Cairo University
Faculty of Veterinary Medicine
Dept. of Biochemistry and Chemistry of Nutrition

Some Biochemical Studies on Immune Response of Chronic Kidney Disease Patients

Presented By

Samar Kamel Mohamed Khalil
B. V. Sc., Cairo University (2003)
M.V.Sc., Cairo University (2010)

For the degree of Ph.D. in Veterinary Sciences
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Under the supervision of

Prof. Dr. Hassan Abd EL-Halim H. Amer
Professor of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Medicine - Cairo University

Prof. Dr. Adel M. El-Behairy
Professor of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Med., Cairo University

Prof. Dr. Mohamad Ali Warda
Professor of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Med., Cairo University

Dr. Mohamad H. Shahin
Head of Laboratory Medicine Dept.
Maadi Armed Forces Hospital

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Approval sheet

This is approved that the dissertation by Vet. Dr. ***Samar Kamel Mohamed Khalil*** to Cairo University for the Degree of Ph.D in Veterinary Science (Biochemistry and Chemistry of Nutrition) has been approved by the examining committee.

Prof. Dr. Mohamed Ahmed Kandel
Professor and Head of Biochemistry and
Chemistry of Nutrition Department
Faculty of Vet. Medicine- Beni Suef University

Prof. Dr. El Said Thabet Awad
Professor of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Medicine - Cairo University

Prof. Dr. Hassan Abd EL-Halim H. Amer
Professor of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Medicine - Cairo University

Prof. Dr. Adel Mohamed A. El-Behairy
Professor of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Medicine - Cairo University

Prof. Dr. Mohamed Ali Ahmed Warda
Professor of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Medicine - Cairo University

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Supervision Sheet

Supervisors

Prof. Dr. Hassan Abd EL-Halim H. Amer

Professor of Biochemistry and Chemistry of Nutrition

Faculty of Veterinary Medicine - Cairo University

Prof. Dr. Adel M. Abo El-Fetouh El-Behairy

Professor of Biochemistry and Chemistry of Nutrition

Faculty of Veterinary Medicine - Cairo University

Prof. Dr. Mohamad Ali A. Warda

Professor of Biochemistry and Chemistry of Nutrition

Faculty of Veterinary Medicine - Cairo University

Dr. Mohamad Hassan Shahin

Head of Laboratory Medicine Dept.
Maadi Armed Forces Hospital

Cairo University
Faculty of Veterinary Medicine
Dept. of Biochemistry and Chemistry of Nutrition

Name : **Samar Kamel Mohamed Khalil**
Nationality : **Egyptian**
Date of birth : **8 / 6/ 1980**
Place of birth : **Cairo**
Specification : **Dept. of Biochemistry and Chemistry of Nutrition**
Thesis title : **''Some Biochemical Studies on Immune Response of Chronic Kidney Disease Patients''.**
Supervisors : **Prof. Dr. Hassan Abd EL-Halim H. Amer**
Prof. Dr. Adel Mohamad A. El-Behairy
Prof. Dr. Mohamad Ali A. Warda
Dr. Mohamad Hassan Shahin

Abstract

Inflammation and oxidative stress are two faces of one coin in end stage renal disease patients (ESRD) on maintenance hemodialysis. Their interconnection induces anemia complicated with erythropoietin hyporesponsiveness. The biochemical basis behind the resistance to erythropoietin therapy with frequent hemoglobinemia, oxidative stress and iron status have not been fully recovered. Two equal groups (40 patients each) of responders and non-responders to recombinant human erythropoietin therapy (higher than 300 IU /kg/wk of epoetin) were used in the study. Hematological and biochemical analysis of collecting blood and serum samples were performed along with serum electrophoretic protein foot printing. The leukocytes DNA fragmentation was used to evaluate the degree of oxidative insult. Erythropoietin (EPO) good responders showed lower erythrocyte malondialdehyde (E-MDA) level; less pronounced DNA fragmentation of circulating leukocytes than poor responder with elevated hemoglobin, albumin, A/G ratio, total iron, and ferritin levels. Contrariwise, lower erythrocyte superoxide dismutase (E-SOD) and catalase activities in EPO poor responder group than good responder one. Other serum constituents and electrophoretic protein pattern showed a no significant difference between the two groups. There were higher levels of inflammatory markers, interleukin-6 (IL-6) and C- reactive protein (CRP) in EPO poor responder than good responder. The present data showed negative correlations between Hb with both IL-6 and CRP levels, indicating a positive correlation between inflammatory markers and severity of anemia. A direct correlation between Hb and antioxidant enzymes (E-SOD and catalase) was noticed, while the inverse correlation with E-MDA was recorded.

Key words: erythropoietin resistance; inflammatory markers; oxidative stress; hemodialysis; anemia of chronic disease.

DEDICATION

To The sole of my father

& the sole of the Eldest Brother' Mustafa'

To My loving & caring Mother

To My Beloved Brother 'Mohamad'

& My Dear Sisters "5N"

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Introduction

The ‘uremic milieu’ is a term that is overused in attempts to explain the multiple organ dysfunction of chronic kidney disease (CKD). In vivo, the concept of a uremic milieu may explain why the level and prevalence of anemia correlate with the severity of the kidney disease. A glomerular filtration rate (GFR) lower than 60 mL/minute/1.73 m^2 has been associated with a higher prevalence of anemia, which reached 75% in some studies **(McClellan *et al.*, 2004)**.

O’Mara, (2008) defined anemia in patients with chronic renal failure according to age and sex as an hemoglobin (Hb) concentration of < 11.5 g/dl in women, < 13.5 g/dl in men ≤ 70 years of age, and < 12 g/dl in men > 70 years of age.

Renal anemia has been regarded as a special form of ‘anemia of chronic disease’ (ACD), in which inappropriate levels of erythropoietin (EPO) is considered as the main cause **(Zarychanski and Houston, 2008)**. **McClellan *et al.*, (2004)** postulated that the specialized peritubular cells that produce EPO are partially or completely depleted or injured as renal disease progresses.

Anemia is universal in CKD primarily due to a relative lack of EPO. Various secondary causes can contribute to anemia, including a deficiency of iron, gastrointestinal bleeding, active blood loss; haemolysis; haemo-globinopathies; aluminum overload; hypothyroidism; severe hyperparathyroidism (elevated parathyroid

hormone (PTH) concentrations); inflammatory conditions (acute and chronic inflammations suppress erythropoiesis in the bone marrow); shortened red blood cells (RBCs) survival due to uremia, and deficiencies of folate and vitamin B12 **(Dowling *et al.*, 2007)**.

Abu-Alfa, (2003) has reported that iron deficiency is a common secondary cause of anemia in CKD, and several factors may contribute to the development and maintenance of this deficiency.

Hemodialysis (HD) patients lose 2 gram of iron per year due to blood trapping in dialyzers, repeated phlebotomy and gastrointestinal losses. This amount corresponds to the iron that normally is absorbed in 1000-2000 days and to more than the half of the total body iron **(Eleftheriadis *et al.*, 2009)**.

Resistance to erythropoiesis stimulating agents (ESAs) has been associated with an increased risk of cardiovascular events and death in CKD patients **(Solomon *et al.*, 2010).** In the same way, **Panichi *et al.*, (2011)** stated that ESAs response reflects the overall health status of the patient and indeed, high ESAs resistance is associated with increased morbidity and mortality.

Although the majority of CKD patients respond adequately to ESAs, 10% of these patients does not adequately respond to the recombinant human erythropoietin (rhEPO) therapy and show a marked resistance to this therapy (**Bárány, 2001** and **Smrzova *et al*., 2005)**.

Additionally, **Cooper *et al.*, (2003)** and **Smrzova *et al.*, (2005)** demonstrated that the reasons for this variability of rhEPO response are unclear. There are a lot of conditions associated with rhEPO resistance, including inflammation, oxidative stress and iron deficiency, as major causes, and blood loss, hyperparathyroidism, aluminium toxicity and vitamin B12 or folate deficiency, as minor causes. However, exclusion of these factors does not eliminate the marked variability in sensitivity to rhEPO (**Macdougall and Cooper, 2002)**.

Several studies reported on levels of lipid peroxidation and antioxidants in HD patients with variable results due to different factors such as dialysis materials (e.g., the kind of HD membrane) (**Malliaraki *et al.*, 2003)**, duration of HD therapy (**Nguyen-Khoa *et al.*, 2001)** and **Ferretti *et al.*, 2008)** and trace elements disturbances.

Inflammatory markers are powerful predictors of mortality after adjustment for other risk factors **(Panichi *et al.*, 2004)**. Inflammation also is responsible for other mortality risk factors, such as anemia, malnutrition, vascular disease, and left ventricular hypertrophy.

Interleukin 6 (IL-6) probably is more related to mortality and is associated with more causes of inflammation than C- reactive protein (CRP) **(Panichi *et al.*, 2004)**.

Patients with end stage renal disease (ESRD) undergoing renal replacement therapy, either hemodialysis or peritoneal dialysis (PD), may face a partial loss of some low molecular- weight plasma factors