Analysis and Interpretation of Iron studies and Vitamin C levels in Paediatric patients with chronic renal failure.

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Paediatrics.

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DECLARATION

I, Tracey Leigh Lutz, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Paediatrics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University

27th October 2009

DEDICATION

To Brett,

For his ongoing support

And encouragement

ABSTRACT

This prospective observational study analysed iron studies and vitamin C levels in patients with chronic kidney disease attending Johannesburg Hospital Paediatric Nephrology Clinic. The rationale behind this study was to determine the extent of iron deficiency among patients in chronic renal failure. Vitamin C deficiency is common among dialysis patients, it is easy to test for and easy to prevent. This study may assist in guiding future management with regards to vitamin C supplementation in patients with chronic renal insufficiency on dialysis.

The study contained 45 patients of which 27 (60 %) were male and 18 (40 %) were female. The ages of the children varied from 2 years 1 month to 19 years and 7 months. The study included patients from all ethnic groups; 9 were Caucasian, 33 African, 2 Indian and 1 Coloured. Two male patients did not have Vitamin C levels analyzed**.**

The patients were divided into 3 distinct groups; firstly those patients on haemodialysis (12 patients), those on peritoneal dialysis (22 patients) and those not yet dialysed (11 patients). In all patients who were not yet on dialysis the GFR ranged between 18.1 and 45 ml/min/1.73m$^{2.}$

There were no statistically significant differences between the three groups when the results of the iron studies were analysed. However, despite iron treatment 26.6 % of patients were iron deficient as indicated by their transferrin saturation which was less than 20 %.

Vitamin C levels were also analysed in this study. Forty one percent of children in chronic renal failure were vitamin C deficient. There was no statistically significant variability

among the three groups. Two patients (4.6%) were noted to be Vitamin C toxic. One of these patients was haemodialysed; the other was not yet on dialysis.

Vitamin C deficiency in chronic renal insufficient patients on dialysis is easily correctable when identified. Vitamin C in specific well documented doses is safe to administer to this group of patients. It will also enhance the absorption of iron and thereby have an indirect effect on anaemia.

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CHAPTER 1

INTRODUCTION

Anaemia and its association with kidney disease was first described in 1836 by Richard Bright. The nature of the anaemia associated with renal disease was initially poorly understood (1), but with time and extensive research it has been shown that the aetiology of the anaemia in chronic kidney disease is multifactorial. Anaemia is an independent risk factor for early death in patients with chronic kidney disease.

Firstly, there is inadequate production of erythropoietin by the failing kidney. This is singularly the most important reason for the anaemia. Other factors which contribute to the anaemia include; inhibition of erythropoeisis by uraemic toxins, excessive blood loss in haemodialysis circuits and increased osmolality which results in reduced red cell survival thereby aggravating the anaemia (2). Certain nutritional deficiencies are also associated with chronic kidney disease. These include Vitamin C (ascorbic acid), iron and folate deficiencies. Intoxications with substances that impair red blood cell development (aluminium and lead) and substances that contribute to haemolysis (copper) can also contribute to the anaemia of end stage renal disease (3).

The anaemia associated with chronic renal failure is usually of a progressive nature with pre-dialysis patients being less severely affected than those on dialysis (4). This suggests a linear relationship between Glomerular Filtration rate and severity of anaemia, the lower the GFR the less erythropoietin is produced by the failing kidney.

Anaemia is usually more common in children with chronic kidney disease than in adults; however, most research in this field use adult subjects. It also seems to be more severe, consequently demanding more frequent blood transfusions. The adverse effects of these transfusions include increased risk of viral infections (HIV, Hepatitis), blood borne diseases, and enhanced sensitisation to histocompatibility antigens, thereby decreasing the success of future renal transplantation as well as iron overload (5).

Anaemia has been closely linked with poor concentration, impaired physical capabilities (exercise intolerance), cardiovascular complications (left ventricular hypertrophy being the most common recorded cardiovascular abnormality), anorexia as well as increased mortality (6). Several studies have documented that a haemoglobin level of 10g/dl or less is associated with a high risk of death when compared with levels of more than 10g/dl (7). Correction of the anaemia has benefits in improving concentration, reducing left ventricular mass index, improving fatigue and depression as well as stimulating an improved appetite.

K/DOQI guidelines have suggested maintaining haemoglobin levels between 11g/dl and 12g/dl and a haematocrit above 33%. According to these guidelines the monitoring of haemoglobin is preferred to haematocrit in the identification and management of anaemia. Haemoglobin measurement is more accurate if a time interval is present between collection and laboratory analysis. Hyperglycaemia also adversely affects the MCV (elevating it) which results in an invalid haematocrit reading. The laboratory variability in the measurement of haematocrit is greater than for haemoglobin. Maintaining a normal haemoglobin level is not always easily achievable. Anaemia (as mentioned above) and polycythaemia are both associated with complications. Polycythaemia has been associated with an increased incidence of thrombosis (including access thrombosis in patients on haemodialysis) and hypertension. Hypertension is a well documented complication of erythropoietin supplementation (8). Erythropoeitin stimulating agents have an increased thrombotic risk through increased inflammation and antifibrinolytic activity (9).

In newborn babies, the liver is the primary site of erythropoietin production. Shortly after birth the kidneys take over this role. Erythropoetin is primarily produced by the cells of the peritubular capillary endothelium of the kidney. It is a 165 amino acid glycoprotein (62 % protein and 38 % carbohydrate); it is responsible for the regulation of red blood cell production. Erythropoeitin induces erythropoeisis by stimulating the division and differentiation of committed erythroid progenitor cells. This accelerated erythropoeisis creates a need for additional iron (10). If available iron reserves are inadequate, optimal therapeutic response to erythropoietin is threatened by iron deficiency (11). The reticulocytes which are released from the bone marrow mature into red blood cells.

Erythropoietin should be supplemented subcutaneously or intravenously to all patients with anaemia and chronic renal failure (12). Correction of the anaemia with erythropoietin increases oxygen delivery to the tissues and thus reduces hypoxia (13). Supplementation of erythropoietin into the peritoneal fluid is also effective, as it is absorbed in significant amounts. The one problem with intraperitoneal administration of erythropoietin is that the dose required is significantly higher which has a negative impact on cost. Comparative studies on the various routes of administration and their effect have not been studied in children. However, we do know that intravenous erythropoietin is well tolerated. Intravenous erythropoietin is administered after a dialysis session and therefore compliance is assured. Subcutaneous administration of erythropoietin is painful and this may affect compliance. Recommended guidelines suggest starting at a dose of around 50-150 U/kg/week in two or three doses. It is well known that younger children need relatively more erythropoietin than older ones (14). If there is an inadequate response (haemoglobin should increase at one g/dl/month) the dose should be increased in stepwise increments of 50 U/kg/week. Patients can either respond to erythropoietin, fail to respond to it or develop a pure red cell aplasia. A pure red cell aplasia is extremely rare and is due to the development of neutralizing antierythropoeitin antibodies.

There are various types of erythropoietin available, erythropoietin alpha (eprex), erythropoietin beta (recormon) and erythropoeitin delta. Darbopoeitin alpha has a longer half life and has not been found to cause a pure red cell aplasia; it is more cost effective due to its longer half-life (15). It is good for the stable control of anaemia in haemodialysis patients (16). A new class of third generation erythropoeitic-stimulating agents have become available; these are continuous erythropoietin receptor activators (CERA). Micera is similar to previous synthetic erythropoietin drugs, except that it is connected to a chemical called polyethylene glycol which makes it last longer in the body. It has the longest half-life of all FDA-approved erythropoeisis stimulating agents. This has two significant advantages; firstly, lower dosing which reduces cost and secondly, less frequent injections for patients which will impact positively on compliance. This drug is currently available and registered for use in different parts of the world. Recombinant erythropoietin is immunologically indistinguishable from the native hormone; it would therefore not be expected to promote an immune response (12).

There are a number of reasons why patients may develop resistance or fail to respond to erythropoietin and they include: the development of an infection, antibodies to erythropoeitin (as mentioned above), vitamin C deficiency, malnutrition (Protein/Energy Wasting), hyperparathyroidism, haemolytic disorders, vitamin B 12 and folate deficiencies and drug ingestion (Angiotensin-Converting Enzyme Inhibitors) (17). The exact mechanism of how ACE inhibitors impair production of erythropoeitin is not clear; however, they are thought to alter tissue oxygenation and affect erythropoeitin production directly through angiotensin II (3). The most important reason for patients to fail to respond to erythropoietin is iron deficiency (18). This can be in the form of true iron deficiency or functional iron deficiency (2).

Iron is an essential element required for growth and survival and plays a role in oxygen transport and various enzyme-catalyzed reactions. Excessive free iron can be dangerous due to its contribution to free radical production.

Iron supplementation forms an integral part of the management of anaemia associated with chronic kidney disease (19). Intravenous replacement and maintenance iron are frequently required in haemodialysis patients (20). Low serum iron has been associated with a poor clinical outcome, including significantly greater rates of mortality and hospitalisation (21*)*. Indications are that the prevalence of iron deficiency in paediatric patients is approximately 25 % which is comparable with that in adults (18). The K/DOQI guidelines (22) suggest supplementing iron if the ferritin is less than 100ng/ml and the TSAT (percent transferrin saturation) falls below 20% in pre-dialysis or peritoneal dialysis patients. In the haemodialysis group absolute iron deficiency is defined as a TSAT of less than 20% and a ferritin of less than

200ng/ml. The TSAT is calculated by multiplying serum iron by 100 and dividing it by the total iron binding capacity.

The Reticulocyte Haemoglobin is also a very effective means of monitoring iron status (23); the reticulocyte haemoglobin content reflects the amount of iron available for haemoglobin production in the bone marrow. Reticulocyte haemoglobin is an accurate and direct measure of the effective iron supply for erythropoesis as it focuses on new red cells just released from the bone marrow. It is not fully understood or investigated whether reticulocyte haemoglobin is influenced by inflammation (24). The testing for this form of haemoglobin is not readily available in all laboratories (25) and as such, it has not been used in this study.

Ferritin is often used as a marker for iron stores. The main concerns with using ferritin are that:

- Firstly, uraemia stimulates a chronic inflammatory response which can elevate ferritin despite inadequate iron stores (26).
- Secondly, in patients who have had multiple blood transfusions hepatic iron stores do not correlate with ferritin levels or serum transferrin.
- Finally, ferritin is an acute phase reactant and can be elevated in response to an infection or an inflammatory process.

Ferritin levels may be normal in patients with a functional iron deficiency; however, they are unable to mobilise iron from these stores rapidly enough to satisfy the demands of the bone marrow (27).

Hepcidin has recently been recognised as a hormone essential to the negative regulation of iron; it also has antimicrobial properties. It is a small peptide produced by the liver as pro-hepcidin; it then undergoes proteolytic cleavage to form hepcidin. Hepcidin acts as an essential iron-regulatory hormone; its production is regulated by anaemia/hypoxia, iron status and inflammation.

When iron is absorbed in the duodenum, ferric iron is reduced to ferrous iron by cytochromes. The proximal duodenal environment allows transport of this ferrous iron into the enterocyte. This iron is then lost in the faeces or transferred to the circulation to bind transferrin. Excess circulating iron is stored, mainly in hepatocytes. This occurs by binding to ferritin. Hepcidin can block iron absorption by the duodenum, iron release from the liver (storage iron) and interrupt the macrophage recycling of iron between red cells and the reticuloendothelial system (28).

In iron overload, hepcidin expression is increased which decreases the absorption of intestinal iron. In iron deficiency the opposite occurs. In anaemia/hypoxia hepcidin expression is decreased which enhances the absorption of iron (28). In anemia of chronic disease or associated with chronic inflammation, hepcidin is increased which interferes with the absorption of iron. This can result in a real iron deficiency if ongoing. Hepcidin also decreases iron availability by increasing sequestration by the reticulo-endothelial system (29).

Serum hepcidin can be measured using an ELISA (Enzyme Linked Immunosorbent Assay). This was developed and validated by Ganz *et al* (30). In healthy volunteers, serum hepcidin concentrations correlated with its urinary levels and with serum ferritin. Hepcidin was low or undetectable in patients with iron deficiency anaemia and increased in cases of inflammation. Pro-hepcidin can also be measured using an ELISA; most clinical studies are based on these levels. Pro-hepcidin appears to be a reliable indicator of hepcidin production.

A study by Tsuchihashi *et al* (31) found no difference in serum pro-hepcidin levels between haemodialysis patients and healthy volunteers. Kulaksiz *et al* (32) found pro-hepcidin levels to be 30% lower in healthy volunteers than in patients on haemodialysis without anaemia. Although various studies have not had the same result outcomes what is clear is that pro-hepcidin levels are increased in haemodialysis patients who are anaemic (Hb <11g/dl). Other confounding factors in using hepcidin as a marker for iron status in haemodialysis patients are that intravenous iron and supplemented erythropoietin all influence hepcidin expression.

Iron can be given in two different forms; orally or intravenously. There are three different types of intravenous iron: iron dextran, sodium ferric gluconate and iron sucrose (33). Supplementation of iron is not completely benign and has been associated with anaphylaxis. Anaphylactic reactions to intravenous iron vary from mild urticaria and other rashes to dyspnoea, hypotension, shock and death. Iron dextran seems to have the highest incidence of anaphylaxis and death. The rationale behind this is that the high molecular weight dextran moiety shares carbohydrate antigens with gastrointestinal organisms. Delayed adverse events included arthralgias, myalgias, fever and headache. There is also potentially an increase in infection associated with the supplementation of iron (34). Iron acts as a growth factor for bacteria; it inhibits neutrophil function (35). Iron contributes to endothelial damage and inflammation when in its free circulating form (hydroxyl). Iron also causes renal tubular damage, with limited data regarding severity (36*).*

Iron requirements are increased in patients with chronic kidney disease due to erythropoietin facilitated iron utilisation and iron sequestration secondary to ongoing chronic inflammation (37). Chronic kidney disease patients have also been noted to have high interleukin 6 levels which results in the modification of iron metabolism. In the absence of erythropoietin, iron accumulates and is redistributed to the reticulo-endothelial system and non haematopoietic tissues. Iron is principally accumulated in hepatocytes and Kupfer cells when erythropoiesis is depressed (38). Iron deficiency is the single most important cause for resistance to erythropoietin. For optimal response to erythropoeitin iron needs to be maintained within the normal range (2).

Studies done have revealed that intravenous maintenance iron in haemodialysis patients has allowed for the reduction in dosage of erythropoietin to maintain blood haemoglobin levels within the normal range. Regular maintenance iron has a lower incidence of iron overload than intermittent iron boluses (37).

In haemodialysis patients intravenous use of iron has resulted in improved results when compared with the oral administration of iron (27). Two possible reasons for this are; firstly, poor compliance with the oral form and secondly, impaired intestinal absorption of iron. These studies are adult based and paediatric data is limited. We do, however, know that certain substances enhance iron absorption i.e. Vitamin C while others inhibit it i.e. tannins in tea and coffee, cereals as well as dairy products.

Oral iron should be administered without food or medication; a dose of 200mg daily is recommended. The oral paediatric dose is 2-3mg/kg/day (22).

Fishbane (34) reviewed iron replacement in non-dialysis patients with established kidney failure. Most studies revealed only a modest superior efficacy of intravenous iron therapy when compared to oral iron. Achieving vascular access was inconvenient in the out-patient setting. Also, intravenous iron causes a transient surge in oxidative stress which has implications on vascular access in the future. Further studies need to be done to determine the best method of administration in this group of patients.

Supplementation of iron to patients with chronic kidney disease has allowed for reduced dosages of erythropoietin – this has two identifiable benefits; firstly cost reduction and secondly improved safety (38). A well documented complication of erythropoietin is hypertension. The erythropoietin affects endothelial and vascular smooth muscle thereby causing hypertension. The hypertension related to erythropoietin can be improved by reducing the dosage of the drug and by changing its route of administration from intravenous to subcutaneous. Blood pressure monitoring is essential as most paediatric patients have hypertension as a result of their underlying renal pathology, which is further exacerbated by the use of certain drugs.

Very little information is available to determine the upper limit of safety of ferritin levels in patients on intravenous iron supplementation. We know that iron accumulates in the heart, liver and pancreas (haemosiderosis) and can be hazardous.

However, it has been noticed that in dialysis patients most iron accumulates in the reticulo-endothelial cells and very little parenchymal damage occurs. Iron overload has also been associated with an increased risk of infection (22). Adult studies have not recommended routine iron administration when the ferritin level is above 500ng/ml. Iron has the potential to accelerate kidney damage in patients with chronic kidney disease not on dialysis therapy (39).

Vitamin C or ascorbic acid is an essential nutrient that is required for the formation of collagen, normal immune function and for the generation of corticosteroids and catecholamines. The most common clinical effects of ascorbic acid deficiency are gingivitis, soft tissue bleeding, fatigue and possible alterations in immune function. These symptoms are fairly non-specific and may be attributed to many different causes.

Recent research into Vitamin C levels in patients with chronic renal failure on haemodialysis has revealed that Vitamin C levels have been found to be subnormal in a significant number of patients. The two reasons for this are poor dietary intake as well as intra-dialysis loss of the vitamin. The intra-dialysis loss of the vitamin is enhanced due to its low molecular weight and its low or absent albumin-binding (40). Ascorbic acid stores depend on a number of factors; such as dietary intake, intracellular distribution, rate of utilization as a free radical scavenger and cofactor in the dihydroxygenase reactions as well as the rate of regeneration from monodehydroascorbate radical and dehydroascorbate. Vitamin C supplementation has been recommended in patients on long term haemodialysis therapy due to its involvement in certain metabolic pathways and for its role as an antioxidant (41). The therapeutic window is however fairly narrow.

Removal of ascorbate by peritoneal dialysis is proportional to the peritoneal creatinine clearance. In peritoneal dialysis patients, low ascorbate levels have been associated with low serum albumin. In one study, there were similar levels in the patients on CAPD (Continuous Ambulatory Peritoneal Dialysis) and CCPD (Continuous Cycling Peritoneal Dialysis) (42).

Vitamin C, being a water-soluble vitamin is excreted by the kidney. If large doses are given to patients with normal renal function the kidney will simply increase the amount excreted through glomerular filtration and active tubular secretion. However, in patients with renal failure this is not possible and it will then accumulate and deposit as oxalate crystals (41). The clinical significance of super saturation in the blood is poorly understood, however we know that tissue oxalate crystal deposition can only occur in a supersaturated state. Oxalate forms as a metabolite from the breakdown of vitamin C. These oxalate crystals deposit in the renal tubules (calculi) as well as in parenchymal tissues. A dose of 120mg of Vitamin C is unlikely to cause oxalosis but sufficient to prevent a deficiency. Administration of larger doses of ascorbic acid is associated with induction of acute free radical generation (43).

CHAPTER 2

MATERIALS AND METHODS

2.1 Study Sample

2.1.1 Geographic and age details

This prospective observational study was carried out in the Department of Paediatric Nephrology at Johannesburg General Hospital from October until December 2005. This Study was approved by the Human Medical Research Ethics Committee of the University of the Witwatersrand, Johannesburg.

Forty-five patients (18 females and 27 males) were entered into the study. The age of the patients included in the study varied from 2 years to 19 years 7 months (mean age was 12 years). The subjects were from diverse racial and ethnic groups. There were 9 white children, 33 black children, 1 coloured child and 2 children of Indian ethnicity.

The inclusion criteria consisted of all the patients who had chronic renal disease that gave consent or assent for the study. All patients 14 years and above gave their own informed consent (assent) to participate in the study; parental consent was obtained for patients under the age of 14 years. All patients, enrolled in the study, and their parents had a good understanding of English and as such it was not necessary to work through an interpreter. There were essentially three groups of patients: those on haemodialysis (12 patients), those on continuous ambulatory peritoneal dialysis (22 patients) and those not yet dialysed (11 patients) but in established renal failure with all the participants having a calculated Glomerular Filtration Rate (GFR) under 40ml/min/1.73m^2 surface area (22). The aetiology of the renal failure was diverse:

- Alport's Syndrome – one case
- Primary Hyperoxaluria – five cases
- Congenital Nephrotic Syndrome – four cases
- Hypoplastic kidneys – two cases
- Dysplastic kidneys – seven cases, two of which had single kidneys
- Posterior urethral valves – five cases one of which was associated with prune belly syndrome
- Focal segmental glomerulosclerosis - four cases
- Rapidly progressive glomerulosclerosis – one case
- Reflux nephropathies of varying grades – two cases
- VATER association with a neuropathic bladder – one case
- Autosomal recessive polycystic kidney disease – four cases
- Haemolytic uraemic syndrome – two cases
- Unknown aetiology of the renal failure – seven cases

2.1.2 Sampling method

Early morning random blood samples were taken on all patients enrolled in the study over a period of 6 weeks. These specimens were taken at the time of regular outpatient clinic follow-up appointments. In the patients on haemodialysis blood specimens were taken at the start of a three hour dialysis session. The blood specimens were all sent to the same laboratory for analysis of a full blood count, a urea and a creatinine, a c-reactive protein and iron studies. A further six millilitres of blood was sent for Vitamin C analysis. The Vitamin C was measured using the White Cell Vitamin C Assay method (see appendix). Only 43 out of the 45 patients enrolled in the study had vitamin C levels performed.

2.1.3 Statistical Methods

2.1.3.1 Fischer's Test

The conventional Fischer's exact test was used to determine non random associations between the categorical variables. This test compared iron levels, transferrin levels and vitamin C levels in patients on haemodialysis, peritoneal dialysis and a group of patients not yet dialysed. A result of < 0.05 was considered statistically significant. The Fischer's exact test was selected in favour of the chi square test due to the small sample size.

2.1.3.2 Box and Whisker Plot

A Box and Whisker plot has been used to display the statistical data. This plot easily identifies the mean, the spread and the overall range of distribution of iron levels, transferrin and vitamin C in the three groups (haemodialysis, peritoneal dialysis and the group not yet dialysed).

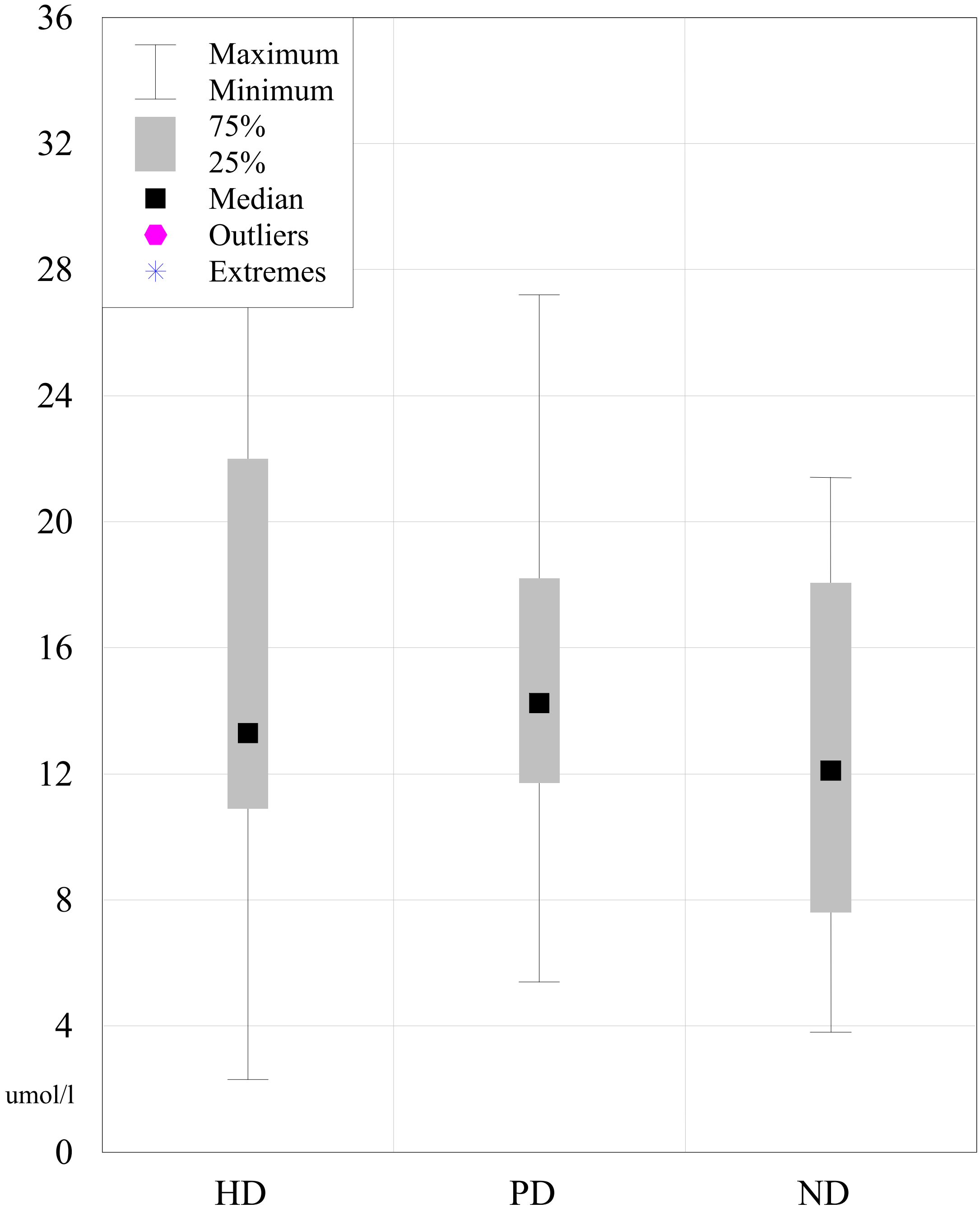
2.2 Statistical Analysis

2.2.1.1. Iron levels

Group	Normal	Abnormal	Total
Peritoneal Dialysis	18 patients 81.82% Range: 11.8 – 27.2 Mean: 16.12umol/l	4 patients 18.18% Range: 5.4 – 9.9 Mean: 7.93umol/l	22 patients 100.00% Range: 5.4 – 27.2 Mean: 14.6umol/l
Haemodialysis	8 patients 66.67 % Range: 10 – 28.9 Mean: 15.9umol/l	4 patients 33.33 % Range: 2.3 – 35.7 Mean: 19umol/l	12 patients 100.00 % Range: 2.3 – 35.7 Mean: 16.9umol/l
Non- Dialysis	8 patients 72.73 % Range: 10 – 21.4 Mean: 15.76umol/l	3 patients 27.27 % Range: 3.8 – 5.2 Mean: 4.57umol/l	11 patients 100.00 % Range: 3.8 – 21.4 Mean: 12.7umol/l
Total	*34 patients* *75.56 %* *Range: 10 – 28.9* *Mean: 15.99umol/l*	*11 patients* *24.44 %* *Range: 2.3 – 35.7* *Mean: 11umol/l*	*45 patients* *100.00 %* *Range: 2.3 – 35.7* *Mean: 14.77umol/l*

Fischer's exact test – p value = 0.605

2.2.1.2. Box Whisker Plot of Iron levels



HD: Group of Patients on Haemodialysis

PD: Group of Patients on Peritoneal Dialysis

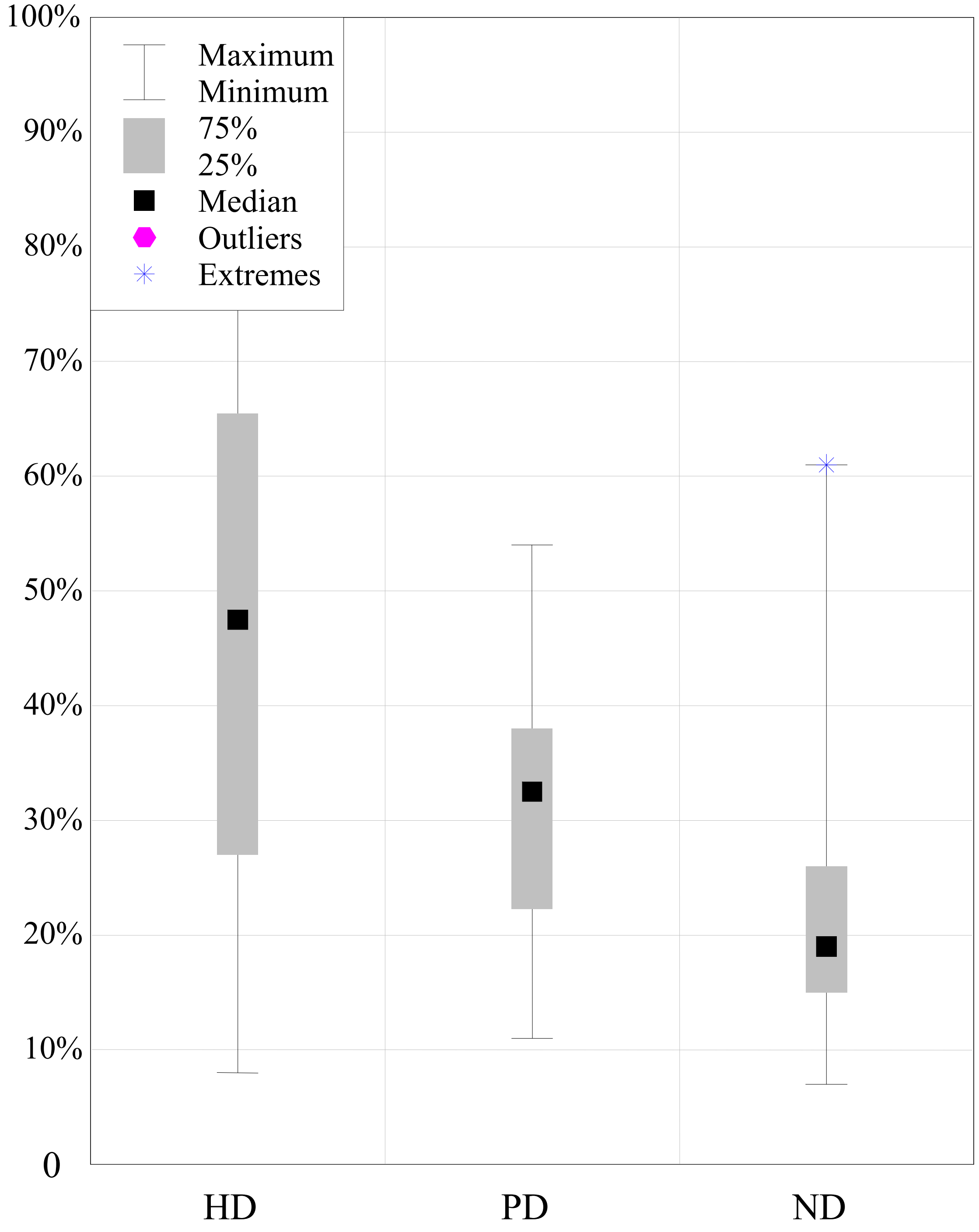
ND: Group of Patients not yet dialysed

2.2.2.1. Transferrin Saturation

Group	Normal	Abnormal	Total
Peritoneal Dialysis	16 72.73 % Range: 22 – 50% Mean: 34.25%	6 27.27 % Range: 11 – 54% Mean: 22.67%	22 100.00 % Range: 11 – 54 % Mean: 31.09%
Haemodialysis	4 33.33 % Range: 27 – 42% Mean: 31.5 %	8 66.67 % Range: 8 – 95 % Mean: 57.13%	12 100.00 % Range: 8 – 95 % Mean: 48.58%
Non dialysis	5 45.45 % Range: 22 – 30% Mean: 26.8%	6 54.55 % Range: 7 – 61% Mean: 22.17%	11 100.00 % Range: 7 – 61% Mean: 24.27%
Total	*25* *55.56 %* *Range: 22 – 50 %* *Mean: 32.3%*	*20* *44.44 %* *Range: 7 – 95%* *Mean: 36.3%*	*45* *100.00%* *Range: 7 -95%* *Mean: 34.08%*

Fischer's exact test – p value = 0.064

2.2.2.2. Box Whisker Plot of Transferrin Saturation Levels



HD: Group of Patients on Haemodialysis

PD: Group of Patients on Peritoneal Dialysis

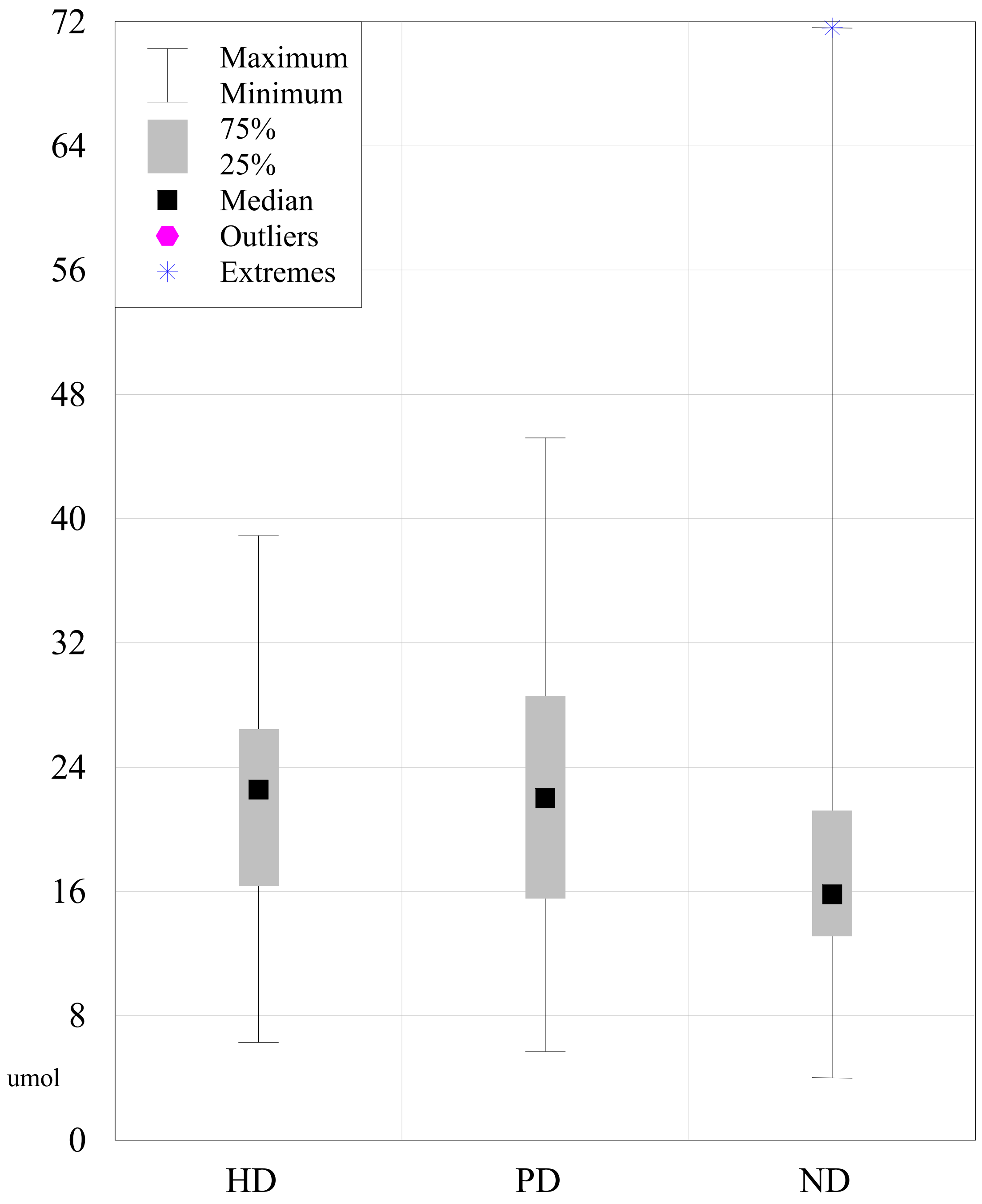
ND: Group of Patients not yet dialysed

2.2.3.1. Vitamin C Levels

Group	Normal	Abnormal	Total
Peritoneal Dialysis	13 59.09% Range: 20.3 – 39.6umol Mean: 26.97umol	9 40.91% Range: 5.7 – 45.2umol Mean: 17.04umol	22 100.00% Range: 5.7 – 45.2umol Mean: 22.9umol
Haemodialysis	7 58.33% Range: 21.7 – 38.9umol Mean: 27.3umol	5 41.67% Range: 6.3 – 19.2umol Mean: 13.14umol	12 100.00% Range: 6.3 – 38.9umol Mean: 21.4umol
Non-dialysis	3 33.33% Range: 21.1 – 28.3umol Mean: 23.5umol	6 66.67% Range: 4 – 71.6umol Mean: 21.06umol	9 100.00% Range: 4 – 71.6umol Mean: 21.8umol
Total	*23* *53.49%* *Range: 20.3 – 39.6umol* *Mean: 26.6umol*	*20* *46.5%* *Range: 4 – 71.6umol* *Mean: 17.26umol*	*43* *100.00%* *Range: 4 – 71.6umol* *Mean: 22.3umol*

Fischer’s exact test – p value = 0.43

2.2.3.2. Box Whisker Plot of Vitamin C Levels



HD: Group of Patients on Haemodialysis

PD: Group of Patients on Peritoneal Dialysis

ND: Group of Patients not yet dialysed

CHAPTER 3

RESULTS

Analyses of Vitamin C and iron levels were done comparing the three different groups: Patients on haemodialysis, peritoneal dialysis and those not yet dialysed but in well established renal failure.

3.1 Iron, Vitamin C and Haemoglobin Results

Study No.	Patient Age	Type of Dialysis	Iron levels (10-30umol/l)	Vitamin C level (20-40umol/l)	Haemoglobin
1	15y5m	PD	12.9umol/l	15.1umol/l	13.2mmol/l
2	12y2m	PD	11.8umol/l	25.8umol/l	5.3mmol/l
3	16y5m	PD	9.9umol/l	21umol/l	8.1mmol/l
4	14y1m	PD	16.8umol/l	21.4umol/l	12.4mmol/l
5	12y 6m	HD	19.7umol/l	21.7umol/l	7.8mmol/l
6	17y1m	PD	10.7umol/l	13.2umol/l	6.5mmol/l
7	16y7m	HD	11.2umol/l	26.6umol/l	11.3mmol/l
8	19y7m	HD	10umol/l	26.4umol/l	5.7mmol/l
9	17y11m	HD	13umol/l	23.4umol/l	7.9mmol/l
10	14y11m	PD	12umol/l	17umol/l	8mmol/l
11	8y8m	PD	21.3umol/l	29.4umol/l	6.2mmol/l
12	13y5m	PD	9.8umol/l	12umol/l	10.5mmol/l
13	15y8m	PD	11.8umol/l	12.3umol/l	12.1mmol/l
14	11y2m	PD	21.1umol/l	32umol/l	9.1mmol/l
15	2y1m	PD	27.2umol/l	45.2umol/l	9.9mmol/l

16	15y8m	HD	28.9umol/l	19.2umol/l	10.8mmol/l
17	13y1m	HD	12.2umol/l	13.3umol/l	7.9mmol/l
18	5y6m	HD	35.7umol/l	38.9umol/l	11.4mmol/l
19	14y4m	HD	32.7umol/l	6.3umol/l	9.4mmol/l
20	10y11m	HD	2.3umol/l	9.5umol/l	6.7mmol/l
21	3y1m	ND	18.5umol/l	21.1umol/l	11.8mmol/l
22	13y2m	PD	14.2umol/l	29.1umol/l	7.3mmol/l
23	12y4m	PD	15.6umol/l	22.6umol/l	7.6mmol/l
24	13y11m	PD	19.7umol/l	33.9umol/l	7.7mmol/l
25	15y	ND	19.9umol/l	28.3umol/l	10.3mmol/l
26	7y5m	PD	11.7umol/l	27.1umol/l	10.7mmol/l
27	12y3m	PD	18.6umol/l	25.8umol/l	8.9mmo/l
28	13y7m	PD	6.6umol/l	22.6umol/l	10.7mmol/l
29	14y9m	ND	12.1umol/l	15.8umol/l	12.0mmol/l
30	12y3m	ND	4.7umol/l	4umol/l	10.3mmol/l
31	15y4m	ND	3.8umol/l	14.1umol/l	10.3mmol/l
32	17y1m	PD	5.4umol/l	5.7umol/l	11.3mmol/l
33	18y7m	HD	18.6umol/l	24.2umol/l	11.4mmol/l
34	13y8m	HD	13.6umol/l	17.4umol/l	6.1mmol/l
35	8y5m	ND	10.5umol/l	21.2umol/l	11.1mmol/l
36	9y8m	PD	14.3umol/l	20.3umol/l	16.3mmol/l
37	6y6m	PD	17umol/l	39.6umol/l	13.4mmol/l
38	13y5m	ND	21.4umol/l	71.6umol/l	8.7mmol/l
39	14y9m	ND	17.6umol/l	Not analyzed	14.4mmol/l

40	10y4m	ND	5.2umol/l	13.1umol/l	10.6mmol/l
41	9y1m	HD	5.3umol/l	30.1umol/l	6.6mmol/l
42	15y11	ND	16.1umol/l	7.5umol/l	14.6mmol/l
43	15y3m	PD	14.9umol/l	17.8umol/l	9.5mmol/l
44	11y4m	PD	18.7umol/l	15.1umol/l	7.6mmol/l
45	17y5m	ND	10umol/l	Not analyzed	12.7mmol/l

HD: Group of Patients on Haemodialysis

PD: Group of Patients on Peritoneal Dialysis

ND: Group of Patients not yet dialysed

3.2 Iron toxicity and deficiency

	Deficient	Toxic
Peritoneal Dialysis	4 (22) (18.2%) Range: 5.4 - 9.9umol/l Mean: 7.93umol/l	0 (22) (0%)
Haemodialysis	2 (12) (16.7%) Range: 2.3 – 5.3umol/l Mean: 3.8umol/l	2 (12) (16.7%) Range: 32.7 – 35.7 Mean: 34.2umol/l
Non-dialysis	3 (11) (27.3%) Range: 3.8 – 5.2umol/l Mean: 4.57umol/l	0 (11) (0%)
Total	*9 (45) (20%)* *Range: 2.3 -9.9umol/l* *Mean: 5.89umol/l*	*2 (45) (4.4%)* *Range: 32.7 – 35.7umol/l* *Mean: 34.2umol/l*

The levels that were considered normal for serum iron were 10-30 umol/l

3.3 Transferrin Saturation

	Decreased	Increased
Peritoneal Dialysis	5 (22) (22.7%) Range: 11-19% Mean: 16.4%	1 (22) (4.5%) 54 %
Haemodialysis	2 (12) (16.7%) Range: 8 – 19% Mean: 13.5%	6 (12) (50%) Range: 53 - 95 % Mean: 71.67%
Non-dialysis	5 (11) (27.3%) Range: 7 – 19% Mean: 14.4%	1 (11) (9%) 61%
Total	*12 (45) (26.6%)* *Range: 7 – 19%* *Mean: 15%*	*8 (45) (17.7%)* *Range: 53-95%* *Mean: 68.1%*

The normal range for Transferrin saturation was 20-50%

3.4 Ferritin Levels

	Decreased	Increased
Peritoneal Dialysis	0 (22) (0.0%)	8 (22) (36.4%) Range: 449 – 1708ng/ml Mean: 838.86ng/ml
Haemodialysis	0 (12) (0.0%)	8 (12) (66.67%) Range: 614 – 3076ng/ml Mean: 1484.1ng/ml
Non-dialysis	1(11) (9%) 29ng/ml	2 (11) (18.1%) Range: 509 – 590ng/ml Mean: 549.5ng/ml
Total	*1 (45) (2.2%)* *29ng/ml*	*18 (45) (40%)* *Range: 449 – 3076ng/ml* *Mean: 1093.5ng/ml*

The normal range for ferritin was 30-400ng/ml

3.5 Vitamin C Levels

	Deficient	Toxic
Peritoneal Dialysis	8 (22) (22.7%) Range: 5.7 – 17.8umol Mean: 13.53umol	1 (22) (4.5%) 45.2umol
Haemodialysis	5 (12) (41.7%) Range: 6.3 – 19.2umol Mean: 13.14umol	0 (12) (0%)
Non-dialysis	5 (9) (55.6%) Range: 4 – 15.8umol Mean: 10.9umol	1(9) (11.1%) 71.6umol
Total	*18 (43) (41.8%)* *Range: 4 – 19.2umol* *Mean: 12.69umol*	*2 (43) (4.6%)* *Range: 45.2 – 71.6umol* *Mean: 58.4umol*

The normal range for Vitamin C levels was 20-40umol/WCC (44).

When looking at the results of the iron studies, more specifically iron levels, ferritin and transferrin saturation results, it is apparent that the non-dialysis group had the highest percentage of patients with biochemical iron deficiency. However, when analysing the statistics for iron levels using the Fishers exact method it was clear that there was no statistical significance between the three groups (p value 0.605).

When the Fishers exact test was performed for the transferrin saturation group the p-value was 0.064 which was marginally significant. A p-value of less the 0.05 would have been considered truly significant. There were two patients who biochemically demonstrated iron toxicity; both these patients were on haemo-dialysis (4.4%). When analyzing the transferrin saturation results, 7 patients were considered to be iron toxic, again the haemodialysis group presenting with the highest number of patients. One of the factors contributing to this finding could have been the time of sampling of the bloods in relation to the timing of the intravenous iron which was unrecorded.

Studying the results showed that there are a number of patients who remain significantly anaemic despite adequate iron levels; this was not one of the aims or outcomes of the study but was merely an observation. Further investigations would be needed to determine the cause of the anaemia.

Analysis of the Vitamin C levels showed that a total of 41.8 % of patients (18 candidates out of a total sample size of 43) assessed in this study were Vitamin C (ascorbic acid) deficient. The highest percentage of patients who were deficient were again noted to be in the non-dialysis group. There was a relatively small sample size in this group which may have accounted for this (only 9 patients). Analysing the statistics using the Fishers exact test showed no statistical significance between the three groups (p-value 0.435). Only two patients displayed Vitamin C toxicity, one in the peritoneal group and one in the non-dialysis group.

CHAPTER 4

DISCUSSION

Patients with chronic kidney disease have many complications; either as a result of the disease itself or as a result of the treatment which we initiate. One of the most common abnormalities found in children suffering from chronic kidney disease is anaemia secondary to iron deficiency. Iron toxicity is not common but does occur.

It is well established that erythropoietin supplementation is essential in the failing kidney. With this is the well documented fact that iron is required for erythropoietin to work effectively. The dose of erythropoietin can also be significantly modified if iron deficiency is prevented.

In this study we showed that a significant percentage of children with chronic kidney disease (on haemodialysis, peritoneal dialysis or not yet dialysed but with established renal failure) are iron deficient. All patients in this study were on iron supplementation when there haemoglobin levels dropped to within the anaemic range. All patients on peritoneal dialysis or not yet dialysed who were anaemic were started on an oral iron formulation; all patients on haemodialysis were receiving intravenous iron.

We need to explain the fact that between 20 and 26.6 percent of children in renal failure remained iron deficient despite iron supplementation. The best explanation for this is two-fold; non-compliance on treatment or inadequate dosing. The side-effects of oral iron include nausea, constipation, abdominal discomfort and tooth discolouration with the liquid forms. Uraemia also diminishes the intestinal absorption of iron even with severely diminished iron stores (45). This would not offer an explanation for the group on haemodialysis who are receiving intravenous iron while on the dialysis machine. We have also not taken into account functional iron deficiency. This is characterized by the presence of adequate iron stores (normal ferritin levels), as defined by the K/DOQI guidelines, but an inability to sufficiently mobilise these stores when erythropoeisis is stimulated by an erythropoeitic agent.

Iron toxicity was also demonstrated in 4.4 % of the patients. The two patients who demonstrated iron toxicity when analysing iron levels were both on haemodialysis. When looking at TSAT results, a higher percentage were toxic (15.5%); with two of the patients falling into this group on haemodialysis having extremely high ferritin levels (3076 and 1863).

A raised ferritin is also associated with chronic inflammation and therefore is not specific for iron metabolism (26); however, in view of a raised TSAT and iron level the raised ferritin results were probably significant in these two patients. A group of patients in this study were noted to have raised ferritin levels despite normal or low iron and transferrin saturation levels; it is in this group that other causes of raised ferritin should be considered i.e. inflammation, infection or neoplasm (26). Infection with raised C - reactive protein levels was found to be the cause in certain individuals.

The analysis of the Vitamin C results revealed some interesting findings. Results indicated that the group displaying the highest percentage of Vitamin C deficiency was in fact the non dialysis group (55.6%). We did not specifically look at the overall nutrition of the patients enrolled in the study; particularly protein energy malnutrition.

Poor nutrition may have in part explained the nutritional deficiencies evident in this particular group of patients. All patients enrolled in the study had their weight and height measured on the day the bloods were sampled. Results indicate that 61 % of the girls had weight values which fell on or below the third centile (11 out of 18), while 74 % of the enrolled boys had weight on or below the third centile (20 out of 27). When evaluating heights 76 % (13 out of 17) girls had measurements that fell on or below the third centile when comparing age equivalents. When reviewing the male group 88.8 % of boys enrolled in the study had lengths recorded on or below the 3rd centile when comparing with other boys their age. It is clear from these findings that although no formal assessment of nutrition was analyzed on this group of patients, there is significant evidence to support malnutrition and poor growth. All patients in chronic renal failure are advised to be on a 'renal diet', with various restrictions. Diet was not explored and considered in this study but clearly this would affect the Vitamin C and iron results.

There was no documented history of when patients received blood transfusions or had a confirmed infection (peritonitis; line sepsis) which would obviously affect their haemoglobin as well as their iron levels. No patients in the study were on Vitamin C supplements; however, the ingestion of over the counter and other prescribed medications were not considered. Certain types of medications ingested may have influenced results; these include Proton Pump Inhibitors, ACE inhibitors and antacids.

Also, analysis of albumin levels in relation to Vitamin C levels would be interesting in particular in patients on peritoneal dialysis where this association has previously been documented (42). The differences in the levels of iron and Vitamin C were not explored in the different ethnic groups due to the small sample size. In future this area could be examined.

CHAPTER 5

CONCLUSION

It was clear from this observational study that a significant number of patients with chronic renal insufficiency are Vitamin C and iron deficient. Intravenous iron supplementation needs to be considered in the patients on peritoneal dialysis or in the group not yet dialysed who remain iron deficient despite oral iron supplementation. A further study could be the generated from this initial study; Vitamin C could be administered in therapeutic doses to children with chronic renal insufficiency while closely monitoring the levels. Analysis of iron and haemoglobin should be repeated after Vitamin C has been administered to see whether there are any significant changes in these values. The volume of fluid exchanges and the percentage dextrose concentration used in patients on Peritoneal Dialysis may affect Vitamin C levels and this information should be documented in follow up studies. Also, if another study were undertaken, careful documentation of nutritional status, diet and albumin levels may reveal further useful information.

APPENDICES

White Cell Vitamin C Assay

Modified from Marchand and Pelletier, International Journal Vitamin Nutrition 1977; 47; pp. 236-247

CMC Solution: 0.9 grams NaCl

1 gram Methyl Cellulose

2 ml Glycerol

Make up to 100ml with distilled water

DNPH Solution: 2.2 % 2:4 dinitro-phenyl hydrazine in 10N H_2SO_4

DNPH Reagent Mixture: 20 volumes DNPH Solution

1 volume 5 % Thiourea

1 volume 0.6 % $CuSO_4.5H_2O$

Method:

Take 6 millilitres of EDTA blood which has been well mixed.

Add 0.6mls 1% CMC solution, mix well and allow to settle.

Take off 2 ml of plasma and place in a 15 ml conical plastic tube. Fill up with normal saline to 12ml mark. Mix Well. Take off 0.5ml for a white cell count.

Spin the rest for 10 minutes at approximately 2000rpm. Decant the supernatant.

Add 1.3ml 5% trichloracetic acid which breaks down the protein. Make sure that the pellet is well in suspension (use an orange stick if necessary). Spin for 10 minutes at 2600rpm.

Take off 1ml supernatant and add 0.3ml of the DNPH reagent mixture to it.

Incubate for 4 hours at 37 degrees Celsius.

Place on ice and add 1.5mls 65% H_2SO_4. Allow to stand for 30 minutes on ice.

Make a blank solution consisting of 1ml of 5% trichloracetic acid and 0.3mls of the DNPH reagent mixture.

Read at 520 nm, using blank as zero.

Standard Curve

Dilute 10g ascorbic acid in 1 litre of 5 % trichloracetic acid (TCA). This is equivalent to 10mg/ml.

Dilute 1ml of the above solution in 100ml of 5 % trichloracetic acid (100ug/ml)

Make dilutions of this in 5% TCA as follows:

100ul in 1.0ml 5% TCA = 10ug/ml

200ul in 2.5ml 5% TCA = 8ug/ml

100ul in 2.0ml 5% TCA = 5ug/ml

100ul in 5.0ml 5% TCA = 2ug/ml

100ul in 10.0ml 5% TCA = 1ug/ml

100ul in 20.0ml 5% TCA = 0.5ug/ml

100ul in 50.0ml 5% TCA = 0.2ug/ml

3. Incubate duplicate 1ml aliquots of each of the above solutions with 0.3ml of the DNPH reagent mixture for 4 hours as for the samples

4. Read at 520nm using a blank to zero as before. Plot the OD against the concentration on normal graph paper.

Calculation

Calculate the concentration of the sample from the standard curve.

Concentration from standard curve (ug/ml) x 1.3 x 100 = ug Vitamin C x 10 leucocytes

White cell count x 11.5

Normal Range: 20 – 40 ug/10 eight leucocytes

CONSENT FOR PARTICIPATION IN IRON AND VITAMIN C STUDY

Dear Parent

My name is Tracey Lutz. I am a paediatric registrar working in research in the renal unit (296) at Johannesburg Hospital, under the supervision of Dr Hahn.

Your child has chronic renal failure and is attending our renal clinic. One of the complications of chronic renal failure is anaemia and because of this your child is on erythropoietin (eprex) and iron supplementation (ferrous fumarate or venofer).

As part of my research I would like to analyze iron studies on all the patients with chronic renal failure. At the same time I would like to take an extra specimen of clotted blood (5mls / one teaspoon measure) to measure vitamin C levels; I would like to invite you to give permission for your child to volunteer to take part in the study. This will be done while your child's routine monthly bloods are being taken, thereby not causing any unnecessary discomfort for your child. Vitamin C is one of the water-soluble vitamins in our bodies that improves iron absorption. It has also been proven to improve the anaemia associated with renal failure when given with iron and erythropoeitin.

The aim of my study is to see whether we are adequately supplementing iron in all our patients. By looking at vitamin C levels we will determine whether our patients in chronic renal failure have subclinical (no signs to see on the patient) deficiency of this vitamin.

At the time of the study we would review your child's file and would record some demographic details and information regarding the history of your child's illness. We will keep all information and results confidential. At no point in time will your child's information and blood results be made public knowledge.

Taking part in the study is voluntary and if you refuse your child's care will not be affected in any way. You can withdraw from the study at any time. Feel free to contact me if you would like to ask more questions.

Thanking you

Tracey Lutz
Contact no. 488-3296 or LR bleep 22628

I ________________________ have read and understood the above information and agree that my child _______________________ may participate in the study.

Signed ____________________________ Date _________________________

Witness ____________________________ Place _________________________

PATIENT CONSENT FOR PARTICIPATION IN IRON AND VITAMIN C STUDY

(Subjects over the age of 14 years)

Dear Patient

My name is Tracey Lutz. I am a doctor working with children in the renal unit at Johannesburg Hospital, under the supervision of Dr Hahn.

You have chronic renal failure and are on certain medications as a result of that. Two of the medications which you are taking are iron and erythropoietin (eprex). These treatments help prevent anaemia which can result in you feeling tired or dizzy.

As part of my research I would like to analyze iron studies which are done on you as part of your routine monthly blood tests. I would also like to take an extra 5mls (one teaspoon measure) of clotted blood to look at vitamin C levels; I would like to invite you to volunteer to take part in the study. Vitamin C may be low in your blood which may worsen your anaemia. I would take this blood at the same time as your routine bloods to avoid any additional discomfort (pain) to you.

At the time of the study we would review your file and would record some details about you (e.g. your age, your gender etc.) and information regarding the history of your illness. We will keep all information and results confidential (secret), so that no person can see this information.

Taking part in the study is voluntary (you can choose whether you want to or not) and if you don't want to take part your medical care will not be compromised. You can withdraw from the study at any time. Feel free to contact me if you would like to ask more questions.

Thanking you

Tracey Lutz
Contact no. 488-3296 or LR bleep 22628

I ________________________ have read and understood the above information and agree to take part in the study.

Signed __________________________ Date ______________________________

Witness__________________________
Place______________________________

DATA COLLECTION SHEET

Analysis of iron studies and vitamin C levels in paediatric patients with chronic renal failure.

DEMOGRAPHIC DETAILS

STUDY CODE NUMBER: ______________________________

AGE/DATE OF BIRTH: ________________________

SEX: ________________________

HOSPITAL NUMBER:_______________________

RACE: Black White Coloured Asian

HISTORY

AETIOLOGY OF RENAL FAILURE:________________________________

BIOPSY YES NO
If Yes results:

DIALYSIS PERITONEAL HAEMODIALYSIS NONE

DURATION OF DIALYSIS (years and months): ____________________________________

EXAMINATION

WEIGHT (KG): _________________ HEIGHT (CM):________________________

BLOOD PRESSURE:____________________________

GLOMERULAR FILTRATION RATE (SCHWARTZ FORMULA):______________________

INVESTIGATIONS

WCC	
HAEMOGLOBIN	

MEAN CELL VOLUME	
HAEMATOCRIT	
IRON LEVELS	
FERRITIN	
TRANSFERRIN	
PERCENTAGE SATURATION (TSAT)	
C-REACTIVE PROTEIN	
VITAMIN C LEVELS	

CURRENT TREATMENT

ERYTHROPOEITIN

TYPE AND DOSE	
ROUTE OF ADMINISTRATION	

IRON

DOSE	
ORAL OR INTRAVENOUS	

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Lutz

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M050807**
PROJECT	Analysis and Interpretation of Iron Studies and Vitamin C Levels in Paediatric Patients with Chronic Renal Failure
INVESTIG ATORS	Dr Lutz
DEPARTMENT	Department of Paediatrics
DATE CONSIDERED	05.08.26
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 05.09.23 **CHAIRPERSON** ....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr D Hahn

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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