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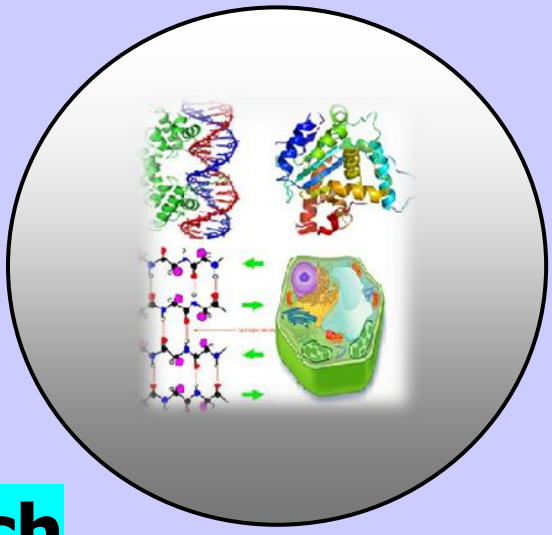

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RESEARCH PAPER

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Erythropoietin and Kidney Diseases: A Review

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ABSTRACT

Erythropoietin also known as Epo, is a glycoprotein hormone that controls erythropoiesis.It is a cytokine for erythrocyte precursors in the bone marrow. Human erythropoietin has a molecular weight of 30.4 kDa. It is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and tubular epithelial tubule. It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood.In addition to erythropoiesis, erythropoietin also has other known biological functions. The primary role of erythropoietin is an essential hormone for red cell production. Without it, definitive erythropoiesis does not take place. Under hypoxic conditions, the kidney will produce and secrete erythropoietin to increase the production of red blood cells by targeting CFU-E, proerythroblast and basophilic erythroblast subsets in the differentiation. Erythropoietin has its primary effect on red blood cell progenitors and precursors by promoting their survival through protecting these cells from apoptosis. The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme renin, the latter of which indirectly acts on the kidney in negative feedback. Diseases of the kidney are diverse, but individuals with kidney disease frequently display characteristic clinical features.Many other disease cases have adverse effects on the kidney which is the major site of production of erythropoietin such hypertension, diabetes, HIV/AIDS.
Keywords: Erythropoietin, Kidney diseases, Erythropoietin receptor, Synthesis, Mechanism of action and Functions.

INTRODUCTION

Erythropoietin also known as Epo, is a glycoprotein hormone that controls erythropoiesis.It is a cytokine for erythrocyte precursors in the bone marrow. Human erythropoietin has a molecular weight of 30.4 kDa. It is produced by interstitial fibroblasts in the kidney in close

association with peritubular capillary and tubular epithelial tubule. It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood.In addition to erythropoiesis, erythropoietin also has other known biological functions. For example, it plays an important role in the brain's response to neuronal injury (Siren *et al.,* 2001). EPO is also involved in the wound healing process (Haron *et al.*, 2003).

Exogenous erythropoietin is produced by recombinant DNA technology in cell culture. Several different pharmaceutical agents are available with a variety of glycosylation patterns, and are collectively called erythropoiesis-stimulating agents (ESA). The specific details for labelled use vary between the package inserts, but ESAs have been used in the treatment of anemia in chronic kidney disease, anemia in myelodysplasia, and in anemia from cancer chemotherapy. Boxed warnings include a risk of death, myocardial infarction, stroke, venous thromboembolism, and tumor recurrence (FDA,2011). Exogenous erythropoietin has been used illicitly as a performance-enhancing drug; it can often be detected in blood, due to slight differences from the endogenous protein, for example, in features of posttranslational modification.

The primary role of erythropoietin is an essential hormone for red cell production. Without it, definitive erythropoiesis does not take place. Under hypoxic conditions, the kidney will produce and secrete erythropoietin to increase the production of red blood cells by targeting CFU-E, proerythroblast and basophilic erythroblast subsets in the differentiation. Erythropoietin has its primary effect on red blood cell progenitors and precursors by promoting their survival through protecting these cells from apoptosis.

Erythropoietin has a range of actions including vasoconstriction-dependent hypertension, stimulating angiogenesis, and inducing proliferation of smooth muscle fibers. It can increase iron absorption by suppressing the hormone hepcidin (Ashby *et al.,* 2010).

Multiple studies have suggested that erythropoietin improves memory. This effect is independent of its effect on hematocrit (Miskowiak *et al.,*2007; Miskowiak *et al.,*2007). Rather, it is associated with an increase in hippocampal response and effects on synaptic connectivity,neuronal plasticity, and memory-related neural network (Adamcio *et al.,* 2008; Adamcio *et al.*,2010). Erythropoietin may have effects on mood (Miskowiak *et al.*,2007).

Erythropoietin has been shown to exert its effects by binding to the erythropoietin receptor (EpoR) (Middleton *et al.,* 1999; Livnah *et al.,*1998).

Erythropoietin is highly glycosylated (40% of total molecular weight), with half-life in blood around five hours. Erythropoietin's half-life may vary between endogenous and various recombinant versions. Additional glycosylation or other alterations of erythropoietin via recombinant technology have led to the increase of erythropoietin's stability in blood (thus requiring less frequent injections). Erythropoietin binds to the erythropoietin receptor on the red cell progenitor surface and activates a JAK2 signaling cascade. Erythropoietin receptor expression is found in a number of tissues, such as bone marrow and peripheral/central nervous tissue. In the bloodstream, red cells themselves do not express erythropoietin receptor, so cannot respond to erythropoietin.

However, indirect dependence of red cell longevity in the blood on plasma erythropoietin levels has been reported, a process termed neocytolysis.

Erythropoietin levels in blood are quite low in the absence of anemia, at around 10 mU/ml. However, in hypoxic stress, erythropoietin production may increase 1000-fold, reaching 10,000 mU/ml of blood. Erythropoietin is produced mainly by peritubular capillary lining cells of the renal cortex, which are highly specialized, epithelial-like cells. It is synthesized by renal peritubular cells in adults, with a small amount being produced in the liver . Regulation is believed to rely on a feedback mechanism measuring blood oxygenation (Jelkam *et al.*, 2007). Constitutively synthesized transcription factors for erythropoietin, known as hypoxia-inducible factors,are hydroxylated and proteosomally digested in the presence of oxygen.

Erythropoietin (Epo) is a complex molecule, which regulates red blood cell production in the bone marrow.Recombinant human erythropoietin (rHuEPO) is commercially available and is widely used for the treatment of anemia. In recent years, additional nonerythropoietic tissue/organ protective properties of erythropoietin have become apparent, in particular for kidneys.

Erythropoietin is a 30.4 kD glycoprotein and class I cytokine consisting of 165 amino acids (Mocini *et al.,* 2007). Erythropoietin has four acidic oligosaccharide side chains (3 N-linked and 1 O-linked) and contains up to 14 sialic acid residues. Its carbohydrate portion contributes 40% of its molecular weight (Mocini *et al.*,2007). The N-linked polysaccharide side chains appear to be important for the biosynthesis and secretion of erythropoietin, enhance its stability in blood, and limit hepatic clearance, thus facilitating the systemic transit of erythropoietin from kidney to bone marrow (Obeagu, 2015).

The variable nature of the sialic acid content gives rise to erythropoietin isoforms with differences in charge. As the number of sialic acid groups on the carbohydrate portion of erythropoietin increase, so does its serum half-life, whereas receptor-binding capacity decreases (Cartlin *et al.,* 2002; Elliot *et al.*, 2004; Middleton *et al.,* 1999; Weidemann and Johnson, 2009). Clearance, however, appears to have a stronger influence on *in vivo* activity than receptor-binding affinity.

Each erythropoietin molecule has two erythropoietin receptor (EpoR) binding sites. There are two affinities of the EpoR for erythropoietin in solution: one of high and one of low affinity (needs 1,000 times the concentration of erythropoietin for activation) (Weidemann and Johnson, 2009).

The kidneys are bean-shaped organs that serve several essential regulatory roles in vertebrate animals.They remove excess organic molecules and it is by this action that their best-known function is performed: the removal of waste products of metabolism.They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance, and regulation of blood pressure .They serve the body as a natural filter of the blood, and remove water soluble wastes,which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the reabsorption of water, glucose, and amino acids.The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme renin, the latter of which indirectly acts on the kidney in negative feedback.

Diseases of the kidney are diverse, but individuals with kidney disease frequently display characteristic clinical features.Common clinical conditions involving the kidney include the nephritic and nephrotic syndromes, renal cysts, acute kidney injury, chronic kidney disease, urinary tract infection, nephrolithiasis, and urinary tract obstruction (Contran *et al*., 2005).

Many other disease cases have adverse effects on the kidney which is the major site of production of erythropoietin such hypertension, diabetes, HIV/AIDS. Various cancers of the kidney exist; the most common adult renal cancer is renal cell carcinoma. Cancers, cysts, and some other renal conditions can be managed with removal of the kidney, or nephrectomy. When renal function, measured by glomerular filtration rate, is persistently poor, dialysis and kidney transplantation may be treatment options. Although they are not normally harmful, kidney stones can be painful, and repeated, chronic formation of stones can scar the kidneys. The removal of kidney stones involves ultrasound treatment to break up the stones into smaller pieces, which are then passed through the urinary tract. One common symptom of kidney stones is a sharp to disabling pain in the medial/lateral segments of the lower back or groin.

The kidneys secrete a variety of hormones, including erythropoietin, and the enzyme renin. Erythropoietin is released in response to hypoxia in the renal circulation. It stimulates erythropoiesis in the bone marrow. Calcitriol, the activated form of vitamin D, promotes intestinal absorption of calcium and the renal reabsorption of phosphate. Part of the renin-angiotensin-aldosterone system, renin is an enzyme involved in the regulation of aldosterone levels.

Many renal diseases are diagnosed on the basis of classical clinical findings. A physician (usually a nephrologist) begins by taking a detailed clinical history and performs a physical examination. In addition to medical history and presenting symptoms, a physician will ask about medication history, family history recent infections, toxic/chemical exposures and other historical factors that may indicate an etiology for the patient's renal disease.Often, some diseases are suggested by clinical history and time course alone. For example, in a formerly healthy child with a recent upper respiratory tract infection and facial/lower limb swelling, findings of proteinuria on urinalysis, a diagnosis of minimal change disease is highly suggested. Similarly, a patient with a history of diabetes who presents with decreased urine output is most likely to be suffering from diabetic nephropathy. Often, such cases do not require extensive workup (such as with renal biopsy). A presumptive diagnosis can be made on the basis of history, physical exam and supportive laboratory studies.

Laboratory studies are an important adjunct to clinical evaluation for assessment of renal function. An initial workup of a patient may include a complete blood count (CBC);serum electrolytes including sodium, potassium, chloride, bicarbonate, calcium, and phosphorus; blood urea, nitrogen and creatinine; blood glucose and glycocylated hemoglobin. Glomerular filtration rate (GFR) can be calculated.

Urine studies may include urine electrolytes, creatinine, protein, fractional excretion of sodium (FENA) and other studies to assist in evaluation of the etiology of a patient's renal disease.

Urinalysis is used to evaluate urine for its pH, protein, glucose, specific gravity and the presence of blood/hemoglobin. Microscopic analysis can be helpful in the identification of casts, red blood cells, white blood cells and crystals.When the kidneys are in disease condition,the level of erythropoietin will be affected.

The anemia of chronic kidney disease (CKD) is, in most patients, normocytic and normochromic. It is principally due to reduced renal erythropoietin (EPO) production and, to a lesser degree, to shortened red cell survival and decreased responsiveness to the hormone.

Anemia can develop well before the onset of uremic symptoms due to end-stage renal disease (ESRD).Although anemia due to renal dysfunction generally develops when the glomerular filtration rate (GFR) declines to <30 mL/min, it can also be observed in those with markedly higher GFRs (such as 60 mL/min) and tends to occur at higher levels of GFR in African Americans than whites.

If left untreated, the anemia of chronic kidney disease is associated with several abnormalities. These include deterioration in cardiac function, decreased cognition and mental acuity, fatigue, and other signs and symptoms. There are also associations with an increased risk of morbidity and mortality, principally due to cardiac disease and stroke.

There is high level of both documented cases of renal diseases and undocumented ones especially in the rural areas.As erythropoietin is produced mainly in the kidney any damage in it can affect the haematocrit level which will affect the general well-being of the patient.The focus of this research is to estimate erythropoietin and haematocrit in diagnosed renal diseases.

ERYTHROPOIETIN (EPO)

Erythropoietin, also known as erythropoetin, erthropoyetin or erythropoietin, is a glycoprotein hormone that controls erythropoiesis, or red blood cell production.It is a cytokine for erythrocyte precursors in the bone marrow. Human erythropoietin has a molecular weight of 30.4 kDa (Obeagu, 2015).

Also called hematopoietin or hemopoietin, it is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and tubular epithelial tubule.It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood.In addition to erythropoiesis, erythropoietin also has other known biological functions. For example, it plays an important role in the brain's response to neuronal injury (Siren *et al.,*2001).Erythropoietin is also involved in the wound healing process (Haron *et al.,* 2003).

When exogenous erythropoietin is used as a performance-enhancing drug, it is classified as an erythropoiesis-stimulating agent (ESA). Exogenous erythropoietin can often be detected in blood, due to slight differences from the endogenous protein, for example, in features of posttranslational modification.

Effects of Erythropoietin on red blood cell

The principal physiological function of erythropoietin is red blood cell production, which results from a tightly controlled proliferation and differentiation pathway (Salahudeen et al.,2008).Early hematopoietic progenitor cells differentiate into burst-forming unit-erythroid cells (BFU-Es). Continuous stimulation with erythropoietin triggers the differentiation of CFU-Es into erythroblasts, which lose their nuclei to form reticulocytes. After a few days, reticulocytes lose reticulin and become erythrocytes (red blood cells). Reticulocytes and erythrocytes stop expressing EpoR and cease being responsive to erythropoietin (Silva *et al.,*1999).

Erythropoietin -binding to EpoRs on erythroid progenitor cells leads to activation of the JAK2-STAT5 signaling pathway and phosphorylation of PI3K and Akt1 (Cazzola *et al.,*2007; Mocini *et al.,*2007).

Akt-mediated phosphorylation of Bad in the Bad-Bcl-xL complex releases the antiapoptotic protein Bcl-xL, which suppresses erythroid progenitor cell apoptosis (Joyeux-Faure *et al.,*2005). Akt also is involved in several pathways that promote cell survival and antiapoptotic effects through inhibition of FOXO3a, inactivation of GSK3β, induction of XIAP, inactivation of caspases, and prevention of cytochrome C release. These effects not only enhance the erythropoietic properties of erythropoietin but appear to be important in the protection of other cell types and may contribute to the reported neuronal and renal protective effects (Cazzola *et al.,*2007).

Nonhematopoietic roles

Erythropoietin has a range of actions including vasoconstriction-dependent hypertension, stimulating angiogenesis, and inducing proliferation of smooth muscle fibers. It can increase iron absorption by suppressing the hormone hepcidin (FDA,2011).

Erythropoietin also affects neuronal protection during hypoxic conditions (stroke, etc.) (Siren *et al.,*2001). Trials on human subjects are not yet reported; if proven to be a viable treatment of heart attack and stroke patients, it could improve the outcome and quality of life. The reasoning behind such a proposal is that erythropoietin levels of 100 times the baseline have been detected in brain tissue as a natural response to (primarily) hypoxic damage.

Multiple studies have suggested that erythropoietin improves memory. This effect is independent of its effect on hematocrit (Miskowiak *et al.,*2007; Miskowiak *et al.,*2007). Rather, it is associated with an increase in hippocampal response and effects on synaptic connectivity, neuronal plasticity, and memory-related neural networks (Adamcio *et al.,*2008; Adamcio *et al.,*2010).EPO may also be an effective treatment for depression (Miskowiak *et al.,*2007; Miskowiak *et al.,* 2007).

Mechanism of action

Erythropoietin has been shown to exert its effects by binding to the erythropoietin receptor (EpoR) (Middleton *et al.,*1999; Livnah *et al.,*1998).

Erythropoietin is highly glycosylated (40% of total molecular weight), with half-life in blood around five hours. Erythropoietin's half-life may vary between endogenous and various recombinant versions. Additional glycosylation or other alterations of erythropoietin via recombinant technology have led to the increase of erythropoietin's stability in blood (thus requiring less frequent injections). Erythropoietin binds to the erythropoietin receptor on the red cell progenitor surface and activates a JAK2 signaling cascade.Erythropoietin receptor expression is found in a number of tissues, such as bone marrow and peripheral/central nervous tissue. In the bloodstream, red cells themselves do not express erythropoietin receptor, so cannot respond to erythropoietin. However, indirect dependence of red cell longevity in the blood on plasma erythropoietin levels has been reported, a process termed neocytolysis.

Synthesis and regulation

Erythropoietin levels in blood are quite low in the absence of anemia, at around 10 mU/ml. However, in hypoxic stress, erythropoietin production may increase 1000-fold, reaching 10,000 mU/ml of blood.

Erythropoietin is produced mainly by peritubular capillary lining cells of the renal cortex, which are highly specialized, epithelial-like cells. It is synthesized by renal peritubular cells in adults, with a small amount being produced in the liver (Obeagu,2015). Regulation is believed to rely on a feedback mechanism measuring blood oxygenation (Jelkman,2007). Constitutively synthesized transcription factors for erythropoietin, known as hypoxia-inducible factors, are hydroxylated and proteosomally digested in the presence of oxygen.

Medical uses

Erythropoietins available for use as therapeutic agents are produced by recombinant DNA technology in cell culture, and include Epogen/Procrit (epoetin alfa) and Aranesp (darbepoetin alfa); they are used in treating anemia resulting from chronic kidney disease, inflammatory bowel disease (Crohn's disease and ulcer colitis) (Liu *et al*.,2013) and myelodysplasia from the treatment of cancer (chemotherapy and radiation), but include boxed warnings of increased risk of death, myocardial infarction, stroke, venous thromboembolism, tumor recurrence, and other severe off-target effects (FDA,2011).

Blood doping

Erythropoiesis-stimulating agents (ESAs) have a history of use as blood doping agents in endurance sports, such as horseracing, boxing, cycling, rowing, distance running, race walking, snowshoeing, cross country skiing, biathlon, and triathlon. The overall oxygen delivery system (blood oxygen levels, as well as heart stroke volume, vascularization, and lung function) is one of the major limiting factors to muscles' ability to perform endurance exercise. Therefore, the primary reason athletes may use ESAs is to improve oxygen delivery to muscles, which directly improves their endurance capacity. With the advent of recombinant erythropoietin in the 1990s, the practice of autologous and homologous blood transfusion has been partially replaced by injecting erythropoietin such that the body naturally produces its own red cells. ESAs increase hematocrit and total red cell mass in the body, providing a good advantage in sports where such practice is banned(Jelkman and Lundy, 2011). In addition to ethical considerations in sports, providing an increased red cell mass beyond the natural levels reduces blood flow due to increased viscosity, and increases the likelihood of thrombosis and stroke. Due to dangers associated with using ESAs, their use should be limited to the clinic where anemic patients are boosted back to normal hemoglobin levels (as opposed to going above the normal levels for performance advantage, leading to an increased risk of death).

Though erythropoietin was believed to be widely used in the 1990s in certain sports, there was no way at the time to directly test for it, until in 2000, when a test developed by scientists at the French national antidoping laboratory (LNDD) and endorsed by the World Anti-Doping Agency (WADA) was introduced to detect pharmaceutical erythropoietin by distinguishing it from the nearly identical natural hormone normally present in an athlete's urine.

In 2002, at the Winter Olympic Games in Salt Lake City, Dr. Don Catlin, the founder and then-director of the UCLA Olympic Analytical Lab, reported finding darbepoetin alfa, a form of erythropoietin, in a test sample for the first time in sports. At the 2012 Summer Olympics in London, Alex Schwazer, the gold medalist in the 50-kilometer race walk in the 2008.

Summer Olympics in Beijing, tested positive for erythropoietin and was disqualified.
Since 2002, erythropoietin tests performed by US sports authorities have consisted of only a urine or "direct" test. From 2000–2006, erythropoietin tests at the Olympics were conducted on both blood and urine . However, several compounds have been identified that can be taken orally to stimulate endogenous erythropoietin production. Most of the compounds stabilize the hypoxia-inducible transcription factors which activate the v gene. The compounds include oxo-glutarate competitors, but also simple ions such as cobalt(II) chloride (Jelkman,2012).
Synthetic erythropoietin is believed to have come into use in cycling about 1990 . In theory, erythropoietin use can increase VO2max by a significant amount, making it useful for endurance sports like cycling.

History of Erythropoietin

In 1905, Paul Carnot, a professor of medicine in Paris, and his assistant, Clotilde Deflandre, proposed the idea that hormones regulate the production of red blood cells. After conducting experiments on rabbits subject to bloodletting, Carnot and Deflandre attributed an increase in red blood cells in rabbit subjects to a hemotropic factor called hemopoietin. Eva Bonsdorff and Eeva Jalavisto continued to study red cell production and later called the hemopoietic substance 'erythropoietin'. Further studies investigating the existence of erythropoietin by K.R. Reissman and Allan J. Erslev (Thomas Jefferson Medical College) demonstrated that a certain substance, circulated in the blood, is able to stimulate red blood cell production and increase hematocrit.This substance was finally purified and confirmed as erythropoietin, opening doors to therapeutic uses for erythropoietin in diseases such as anemia (Jelkman,2007).
Haematologist John Adamson and nephrologist Joseph W. Eschbach looked at various forms of renal failure and the role of the natural hormone erythropoietin in the formation of red blood cells. Studying sheep and other animals in the 1970s, the two scientists helped establish that erythropoietin stimulates the production of red cells in bone marrow and could lead to a treatment for anemia in humans. In 1968, Goldwasser and Kung began work to purify human erythropoietin, and managed to purify milligram quantities of over 95% pure material by 1977. Pure erythropoietin allowed the amino acid sequence to be partially identified and the gene to be isolated (Jelkman, 2007). Later, an NIH-funded researcher at Columbia University discovered a way to synthesize erythropoietin. Columbia University patented the technique, and licensed it to Amgen. Controversy has ensued over the fairness of the rewards that Amgen reaped from NIH-funded work, and Goldwasser was never financially rewarded for his work.
In the 1980s, Adamson, Joseph W. Eschbach, Joan C. Egrie, Michael R. Downing and Jeffrey K. Browne conducted a clinical trial at the Northwest Kidney Centers for a synthetic form of the hormone, Epogen, produced by Amgen. The trial was successfu l (Chapman,2012).
In 1985,Lin et al isolated the human erythropoietin gene from a genomic phage library and were able to characterize it for research and production (Ahmet,2005).Their research demonstrated the gene for erythropoietin encoded the production of erythropoietin in mammalian cells that is biologically active in vitro and in vivo.There is industrial production of recombinant human erythropoietin (RhEpo) for treating anemia patients .
In 1989, the US Food and Drug Administration approved the hormone Epogen, which remains in use today.

The structure of the Erythropoietin molecule

Erythropoietin is a 30.4 kD glycoprotein and class I cytokine consisting of 165 amino acids (Mocini *et al.,*2007). Erythropoietin has four acidic oligosaccharide side chains (3 N-linked and 1 O-linked) and contains up to 14 sialic acid residues. Its carbohydrate portion contributes 40% of its molecular weight (Mocini *et al.,*2007) . The N-linked polysaccharide side chains appear to be important for the biosynthesis and secretion of v, enhance its stability in blood, and limit hepatic clearance, thus facilitating the systemic transit of erythropoietin from kidney to bone marrow .

The variable nature of the sialic acid content gives rise to erythropoietin isoforms with differences in charge. As the number of sialic acid groups on the carbohydrate portion of erythropoietin increase, so does its serum half-life, whereas receptor-binding capacity decreases (Catlin *et al.,*2002; Elliott *et al.,*2004; Middleton et al.,1999). Clearance, however, appears to have a stronger influence on in vivo activity than receptor-binding affinity.

Each erythropoietin molecule has two erythropoietin receptor (EpoR) binding sites. There are two affinities of the EpoR for erythropoietin in solution: one of high and one of low affinity (needs 1,000 times the concentration of erythropoietin for activation)(Weidemann and Johnson,2007).

Physiological stimuli for Erythropoietin production/release

Approximately 90% of systemic erythropoietin in adults is produced by peritubular interstitial fibroblasts in the renal cortex and outer medulla of the kidney. A feedback mechanism involving oxygen delivery to the tissues appears to regulate erythropoietin production (Jelkmann *et al.,* 2007). Hypoxia-inducible factor (HIF) regulates transcription of the erythropoietin gene in the kidney, which determines v synthesis. This process is dependent on local oxygen tension. HIF is quickly destroyed in well-oxygenated cells through ubiquitylation (tagging for degradation in the proteasome) by the von Hippel-Landau tumor suppressor protein (pVHL), but when oxygen delivery decreases, pVHL ceases its proteolysis of HIF, increasing the levels of HIF, which subsequently increases erythropoietin production (Diskin *et al.,*2008; Bahlmann and Fisher,2009).

Structure of Erythropoietin receptors

The erythropoietin receptor (EpoR) is a 66 kD membrane glycoprotein typically consisting of 484 amino acids and 2 peptide chains; it belongs to a large cytokine and growth factor receptor family (Obeagu, 2015). Binding studies have demonstrated that the EpoR has different affinities for erythropoietin and that EpoR isoforms with higher affinity for erythropoietin may be responsible for the erythropoietic effects of Erythropoietin, whereas isoforms with a lower affinity for erythropoietin binding may have nonerythropoietic effects, such as tissue protection (Johnson *et al.,* 2006).

The cytoplasmic domains of the EpoR contain a number of phosphotyrosines that are phosphorylated by the activation of a member of the Janus-type protein tyrosine kinase family (JAK2), which is bound to the common beta subunit of the EpoR (Percy *et al.,*2008). In addition to activating the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and protein kinase B (Akt) pathway, phosphotyrosines also serve as docking sites for signal transducer and activators of transcription (STATs), such as STAT5.

Dephosphorylation of JAK can be induced by phosphatase with the consequent internalization and degradation of the Epo/EpoR complex, which marks the end of erythropoietin activity. This prevents overactivation, which may lead to excessive erythrocytosis (Li *et al.,*2004).

The main pathways of the effects of erythropoietin. The intracellular domain of the EPOR contains phosphotyrosines, which are phosphorylated by activation of a member of the Janus-type protein tyrosine kinase family (JAK2) bound to the EpoR.

Erythropoietin (Epo) is present in low amounts in the circulation under homeostatic conditions,wheras erythropoietic stress,such as hypoxia or anaemia,can stimulate a dramatic increase in erythropoietin production in the kidney, leading to a significant rise in circulating hormone amounts and subsequently increased erythropoiesis. Erythropoietin stimulates red blood cell production by binding and activating a high affinity receptor (EpoR) that is expressed predominantly on the surface of immature erythroid cells. EpoR is a member of the type 1 cytokine receptor superfamily,sharing specific structural motifs with other members of this receptor family including 2 extracellualr immunoglobulin-like domains,similarly spaced cysteine residues, and the sequence WSXWS.Signal transduction throgh the EpoR is initiated by ligand binding,which induces a dimerisation and/or reorientattion of EpoR monomers. Within a dimeric receptor structure,a process that remains poorly understood despite its clear importance in terms of developing erythropoietin agonists. The general mechanisms used by EpoR to activate intracellular signal transduction pathways are shared by other members of the type I & ii cytokine receptor families,namely ligand –dependent oligomerisation and/ or structural orientation of clustered receptor molecules (Stephanie, 2011). The predominant pathway activated by EpoR and other cytokine kinase are constitutively associated with the membrane-proximal regions of cytokine receptor intacellular domains and are activated upon lignad binding and receptor reorientation. The EpoR associates selectively with the Jak 2 kinase. After EpoR activation, Jak2 phosphorylates tyrosine residues in the intracellular region of the EpoR,providing docking sites for signaling molecules with phosphtyrosine binding motifs, including the signal transducer and activator of transcription protein STAT5, which mediates the principal intracellular signaling pathway elicited by the EpoR.

Molecular structure of the Erythropoeitin receptor (EpoR)

The 3-dimensional structure of the EpoR extracellular region was first determined at 2.8 A resolution in complex with the agonist peptide EMP1 and later in complex with erythropoietin at 1.9 A resolution. Both approaches showed that the erythropoietin extracellular region comprises 2 immunoglobulin-like domains, each formed by a β-sandwich-like structure containing 7- β-strands. The membrane-distal(D1) domain and membrane-proximal (D2) domain are linked by a short hinge and are oriented at approximately 90 degrees to one another. The D1 domain contains the 4 conserved cysteine residues,which form 2 intracellular disulphide bridges that stabilise the EpoR WSXWS motif,this motif seems to stabilise the EpoR teriary structure.

Th EpoR: Epo complex revealed that Epo has 2 discrete binding sites for the EpoR. One binding interface of the ligand governs a high affinity interaction with the receptor,comprising a hydrophobic core surrounded by hydrophylic residues, a motif that has been referenced as a hot spot in terms of directing cytokine:

cytokine receptor interactions.The high affinity site ehibits a dissociation constatnt of aproximately 1µm and is thought to contribute the majority of the ligand binding energy.A second binding site that uses a distinct set of determinant residue on erythropoietin as well as EpoR has an affinity approximately 1000-fold lower. Thus,a sequential binding model for lignad-mediated activation of EpoR has been proposed, in which ligand interacts first via the high affinity interaction with the second EpoR monomer. Despite the asymmetry of this complex both monomers seem to be functionally similar in terms of activating signal transduction(Zang *et al.,*2009).Moreover,interactions between the transmembrane and membrane proximal cytoplasmic domains of EpoR monomers facilitate dimerisation and/or stabilisattion of the EpoR dimeric complex.The importance of the asymmetric complex and the dimerisation model or receptor activation is supported by observation that an erythropoietin molecule mutated in the site 2 region (R103 A) or EpoRs mutated at the binding region for erythropoietin site 1 or site 2 fail to elicit receptor signaling in haematopoietic cells(Zang *et al.,*2009). The erythropoietin R103A mutant also has provided an opportunity to characterise the EpoR complex on nonhaematopoietic cells. Cytoprotective activities orf erythropoietin on the differentiated neuroblastoma cell line were suppressed considerably with erythropoietin R103A or via RNA-mediated interference of EpoR expression,indicating that survival signaling elicited by erythropoietin on neuronal cells is most likely due to low levels of the EpoR expressed in the configuration of the haematopoietic receptor(Um *et al.*,2007).

The dimeric EpoR structure formed by interaction with erythropoietin or the EMP1 agonist differ in several aspects,likely explaining the fact that EMP1 exhibits reduced potency in terms of EpoR activation,as EMP1 is required at significantly higher concentrations than erythropoietin to elicit erythropoietic responses. The angle between the D1 domains differs in each EpoR:lignad complex.Moreover,the EpoR:Epo complex shows the D2 domain positioned within the same plane while they are twisted at an approximately 45-degree angle in the EpoR:EMP1 structure. This distinction may affect the ability to activate the associated Jak2 kinase,as a particular orientation may be favoured for full Jak2 activation via autophosphorylation. Hence,efficient erythropoietin agonists will likely need to more closely mimic the ligand-occupied receptor orientation (Stephanie ,2011).

Role of dimerisation in EpoR Activation

The activation mechanism for EpoR was elucidated first through investigation of a constitutively acting form of the EpoR,which was isolated from a retroviral transduction screen in the interleukin 3-dependent murine cell line Ba/F3 by virtue of its ability to support cytokine-independent proliferation. Initially, the biochemical basis for the constitutive activity was unclear; however, a point mutation at residue 129 in the extracellular region that rendered an arginine to cysteine substitution(EpoR R129 C) suggested the possibility that aberrant disulphide bond formation was involved. The constiutive actiivity of EpoR R 129 C is attributed to acquisition of cysyteine and not loss of arginine. Studies with the constitutive EpoR provided a paradigm for the process of type 1i and ii cytokine receptor activation because it was subsequently recognised that oligomerisation or structural reorientation of receptor subunits was a common activation mechanisms within the cytokine receptor family.

Equilibrium binding experiments withiodinated erythropoietin demonstrated that Epo:receptor complexs containing either wild –type EpoR or EpoR R129 C where governed by a single affinity, indicating that the 3-dimensional structure of EpoR R 129 C is similar to the wild-type receptor, at least within Epo-binding domain.This suggested that covalent dimerisation of EpoR R 129 C mimics the Epo/EpoR confirmation,further supporting the role for receptor dimerisation in the activation process. EpoR R 129 C stimulates Epo-independent colony forming unit-erythroid (CFU-E) development, as judged by in vitro assays,indicating that it supports erythroid proliferation and dimerisation. Mice infected with retrovirus carrying EpoR R129C develop erythropoiesis and splenomegaly and show increased amounts of circulating red blood cells, demonstrating that EpoR R129C stimulates expansion of the erythroid compartment in vivo and indicating deregulation of homeostatic mechanism most likely due to constitutive signaling of EpoR R129C. This, EpoR R129C seems to mimic the biological activity of the Epo:EpoR complex in terms of directing proliferation and differentiation of red blood cell precursors, without perturbing the reythroid developmental programme. Interestingly, overexpression of EpoR R129C in haematopoietic progenitor cells can enhance the generation of other myeloid lineages, early evidence that suggest redundancy in the signal pathways between blood cell growth factors.EpoR dimerisation by bivalent antibodies,analysis of chimeric receptor molecules,or biochemical studies of the purified poR extarcellular region further supported the idea that receptor clustering is an important step in the activation process. The EpoR; Epo complex is a dimeric receptor occupied by a single Epo molecule in a 2:1 EpoR:Epo ratio.EpoRs seem to be transported to the plasma membrane,where ligand activation induces a confirmation change in the orientation of receptor subunits without the dimer.

The quarternary structure of the EpoR has important implications for haematopoiesis in humans as it suggests that EpoR-mediated signal transduction could be altered in individuals with heterozygous mutation of the EpoR gene resulting from inherited or acquired events.Individuals from an extended Finnish family with dominant benign familial erythrocytosis provide such an example;unfortunately, in this instance,the phenotype is mild.Certain individuals within this Finnish family wrer found to have enhanced erythrocytosis, accompained by increased haematocrits and haemoglobin amounts. Erythroid progenitor cells isolated from these individuals demonstarte hypersensitivity to erythropoietin in culture,as judged bt their ability to undergo effective proliferation and differentiation in reduced erythropoietin amounts in vitro,compared with progenitor cells from unaffected individuals.The individual aexhibiting erythrocytosis possess a mutation in one copy of the EpoR gene,which generates a premature stop codon and truncated receptor isoform lacking approximately 70 amino acids from the carboxy-terminus.This truncated EpoR is missing an important negativel regulatory region in the cytoplasmic domain that is responsible for recruitment of haematopoietic cell phosphatase 1,which has beeen shown to suppress signaling from the EpoR as well as other cytokine receptors upon its association with activated receptors complexes. Assuming both wild-type and mutant EpoR alleles are coexpressed in individuals may express different EpoR complexes versus those with only wild-type EpoR. The EpoR complexes may include homdimers of the truncated EpoR and heterodimers of wild-type and mutant EpoRs,which may alter receptor signal transduction resulting in hypersensitivity to erythropoietin and mild erythropoiesis.

Cytokine-dependent cells that were engineered to express both wild-type and truncated EpoR mimicking the Finnish mutation,or similarly truncated EpoR expressed in affected members of a Sweddish family with dominant erythrocytosis, exhibited enhanced Epo-mediated signal transduction and cellular proliferation compared with cells expressing only wild –type EpoR. In both Finnish and Sweddish families,the EpoR mutation seemsto be inherited with Mendelian frequencies. Moreover, the EpoR mutation functions in a dominant manner relative to the wild-type allele of the EpoR gene (Stephanie, 2011).

Roles for EpoR-mediated Jak 2 and STAT 5 signaling in erythropoiesis

One of the aerlist detectable signaling events elicited upon EpoR activation is tyrosine phosphorylation of several intracellular proteins. Because the receptor lacks a kinase domain within its cytoplasmic region, these results indicated that protein tyrosine kinase function is carried out by a distinct factor.Subsequently, the Jak2 protein tyrosine kinase was identified as associating with the EpoR and serving as the principal kinase involved in mediating Epo-responsive signal transduction. Jak2 is constitutively bound to the EpoR intracellular region and seems to provide a chaperone function for newly assembled EpoR molecules, aiding their transit through the secretory pathway from the endoplasmic reticulum to the plasma membrane.

Significantly, Jak 2 function is important in human erythropoiesis. A mutation within the Jak 2 pseudokinase domain rendering a valine to phenylalanine substitution at residue 617 (V617F) and hormone-independent kinase activity of myeloproliferative disorders (MPDs) including polycythaemia vera. In vitro and in vivo approaches to study Jak2V617F show that this mutant protein mediates erythropoietin-independent erythroid progenitor growth and development as well as erythroid cell expansion in vivo. (The EpoR contains 8 tyrosine residues within te membrane –distal portion of cytoplasmic tail,docking sites for intracellular signaling molecules including the transcription factors STAT 5 A and STAT 5 B, the p 85 subunit of phodphoinositol $3^{/}$ kinase (PI3K), the cytokine suppressor CIS, and the phosphatase SHP-1. Tyrosine phosphorylated Jak 2 also seems to interact directly with STAT 5 A and STAT 5 B, indicating that it can serve as a scaffold for signal protein activation in addition to its enzymatic role in EpoR signal transduction.Recruitment of signaling molecules in proximity to Jak 2 in the EpoR complex enables their tyrosine phosphorylation,which is an important step in subsequent activation of their respective signaling cascades.The STAT5A and STAT 5 B proteins are predominant signal transducers for EpoR; these proteins are activated within seconds of erythropoietin binding and accumulate in the nucleus to mediate erythropoietin-responsive transcription.

Erythropoietin assay

A classification of anaemia has been propsed around the concept of adequate or inadequate erythropoietin response to degree of anaemia. There are several problems with the use of erythropoietin levels in the management of patients. The interpretation of an erythropoietin level must take into account the degree of anaemia at the time of measurement.Commercial assay results do not take this into consideration; hence,clinicians must have some familiarity with mathematical corrections, such as observed / predicted ratios.

All anaemic patients with cancer or on renal dialysis have erythropoietin levels taht are inadequate for the degree of anaemia, measuring erythropoietin levels is not useful in these settings. Furthermore,guidelines recommend that erythropoietin therapy be instituted before haemoglobin levels fall below 10g/dl, a level is not valid. The erythropoietin assay may be most useful as a determinant of response to therapy in certain patients,such as those with myelodysplasia (Stephanie, 2011).

The proliferative state of bone marrow erythroid cells affects erythropoietin levels,as does iron status,haemolysis,and chemotherapy-induced endothelial damage. Transferrin has helped in the understanding of the relationship between haemoglbin level and serum erythropoietin.Before haemoglobin level increased,erythroid activity measured by transferrin receptor (TFR) reappeared. The increased clearance of erythropoietin is probably related to an influx of early red blood cell precursrors into CFU-E from primitive erythroid burst-forming units (BFU-E). CFU-E has a higher concentration of erythropoietin receptors than BFU-E, so late BFU-E throgh the proeythroblast statge defines the narrow window of erythroid cellular compartment that is erythropoietin responsive.

Post-receptor (intracellular) effects of Erythropoietin

There are a number of common pathways through which erythropoietin exerts its erythropoietic effects that also appear to confer tissue protection. Erythropoietin binds to two EpoRs, which become joined as a homodimer and change. This activates JAK 2, which is bound to the common beta subunit of the EpoR (Percy *et al.,* 2004) and leads to phosphorylation of tyrosine residues of the EpoR, which activates a number of signaling pathways.

Erythropoietin classically signals through the "signal transducer and activator of transcription 5" (STAT-5) pathway. The STAT proteins are direct substrates of Janus kinases (JAKs), which results in tyrosine phosphorylation of the STATs as well as phosphorylation of the phosphatidylinositol 3-kinase (PI3K) and subsequent phosphorylation of Akt.

The principal component of pathways that promote anti-apoptotic effects is Akt, which inactivates caspases, the major mediators of apoptosis, mitochondrial dysfunction, and subsequent release of cytochrome C (Rusai *et al.*,2010). Erythropoietin's ability to maintain cellular integrity and prevent inflammatory apoptosis is closely linked to maintenance of mitochondrial membrane potential, modulation of Apaf-1, inhibition of cytochrome C release, and inhibition of caspases. Recent data also indicate that serum and glucocorticoid-regulated kinase-1 (SGK1) may contribute to the mediation of erythropoietin's renoprotective effects (Myklebust *et al.,*2009).

Apoptotic pathways influenced by erythropoietin. Activated STAT5 promotes transcription of promitogenic and antiapoptotic genes associated with apoptotic regulation and cytoprotection. Akt promotes cell survival and antiapoptotic effects by (Mocini *et al.*,2007)) inhibiting forkhead .

The phosphorylation of mitogen-activated protein kinases (MAPKs) appears to contribute to the cell protection erythropoietin confers (Boissel *et al.,*1993). Protein kinase C (PKC) also is involved in inhibition of apoptosis and cell survival. It regulates the erythropoietin-induced erythroid proliferation and differentiation (Miller *et al.,* 1999) and interferes with phosphorylation of the EpoR, making it a likely upstream modulator of the EpoR.

Erythropoietin may be involved in modulation of cellular calcium homeostasis by increasing calcium influx (Figueroa *et al.,* 2002). Nuclear factor-kappaB (NF-kB), a mediator of inflammatory and cytokine response, is implicated in erythropoietin signaling. The cytoprotection of erythropoietin partly depends on Akt and subsequent NF-kB activation. NF-kB plays a role in the release of erythropoietin during HIF-1 induction; Akt can increase NF-kB and HIF-1 activation with resultant increase in erythropoietin expression (Yang *et al.*,2003).

Finally, induction of heat shock protein 70 (HSP70) by erythropoietin is related to renal protection in ischemic kidneys (Lui *et al.*,2007). HSP70 prevents apoptosis by inhibiting movement of apoptosis inducing factor (AIF) to the nucleus (Beere *et al.,*2000)and by preventing Apaf-1/cytochrome C binding in the cytosol (Elliott *et al.,*2008).

The pleiotropic effects of Erythropoietin

The tissue protective or "pleiotropic" effects of erythropoietin beyond erythropoiesis have been shown in the kidney in many animal and some clinical studies.

Its tissue protective effects may be elicited through the EpoR homodimer via JAK2-STAT5 activation and inhibition of apoptosis or may be mediated by a second EpoR isoform heterodimer composed of an EpoR monomer and the cytokine receptor, common beta subunit (CD-131). For example, carbamylated v (CEPO) does not bind to the classical EPOR isoform and is devoid of hematopoietic activity; however, it can provide tissue protection in the kidney, supporting the existence of a heteroreceptor erythropoietin isoform, which mediates tissue protection (Westenfelder *et al.,*1999). It is clear that the relationship of erythropoietin with its receptor is extremely complex. Therefore, further investigation is required to fully understand the EpoR heterodimer isoform, and the mechanisms and pathways involved in its tissue protective activity.

Animal and in vitro studies

Many animal studies have shown that erythropoietin administration protects kidney tissue from damage and improves renal function in ischemia-reperfusion (IR) and contrast-induced injury models of AKI (Ates *et al.,*2005; Esposito *et al.*,2009; Forman *et al.,*2002; Gong *et al.,* 2004; Imamura *et al.*, 2008; Imamura *et al.,*2007; Johnson *et al.*,2006; Kiris *et al.*,2008) in which erythropoietin reduced kidney dysfunction by decreasing apoptosis. In addition, erythropoietin has been shown to reduce the expression of proinflammatory mediators, TNF-alpha and IL-2, in IR renal injury and reverse the effect of endotoxin on the antioxidant, renal superoxide dismutase (SOD). These anti-inflammatory properties of erythropoietin also suggest involvement of the NF-kB pathway in its kidney protection.

Animal studies of erythropoietin in nonischaemic models of Acute kidney injury

CEPO: The administration of carbamylated erythropoietin (CEPO), which does not bind to the classical EpoR, also provides renal tissue-protective effects. In an IR rat model, CEPO markedly reduced apoptosis and increased tubular epithelial cell proliferation. Moreover, CEPO was more protective against IR injury to tubular epithelial cells than erythropoietin in this study. In an in vitro model performed by the same team, CEPO promoted more capillary formation than erythropoietin and also appeared to protect the kidneys from IR injury by promotion of angiogenesis (Vaziri *et al.,* 1994). This protective effect requires mitogenesis and endothelial progenitor cell differentiation, proliferation, and migration.

Erythropoietin activates endothelial nitric oxide synthase, and this effect on the endothelium may be critical for the renal tissue protective effects of erythropoietin. Erythropoietin is an extremely potent stimulator of endothelial progenitor cells, whose function is partly dependent on nitric oxide bioavailability. Endothelial progenitor cells appear to be involved in endothelial recovery after injury. Erythropoietin limits AKI in part by stimulating vascular repair and by mobilizing endothelial progenitor cells and increasing tubular cell proliferation (Westenfelder *et al.,*1999). These findings suggest that erythropoietin may exert a protective effect via an interaction with the microvasculature.

Angiogenesis and erythropoietin's renoprotective effects may be influenced by vascular endothelial growth factor (VEGF). Nakano and colleagues found that the vascular Epo/EpoR system promoted postischemic angiogenesis by upregulating the VEGF/VEGF receptor system, both directly by promoting neovascularization and indirectly by mobilising endothelial progenitor cells and bone marrow-derived proangiogenic cells (Nakano *et al.,*2007). It appears that angiogenesis is impaired and blood vessels are less responsive to VEGF in the absence of EpoR.

Erythropoietin in Acute kidney injury

Acute kidney injury is common in critically ill patients (Bagshaw *et al.*,2008; Ostermann and Chang,2007) and is independently associated with increased mortality, and with prolonged length of stay. It escalates both the human and financial costs of care. Therefore, it seems desirable to investigate treatments with potential to ameliorate or prevent Acute kidney injury. Some injury pathways for Acute kidney injury in the critically ill include exposure to endogenous and exogenous toxins, metabolic factors, ischemia and reperfusion insults, neurohormonal activation, inflammation, and oxidative stress. Of these, ischemia-reperfusion may be the most common. Erythropoietin can prevent or reduce injury and assist renal repair and recovery through limitation of apoptosis, promotion of neovascularization, anti-inflammatory action, and tissue regeneration.

Investigation of potential treatments for acute kidney injury has had limited success to date; however, from the results of animal and some limited preliminary human studies, therapeutic use of erythropoietin seems promising for those "at risk" for acute kidney injury.

Clinical trials of Erythropoietin in acute kidney injury

One randomized, placebo-controlled, clinical trial of preoperative erythropoietin in 71 patients who underwent elective coronary artery bypass graft (CABG) surgery reported renoprotective effects (Song e*t al.,*2009). EPO was given at a dose of 300 U/kg IV immediately preoperatively and was associated with a reduction in the incidence of AKI from 29% to 8% ($p = 0.035$) and improved postoperative renal function as indicated by a smaller increase in SCr (% increase at 24 hours of 1% vs. 15%, $p = 0.04$) and a smaller decline in estimated GFR (% change at 24 hours of +3% vs. -5%, $p = 0.04$) postoperatively(Song *et al.*,2009).

POTENTIAL RISKS OF Erythropoietin

A. Pure red cell aplasia

Despite the numerous benefits of erythropoietin, there are some risks. Pure red cell aplasia is a rare adverse event, which is characterized by anemia, low reticulocyte count, absence of erythroblasts, resistance to erythropoietin, and neutralizing antibodies against erythropoietin (McKoy *et al.*,2008). This is an extremely rare complication.

B. Cancer patients

Erythropoietin administration in patients with cancer has been associated with increased mortality and enhanced tumor growth (Bohlius *et al.*,2008). The underlying mechanisms remain uncertain, but patients with certain malignancies may be in a hypercoagulable state, making erythropoietin administration unadvisable.

C. Thrombosis

Recent studies and clinical trials have found an increased rate of thrombosis with EPO, which has mainly been observed in patient groups with higher than conventional levels of hemoglobin (> 120 g/L). Exclusion of patients with hemoglobin >120 g/L from clinical trials of erythropoietin minimizes the risk for thrombosis. Nonetheless, systematic assessment for thrombosis should be performed in any erythropoietin trials of critically ill patients because they have an increased risk for thrombosis.

D. Hypertension

Hypertension occurs in approximately 30% of patients who receive long-term erythropoietin treatment and appears to involve increased endothelin release, upregulation of tissue renin and angiotensin production, changes in the balance of vasoactive substances (prostaglandin/prostacyclin/thromboxane), and an elevation of calcium by erythropoietin (at least in chronic kidney disease) that impairs the vasodilating action of nitric oxide. It is advisable that patients with uncontrolled hypertension do not participate in trials of erythropoietin in acute kidney injury.

Carbamylated erythropoietin, a cytoprotective, nonerythropoietic derivative of erythropoietin may not exhibit the same risks as erythropoietin and holds great interest as a future tissue-protective therapy. However, it requires further experimental testing before it can be safely evaluated in clinical trials.

Clinical information

Normally, erythropoietin levels vary inversely with hematocrit if the kidney is not adversely damaged. Hypoxia stimulates erythropoietin release, which, in turn, stimulates bone marrow erythrocyte production. High blood levels of red blood cell, hemoglobin, hematocrit, or oxygen suppress the release of erythropoietin.

Primary polycythemia (polycythemia vera) is a neoplastic (clonal) blood disorder characterized by autonomous production of hematopoietic cells. Increased erythrocytes result in compensatory suppression of erythropoietin levels. Findings consistent with polycythemia vera include hemoglobin >18.5 gm/dL, persistent leukocytosis, persistent thrombocytosis, unusual thrombosis, splenomegaly, and erythromelalgia (dysesthesia and erythema involving the distal extremities).

Secondary polycythemias may either be due to an appropriate or an inappropriate increase in red cell mass. Appropriate secondary polycythemias (eg, high-altitude living and pulmonary disease) are characterized by hypoxia and a compensatory increase in red cell mass. Erythropoietin production is increased in an attempt to increase the delivery of oxygen by increasing the number of oxygen-carrying red blood cells. Some tumors secrete erythropoietin or erythropoietin-like proteins; examples include tumors of the kidney, liver, lung, and brain. Such increases result in inappropriate secondary polycythemias.

Abnormal erythropoietin levels also may be seen in renal failure. The majority of erythropoietin production is in the kidneys. Therefore, chronic renal failure may result in decreased renal erythropoietin production and, subsequently, anemia. In addition to the kidneys, the liver also produces a small amount of erythropoietin. Thus, anephric patients have a residual amount of erythropoietin produced by the liver.

Chronic renal failure patients, as well as patients with anemia due to a variety of other causes including chemotherapy, HIV/AIDS, and some hematologic disorders may be candidates for treatment with recombinant human erythropoietin. Recombinant erythropoietin compounds used to treat anemia include epoetin alpha and darbepoetin. Epoetin alpha is a 165 amino acid glycoprotein produced in mammalian cells and has an identical amino acid sequence to natural human erythropoietin. It has 3 oligosaccharide chains and a molecular mass of 30.4 kDa. Darbepoetin alpha is a 165 amino acid glycoprotein that is also produced in mammalian cells. It has 2 additional N-linked oligosaccharide chains and a molecular mass of 37 kDa. There are no specific assays for measuring recombinant erytropoietin compounds. Drug levels can only be roughly estimated from the cross reactivity of the compounds in erythropoietin assays.

Because results obtained with 1 commercial erythropoietin assay may differ significantly from those obtained with any other, it is recommended that any serial testing performed on the same patient over time should be performed with the same commercial erythropoietin test.

Heterophile antibodies may interfere in this assay. Lower erythropoietin levels than expected have been seen with anemias associated with the following conditions: rheumatoid arthritis, AIDS, cancer, ulcerative colitis, sickle cell disease, and in premature neonates.

After allogeneic bone marrow transplant, impaired erythropoietin response may delay erythropoietin recovery.

Patients with hypergammaglobulinemia associated with multiple myeloma or Waldenstrom disease have impaired production of erythropoietin in relation to hemoglobin concentration. This has been linked to increased plasma viscosity.

There is some diurnal variation in erythropoietin levels. For optimal results in serial patient monitoring, all specimens should be collected at the same time of day. The diurnal variation is minimal in normal individuals (<20%), but in hospitalized patients with a variety of illnesses, as well as ambulatory patients with chronic lung disease, serum erythropoietin concentrations can be 20% to 60% higher at night than early in the morning. This phenomenon is most pronounced in patients with erythropoietin levels within approximately 2-times the upper limit of the normal population reference interval(Casadeval,2003;Fisher,2003;Strippoli *et al.,*2005).

Erythropoietin in health and disease

Under normal steady-state conditions, the concentration of circulating erythropoietin is that amount necessary to maintain the red cell mass and to replace senescent and dying cells (Obeagu, 2015). Sensitive radioactive assays of erythropoietin have revealed this level normally to be approximately 10 to 25 mU/mL (Cotes,1982). However, serum titers can vary considerably. For example, at a hematocrit of 30%, erythropoietin levels can range from 50 to 500 mU/mL.

It was once believed that the overall inverse relationship between serum erythropoietin and hematocrit in anemic patients was lost oncehematocrit fell below 33%, (Nielson,1982) and it now seems that the inverse correlation between serum erythropoietin level and hematocrit holds in anemic patients even if hematocrit is greater than 33%. Despite the difficulty in assigning a strict value to normal erythropoietin levels, it is generally recognized that patients with diseases associated with varying degrees of anemia manifest erythropoietin levels outside the generally accepted range. For example, endocrine renal function decreases in parallel with excretory renal function resulting in erythropoietin deficiency and anemia once creatinine clearance falls below 40 mL/min/1.73 m2.(Radtke *et al.*,1979). However, even severely diseased kidneys are capable of producing some erythropoietin; despite the deficiency, the inverse relationship between erythropoietin level and hematocrit remains intact, although it functions at a lower level. Ninety percent of patients with the myelodysplastic syndrome present with anemia. However, their anemia is not secondary to erythropoietin deficiency as their renal function usually is intact. The myelodysplastic syndrome comprises a group of disorders in which a clonal abnormality of hematopoietic stem cells exists that may progress to an acute leukemia state. Ineffective hematopoiesis is an early feature of myelodysplastic syndrom and causes the associated anemia, but may occur in nonanemic patients. In contrast to the situation in chronic renal failure, there is only a weak inverse correlation between hematocrit and erythropoietin level despite the presence in many myelodysplastic syndrome patients of active erythropoiesis and even erythroid hyperplasia. While erythropoietin levels vary greatly in myelodysplastic syndrome patients for the same or similar hemoglobin concentrations,often serum erythropoietin levels are elevated, but there is not the expected close relationship of erythropoietin level with the degree of anemia. Jacobs et a1 (Jacobs *et al.,*1989) suggested that these observed erythropoietin levels

may be secondary to reduced utilization by erythropoietin responsive cells. Interestingly, the highest erythropoietin levels are seen in those patients with erythroid hypoplasia. Furthermore, patients with sickle cell anemia have low erythropoietin levels for their degree of anemia. Sherwood et a1. (1986) propose a mechanism of ongoing renal damage interfering with the synthesis of erythropoietin or with the function of the putative oxygen sensor.

Human immunodeficiency virus (HIV) infection is associated with defects in hematopoiesis, including decreased proliferation of hematopoietic progenitor cells and increased destruction of mature cells. These events may be secondary to the influence of HIV infection of progenitor cells on bone marrow stromal elements. 4 Regulatory cytokines also are disturbed; thus, hematopoietic cytopenias are common. In patients with acquired immunodeficiency syndrome (AIDS) treated with zidovudine, which causes additional significant bone marrow suppression, two distinct types of anemia can be observed: one associated with macrocytosis and low serum erythropoietin and the other with normocytic red blood cells and high serum erythropoietin. This distinction becomes important when considering treatment of such patients with exogenous erythropoietin. Polycythemia rubra vera is a myeloproliferativedisorder marked by autonomous overproduction of erythrocytes and variable overproduction of granulocytes and platelets. Evidence suggests there is an acquired sensitivity of erythroid precursors to erythropoietin, and erythropoietin levels in polycythemia rubra vera are usually low.

Because the life span of red blood cells remains unchanged during pregnancy, (Pitchard and Adams,1960) this change in red blood cell mass is most likely secondary to decreased erythropoiesis. Although absolute erythropoietin levels increase over nonpregnant values throughout pregnancy and correlate with hematocrit in the third trimester, delivery, and postpartum, no such correlation is evident earlier in pregnancy. Erythropoietin levels remain relatively low for the degree of anemia at this stage, thus likely accounting for the observed decrease in erythropoiesis and total red blood cell mass seen early in pregnancy. Nevertheless, these observed alterations in the rate of erythropoiesis are adaptive and require no therapeutic intervention, as may be the case in the aforementioned conditions.

Chronic kidney disease

The kidneys are vital excretary organs and are central to fluid,electrolyte and acid-base homeostasis in humans.Dmage of the kidneys has serious implications for systemic functions,growth and existence.Irreversible damage that compromises the ability of the kidneys to sustain bodily functions,normal growth and lives as occurs in end stage renal disease poses great challenges of renal replacement strategies and other management modalities.

World Health Organisation (WHO) statistics reveal that the death rate from intrinsic kidney and urinary tract diseases was one million in the year 2002,ranking twelfth on the list major causes of death(WHO,2003).The prevalence of impaired kidney function was greatly underesitimated in the past.The prevalence of impaired kidney function was estimated to range 10% and 205 of the adult population in most countries (WHO,2003).The incidenece of Chronic kidney disease is increasing globally . The National kidney foundation estimates that 20 million Americans have chronic kidney disease and at least a further 20 million people have an increased risk (Johnson *et al;* 2004).

In developing countries like Nigeria, the prevelance of preventable renal diseas is not known.

According to the definition provided by the Kidney Disease Outcome Quality Initiative (KDOQI), the presence of chronic kidney kidney disease should be established based on the occurance of kidney damage and the level of kidney function, regardless of the specific diagnosis of diseases and condisions causing the damage. The diagnosis of chronic kidney disease is based on the evidence of kidney damage and / or reduced kidney lasting at least three months. Initial evidence of kidney damage or a reduction in kidney function can be detected through routine blood or urine testing. The most common indicators of kidney damage are proteins in the urine (proteinuria or albumiuria), blood in the urine and raised levels of urea or creativiue in the blood . However, the reliable marker of kidney damage is the persistence of albumin in the urine. The ratio of albumni to creatimine (ACR) obviates the need for 24-hours urine collection to assess proteinuria, and it corrects well with kidney function . In resource poor-poor countries like Nigeria, this is a useful screening tool to detect chronic kidney disease, Especially in family practice clinics.

Chronic kidney disease (CKD) is consideredas a rapidly growing global health problem, characterize by progressive destruction of renal mass with irreversible sclerosis and loss of nephon. In case of end-stage renal disease and/ or chronic renal failure, kidney transportation is a treatment of chronic for patients through a surgical procedure to take over the task of purifying the blood and remove the waste materials.

The kidney Disease Outcome Quality Initiative designates 5 stages of chronic kidney disease, with stage 5 being ESRD, the point at which patients' loss of kidney function need dialysis or kidney transplant. Patients at too higher risk for chronic kidney disease include patients with diabetes, hypertension,or chronic kidney disease . Most people with chronic kidney disease die of a co-morbid condition, usually cardiovascular disease, before experiencing complete kidney failure requring dialysis or transplantation. However,the onset and progression of chronic kidney disease are highly preventable and early treatment of complications can significantly improve long-term patient outcomes.

Haematological disturbance such as anaemia is considered as a frequent complication occurs in Chronic kidney disease and is associated with morbidity and mortality and a decline in quality of life (Weiss *et al*.,2005). The severity of anaemia is directly proportional to the degree of renal function. One of the important aspects of chronic kidney disease management is the correction of anaemia and the maintenance of haemoglobin level by using Erythropoietin-stimulating agents (ESAs). Also,the measurement of erythropoietin level is useful only for anaemic patients with haemoglobin level of <10g//dl. However,any interpretation of erythropoietin level in anaemia of chronic disease with a haemoglobin level less than 10g/dl must take into acount the degree of anaemia(Aronoff *et al.,*2009).Anaemia is a common feature of chronic kidney disease.The physiologic response to anaemia include vasodilation, increased venous return,cardiac output.Observational studies in haemodialysis patients have found anaemia to be associated with higher mortality in populations in which the vast majority of patients had haemoglobin levels between 6 and 12g/dl .

The anaemia of chronic kidney disease is in most patients,normocytic and normochromic.It is principally due to reduced renal eryhtropoietin(Epo) production and,to a lesser degree,to shortened red cell survival and decreased responsiveness to the hormone.Anaemia can develop well before the onset of uremic symptoms due to end-stage renal disease (ESRD). Although, anaemia due to renal dysfunction generally develops when the glomerular filteration rate (GFR) declines to <30ml/min. It can also be observed in those with markedly higer GFRs (such as 60 ml/min) and tends to occurs at higher levels of GFR in African Americans than whites.

If left untreated, the anaemia of chronic kidney disease is associated with several abnormalities. These include deterioration in cardiac function, decresed cognition and mentally acuity, fatigue, and other signs and symptom. There are also associations with an increased risk of morbidity, principally due to cardiac disease and stroke.

The primary therapeutic options for the anaemia of chronic kidney disease include red blood cell transfusion, erythropoietin-stimulating agents (ESAs), and to a much lesser degree, androgens.

Chronic renal failure

Chronic renal failure is a syndrome characterised by progressive and irrervisible deterrioration of renal functtion due to slow destruction of renal parenchyma, eventually terminating in death when sufficient numbers of nephrons have been damaged. Acidosis is the major problem in Chronic renal failure with development of biochemical azotemia and clinical uraemia syndrome (Charlse, 2004).

Renal diseases are associated with a variety of haemopoietic changes. Anaemia parelles the degree of renal impairment and its most important cause is failure of renal eryhtropoietin secretion. Other factors include chronic blood loss,haemolysis and bone marrow suppression by retained uremic factors. The success of erythropoietin therapy in correcting the anaemia of chronic renal failure has led to substantial clinical experience and knowledge in eryhtropoietin,iron metabolism,and erythropoisis.A distinguishing characteristic of the anaemia in patients undergoing chronic renal dialysis is the presence of a normal mean corpuscular volume (MCV) in 85% of the patients and hypochromia in 96% of the patients(Lawrence *et al.,*2000). Hyporesponsiveness to erythropoietin therapy is a common phenomenon in these patients (Means, 1999, Tarng *et al.,*1999) because of variety of co-morbid conditions, particularly aluminium toxicity and non deficiency. Anaemia patients undergoing dialysis may have suboptional responses to oral non therapy for several reasons.under basal conditions, their absorbtion levels of food iron and therapeutic oral iron are similar to levels in normal subjects. During erythropoietin therapy,the absorbtion of iron increases as much as 5times. During erythropoietin therapy,the absortion of iron increases as much as 5times. neverthless, external iron loss,including loss from leomodialysis and blood testing, exceed gastrointestinal symptoms is problematic, and significantly reduced iron absorbtion may occur with some newer iron formulation (lawrence et al., 2000) iron restricted erthropoiesis is evident by clinical responses to ascorbate supplementation,thought to facilitate the release of iron from recticuloendothelial stores and increased iron use by erythron(Tarng *et al.,*1999), and by the success of intravenous iron therapy in reducing erythropoietin dosage. Because anaemia is a determinant of life expectancy in patients on dialysis for chronic diseases ,intravenous iron administration has become standard therapy for many patients recieving erythropoietin therapy.There is decrease of red blood cell count in chronic kidney disease.Primary cause of decrease Red blood cell count in chronic renal failure is impaired erythropoietin production and other factors which suppress marrow erythropoiesis and shortened red cell survival.Erythropoietin is the hormone which is the major humoral regulator of red blood cell production and helps to maintain the viability of red blood cell by retarding in CFU-Es. In the absence of erythropoietin, DNA cleavage is rapid and leads to cell death.

Red blood cell survival is decreased in uremic patient's in proprtion to the blood urea nitrogen concentration and, it improve significantly after intensive haemodialysis.Uremic plasma increases the expression of phosphatidylserine on the outer cell surface in red blood cells.This enhances the recognition of damaged red blood cells by macrophages,leading to their subsequent destruction and decreased survival.

The haematolytic factor implicated in decreased red blood cell survival is presumed to be a toxic substance normally excreted or metabolised by the kidneys,one such substance is guanidine and its derivatives which appear to be a subset of the many retained metabolites,adversely affect erythrocyte survival. There is a decraese in haemoglobin concentration and haematocrit in chronic renal failure patients.The haemoglobin concentration and haematocrit generally provide an accurate reflection of the extent to which the circulating red cell mass is reduced.In chronic renal disease because of impaired erythropoietin secretion,increased destruction of red blood cells, leads to a fall in red blood cell count,which reduces the haemoglobin concentration and haematocrit(Emmanuel *et al.*,2004).

An inverse relationship between serum or plasma erythropoietin levels with haemoglobin (Hb) concentration and haematocrit (PCV) normally exist. As the haemoglobin and haematocrit decrease the erythropoietin level rises.

Erythropoietin :anaemia of chronic kidney disease among predialysis and peritoneal dialysis patients

The anemia of chronic kidney disease (CKD) is, in most patients, normocytic and normochromic. It is principally due to reduced renal erythropoietin (EPO) production and, to a lesser degree, to shortened red cell survival and decreased responsiveness to the hormone.

The enormity of chronic kidney disease in Nigeria

The magnitude of the problem of chronic kidney disease (CKD) is enormous, and the prevalence of kidney failure is rising. Currently, chronic kidney disease is emerging as a worldwide public health problem. The World Health Report 2002 and Global Burden of Disease project reports show that diseases of the kidney and urinary tract contribute to the global burden of diseases—with approximately 850,000 deaths every year and 15,010,167 disability-adjusted life years. Globally, they represent the 12th cause of death and 17th cause of disability (WHO,2006). This may however be an underrepresentation of the contribution of chronic kidney disease to global burden of disease.

Apart from the effect on kidney function per se, kidney damage is a major determinant for the development of progression of accelerated atherosclerosis, ischaemic vascular disease, and cardiovascular death . Individuals with even the earliest signs of chronic kidney disease are at increased risk of cardiovascular disease and may die long before they reach end-stage renal disease. The burden of chronic kidney disease is therefore not limited to its impact on demand of renal replacement therapy (RRT); it is paralleled by the huge cost of provision of health care services for these patients. The cost of care includes not only the direct cost of dialysis and transplant services but also indirect cost like man hours lost at the workplace.

In Nigeria, the situation is such that chronic kidney disease represents about 8–10% of hospital admission (Akinsola *et al.,*1989) . This may be a huge underrepresentation of the true situation. It is well known that chronic kidney disease is underrecognized and underdiagnosed, patients with end-stage renal failure (ESRD) are thought to represent the tip of the iceberg of the entire burden of chronic kidney disease (Levey e*t al.,*2002;Bello *et al.,*2005). This is more so in developing countries where patients often present late or not at all to health facilities for several reasons which range from prohibitive cost of health care services to use of alternative treatment like spiritual healing and traditional/native healers (Ojogwu and Anah,1982; Kadiri *et al.,*1999) .

The cost of management of ESRD is prohibitive (Ijoma *et al.,*1998). In developing countries in places where RRT is available it is unaffordable by most patients. In Nigeria as in most other developing countries, there is no social security system or health insurance scheme in place to assist the patient, and the burden is borne solely by the patient and relatives.

With this background, this study was carried out to highlight the plight of patients with ESRD in a typical developing country and to underscore the need for preventive measures, early detection, and intervention to stem the rising prevalence and to alleviate the burden of the disease.

Our environment (Naicker, 2003; Alebiosu *et al.,* 2006). Hypertension runs a more aggressive cause in blacks and also hypertensive nephrosclerosis is more common in blacks (Gibbs *et al.,*1999). Diabetic nephropathy is also commoner in blacks and runs a more aggressive course and often occurs at a young age than in Caucasians (Young *et al.,*2005). The brunt of HIV pandemic is borne by Sub-saharan Africa and many of such patients develop ESRD from HIVAN and other glomerulopathies (Allison, 2009). Use of nephrotoxic agents including traditional/native medication, mercury-containing soaps/skin lightening creams, is rampant in the study community (Ulasi *et al.,* 2005). Awareness of deleterious effect of these agents is low in these areas. However, because of late presentation of our patients, the full impact of ESRD from use of nephrotoxic agents is not quite obvious, as earlier and mild forms of the disease do not present to hospitals. Chronic haemodialysis became available in Nigeria in late 1981, and by 2003 there were 27 dialysis units in the country. By 2006, with a population of the nation estimated at about 130 million there were 84 nephrologists and 56 dialysis units (Naicker,2009). These units are mainly located in urban areas and are run with refurbished poorly maintained machines.

Diabetic nephropathy

In the UK,as in the rest of the Western World, diabetes is the most prevalent cause of renal failure. The prognosis of diabetic nephropathy has improved,there remains an excess mortality of 70-100 times that of an otherwise matched population (Chantrel *et al*.,1999). Survival rates on dialysis remain poor, with up to 33% of patients who require renal replacement thaerpy,morbidity as assessed by hospitalisation is 2-3 times greater than for nondiabetic patients with end-stage renal failuer (Katherine *et al.,* 2005).This excess of morbidity and mortality in part relates to the high incidence of cardiovascular disease in this patient.The identification of mechanisms underlying modifiable factors taht may prevent or slow progression or improve patient survival in diabetic nephropathy commonly have a greater degree of anaemia for their degree of renal impairment than those presenting with other causes of renal failure, and anaemia develops earlier in these patients than in those with renal impairment from other causes(Thomas *et al.*,2003).Anaemia has been identified as one of teh risk factor for the need for renal replacement therapy in diabetes(Cusick *et al.,*2004). Anaemia has a negative impact on patient survival,and is considered to be an important cardiovascular risk factor associated with renal disease. Understanding the pathogenesis of anaemia associated with diabetes and nephropathy may thereby lead to opportunities for developing interventions to optomise outcomes in these patients. Many factors have been suggested as the reason for the earier onset of anaemia in patients with diabetes, including severe sympathetic denervation of the kidney and loss of appropriate erythropoietin production;damage to the renal interstitium,systemic inflammation; and inhibition of erythropoietin release. It has also been shown that a normochromic,normocyttic anaemia can occur before evidence of renal impairment is present (Kathrine *et al.,*2005).

Pathophysiology of anaemia in diabetics with chronic kidney disease

There are several factors which have been implicated in the development of anaemia in chronic kidney disease which include erythropoietin deficiency, iron deficiency, decreased

lifespan of red blood cells, chronic blood loss,secondary hyperparathyroidism, chronic inflammation, oxidative stress, nutritional folate deficiency, uremia and chronic suppression of erythropoiesis (McFarlane *et al.,* 2008; Dharawhat *et al.,*2009). Diabetes exercabates many of these factors, leading to a higher degree of anaemia in patients with diabetic nephropathy than in patients with kidney disease from other causes.

Erythropoietin deficiency

Erythropoietin (Epo) is a glycoprotein hormone that regulates proliferation,differentiation and maturation of red blood cells. Erythropoietin is produced by the peritubular capillary cells within the kidney,and this process is mediated by oxygen availability.In normal kidneys, erythropoietin increases in proportion to the degree of anaemia. Relative deficiency of erythropoietin is the most important cause of anaemia in patients with chronic kidney disease. The ability of the kidneys to produce erythropoietin is not impaired in renal disease. The absolute value of erythropoietin can be in the normal or even high,so measuring erythropoietin levels does not aid clinical mangement. However,erythropoietin levels will be appropriately low relative to the degree of anaemia,resulting in a functional erythropoietin deficiency. This is due to the uncoupling of erythropoietin synthesis from haemoglobin concentration so that the protein is no longer upregulated by anaemia (Thomas,2007). In the diabetic kidney tubulointerstitial dysfunction is observed early in the course of disease (Thomas et al.,2003). This could cause disruption of the intricate signaling mechanism between the capillaries, interstitial fibroblast and tubular cells regulating production,thus contributing to the uncoupling of erythropoietin synthesis from haemoglobin level.Diabetes also negatively affects hypoxia-inducible factor(HIF),a transcription factor that plays a crucial role in regulating the renal response to hypoxia.HIF regulates the transciptional activation of many oxygen-sensitive genes,including erythrpoietin.Hyperglycaemia has been shown to inhibit stabilisation of the HIF protein (Catrina *et al.,*2004).

Autonomic dysfunction has also been suggested as another factor taht may contribute to erythropoietin deficiency in diabetic patients. In experimental models,erythropoietin production is impaired when the kidney is denervated. Also,patients with primary disorders of the autonomic nervous system have blunted production or erythropoietin and a high risk of developing anaemia.

Decreased red blood cell lifespan

Patients with renal disease have a 30-70% reduction in red blood cell lifespan. Blood from uremic donors transfused into normal recipients results in normal red blood cell survival, implying that the uremic environment of patients with chronic kidney disease is the underlying cause of this phenomenom. (Ly *et al.*,2004).

The red blood cells of the patients with diabetes are metabolically and functionally abnormal.These changes contribute to reduced erythrocyte survival in diabetic patients to a greater degree than in nondiabetic patients with a similar degree of renal impairment (Manodori and Kuypers, 2002).

Iron deficiency

Uremic causes platelet dysfunction,putting patients with chronic kidney disease at incraesed risk of bleeding and non loss.Patients on haemodialysis in particular are prone to losing iron through blood trapping in dialysis machine and repeated phlebotomy.

It has been shown that patients with chronic kidney disease have impaired absorption of dietary iron. Transferrin is a preotein that delivers iron from the gastrointestinal tarct and the reticuloendothelial sysytem into the bone marrow to be utilised by maturing erythrocytes.Patients with chronic kidney disease have decreased levels of transferrin, impairing iron mobilization (Nurko,2006).

The overall prevalence of iron deficiency in patients with diabetes is not significantly different from that in the general adult population.However,normal iron indices do not prelude the patients from achieving benefit with iron supplementation.In particular,patients on dialysis are often found to have a functional iron deficiency,in which their iron studies are normal but their anaemia improves with parenteral iron supplementation.

Chronic inflammation and oxidative stress

Anaemia of chronic inflammation is characterised by an impairment of the ability to release iron from the hepatocytes and macrophages of the reticuloendothelial system. Patients with chronic kidney disease exhibit a generalised increase in the inflammatory response due to a variety of factors, including decreased clearance of inflammatory cytokines, volume overload,oxidative stress and their underlying comorbid conditions.

Although, decreased glomerullar filteration rate (GFR) and decreased iron stores are major contributing factors to anaemia in diabetic patients, erythropoietin deficiency and inflammation are becoming a leading factors in explaining the high prevalence of anaemia in diabetes with chronic kidney disease. These factors lead to anaemia and lead to heart failure,cardiomyopathy and myocyte death (National Kidney Foundation, 2006).

CONCLUSION

The presence of anaemia in patients with chronic kidney disease has a wide range of clinically important consequences. Some of the symptoms that were previously attrributed to reduced renal function are,in fact, a consequence of anaemia.These include,in particular,reduced physical performance, fatigue, shortness of breath, loss of appetite, insomnia, sexual and cognitive function disorders and reduced immunological reactivity. Anaemia contributes to increased cardiac output,the development of left ventricular hypertrrophy, angina, and congestive haert failure. A relative lack of erythropoietin (Epo) is considered to be the main cause of the devlopment of renal impairment. Erythropoietin is a hormone produced by specialised type 1 interstitial fibroblasts in the cortex and outer layer of the renal medulla.It is crucial factor that regulates the size of the red blood cell system.The main stimulus for elevated synthesis of erythropoietin is tissue hypoxia,which normally leads to an exponential increase in serum erythropoietin levels.The study showed that there was decrease Epo and PCV level in the renal diseases studied.The depression of erythropoietin level were more pronounced in chronic kidney disease subjects before dialysis(predialysis) and improved on dialysis but for the diabetic nephropathy subjects maintained negative correlation when Epo and PCV were correlated in the subjects and chronic kidney disease subjects on predialysis and dailysis showed perfect and positive relationship respectively showing that they do not follow the established inverse relationship in pathological conditions. These subjects need urgent attention to restore the function of the kidneys.

There should be more enlightment compaign to the public on the preventive ways of kidney disease such as reduced intake of nephrotoxic drugs such as NSAIDs, toxic chemicals,excessive use of herbal therapies. Those who are diabetic should be monitored early. There should be serious prevention of urinay tract infection. The blood pressure of the patients should be monitored and controlled to avoid causing damage to the kidney which may affect the level of erythropoietin.More research should be done on the differnet stages of renal diseases and theerythropoietin and PCV level in the patients.Before a patient with renal disease start receiving exogenous stimulating agents such as darbepoietin,the Epo level of the patient should be established and monitored regularly to avoid causing unnecessary harm to the patient.The government and non-governmental organisations should help to build better hospitals in the rural areas and well equiped with enogh required specialists.

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