INTRODUCTION

Anemia of Chronic Kidney Disease

Anemia in patients with chronic kidney disease (CKD) is a common complication that has been associated with poor outcomes such as cardiovascular complications and mortality.[1] It is defined by the National Kidney Foundation (NKF) as a haemoglobin (Hb) concentration less than 12g/dl for women and less than 13.5 g/dl for men.[2]

Pathophysiology:

Decline in functional renal tissue in CKD patients decrease the hypoxia related erythropoietin (EPO) production.[3]Another factor is systemic inflammation, where production of inflammatory mediators, such as interleukins and tumor necrosis factor, blunt the effect of EPO on the bone marrow. Other factors include platelet dysfunction which leads to an increased risk of gastrointestinal bleeding, shortened erythrocyte survival time (30%-60% of the normal 120 days) and hemolysis secondary to uremic toxin accumulation. Finally, malnutrition and deficiencies of iron, folate, and vitamin B_{12} have been found to cause a reduction in Hb concentration.[4]

Laboratory methods for diagnosis of anemia of CKD:

Complete blood count:

Typically the anemia of EPO deficiency is normocytic (normal mean corpuscular volume MCV) and normochromic (normal mean corpuscular haemoglobin concentration MCHC).[5] Iron deficiency usually initially manifests as a falling MCV accompanied by a rising red cell distribution width (RDW).[6]

Total iron binding capacity (TIBC):

TIBC measures the maximum amount of iron the blood can carry, which indirectly measures transferrin since it is the most dynamic iron carrier; in anemia of CKD with absolute or functional iron deficiency the TIBC level is low. [5]

Transferrin saturation (TSAT):

TSAT is a measure of circulating iron which is available for delivery to the bone marrow. A TSAT of less than 16% in an anemic patient with CKD is consistent with functional or absolute iron deficiency.[5]

Serum Ferritin:

Serum ferritin is the early sensitive laboratory test for the diagnosis of iron deficiency as it may decrease before a decrease in the serum iron level is detected. Every 1 ng/ml of serum ferritin corresponds to 8-10 mg storage of iron.[6]

Ferritin levels below 30-300 ng/ ml in males and 10-200 ng/ ml in females are consistent with low total iron stores and, along with a low TSAT, are diagnostic of

absolute iron deficiency. Functional iron deficiency is seen when the ferritin levels are higher than normal ranges and coincide with low TSAT.[5]

Simple clinical cut off can guide management of anemic subjects; serum ferritin <15 ng/ml rules in iron deficiency. In iron deficiency patients with normal kidney function, absolute iron deficiency is characterized by low serum ferritin concentration (< 30 ng/ml), while in CKD patients it is 100 ng/ml due to the presence of chronic inflammation that increases serum ferritin levels approximately three fold. [7] The Kidney Disease Outcomes Quality Initiative (KC/DOQI) guidelines recommend serum ferritin levels >200 ng/ml for adult hemodialysis patients.

Ferritin is an acute-phase reactant that is elevated in inflammation, infection, malignancy and chronic renal failure.[6]

Reticulocyte count:

Reticulocytes are released into the circulation about two days prior to maturation into red blood cells. Reticulocytes count assesses the number and percentage of reticulocytes circulating in the blood. It helps distinguishing red cell underproduction from anemia caused by red blood cell loss or destruction. With inadequate or deficient EPO production in patients with CKD, the reticulocytes count would be expected to be low. If a patient is found to have an elevated count, an evaluation for hemolysis or extravascular blood loss is expected.[5]

Reticulocyte Haemoglobin Content:

The reticulocyte haemoglobin content (CHr) reflects the amount of iron available to the bone marrow for incorporation into new RBCs since reticulocytes life span is only 1- 2 days. The sensitivity and specificity of this test are comparable to those of serum ferritin.[6]

Causes of anemia in CKD:

The association of anemia with the kidney is important because this organ is responsible for both sensing oxygen availability to tissues and for releasing erythropoietin (Epo) into the circulation.[8]

The most important causes of the anemia of in CKD:

1- ***Decreased Erythropoietin (EPO) production***: The patients with ESRD had values well below the plasma Epo levels of patients with comparable degrees of anemia but without renal failure, and most had values similar to normal subjects.[9]

Erythropoietin is the main regulator of erythropoiesis. [10] It is produced by peritubular cells in the kidneys of the adult and hepatocytes in the foetus. Small amounts of extra-renal EPO are produced by the liver in adult human subjects. [11]

Approximately 90% of the hormone erythropoietin is produced by the kidneys. Under normal physiological conditions, hypoxia in the kidney leads to an increase in the production of EPO, which subsequently stimulates erythropoiesis. The main regulator of EPO production is hypoxia, and there are no stores of preformed EPO. [3]

The primary target cell for EPO in the bone marrow is the colony forming unit crythroid (CFU-E). EPO acts synergistically with stem cell factor (SCF), granulocyte macrophages colony-stimulating factor (GM-CSF), IL-3, IL-4, IL-9 and insulin-like growth factor-1 (IGF-1) to cause maturation and proliferation from the stage of the burst-forming unit erythroid (BFU-E) and CFU-E to the normoblast stage of crythroid cell development. Thus, EPO acts primarily on apoptosis to decrease the rate of cell death in erythroid progenitor cells in the bone marrow.[11]

The longer erythropoietin remains in the circulation, the greater it stimulates erythroid progenitor cells located in the bone marrow.[12]

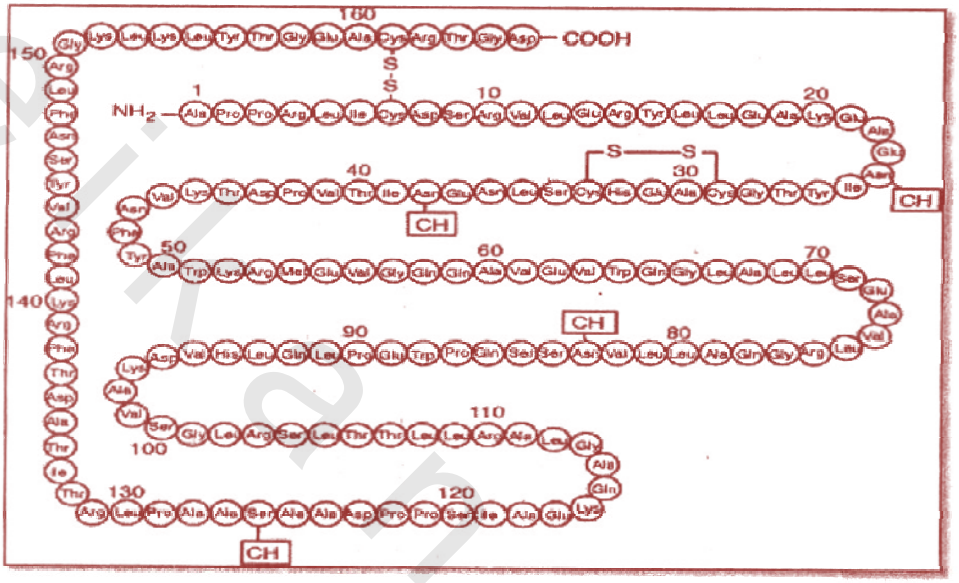

Figure (1): The primary structure of erythropoietin showing the circulating form of 165 amino acids. Two disulfide bonds tether the molecule together between cysteine 29 and 33 and cysteine 6 and 161. Three N-linlced sugars are present at asparagines 24, 38, and 83, and one O-linked sugar is present at serine 126.

• Erythropoietin receptor:

EPO binds to a specific transmembrane receptor, the EPO receptor. It is expressed primarily on erythroid cells. It consists of an extracellular, a transmembrane, and an intracellular domain and is expressed as protein that ranges from 66 to 78 kDa and exists as a preformed dimer. [11] Binding of EPO to the EPO receptor is essential for the production of mature RBC. The EPO receptor belongs to the hematopoietic cytokine receptor superfamily. The EPO-bound receptor is a dimer, where two monomeric receptors become a dimer after the binding of EPO, resulting in signal transduction. [13] Binding of ligand shifts the receptor from an inactive to an active conformation.[14] (Fig. 2).

Activation of the EPO receptor is transient; it is rapidly deactivated by down regulating mechanisms, including receptor internalization and degradation.[13]

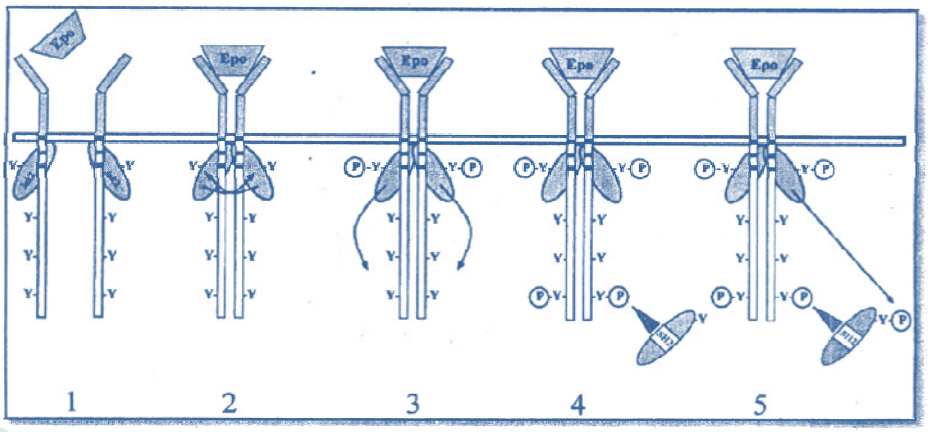

Figure (2): **The First step in EPO activation of the reccptor is dimerization** (1), the preassociated JAK2 kinases are in close contact and activated by transphosphorylation (2), the tyrosine residue of EPO reccptor are then phosphorylated (3,4), providing docking sites for intracellular signalling proteins (5). [11]

Decreased Epo production, shortened red cell survival,[15] and retained inhibitors or toxic metabolites in ESRD inhibit erythropoiesis [16,17,18]and interfere with erythroid marrow function. Other recognized potential complications impairing marrow function are iron or folate deficiency [19,20], aluminum toxicity [21-23], and the osteitisfibrosa associated with hyperparathyroidism .[24]

There are several clinical settings that suggest strongly that substances retained in ESRD interfere with erythroid marrow function. The most convincing evidence was provided by Caro who showed elevated levels of bioactive Epo in the plasma of a number of patients with ESRD [9], This finding implies that the marrow is unable to respond to the circulating Epo in these individuals. Furthermore, a few patients placed on hemodialysis[25] or switched to CAPD have had a significant improvement in hemoglobin and hematocrit in the absence of dramatic changes in plasma Epo levels, suggesting that an inhibitor was removed by dialysis. [26]

In contrast to these observations, there are several suggestions why inhibitors, if present, are of little physiological relevance. First, approximately 3% of dialysis patients normalize their hematocrits spontaneously.[27] Second, those patients who develop a hemolytic-uremic syndrome have high circulating Epo levels, may require dialysis, and yet erythropoiesis typically is increased dramatically. Third, bilateral nephrectomy in a stable patient maintained by hemodialysis results in a decrease in effective erythropoiesis and a fall in hematocrit. This last finding would suggest that even the low levels of Ep produced by the residual diseased kidneys were capable of maintaining red cell production andthat the removal of this source of Epo resulted in further impairment of erythropoiesis. [28]

2-Iron deficiency

Iron metabolism: Iron is an essential micronutrient for all mammalian cells. All iron containing proteins have a vital role in energy metabolism, cell proliferation and DNA repair, so that severe iron deficiency results in inhibition of cell proliferation and cell death.[29]

In an adult male, the average loss of 1 mg of iron per day must be replaced by dietary sources. [30] Average diet provides 10-20 mg of iron daily, which is found in heme (10%) and non heme (ionic 90%). Only 1-2 mg is absorbed daily under normal circumstances. [31] Regulation of absorption of dietary iron from the duodenum plays a critical role in iron homeostasis in the body. [32]

Distribution of iron in the body:

In an average body weight adult (70 kg), total body iron is about 50 mg/kg in males, and 40 mg/kg in females.[33] (Table 1)

Table (1): Iron compartments in average man

Compartment	Iron content (mg)	Pcrccnt of total body iron
Haemoglobin iron	2000	67
Storage iron (ferritin, Hemosiderin)	1000	27
Myoglobin iron	130	3.5
Labile pool	80	2.2
Other tissue iron	8	0.2
T ransport iron	3	0.08

• iron Storage :

Serum ferritin is correlated with total body iron stores. However the level of serum ferritin is affected by acute and chronic inflammation and infections as ferritin is a positive- acute phase protein.[34] Its normal range is 30-300 ng/ml in males, 10-200 ng/ml in females.[35]Hemosiderin is another storage form of iron.[36] It contains approximately 25-30 percent iron. When the storage capacity of ferritin is exceeded, iron will deposit adjacent to the ferritin-iron complex. In contrast to ferritin, hemosiderin is insoluble in aqueous solutions. It is found predominantly in cells of the liver, spleen and bone marrow.[37]

Iron absorption, transport, and excretion:

Absorption of dietary iron is carried out by mature villus enterocytes of duodenum and proximal jejunum.[38] Dietary iron is presented to the duodenum either as ferric iron (Fe^{+3}) non-heme (90%) complexed with macromolecules such as ferritin or in the form of heme or heme containing proteins (10%).[31]

Acidic pH of gastric secretion facilitates enzymatic reduction of Fe^{3+} to its Fe^{2+} form by duodenal cytochromes,.[29,39] Vitamin C in food also favours reduction of Fe^{3+} to Fe^{2+}.[36]

Once inside the absorptive enterocytes, iron may be used by the cell, stored as ferritin, or transferred across the basolateral membrane to reach the plasma. Iron that remains in the form of ferritin, where the enterocytes completes its limited life cycle will

be sloughed with the senescent cell and will leave the body through the gastrointestinal tract. This process represents an important mechanism of iron regulation.[39]

Passage of iron across the basolateral membrane appears to be carried out by iron exporter protein; ferroportin (Fp).[40] (Fig. 3) it uses ferrous iron as a substrate exporting iron into the plasma [41], and is found in all cell types as mature duodenal enterocytes, macrophages, hepatocytes, placental trophoblasts cells of the central nervous system. In humans with reduced Fp function, iron rapidly accumulates in macrophages (such as the Kupffer cells) and in parenchymal cells of the liver, intestinal iron absorption is decreased and plasma iron level fells.[42]

As ferroportin transports iron across the enterocyte membrane in the ferrous form and circulating transferrin binds ferric iron, so oxidation is required [41], which is accomplished by a multicopper protein called hephaestin (HEPH). ferroportin expression is down regulated by hepcidin to control the intestinal absorption of iron.[43]

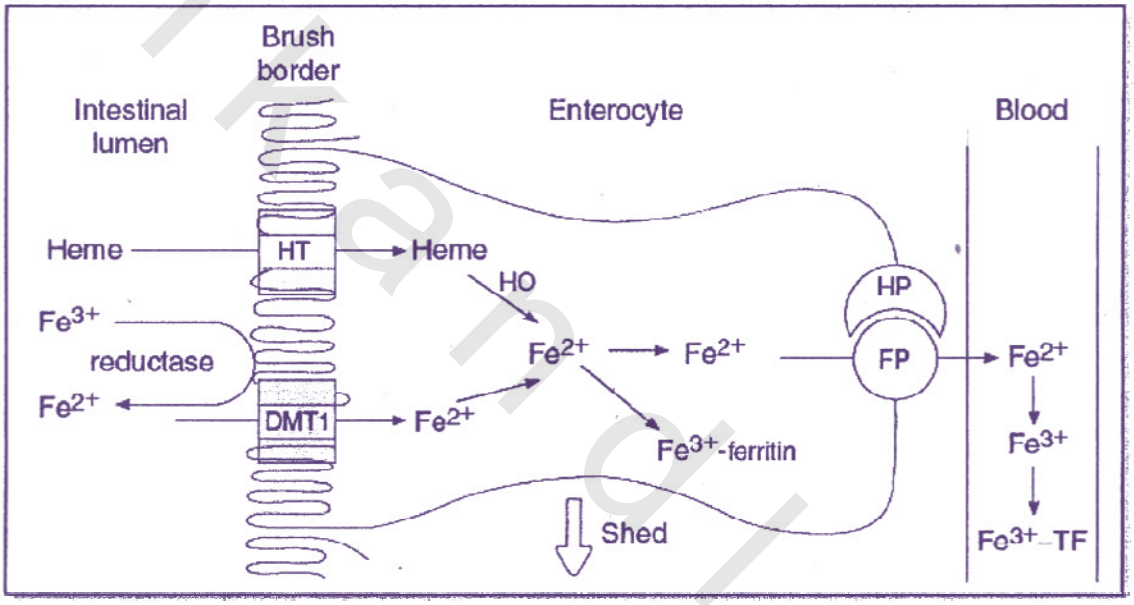

Figure: (3): Absorption of iron [36]

There are three types of iron homeostasis related anemia have been identified in dialysis patient. [44]

a. **Absolute** iron deficiency occurs due to decreased total body iron stores. It is correlated with serum ferritin levels less than 100 ng/ ml in non-dialyzed patients and less than 200 ng/ml in dialyzed patients (70) and serum transferring saturation (TSAT) less than 20%. However transferring saturation more than 20% or a serum ferritin level more than 200 ng/ml does not exclude iron deficiency in end stage renal disease (ESRD) patients.[45] This situation is common in hemodialysis patients due to low grade but frequent blood losses and also due to high doses of erythropoiesis stimulating agents (ESAs) that may over stimulate erythropoiesis to exceed the maximum capacity of liver iron stores.[46]

b. **Functional** iron deficiency which is correlated with serum ferritin levels higher than 100 ng/ml and serum TSAT less than 20%. In this situation, iron stores are normal but

the bone marrow receives more circulating iron from transferring than the iron output from the iron stores. It is characterized by decreased iron and iron-binding capacity (transferring), increased ferritin and the presence of iron in bone marrow macrophages, indicating impaired mobilization of iron from stores. This is mainly due to the effect of cytokines that may impair iron metabolism. Also increased ferritin and decreased transferring shunts iron to the reticuloendothelial storage pool, preventing delivery to erythroid precursor.(46)

c. **reticuloendothelial blockage**, which usually occurs in the setting of acute or chronic inflammation or infection. It can be considered as extreme form of functional iron deficiency and is associated with increased; CRP levels, TSAT less than 20 % and normal to high ferritin levels.(44)

Hepcidin

The peptide hormone hepcidin is the key regulator of systemic iron homeostasis.It is a cysteine-rich cationic peptide hormone that was isolated from human urine and blood and was found to exhibit antibacterial and antifungal activity.[47] Since it was produced by the liver and has antimicrobial properties, it was named liver-expressed antimicrobial peptide-1 (LEAP-1). Later it was named hepcidin *(hepatic bactericidal protein*).[48]

• Gene:

Hepcidin is a product of a 2.5 kb (kilo base pair) human hepcidin gene (hepcidini; antimicrobial peptide LIAMP), consisting of three exons and two introns, located on the ' long arm of chromosome 19 (NCBI Gene ID 57817).[49] It is predominantly expressed in adult liver, restricted to the periportal region of hepatocytes and appears to be dependent on hepatocyte differentiation status.[50] Expression was also detected in heart, brain, kidney and skeletal muscles but to a lesser extent.[49]

The human hepciclin gene encodes a precursor protein of 84 amino acids (aa), pre-prohepcidin. During its export from the cytoplasm, the pre-prohepcidin undergoes enzymatic cleavage, resulting in the 64-aa prohepcidin peptide, which is transported through the hepatocyte basolateral membrane into the circulation.[51] Then, the 39 aa pro-region peptide is posttranslationaly removed by a furin-like proproteinconvertase resulting in the mature bioactive hepcidin-25 (25 aa form). [52]

• Structure: (figure 4)

The structure of the bioactive 25-aa form of hepcidin is an 8 cysteine containing peptide forming a hairpin-shaped molecule with a distorted B-sheet, stabilized by four disulfide bridges between the two anti-parallel strands in a ladder like configuration, including an unusual disulfide bond that connects two adjacent cysteines near the turn of the hairpin. (Fig. 4) This disulfide bond is located in the vicinity of the hairpin loop which points to a possible crucial domain in the activity of the molecule.[53] Structure-function studies on hepcidin have shown that the iron regulating bioactivity is almost exclusive due to the 25 aa peptide, suggesting that the five N-terminal amino acids are essential for this activity.[54]

Three dimensional nuclear magnetic resonance (3D-NMR) structure studies also showed that hepcidin is an amphipathic peptide similar to most antimicrobial peptides.[54]

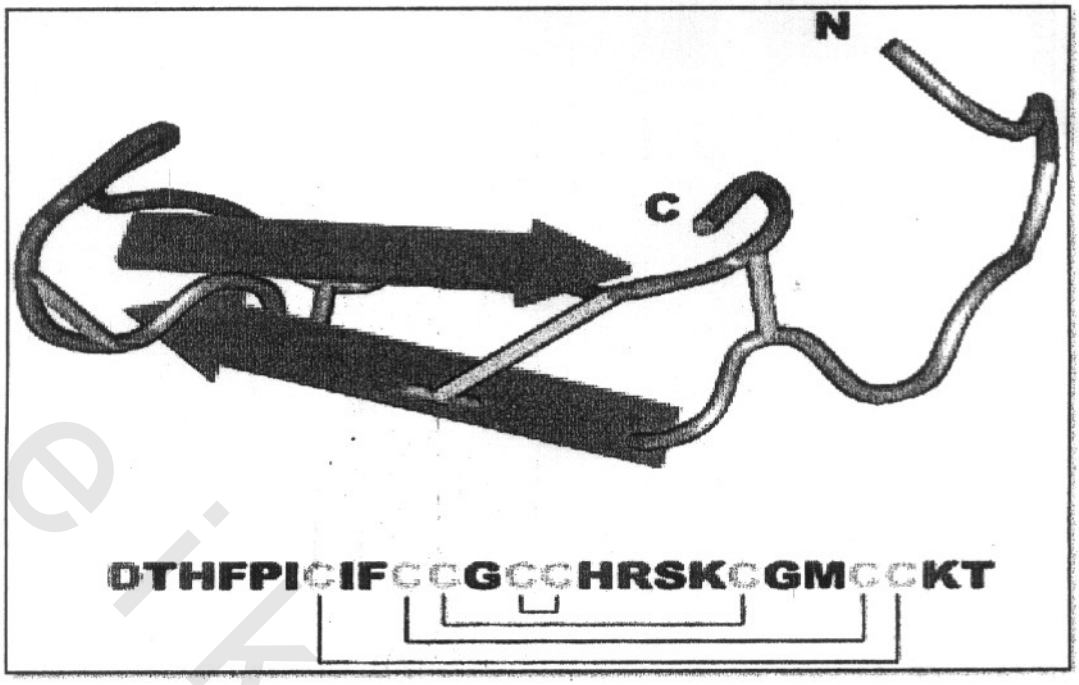

Figure (4): Amino acid sequence and a model of the major form of human hepcidin. The amino and carboxy termini are labeled as N and C, respectively. Disulfide bridges are in yellow, basic amino acids in blue, and acidic in red. The pattern of disulfide linkages between the 8 cysteines is also shown in the amino acid sequence. (54)

Function:

The main hepcidin function is homeostatic regulation of iron metabolism and mediation of host defence and inflammation. It was suggested that hepcidin plays a role as a negative regulator of intestinal iron absorption and iron release from macrophages.(55)

Hepcidin controls intestinal iron absorption by regulating ferroportin (Fpn) expression on the basolateral membrane of enterocytes.(4) It binds to Fpn inducing its internalization and degradation. Once internalized, the phosphates are removed, and Fpn is reacted upon by ubiquitin resulting in its ubiquitination. Once ubiquitinated, Fpn is trafficked through the multivesicular body (MVB) for degradation in the lysosomes.(43) (Fig. 5)

The net effect of hepcidin is the diminished absorption of dietary iron, sequestration of iron in macrophages and in hepatic stores. (55)

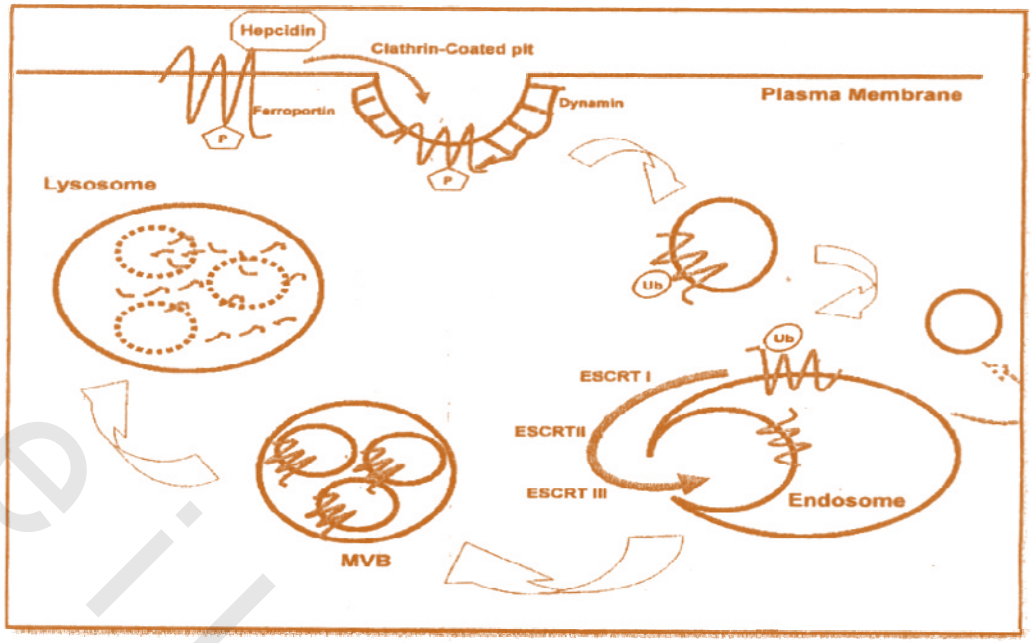

Figure (5): Model for Fpn internalization and degradation. Hepcidin binds Fpn at plasma membrane where Fpn is tyrosine phosphorylated. Once Fpn is internalized, the phosphates are removed, and Fpn is ubiquitinated, which targets it to the MVB for degradation in the lysosomes. [43]

• Hepcidin Regulatory Pathways: (Fig. 6)

Regulatory pathways that control liver hepcidin production interact with each other for maintenance of iron homeostasis.[53]

a- Iron store-related regulation:(direct relation with iron)

When iron stoics are adequate or high, marked by increased iron saturation of transferrin, the liver produces hepcidin, which circulates to the small intestine causing internalization of ferroportin (Fpn) that blocks the iron transfer pathway. [56] When iron stores are low, hepcidin production is suppressed, Fpn is displayed on the basolateral membrane of the enterocytes, and the transport of iron takes place. Liver detects any changes in diferricTf levels via TfR2. This modulates liver hepcidin gene expression. A decrease in diferricTf/TfR ratio reduces hepcidin production and vice versa.[57]

b- Erythropoietic activity driven regulation:

Erythropoiesis is controlled by hypoxia-induced erythropoietin (EPO) production by the kidney. When oxygen delivery is inadequate, the homeostatie response produces more erythrocytes, by signalling the kidney for production of EPO. As erythropoiesis increases, substantial quantities of iron will be used to make haemoglobin for the new erythrocytes. This will cause a fall in scram iron level followed by a fall in tissue iron content and this could be a signal for the down-regulation of hepcidin. [58]

It was also reported that EPO down-regulates the liver hepcidin gene expression. This suggests that hypoxia acts on both erythropoiesis induction and hepcidin gene down-

regulation through EPO. A decrease in hepcidin enables more iron absorption from the intestine and from the stores in liver and macrophages.[59]

The hepcidin promoter contains several binding sites for hypoxia-inducible factor (HIF). It is one of the most important factors in the cellular response to hypoxia, which transcriptionally activates genes encoding proteins that mediate adaptive responses to reduced oxygen availability. HIF target genes play critical roles in metabolism, angiogenesis, cell proliferation, and cell survival. Examples of HIF target genes include glucose transporter 1 (*GLUT1*), and *EPO,* and it is possible that the mcchanisms of hypoxic regulation of hepcidin turns out to be transcriptional.[60]

c- Inflammation related regulation:

During inflammation, proinflammatory cytokines are produced by macrophages and monocytes including interleukin-6 (IL-6), IL-ip, tumour necrosis factor-a (TNF-a), interferon-y, and transforming growth factor-B (TGF-B). These proinflammatory cytokines stimulate acute-phase protein production, with IL-6 the major inducer.[49]

During infection and inflammation, hepcidin synthesis is markedly increased. It was reported that the proinflammatory cytokine IL-6 and IL -1.are a key inducer of hepcidin synthesis during inflammation [61,62] while TNF-a has no effect. [63] IL-6 directly regulates hepcidin through induction and subsequent promoter binding of signal transducer and activator of transcription3 (STAT-3) by the induction and bindingto the hepcidin promoter. Hence, during inflammation, infection and cancer, elevated hepcidin results in decreased iron release from enterocytes, hepatocytes and macrophages, leading to fall in serum iron and diminished availability of iron for bacteria and cancer cells.[64]

Iron is essential for the growth and metabolism of many microorganisms.[65] Bacteria require iron for the production of the superoxide dismutase that protects them from host oxygen radicals. [66] Bacteria develop siderophores (high-affinity iron-binding molecules) retrieving iron from transferrin or lactoferrin of the host. The host develops a defence mechanism to decrease the iron level available to the microorganisms by increasing production of iron-binding proteins (i.e. transferrin), fall in dietary iron absorption, raise in iron-storing proteins, and release of apolactoferrin from neutrophils. Hepcidin, by inducing sequestration of iron in macrophages, robs bacteria of this element. [67]

Hepcidin is one of the defensins which are antimicrobial peptides produced by cells of epithelial lining.[68]Hepcidin, like other defensins, is an antimicrobial peptide killing them on contact. However, because it is produced by the liver, it has not been found to have chemotactic properties. Therefore, hepcidin wards off infections, in part as a defensin, acute-phase protein and by causing hypoferremia.[69,70]

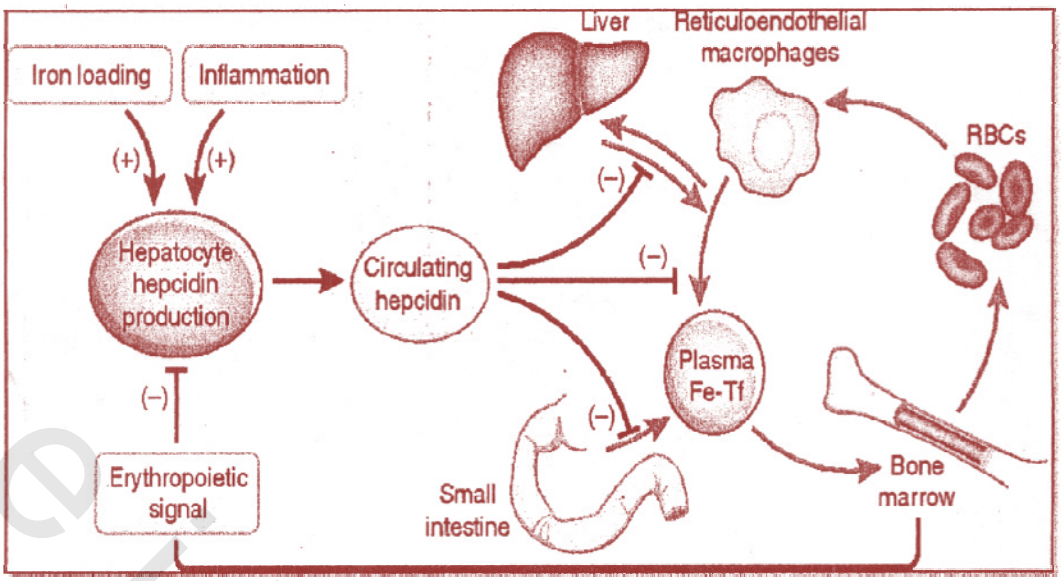

Figure (6): Hepcidin as the main regulator of systemic iron homeostasis

Hepcidin production in patients with CKD depends on iron status, inflammation, anemia, hypoxia and EPO-level (endogenous or exogenous).[71] (Fig. 7).

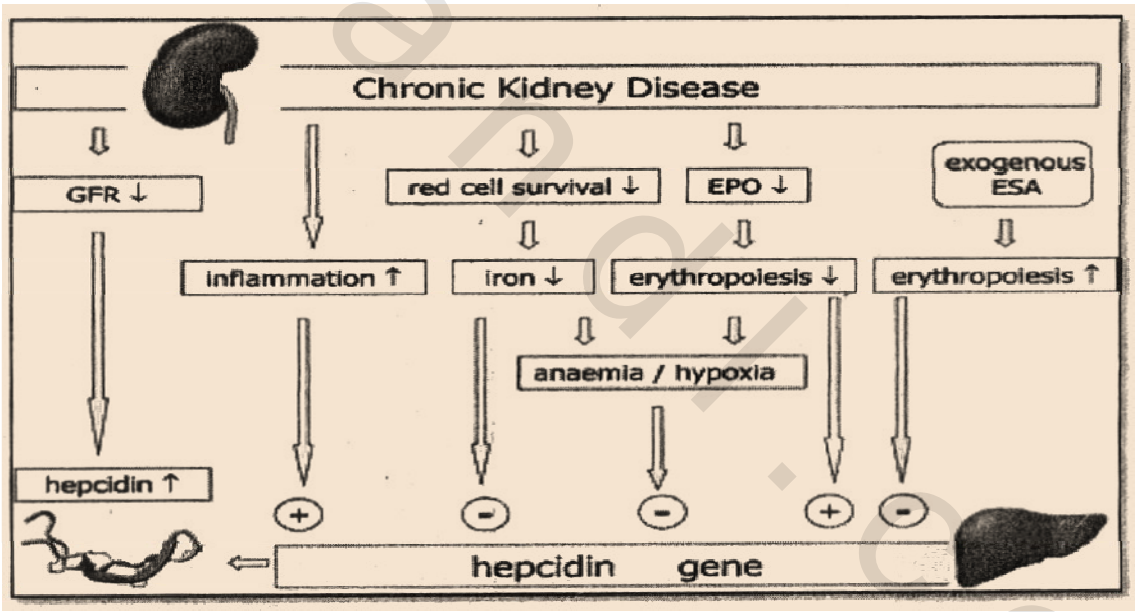

Figure (7): **Regulation of hepcidin in chronic kidney disease. The combined effect of the various pathways determines hepcidin levels.** [71]

In CKD, hepcidin levels was found to be persistently high, probably due to decreased excretion, chronic inflammatory state, or due to induction of hepcidin expression by dialysis and insufficient removing by dialysis. It was suggested that inflammation-related dysregulation of hepcidin expression might cause a functional iron deficiency and a subsequent erythropoietin hyporesponsiveness in patient with CKD. [49]

Various studies have shown a correlation between serum hepcidin and ferritin: serum levels of both parameters are elevated in low grade inflammatory disease such as chronic

kidney disease. Hepcidin directly reflects iron available and needs for erythropoiesis. Moreover, hepcidin integrates the input from inflammatory and erythropoietic pathways and better reflects the status of iron homeostasis than single parameters such as TSAT. Thus hepcidin has a superiority over ferritin in guiding anemia treatment in patients with CKD.[71]

Treatment with rHuEPO in hemodialysis (HD) patients showed higher levels of hepcidin. This was caused by the stimulus of inflammation for hepcidin synthesis which is stronger than the inhibitory effect of rHuEPO. In addition, the use of large amounts of rHuEPO may lead to increased iron utilization by the bone marrow, resulting in depletion of iron stores and ultimately decreased intracellular iron, leading to higher hepcidin levels.[72]

Expected role of hepcidin in anemia of CKD:

Absolute iron deficiency in dialysis patients may be due to the known effect of hepcidin on iron absorption.[26] Also increased hepcidin can aggravate functional iron deficiency by decreasing the release of stored iron through fcrroportin down regulation.[73] Another mechanism is reticuloendothelial iron stores blockage, possibly caused by hepcidin, where there is no release of iron to transferrin. Because of all these factors resistance to EPO therapy can easily develop.[44]

Treatment of anemia associated with CKD:

ESA therapy in the anemia of ESRD:

treatment of anemia has been shown to result in improvements in exercise capacity, reduces hospitalization and mortality rates.[74] Erythropoiesis-stimulating agents (ESA) have been available for almost two decades and remain the central strategy for treatment of anemia in patients with CKD. The use of ESA in management of renal anemia has been shown to improve survival, reduce cardiovascular morbidity and enhance quality of life.[75]

The European Best Practice Guidelines (EBPG) for the management of anemia of CKD specified that, within the recommended Hb target range (more than 11 g/dl, not exceeding 13 g/dl), the exact patient target Hb level should be defined on an individual basis, taking into account gender, age, ethnicity, and activity. In haemodialysis (HD) patients higher Hb concentrations are undesirable due to risks arising from post-dialysis haemoconcentration.[46,76]

Adequate iron stores are essential for achieving maximum benefit from the ESA therapy. Because the demand for iron by the erythroid marrow exceeds iron stores once ESA therapy has been initiated, iron supplementation is essential, or iron deficiency would occur. That is why the optimal treatment of anemia due to CKD is erythropoiesis stimulating agents (ESA) and iron therapy.[2] Serum level of EPO in normal persons ranges between 3-30 mlU/ml. Serum levels of EPO in patients with CKD were found to be about five times as high than in normal human subjects. Though EPO deficiency is the primary cause of anemia of CKD, the uremic state may blunt the bone marrow response to EPO.[11]

Iron therapy in the anemia of ESRD:

ESAs, the recommended therapy for ESRD related anemia, increase the need for iron, as they stimulate the synthesis of 2 million new red cells/second.[77] Evidence now proves that adequate iron availability increases erythropoiesis and reduces ESA requirements. [78] According to **K-DOQI guidelines *(*2006**)** iron status should be evaluated every month during initial ESAs treatment, at least every 3 months during stable ESAs treatment. In clinical practice, no single test adequately monitors iron stores or availability. Serum ferritin is the only available blood marker of storage iron, but it is more reliable in non-dialytic patients than in those who underwent hemodialysis. Tests reflecting the adequacy of iron for erythropoiesis include transferrin saturation (TSAT). [79)]

TSAT and serum ferritin are undoubtedly the most available serum test, but both show acute-phase reactivity and are poorly decodable for assessment of iron status in such state as chronic disease, malnutrition and inflammation; furthermore they exhibit also diuturnal fluctuations.[80] Therefore, there is an urgent requirement for novel markers more specific for iron deficit, especially in CHD patients, in which occult infections and malnutrition play a major role in determining response to therapy and influencing mortality. [44]

In patients undergoing ESAs therapy, interpretation of iron status tests should incorporate consideration of the Hb level and ESAs dose, in order to provide information important to medical decision making, because they elucidate the status of both external iron balance (net loss or gain of iron) and internal iron balance (disposition of iron between stores and circulating red blood corpuscles). [79]

For example, decreasing ferritin levels in the presence of a stable or decreasing Hb level may signify external iron loss, and so iron therapy is indicated. Conversely, decreasing ferritin levels in the presence of increasing Hb denotes an internal shift in iron from stores to Hb, as would be expected in a patient responding to ESA therapy: if iron status remains within the target range, additional iron administration may not be required. Finally, an increase in ferritin levels accompanied by a decrease in TSAT suggests inflammation-mediated reticuloendothelial blockade and may be accompanied by a decrease in Hb and increase in ESA dose. **K-DOQI guidelines *(*2006*)*** suggest that iron supplementation should be administered during ESA treatment to maintain serum ferritin >200 ng/ml and TSAT >20%, in CHD and serum ferritin >100 ng/ml and TSAT >20% in ESRD or in patients in peritoneal dialysis. The upper limit of serum ferritin besides which there is no recommendation to routinely administer iron was set as 500 ng/ml. When ferritin level is greater than 500 ng/ml, decisions regarding iron administration should weight ESA responsiveness, Hb and TSAT level, and the patient's clinical status.[79] The preferred route of iron administration in CHD patients is by intravenous (IV) infusion, since iron absorption from the gastrointestinal tract may be impaired in uremic patients.[81] likely because of high hepcidin levels. In ESRD or peritoneal dialysis patients, iron can be administered either orally or IV. Available intravenous iron formulations include iron dextran, sodium ferric gluconate, and iron sucrose: all these forms may be associated with acute adverse effects, including hypotension with or without anaphylactoid reaction*(82),*

The pathogenesis may differ depending on the type of IV iron: anaphylactoid reactions appear to occur more frequently with iron dextran and high molecular weight forms *(83),* whereas labile or free iron reactions occur more frequently with non dextran

forms of iron. [83,84] Thus, resuscitative medication and personnel trained to evaluate and resuscitate anaphylaxis should be available whenever a dose of iron dextran is administered. As a result, FDA has issued a ‘‘black box” warning, recommending that patients undergo a 25 mg test dose the first time the drug is given. If a patient does not have an adverse reaction to this dose, he/she is less likely to have an anaphylactic reaction to the therapeutic dose of iron dextran, but fatal anaphylactic reactions still occur with an uneventful test dose. [5]

***Hepcidin targeting therapies for the management of the anemia of ESRD* :**

Since over production of hepcidin plays a key role in the pathophysiology of anemia during inflammation and chronic diseases. Development of hepcidin antagonist would possibly be very useful for the treatment of anemia by facilitating iron redistribution from macrophages to erythroblasts.[50]

Hepcidin antagonists could be used as a complement for iron supplementation therapy and they might be useful as a supplement to ESA therapy, particularly for patients with lower response to them. Also hepcidin antagonists may be beneficial in the treatment of anemia associated with inflammation when the primary disease is refractory to therapy.[49]

The approaches under study include neutralizing anti-hepcidin antibodies and ESAs can directly or indirectly inhibit hepcidin release [85,86] . In a mouse model of anemia of chronic disease (ACD) caused by injections of heat-killed *Brucella Abortus* , neutralizing antibodies (Abs) directed against hepcidin were able to restore the reticulocyte response and normal Hb levels in combination with ESAs, whereas ESAs alone, and importantly ESAs plus IV iron, were ineffective. Furthermore, over-expression of hepcidin in mice was sufficient to hamper the erythropoietic response to ESAs. It is therefore conceivable that administration of antihepcidin antibodies could restore ESAs susceptibility in the roughly 10% of CHD patients, who display ESAs resistance due to chronic inflammation and high hepcidin levels.[85]

Anti-cytokines antibodies (Abs), such as those neutralizing IL-6, a major inducer of hepcidin during inflammation would likely reduce hepcidin transcription and inflammation-related ESAs resistance at the same time, but potential side-effects (altered immune function) will be again a limiting factor for their clinical utilization.

In sum, the quantitative assay of serum hepcidin is a better parameter to predict and monitor the response to ESA and iron treatment in patients with CKD. [86]

Renal replacement therapy (RRT)

Since the beginning of maintenance therapy for end stage renal disease (ESRD) through dialysis or transplantation, the number of patients treated for terminal kidney failure worldwide has continued to grow at a rate that is far in excess of the growth rate of the general population. By 2001, more than 1 million patients were reported worldwide to receive dialysis treatment alone, with the numbers growing at an annual global average rate of 7% [87]. The main factors contributing to the continued growth are the universal ageing of populations, multi-morbidity, higher life-expectancy of treated ESRD patients and increasing access of a generally younger patient population to treatment in countries in which access had previously been limited.[88-89]

3 types of RRT are known: kidney transplantation, peritoneal dialysis and haemodialysis,

Kidney transplantation

It is considered the treatment of choice for many patients with severe chronic kidney diseases because quality of life and survival are often improved , compared to patients who use dialysis . a kidney can be taken from a living relative, a living unrelated person, or from a person who has died (deceased or cadaver donor). Patients who undergo transplantation are free of the time – and energy – consuming requirements of dialysis .however, after transplantation , patients must take immunosuppressive medications and are monitored for signs of organ rejection and this is for the life time of the patient.[90.91]

Peritoneal dialysis

It is typically done by the patient or family member at home. It requires that someone be trained in the use of equipment and that the patient have a catheter surgically inserted in the abdomen .the catheter is made of a soft, flexible material and is usually placed near the umbilicus. The patient may receive general or local anesthesia during the insertion procedure. It requires several hours per day to perform exchanges.[92]

History of peritoneal dialysis:

The first attempt of peritonel dialysis was in 1923 by Ganter, a German clinical investigator. [93]

In 1970, peritoneal dialysis became important in treatment of end stage renal disease when it was found that certain groups of patients seemed to tolerate peritoneal dialysis better than haemodialysis.

The modern apply of clinical peritoneal dialysis started in 1959 with the introduction of the single catheter method and dialysis solution.[94] Peritoneal dialysis can be used in treatment of acute renal failure and end stage renal disease.[95]

Unlike haemodialysis, there is no direct contact between blood and membrane paper but diffusion of solutes occurs from peritoneal capillaries to the dialysis fluid which is size selective, considered to occur through a system of pores through which low molecular weight solutes as urea, creatinine and glucose diffuse through these pores, as well as large molecular weight substances such as proteins.[96]

Peritoneal dialysis is applied by introduction of catheter midway between umbilicus and symphysis pubis , inflow of dialysis solution into the peritoneal cavity this solution should have a sodium , chloride , magnesium and potassium chloride can be added.

Indication for peritoneal dialysis : [97]

1- Acute renal failure: Which is considered as a major indication for peritoneal dialysis.

2- Chronic renal failure: As in cases of vascular access failure ,children, patients with preexisting cardiovascular diseases as congestive or ischemic heart diseases ,patients with severe bleeding risks ,home dialysis and old patients above 65 years.

Contraindications :

1- Relative contraindications: [98] fresh intra abdominal foreign bodies , peritoneal leaks, body size limitation ,inflammatory or ischemic bowel diseases ,abdominal wall or skin infection, severe malnutrition and frequent episodes of diverticulitis.

2- Absolute contraindication:[99]intestinal obstruction,recent abdominal or retroperitoneal surgery ,extended intra abominal malignancy

Indications switching from peritoneal dialysis to haemodialysis [100]

Consistent failure to achieve target Kt/V urea and creatinine clearance,inadequate solute transport or fluid removal,unacceptably frequent peritonitis, severe malnutrition resistant to aggressive management and development of technical or mechanical problems such as catheter malposition.

Advantages of peritoneal dialysis:[101]

More quickly initiated than hemodialysis as there is no need dialyzing machine, anticoagulants are not necessary,no need for vascular cannulization and since chemical and fluid exchanges occur more slowly there is less on internal organs .

Peritoneal dialysis schedules: [102]

a) **Acute schedule:** Peritoneal dialysis solution is instilled and drained every 30 minutes to 2 hours, the usual treatment length is 48-72 hours.

b) **Chronic schedule: Modalities of peritoneal dialysis :** Several different techniques available for perfoming peritoneal dialysis (PD) .

Continuous ambulatory peritoneal dialysis (CAPD)

It is the most popular form of PD .it only requires the disposable solution bags and a line to connect the bag to the patient's catheter. This regimen involves multiple exchanges during the day (usually three) followed by an overnight dwell. A modification involves one nighttime exchange with an exchange device, resulting in two overnight exchange three exchange during the day, it has the advantages of low cost , freedom from machines and its ability to mainitain a strady physiological state, control fluid volume and blood pressure in most patients and simplify the control of glycerin in diabetic patients through the use of intra- peritoneal insulin. [103]

Continuous cyclic peritoneal dialysis (CCPD)

It allows greater flexibility in the number and volume of exchanges during the night with a long day time dwell, also large volumes are better tolerated in the supine position. All connections and preparations of equipment usually takes place at bed time a. minority of those undergoing CCPD do not have a day time dwell. *(104)*

Intermittent peritoneal dialysis (IPD)

It generally consists of frequent short cycles performed over 12-15 hours per session , three times weekly. Treatment periods("wet abdomen) alternation with times during which the peritoneal cavity has been drained of dialysate (" dry" abdomen) over 50 hours of dialysis are required to attain clearance similar to CAPD . however , even higher target doses of dialysis are recommended because of the peaks in BUN occurring on the off-dialysis days. For these reasons, this form of PD is not recommended for chronic use. If IPD is practiced on a nightly bassis it is referred to as nocturnal IPD(NIPD) NIPD is mostly reserved for patients with high solute transport and limited ultrafiltration as the short cycles can achieve better ultrafiltration than the longer cycles of CAPD or CCPD. *(105)*

Tidal peritoneal dialysis (TPD)

The principle purpose of TPD was to enhance clearance of small solutes by reducing the normal loss of dialysis time that is associated with the inflow and drainage of solution of the intermittent technique. It consists of exchanges in which the peritoneal cavity always contains at least some dialysis of exchanges in which the peritoneal cavity always contains at leats some dialysis (usually one – half full) , a feature that improves comfort and facilitates drainage in some patients. *(106)*

Continuous flow peritoneal dialysis (CFPD)

It uses the continuous technique and two separate catheters or a double lumen catheter. It is generally performed with high dialysis flow rates. PD fluid is infused into the peritoneal cavity through an inflow catheter while the outflow catheter is clamped. Once the desired fill volume is achieved, the outflow catheter is opened and the inflow and outflow are maintained relatively constant at high flow rates. The requirement of special peritoneal access and large volumes of solution had impeded its clinical application .there has been a renewed interest in this technique because of the increased recognition of frequently inadequate clearance with other PD modalities and the introduction of technical innovations that may make CEPDfeasible. One crossover study of five patients with nightly intermittent PD and nightly tidal PD.additional study is required to better understand the practicality of this technique. *(107,108)*

Effect of CAPD on anemia:

Continuous ambulatory peritoneal dialysis (CAPD) has been reported to produce an improvement in the anemia associated with endstage renal disease as indicated by the hematocrit (109-112). Fisher (113) reviewed the major factors involvedin the mechanism of the anemia of chronic renal failure including erythropoietin deficiency, inhibition of erythropoiesis, and shortened red cell life span. In vitro membrane dialysis has been shown to partially remove inhibitors of erythroid progenitor cell formation including inhibitors of both CFU-E (colony forming unit-erythroid) and BFU-E (burst forming unit-erythroid)

formation.[114-115]The improvement in anemia in patients on CAPD is associated with a much greater clearance of middle molecular weight substances and a less efficient removal of small molecular weight substances in comparison to hemodialysis. [109] These observations have led several investigators to suggest that uremic toxins responsible for inhibition of erythropoiesis are middle molecular weight in size and that it is the difference in relative clearances of these inhibitors of erythropoiesis by HD and CAPD which is responsible for any difference in effect of the two forms of dialysis on the anemia of renal failure.[110,111]

A recent report showed that the increase in hematocrite of patients starting CAPD reflects a decrease in plasma volume in addition to an increase in red cell mass.[116]

The often greater improvement in hematocrit levels during the first 6 months of CAPD treatment in comparison to HD has been reported to be due to a reduction in plasma volume in addition to an increase in red cell mass.[117]

The relatively high incidence of both orthostatic hypotension and symptoms of volume depletion in CAPD patients [110] suggests that the plasma volume can be influenced by CAPD treatment.

The role of erythropoietin in CAPD is still controversial and has been investigated in a very limited number of patients.[118]

Whereas Lamperi et al.[118]could not find a correlation between the increase in hematocrit and changes in serum erythropoietin levels, Zappacosta, Caro, and Erslev[26] observed that only patients with high erythropoietin levels under CAPD could normalize their hematocrit.

The foregoing explanations do not discount the possibility that other factors such as less extracorporeal blood loss and improvement of hemolysis, due to the absence of extracorporeal circulation are also playing a role in the better control of the anemia in CAPD patients. [109]

Reports about differences in the degree of renal aneamia between heamodialysis and peritonel dialysis patients are contradictory. [111]

Most of the studies show higher heamoglobin \ heamatocrite levels, a lower transfusion frequency and\or lower erythropoitine requirement in peritoneal dialysis patients compared with heamodialysis patients.[119-121]

Among several other factors, the lower extent of iron loss and therefor alower incidence of iron defecincy during peritoneal dialysis treatment may be one reason for the reported differences.[121,122]

Despite the lesser extent of blood loss, iron defecincy also play amain role in peritoneal dialysis patients. [109]

In study of non erythropoietin treatment peritoneal dialysis patients showed that marrow iron scores were significally lower in those not receiving iron supplementation as compared with iron- treated patients.[123]

One reason of iron deficiency in peritoneal dialysis patients not reciving erythropoitine may be the improved erythropoieses following initiation of peritoneal dialysis treatment. Berry et al domenstrated an improvement of plasma iron clearances and an increased uptake into red blood cell after starting peritoneal dialysis.[124]Salttissietal showed asignifecant increase of serum heamoglobin and red cell mass after initiatine CAPD treatment .[125]

Haemodialysis

Requires a machine that is connected to the patient using a surgically created path called a vascular access, the best type is the arteriovenous fistula (AVF), if not possible to construct and AVF, a graft can be used. A third type of access, a central venous line can be placed but it is more prone to complications and so it should be used if no other route is available. Hypotention, shortness of breath, abdominal cramps, nausea and vomiting are among complications.[126]

Over the last three decades, it has been demonstrated that haemodialysis (HD) is an efficient therapy, capable of supporting the life of more than one million chronic kidney disease stage V (CKD-V) patients worldwide. However, the success story of renal replacement therapy (RRT) is not complete. High morbidity and high mortality rates of CKD-V patient son supportive therapy are still a challenging problem. In addition, it is frustrating for clinicians to observe that, in spite of major technical advances (high-flux membrane, bicarbonate-buffered dialysis fluid, ultrafiltration-controlled machine, bio-compatible material, etc.) and many therapeutic achievements(correction of haematological and metabolic disorders such as anaemia by recombinant human erythropoietin(rHuEPO), hyperlipidaemia, hyperphosphataemia, hyperparathyroidism, etc.), there has been no significant progress in patient survival. The increasing prevalence of dialysis-related pathology, including beta 2-microglobulin(β_2m)-amyloidosis, accelerated atherosclerosis, left ventricular hypertrophy, ageing and malnutrition in long-term treated patients, is another sign of the partial failure of RRT. Although the precise causes of this pathology are not completely clear, there are several mechanisms that contribute to these poor outcomes.[127]

Four main factors are combined in uraemic patients:

- Relatively poor clearance for middle-size solutes leads to the accumulation of middle and large uraemic toxins.
- Poor biocompatibility of the dialysis system is responsible for periodic activation of circulating cells and protein systems, resulting in a microinflammation state.
- The un-physiologic profile of intermittent treatment leads to permanent instability of the internal milieu, with alternating ‘peaks and valleys’.
- The partial and incomplete correction of the ‘body’ composition that exposes CKD-V patients to a permanent pro-oxidative and carbonyl stress condition.[127]

The two main processes in HD are: diffusion and convection, Compared to the diffusive dialysis process, convective process in HD is associated with more effective removal of larger molecules because of the “solvent drag” effect. This quality is exploited by the technique of hemofiltration (HF), which involves forced removal of large amounts

of fluid from the intravascular space and replacement by a similar volume of an appropriate electrolytic solution. The efficacy of removal of small molecules by HF is lower than by conventional HD. A combination of HD and HF in the form of hemodiafiltration (HDF) offers the highest solute removal of both small and large molecules.[128]

Although HF and HDF have been utilized since the 1970s, there is renewed interest in these methods.[129] They are better at larger-molecule removal than HD.[130] Their main clinical advantages are superior hemodynamic stability, fewer symptoms during treatment, improved blood pressure control, and better clearance of middle and large sized molecules.[131] The main disadvantage of HDF is the significant expense of producing large volumes of sterile replacement solution. Newer techniques lower costs by implementing "online" production of endotoxin-free replacement solution from dialysate. This will probably increase utilization of HF and HDF.[132](Figure 8)

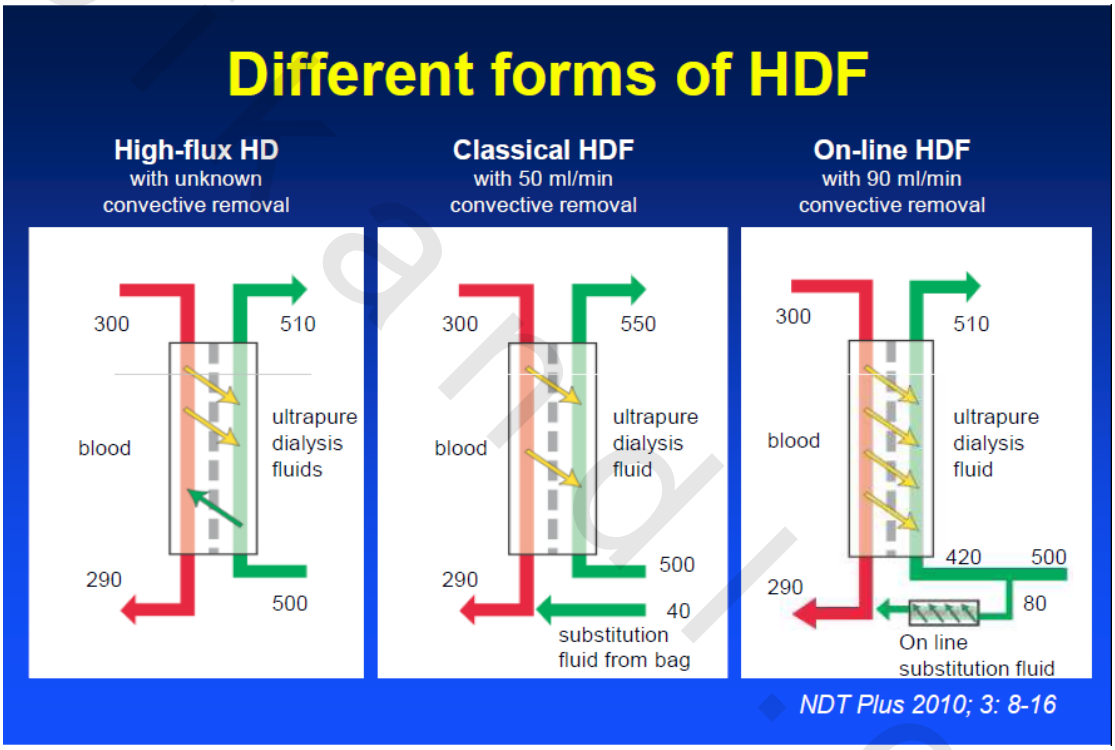

Figure (8): Different forms of HDF

Conventional diffusive-based dialysis modalities, including high-flux HD, are limited in clearing middle-size uraemic toxins and restoring metabolic balance in CKD-V patients.[133]

Convective methods mimicking glomerular filtration of native kidneys are required to enlarge the molecular weight spectrum of solutes removed during the dialysis session, with the intent of maximising toxin removal.[133-136]

Indications for HDF

- Hyperphosphatemia.
- Anemia.

- Infectious Complications.
- Joint Pain.
- Dialysis-associated Amyloidosis.
- Cardiocirculatory Instability.
- Neurological Complications.

Online haemodiafiltration (OL-HDF) provides several advantages in this context by combining diffusive and enhanced convective clearances. It offers an efficient modality to clear small- and middle-size uraemic toxins by using ultrapure dialysis fluid and high-flux synthetic membranes; it offers the most biocompatible dialysis system by providing a virtually unlimited amount of sterile dialysis fluid by cold sterilization filtration; it offers an economical and viable method to achieve high-efficiency haemodiafiltration (HDF)(high fluid volume exchange) therapy; and by keeping the dialysis machine with all in-built technical options(fluid-balancing system, thermal balance, online ionic dialysance monitoring, etc.), it offers the best of the technical options in RRT.[136]

Online Hemodiafiltration (OL-HDF) technique :

The high cost of commercial replacement fluids (bags) stimulated the development of this novel technique. Fresh ultrapure dialysate from the dialysate inlet line is processed with multiple filtration steps and reinfused as replacement fluid. Large amounts of inexpensive replacement solution are generated and HDF can be performed with very high fluid turnover (up to 30–40 l/session). Fluid can be reinfused in either pre- or postdilution mode, or both, in different proportions. Today, online hemodiafiltration (OL-HDF) provides the more efficient and the most biocompatible modality of renal replacement therapy for chronic kidney disease (CKD) patients. By combining diffusive and enhanced convective clearances, OL-HDF offers the highest instantaneous solute clearances over a wide molecular weight range of uremic toxins.[137-139] By reducing the hemo incompatible profile of the dialysis system, OL-HDF reduces exposure to the chronic microinflammation state of CKD patients.[140,141] High-efficient OL-HDF is now a well-established treatment modality with an increased prevalent use in CKD patients.[142-144]

The clinical beneficial effect of OL-HDF: .[144]

Several reports have shown that regular use of high efficiency HDF has beneficial effects in long-term dialysis patients. Improvement of dialysis session tolerance is repeatedly reported with high-efficiency HDF modalities.

- Incidence of hypotensive episodes is reduced with HDF methods and new technical options. This property is particularly important in hypotensive prone and cardiac patients. This effect has been related to a positive vasomodulation effect involving several factors, including a negative thermal balance, a high sodium concentration of the substitution fluid and the removal of vasodilating mediators.[145]
- Post-dialysis fatigue is reduced with OL-HDF. This is particularly useful in elderly, diabetic and other high-risk patients.
- A better control of arterial pressure has been reported with high-efficiency convective therapies. This beneficial effect is mainly due to the intradialytic haemodynamic stability that permits the sodium fluid balance to reach ‘dry weight’ and be adequately restored. Time duration of sessions and compliance to sodium diet restriction also

facilitates achievement of this primary objective in dialysis targets. This positive effect is associated with better anaemia correction and an adequate blood pressure control, suggesting that the effect may be linked to specific properties of OL-HDF.[146,147]

- Phosphate removal has been reported to be significantly enhanced by OL-HDF and high-efficiency convective therapies. Indeed, the higher phosphate removal rate observed with OL-HDF is not sufficient to adequately control blood phosphate concentrations. Adequate phosphate removal is limited in HDF by the high inter compartmental mass transfer resistance, meaning that phosphate binders are required during the interdialytic period.[148]
- High-efficiency OL-HDF based on the regular use of ultrapure dialysate reduces the microinflammation of the HD patient. Based on sensitive markers of the acute phase reaction (C-reactive protein (CRP), interleukin (IL)-1 and -6, IL1-receptor antagonist and IL6-receptor antagonist (IL1-RA and IL6-RA), albumin), several prospective studies have shown that the behaviour of these markers remains stable in the normal range over time in OL-HDF.[149,150] Preventing inflammation is crucial in order to reduce the incidence of dialysis-related complications in long term dialysis patients.[151]
- Caloric and/or protein malnutrition is commonly observed in about one-third of dialysis patients. Several recent studies have shown that the use of high-flux membranes has a positive impact on the nutritional state, compared with low-flux membranes.[152]
- Serum albumin tends to increase when patients are treated with high-flux membranes, as well as dietary protein intake. Indeed, it must be recognized that this positive effect may result from the combination of using highflux membranes with ultrapure dialysate, and possibly with the removal of anorexia-inducing uraemic toxins via convective flux.[153]
- The regular use of high-flux membranes with enhanced convective clearances has been shown to improve lipid profile[154] and to reduce oxidative stress and advanced glycation end-products (AGE) in CKD-V patients.[155,156] Indeed, this beneficial effect may be partly due to the improved biocompatibility of the dialysis system, due to the combined use of synthetic membrane and ultrapure dialysate.[157]
- Neuropathy is a rare complication of RRT that is considered a late marker of inadequate dialysis. It has been reported that intensification of treatment with high-flux convective methods is capable of correcting this neuropathy.[158]
- Renal anaemia commonly observed in HD patients requires erythropoietin (EPO) use in 80–100% of patients. Although controversial, it has been reported that high-efficiency convective therapies are able to improve anaemia and to reduce EPO needs.[159] This positive effect was particularly well illustrated when patients were switched from low-flux to high efficiency HDF modalities or to HD, using high-flux protein-leaking membranes.[160,161] These observations suggest that high-flux convective methods may remove protein-bound EPO inhibitor substances.
- β_2m-amyloidosis is a major disabling complication of long-term HD-treated patients. Using carpal tunnel syndrome as a crude and first manifestation of β_2 amyloidosis, it is commonly accepted that its incidence reaches 50% at 10 years and 100% at 20 years with conventional low-flux HD treatment. .[162]

Effect of O;-HDFon anemia:

To what extent the mode of dialysis influences anemia is yet controversial , long – term effects of high volume OL HDF on uremia and dialysis- related pathology have not been extensively studied ,and there are only a few data concerning anemia.[162,163]

The pathogenesis of renal anemia was dominated for decades by the question of whether primarily due to relative EPO deficiency or uremic inhibition of erythropoiesis.[164] the relative deficiency in EPO is considererd the main cause of renal anemia. In addition, factors other than EPO deficiency seem to contribute to an impairment of the erythropoiesis; several of them have received attention (iron deficiency, inflammatory mediators, parathyroid hormone, etc), but the role of specific uremia-associated inhibitors of erythropoiesis has remained un clear. [165,166] Although direct proof is lacking this does not exclude that a variety of different molecules accumulating in the uremic state may contribute to impaired erythropoiesis. Once HDF is started, the patient experience a significant increase in hematocrite in the absence of an improvement of endogenous EPO.[25]

OL HDF combines convection and diffusion in a single therapy. It provides superior solutes removal over a wide molecular weight rang. a higher removal of medium- and large-size molecules may prevent the accumulation of factors that contribute to some of the morbidity related to chronic HD, anemia included. The impairment of erythropoiesis could be more limited, leading to higher hematocrite levels and lower rHuEPO doses.[162]

It is important to note that factors other than the dialyser may influence the biocompatibility, i.e., the purity of dialysate. OL HDF uses ultrapure dialysate and reinfusate with the aim of sterility and a pyrogenicity and thus contributes to a reduced pro inflammatory cytokine production, improving anemia .[167]

Online hemodiafiltration could improve erythropoietin response as a result of the increased removal of medium- and large-sized molecules. Bonforte et al.[168] showed an improvement in anemia in 32 patients with high-volume online replacement fluid. Osawa et al. [169] were able to lower the erythropoietin dose with hemodiafiltration. Maduell et al.[170] reported improved correction of anemia in 37 patients with lower erythropoietin doses when conventional hemodiafiltration (4 liters) was switched to online hemodiafiltration (24liters), This was attributed to the higher convection volumes, although there were also significant differences in urea clearance. Ward et al. [162] and Wizemann et al. [171] could not confirm these observations in 24 and 23 patients, respectively, treated with online hemodiafiltration compared with 21 patients treated with high-flux hemodialysis and 21 patients treated with low-flux hemodialysis.

The recently published European randomized controlled MINOXIS trial (Modulation of inflammation and oxidative Stress by high-flux hemodialysis) did not show any difference in hemoglobin levels, ESA dose or ESA index (i.e. ESA dose normalized to body weight and hemoglobin level) between treatment with high-flux versus low-flux HD for 12 months. these data indicate that adding a relatively low convective volume to conventional dialysis appears notto be beneficial with respect to ESA resistance. [172]

Simultaneously with the CONTRAST study, a Turkish study on HDF show equal Hb levels were observed in patients treated with online HDF and high-flux HD.[173] Enric Vilaretal [174] found no advantage of HDF over high-flux HD in anemia management.

Effect of OL-HDF on infection:

Uremic patients have a significant risk of infectious complications. Indeed, these complications are the first cause of hospitalization and the second cause of death in hemodialysis patients. Several granulocyte-inhibiting proteins are present in uremic patients, which may contribute to the high incidence of infectious complications. Degranulation-inhibiting protein I and granulocyte inhibitory protein II inhibit in vitro glucose uptake and chemotaxis of polymorphonuclear leukocytes. Factor D decreases the complement-mediated clearance of immune complexes and inhibits granulocyte degranulation. All these uremic toxins are better removed with high-volume hemodiafiltration. [162, 175]

Effect of OL-HDF on iflammation:

Several papers have reported that most CKD patients have a subclinical microinflammatory state with high serum levels of some proinflammmatory/proatherogenic cytokines and accumulation in peripheral blood of activated mononuclear cells that prolong their lifespan. Markers of inflammation such as C-reactive protein and cytokines are independent predictors of all-cause and cardiovascular mortality in these patients.[176,177]

By combining ultrafiltration (convective clearances for removing larger solutes) with diffusion (for removal of small solutes), hemodiafiltration (HDF) offers a highly effective dialysis modality expanding the spectrum of removed uremic toxins from small to middle-sized molecular solutes. The online HDF using high fluid substitution allows a greater clearance of large uremic toxins.[162,178] The mortality of dialysis patients remains elevated despite advances in dialysis technology, significant improvement in dialysis quality and better global care of patients. On one side, it is interesting to note that a preliminary report from the international Dialysis Outcomes and Practice Patterns Study (DOPPS) has shown that patients undergoing HDF had a reduced risk of death compared to those treated by conventional HD. The spectrum of eliminated uremic toxins together with the adoption of ultrapure dialysate may all contribute to explain the reduction of the chronic inflammation. The latter has been associated with an elevated number of circulating monocytes, an increased percentage of mature pro-inflammatory monocytes and an overproduction of interleukin (IL1), tumor necrosis factor and (IL-6), without the ability to synthesize the anti-inflammatory cytokine (IL-10).[179]