, Transferrin saturation, and hematocrit levels (Shaefer R et al, 2002).

2. 5 Evaluation of iron status in hemodialysis patients

In patients with normal kidney function, absolute iron deficiency is characterized by low serum ferritin concentration (<30 μg/l). The ferritin cut-off level for absolute iron deficiency in CKD patients is 100 μg/l by the experience that chronic inflammation

increases serum ferritin levels approximately three-fold (Fishbane *et al*., 1997). The Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines recommend serum ferritin levels >200 μg/l for the adult hemodialysis patient population. The European Best Practice Guidelines define the optimal range for serum ferritin as 200–500 μg/l in adult patients with ESRD. A normal ferritin level (≥100 μg/l) cannot exclude iron deficiency in uremic children, but a serum ferritin <60 μg/l is a specific predictor of its presence. An upper ferritin level of 500 μg/l is recommended for adults and children with CKD (Mittman *et al*., 1997). Serum ferritin is an indicator of storage iron. Iron deficiency is accompanied by reductions in serum iron concentration and transferrin saturation (TSAT) and by elevations in red cell distribution width, free erythrocyte protoporphyrin concentration (Smith *et al*., 2001), total serum iron binding capacity (TIBC), and circulating transferrin receptor. Serum soluble transferrin receptor, however, reflects ongoing erythropoiesis but not iron availability in ESA-treated chronic dialysis patients (Maconi *et al*., 2009).

2.6 Erythrocyte and reticulocyte indices in hemodialysis patients

Erythrocyte and reticulocyte indices, such as the percentage of hypochromic red blood cells and the reticulocyte hemoglobin content (CHr) provide direct insight into bone marrow iron supply and utilization. Determination of the percentage of hypochromic red blood cells, i.e., those with a cellular hemoglobin concentration <28 g/dl, provides important information on functional iron deficiency in ESA-treated dialysis patients (Mittman *et al*., 1997). Found that hypochromic red blood cells >6% are the best marker to identify adult ESRD patients who will have the best response to intravenous iron. CHr has been proposed as a surrogate marker of iron status and as an early predictor of response to iron therapy in adult dialysis patients (Maconi *et al*., 2009). Combined use of CHr and high-fluorescence reticulocyte count predicts with a very high sensitivity and specificity the response to intravenous iron in adult dialysis patients. There are, however, only few studies in the pediatric renal literature on the use of CHr. In children with ESRD (Shaefer *et al*., 2002), an increase from baseline CHr levels was observed in response to oral and intravenous iron, but cut-off values for the use of CHr in the pediatric CKD population are not clear. This measure has proven to be of value with adult ESRD patients. Detection of both absolute and functional iron deficiency is important because iron deficiency is the most common cause of hypo responsiveness to ESAs (Perna A.F et al, 1997). In clinical practice, an

increased erythropoietic response to iron supplementation is the most widely accepted reference standard of iron deficient erythropoiesis. For pharmacological therapy of iron deficiency, both oral and parenteral iron preparations are available. Intravenous iron is more effective than oral iron supplementation, at least in CKD patients (Fishbane *et al*., 2001). Iron is not only a prerequisite for effective erythropoiesis but also an essential element in all living cells. Elemental iron serves as a component of oxygen-carrying molecules and as a cofactor for enzymatic processes. Its redox potential, however, limits the quantity of iron that can be safely harbored within an individual.

2.7Erythropoietin

Also known as haematopoietin or haemopoietin, is a glycoprotein cytokine secreted by the kidney in response to cellular hypoxia; it stimulates red blood cell production (erythropoiesis) in the bone marrow. Interstitial cells, located in the peritubular capillary bed of the kidneys, are the main site of erythropoietin (EPO) production. In addition, some extra renal production can occur in certain situations, mainly by the hepatocytes. Normally, the production is regulated by a feedback mechanism involving an oxygen sensor that monitor the oxygen level in the vicinity of the EPO producing cell. The key mediator in this system is hypoxiainducible factor (HIF), a transcription factor produced in the kidney and the liver. Hypoxia leads to increased level of HIF by stimulating the production and inhibiting the degradation, which in turn stimulates EPO production (Egrie *et al*., 2001). Furthermore, new evidence has been provided that HIF plays a more general role in cellular adaption to hypoxia, involving even the regulation of iron metabolism genes, such as the hepcidin gene, supporting a role for HIF in the coordination of EPO synthesis with iron homeostasis. The mechanisms behind renal anemia are not entirely understood. The explanation is not simply that the EPO production falls secondary to cell damage, because serum EPO is often normal or slightly elevated in renal failure, even in patients with ESRD (Cheetham *et al.*, 1998). However, in comparison to anemic patients with normal kidney function, EPO production in patients with renal failure does not respond adequately to the fall in hemoglobin. Accordingly, the EPO levels are lower than in patients with similar degree of anemia, but without renal failure. Thus, it seems that the regulation of EPO production is impaired and the EPO-producing cells are unable to respond adequately to the signals triggered by the oxygen tension. A well-known

observation is that patients with renal failure (Ng T., Marx G et al, 2003), staying at high altitude, have increased EPO production and lower ESA requirement. This is interesting and it has been implied that hypoxia can trigger an extrarenal EPO production or activate unused production capacity of EPO in the deceased kidneys (Drueke *et al*., 2001).

2.7.1 Erythropoietin use in hemodialysis patients

In patients with renal failure, severe anemia and associated fatigue, cognitive and sexual dysfunctions have a significant impact on the quality of life. Anemia also represents an important etiological factor in the development of left ventricular hypertrophy. An inadequate production of a glycoprotein hormone, erythropoietin (EPO), is the major cause of anemia in presence of a reduction in the glomerular filtration rate. EPO is the primary regulator of the growth and survival of the erythroid progenitor. The treatment of anemia in chronic renal failure has been revolutionized by the introduction of recombinant human EPO. The vast majority of patients responds very well to treatment, although 5-10% of patients shows some resistance to EPO, the most common cause of which is iron deficiency. Several studies have recently been started in order to investigate the effects of preventing renal anemia from ever developing in uremic patients. The hemoglobin concentration target in pre-dialysis and dialysis patients is the subject of continuous re-assessment (Santoro A *et al.*, 2005).

Optimization of erythropoietin therapy includes awareness of target hematocrit and hemoglobin, defining the renal anemia management period (RAMP), drug dosage and mode of application and significance of adjuvant therapy. Anemia should be treated early during the course of renal failure, even when GFR falls below 50 ml/min. According to dialysis outcomes quality initiative (DOQI) guidelines, target values are 0.33-0.36 L/L for hematocrit and 110-130 g/l for hemoglobin. Early administration is recommended especially in high-risk patients: the elderly, diabetics and those with coronary artery and peripheral artery diseases. The reasons for inadequate erythropoietin response are unrecognized bleeding, iron deficiency and infection/inflammation. Adverse events are very rare and predictable; they can be avoided with careful dosage and follow-up of patients. In conclusion, EPO-therapy is

well established and efficient for renal anemia in dialysis and pre-dialysis patients (Dimković, 2001).

2.7.2 Iron deficiency in patients on erythropoietin therapy

Inadequate iron availability, either because of absolute or functional iron deficiency, is now the most common yet easily treatable cause of a suboptimal response or rHuEPO (Vecchi *et al.*, 2000). The overriding importance of iron sufficiency, its accurate recognition and the need for intravenous rather than oral iron supplementation are areas of intense current investigation and debate. About 1 gm of iron is consumed by production of new RBCs during the first month of EPO therapy, and it is estimated that between 0.5-3 gm of iron per year is lost just from dialysis-associated blood losses (Maconi *et al.*, 2009). Adequate oral replacement of iron is often not possible or successful in dialysis patients for reasons, hence the need for immediately bio available intravenous iron. EPO doses in individual or groups of dialysis patients may be reduced by 32-70 with use of intravenous iron therapy. Indeed, in new ESRD patients, it is prudent to correct overt iron deficiency before starting EPO therapy, as this alone may suffice to correct anemia initially. Oral iron should be discontinued once an intravenous preparation is started, as intestinal regulation assures that little or no iron is absorbed from the gut once IV iron is administered (Smith *et al*., 2001).

2.8 Rationale & Objectives

2.8.1 Rationale

Chronic kidney disease (CKD) is considered a public health problem worldwide with high incidence and prevalence rates. In end-stage renal disease (ESRD), renal function must be replaced by dialysis or renal transplantation (Schoolwerth *et al*., 2006). In Sudan, the number of patients on dialysis has increased gradually over the years. Iron deficiency and Anemia are frequent complications in chronically hemodialyzed patients. The main cause is erythropoietin (EPO) deficiency due to impaired kidney function. Dialysis patients received erythropoietin (EPO) therapy which is insufficient to replace normal iron during times and then they require intravenous iron therapy (Smith *et al*., 2001). This study carried to asses of iron Profile & reticulocyte count among Sudanese dialysis patients.

2.8.2 Objectives

2.8.2.1 General objective

To asses of iron profile, hemoglobin and reticulocyte count among Sudanese hemodialysis patients.

2.8.2.2 Specific objectives

1-To evaluate the ability of erythropoietin injection (Eprex) in compensating the haemoglobin level.

2-To compare the mean of iron profile and reticulocyte count in hemodialysis patient with the mean of normal healthy individual.

CHAPTER III

MATERIALS & METHODS

3. Materials and Methods

3.1 Study Design

This study was retrospective analytical case control study and hospital based study.

3.2 Study Area

This study was performed in Bashaier hospital at Khartoum State - Sudan

3.3 Study Duration

This study was performed during period from April 2019 to January 2020.

3.4 Study Population

This study was performed in hospitalized hemodialysis patients.

3.5 Inclusion Criteria & Exclusion Criteria

-Dialysis patients over 40 years of age, being treated with rHuEPO at the study area during the study period who completed questionnaires and collecting data & Individuals at the same age, with normal lipid profile, were selected as (control-group), were included in this study.

-We excluded Pregnancy, individuals who had laboratory evidence of malaria in previous one month, Chronic illnesses like chronic liver disease, liver cirrhosis, chronic renal failure, tuberculosis, Blood disorders like thalassaemia, acute leukemia, haemoglobinopathies, clotting disorders, Gastric carcinoma or other cancers, Operated upon for gastrostomy, Patients who are on radiotherapy, chemotherapy, Patients taking medicine causing folic acid deficiency like Gabapentin, Methotrexate, Trimethoprim and Pyrimethamine.

3.6 Sampling

Under aseptic and antiseptic precaution, blood specimens was collected. The blood were collected in EDTA tube a plain tube at room temperature and centrifuged the plain tube for 10 minutes at 3500rpm. All the precautions were taken in accordance with the Clinical and Laboratory Standards Institute criteria.

3.7 Data Collection

Data were collected from direct questionnaire, and then entered the data for group into an excel sheet.

3.8 Sample Size

Sixty subjects including: (thirty Dialysis patients at the study area who completed questionnaires and collecting data & thirty healthy individuals as control group).

3.9 Sampling Tool

The data were collected direct using questionnaire.

3.10 Principles

3.10.1 Serum iron

Iron dissociated fom its Fe-111 transferrin complex by addition of acidic acid buffur containing hydroxylamine which reduce Fe-111 to Fe-11.

3.10.2 Total iron binding capacity

Excess of iron as FeCl3 is added to serum. Any iron which doesn't bind to transferring is removed with exess MgCo3.

3.10.3 Transferring saturatin

Serum Fe3+transferring complex is dissociated by addition of an aetic buffer containing hydroxylamine which reduce Fe3+ to Fe2+.

3.10.4 Serum ferritin

Its immune-turbidmetry, latex bound ferritin antibodies react with the antigen in sample to orm an antigen/antibody complex measured turbidmetrically.

3.10.5 Reticulocyte count

Cont is based on the property of ribosomal RNA to react with isotonic solution of supravital stain such as new methylene blue or brilliant crystal blue.

3.10.6 Hemoglobin

Blood is diluted in solution containing potassium ferricyanide and potassium cyanide. Potassium ferricyanide oxidizes the iron in heam to the ferric state to form methemoglobin, which is converted to hemoglobincyanide by potassium cyanid.

3.11 Quality Controls

Multi calibrator was used to evaluate the working solutions and to evaluate the testing samples. All Precautions and quality issues was issued as manufacture instructions

3.12 Estimation of iron profiles and reticulocyte count

Serum total iron and TIBC concentrations were estimated by enzymatic method in fully Automated Biochemistry Analyzer (Mindray BS380, china). Serum total Ferritin concentrations were estimated by specific enzyme-linked immunosorbent assay (ELISA) kit method in fully Automated Biochemistry Analyzer (TOSOH AIA 2000, Japan) and manual reticulocyte count by normal light microscope.

3.13 Reference value of iron profiles and reticulocyte count

Total Iron	40-----180 mcg/dl
TIBC	250----100 mcg/dl
Ferritin	50-----100 ng/ml

Transferrin saturation % 25-----35%

Reticulocyte count 0.2-----2%

3.14 Ethical Consideration

An ethical permission was obtained from relevant authorities. Samples was collected after full approval of the patients and laboratory administrations. This study was approved by the ethical committee of Faculty of Medical Laboratory Science of Sudan University of Science & Technology.

3.15 Data Analysis

For analysis of data, Statistical Package for Social Sciences software, version 23.0 (IBM SPSSInc., Chicago, IL) was used. Initially, all information gathered via questionnaire then coded into variables. statistics Independent T-test with p-value less than 0.05 was considered statistically significant.

CHAPTER IV

RESULTS

4.Results

Sixty subjects were enrolled in this study, thirty patients undergoing haemodialysis' therapy and dialyzed tow times weekly, being treated with rHuEpo, The ages reflected the populations of the hospitals involved in the study and ranged from 40 to 70 years old. Mean age of Dialysis patients 52.4±9.6 compared to 30 control subjects with mean age 38.8±14.5.

Table 4.1 Shows the Mean Serum Iron levels in Dialysis patients compared to control group, and significant decrease of serum iron in Dialysis patients compared to healthy individuals (p value=0.01, 0.00,)

	Mean	**S.D**	***P.value***
s.iron control	1.47	0.5	0.00
s.iron case	1.07	0.2	0.01

Table 4.2 Shows the Mean T.saturation in Dialysis patients compared to control group, and significant decrease of T.saturation in Dialysis patients compared to healthy individuals (p value=0.01, 0.00,)

	Mean	**S.D**	***P.value***
T.saturation control	1.03	0.1	0.00
T.saturation case	1.27	0.4	0.01

Table 4.3 Shows the Mean Serum Ferritin levels in Dialysis patients compared to control group, and significant increase of Ferritin levels in Dialysis patients compared to healthy individuals (p value=0.01, 0.00,)

	Mean	**S.D**	***P.value***
Ferritin control	1.13	0.3	0.00
Ferritin case	1.80	0.4	0.01

Table 4.4 Shows significant increase of hemoglobin levels when the Mean Hemoglobin levels in the mid of trail compared to the end of trial. (p value=0.01, 0.00)and

	Mean	**S.D**	***P.value***
End trial	1.47	0.5	0.00
Med terial	1.40	0.4	0.01

Table 4.5 Shows the Mean of retic count in Dialysis patients compared to control group, and significant increase of retic count in Dialysis patients compared to healthy individuals (p value=0.01, 0.00,)

	Mean	**S.D**	***P.value***
Retic control	1.33	0.4	0.00
Retic case	1.07	0.2	0.01

CHAPTER V

DISCUSSION, CONCLUSION&RECOMMENDATIONS

5. Discussion, conclusion & Recommendation

5.1 Discussion

Chronic kidney disease (CKD) is considered a public health problem worldwide with high incidence and prevalence rates. In end-stage renal disease (ESRD), renal function must be replaced by dialysis or renal transplantation (Schoolwerth *et al*., 2006). In Sudan, the number of patients on dialysis has increased gradually over the years.

This study found the mean age of dialysis patients in Sudan was 52.4±9.6 years this findings agreed with (Brunkhorst R *et al.*, 2004) reported the age of dialysis patients ranged 21-91 years with mean age 58.9 years.

Iron deficiency is a frequent complication in chronic hemodialysis patients because of the significant blood losses associated with this technique. The topic became more important after 1986 when rHuEPO became available and most patients with the anemia of ESRD could be treated successfully with the drug reported by old previous studies.

The presented study reported a significant decrease of serum level of total iron and Transferrin saturation in dialysis patients treated with rHuEPO compared to healthy individuals and the significant increase of Ferritin level, agreed with study held by (wish, 2006) who justify main cause of the anemia is the inflammatory state may inhibit the mobilization of iron from reticuloendothelial stores. Also the presented study agreed with study held by (coney *et al.*, 2007) who justified the increasing of the ferritin in response to the intravenous iron medication. Reported a significant increase of reticulocyte count in the presented study agreed with study held by (Talwar VK *et al.*, 2002).

The presented study reported a significant increase of the hemoglobin levels in hemodialysis patients treated with rHuEPO agreed with study held by (Nicaise C *et al.,2002).*

5.2Conclusion

This study concluded:

- Significant decrease of serum level of total iron and Transferrin saturation in Dialysis patients treated with rHuEPO compared to healthy individuals
- Significant increase of TIBC, Ferritin and retic count in Dialysis patients treated with rHuEPO compared to healthy individuals.
- rHuEPO considered an effective medication in the treatments of anemia of CKD but presence of other side elements will interfere such as blood transfusion and IV iron

5.3Recommendations

This study recommends that:

-Blood transfusion is only in very deteriorating cases to avoid the problem of iron accumulation as much as possible.

-conduct this study in better ways like MRI to monitor iron storage.

-Use iron chelating agents to prevent iron deposition from Occurring in the internal organ.

- Do other studies on the rHuEPO, to see if it has any side effect on the patient`s health.

- Until now, kidney transplantation is the best solution.

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Appendix (1) Questionnaire

Sudan University of Science &Technology

Graduate Collage

Department of Hematology and Immunohematology

Questionnaire

Date\ \ \2019

Patient's Number :( )

Name:……

Age ……..……years

Gender: Male ☐ Female ☐

Residence: ……...

Date of first hemodialysis: ……..

Hemodialysis times per week:……

Laboratory Investigations:

1-Total Iron…… mcg/dl

2-TIBC…… mcg/dl

3-Ferritin……ng/ml

4-Transferrin saturation……. %

5- Reticulocyte count ……%

Appendix (2) TOSOH AIA2000

Appendix (3) Mindray BS-380

Appendix (4) Erythropoietin injection