CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1 Introduction

Chronic kidney disease (CKD) is considered a rapidly growing global health problem, characterized by progressive destruction of renal mass with irreversible sclerosis and loss of nephrons. In case of end stage renal disease and/or chronic renal failure, kidney transplantation is the treatment of choice for patients through a surgical procedure to take over the task of purifying the blood and remove the waste material (Wasti *et al*., 2013).

Hematological disturbances such as anemia is considered a frequent complication that occurs in chronic kidney disease. The severity of anemia is directly proportional to the degree of renal function. One of the important aspects of management is the correction of anemia and maintain of Hb level by using erythropoietin stimulating agent (ESA) (Aronoff and Kalantar, 2009).

Erthropoietin was the first human hematopoietic growth factor to be identified, this glycoprotein hormone stimulates erythropoiesis and can cross the placental barrier between the mother and the fetus (Turgeon *et al*., 2012).

There are factors which enhance the chance of suffering renal failure like diabetis mellitus, hypertention and family history. ABO blood group was found to be associated with many disease like peptic ulcer and gastric cancer. So this study will investigate the possiple association with ABO blood group and chronic renal failure (Massimo and gluseppe, 2015).

1.2. Rationale

Chronic renal failure is considered a health problem in Sudan and might cause many other complications including anemia. So the study is to evaluate the complete blood count (CBC) in patients with chronic renal failure under haemodialysis.

The ABO blood group was reported to be associated with many diseases, so this work will investigate the possible association of ABO blood group with chronic renal failure.

1. 3. Objectives

1.3.1. General Objective

To evaluate the haematological parameters changes and investigate the relationship of ABO blood Group with chronic renal failure.

1.3.2. Specific Objectives

a) To measure hemoglobin, RBCs, PCV, MCV, MCH, MCHC, platelets and white blood cells count in chronc renal failure patients and control.

b) To determine the frequency of ABO phenotypes among patients with chronic renal failure.

c) To study distribution of the patients according to gender and age.

d) To determine duration and frequency of dialysis.

e) To investigate the medication taken by the patient.

CHAPTER TWO
LITERATURE REVIEW

2.1. General aspects of haemopoiesis

Haemopoiesis is the process of blood cell formation under control of the haemopoietic growth factor. In the first few weeks of gestation the yolk sac is the main site of haemopoiesis , then after six week until seven month of fetal life the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about two weeks after birth. The bone marrow is the most important site from six to seven months and continue to adult life. In infancy all the bone marrow is haemopoietic but during childhood there is progressive fatty replacement of marrow througout the long bones so that in adult life haemopoietic marrow is confined to the central skeleton proximal ends of the femurs and humeral (Hoffbrand and Moss, 2011).

2.1.1. Regulation of haemopoiesis

Haemopoiesis starts with stem cell division in which one cell replaces the stem cell (self renewal) and the other is committed to differentiation. The cell lineage which is selected for differentiation depends on the external signals received by progenitor cell. This signals include the growth factors which are glycoprotein hormones that regulate the proliferation and differentiation of haemopoietic progenitor cells and the function of mature cells. The major source of most growth factors are the stromal cells (Hoffbrand and Moss, 2011).

2.1.1.2. Erythropoiesis

Erythropoiesis is the process of red blood cells production, starts from the stem cell through the progenitor cells colony-forming unit granulocyte, erythroid, monocyte and megakariocyte (CFU-GEMM), burst forming–unite erythroid (BFU-E) and colony forming unite erythroid (CFU-E) to the first recognizable erythrocyte precursor in the bone marrow (pronormoblast) which give normoblasts by number of cell divisions. Here the synthesis of haemoglobin takes place (65%), the nucleus is finally extruded from the late normoblast within the marrow and a reticulocyte stage results which still contain some ribosomal RNA and is still able to synthesizes haemoglobin. A reticulocyte slightly larger than mature erythrocyte,spends 1-2 days in the bone marrow and also circulates in the peripheral blood for 1-2 days before maturing, when the RNA is completely lost.A single pronormoblast usually gives rise to 16 mature red cells, nucleated red cells are not present in normal human peripheral blood only they appear when exstramedallery erythropoiesis occurs or with some marrow disease. The normal rang of red blood cell count $4.5\text{-}6.5\times10^{6}$/ µL (Men) and $3.9\text{-}5.6\times10^{6}$/ µL (women) (Hoffbrand and Moss, 2011).

2.1.1.3. Leucopoiesis

The white blood cells (leucocytes) may be divided into granulocyte and a granulocyte. Granulocyte which include three types of cells; neutrophils (polymorphs), eosinophils and basophils. The a granulocytes are monocytes which are phagocytic, and the immunocytes include the lymphocytes (Hoffbrand and Moss, 2011).

The blood granulocyte and monocyte are formed in the bone marrow from common precursor cell in the granulopoietic series progenitor cells, myeloblast, promyelocyte, and myelocyte from mitotic pool while the metamyelocyte, band

and segmented graunlocytes make up a post-mitotic maturation compartment. In the blood- stream there are two pools (circulating pools and marginating pools) usually of about equal size. Monocytes circulating for 20-40 hours then transformed in the tissue to macrophages with lifespan several month or even years. Many growth factors are involved in this maturation process including interleukin (IL1,3,5,6,11), granulocyte macrophage-colony stimulating factor (GM-CSF), granulocyte-CSF(G-CSF) and monocyte-CSF (M-CSF) and tumor necrosis factor (TNF). The maturation of granulocytes is completed by appearance of secondary granules (specific) and lobulation of nucleus. Lymphocyte are the immunologically competent cells that assist the phagocytes in the defense of the body against infection and other body invasion which include T and B lymphocytes and natural killer (NK) cells. In postnatal life the bone marrow and thymus are the primary lymphoid organs in which lymphocytes develop. During maturation of lymphocytes antigen-receptor gene arrangements take place to fillfull their function (Hoffbrand and Moss, 2011).

Table (1) Normal range of WBCs and differential count: (Hoffbrand and Moss, 2011)

Type of cell	Blood count
Total leucocyte	4.00-11.00×10^3 /µL
Neutrophils	2.5-7.5×10^3 /µL
Eosinophils	0.04-0.4× 10^3/µL
Monocytes	0.2-0.8×10^3 /µL
Basophils	0.01-0.1× 10^3 /µL
Lymphocytes	1.5-3.5× 10^3/µL

2.1.1.4. Thrombopiesis

Platelets are produced in the bone marrow by fragmentation of the megakaryocytes. The precursor of the megakaryocyte –megakaryoblast- arises by the process of differentiation from the haemopoietic stem cell with normal count (150.000.-400.000×10^3/µL) and life span about 7-10 days. Platelets have specific structure, the glycoproteins of the surface coat are particulary important in the platelets reaction of adhesion and aggregation during haemostasis. Thrombopoietin is the major regulator of platelets production and is conistitutively produced by the liver and kidney.

Thrombopoietin increases the number and rate of maturation of megakaryocytes via specfic receptor (Hoffbrand and Moss, 2011).

2.2. The kidney

Are a pair of bean-shaped organs located at the bottom of the rib cage in the right and left sides of the back. Each kidney contains about one million function units called nephrons . A nephron consists of a filtering unit of tiny

blood vessels called a glomerulus, attached to a tubule. When blood enters the glomerulus, it is filtered and the remaining fluid passes along the tubule. In the tubule chemicals and water are either added to or removed from this filtered fluid according to the body`s needs, with the final product being the urine excrete (Mitchell *et al.*, 2007).

2.2.1. Kidney function

The kidneys principle role is elimination of waste materials and the regulation of the volume and composition of body fluid; also the kidney is the main source of erythropoietin, renin, prostaglandins, and is essential for vitamin D formation, and small molecular weight protein. Although the body is equipped with two kidneys, one reasonably healthy kidney can do the function, if the other is damaged or removed. The kidneys receive blood from the aorta, filter it and send it back to the heart with the right balance of chemicals and fluid for use throughout the body. The urine created by the kidneys is moved out of the body via the urinary tract (Mitchell *et al.*, 2007).

2.2.1.1. Erythropoietin

Is a heavily glycosylated polypeptide of 165 amino acids with a molecular weight of 34 KDa. Normally, 90% of the hormone is produced in the peritubular interstitial cells of the kidney and 10% in the liver and elsewhere. There are no performed stores and stimulus to erythropoietin is the oxygen tension in the tissues of the kidney. Hypoxia induces hypoxia-inducible factors which stimulate erythropoietin production.

Erythropoietin production therefore increases in anemia, when haemoglobin for some metabolic or structural reason is unable to give up oxygen normally.

Erythropoietin stimulates erythropoiesis by increasing the number of progenitor cells committed to erythropoiesis.

Plasma erythropoietin levels can be valuable in clinical diagnosis.They are high if a tumour secreting erythropoietin is causing polycythaemia but low in severe renal disease or polycythemia vera (Hoffbrand and moss, 2011).

2.2.2. Chronic renal failure

It is irreversible loss of the excretory capacity of the kidney which occurs over an extended period of time for months to year. It is characterized by, the kidneys inability to excrete waste products, maintain fluid and electrolyte balance and produce hormones, leads to end stage renal disease, which is a complete failure of the kidney, it usually occurs when despite treatment, chronic renal failure progress to the point where the kidneys can no longer sustain life. Chronic renal failure refer to a progressive an irreversible loss of renal function (Abbound and Hennch, 2010).

The recent kidney disease out comes quality initative (KDOQI) guidelines have classification CRF into five stages using the term GFR as:

Stage 1 Normal or increased GFR but some evidence of kidney damage reflected by microalbuminuria, proteinuria, haematouria or histological changes. Stage 2 Kidney damage with mild decrease in GFR. Stage 3 Moderate decrease in GFR. Stage 4 Severe decrease in GFR. Stage 5 when renal replacement therapy in the form of dialysis or transplantation (Abbound and Hennch, 2010).

2.2.2.1. End Stage Renal Disease

The point at which patients loss of kidney function need dialysis or kidney transplant. Patients at higher risk for CKD include patients with diabetes, hypertension, or a family history of hypertension**,** diabetes, or CKD (Peter., *et al*.,2007**).** ESRD causing significantly higher cardiovascular morbidity and mortality, compared with the general population, this because of the uremic cardiovascular factors. The result is that there will be a growing number of ESRD patients being manage with the intensive care unit (ICU) setting (Sara, 2008).

2.3. Basic facts about blood groups and their importance in clinical medicine

Human red blood cells contain on their surface a series of glycoprotein and glycolipids which constitute the blood group antigens. The development of these antigens is genetically controlled; they appear early in fetal life and remain unchanged until death (Daniels, 2002).

The discovery of ABO blood group, over 100 years ago remain of prime importance in transfusion medicine they are the most immunogenic of all the blood group antigens. The most common cause of death from a blood transfusion is a clerical error in which an incompatible type of ABO blood is transfused (Daniels, 2002).

The ABO system consists of four main groups, A, B, AB and O, which are determined by the presence or absence on the red cell of two antigens A and B. The antigens are under the control of three allelic genes, A,B and O, situated on the long arm of chromosome 9. Group A red cells possess the A antigen,

group B cells possess the B antigen, group AB cells possess A and B antigens , and group O cells possess neither A nor B. The serum of an individual contains antibodies against the antigens lacking in the person's red cells. Thus, as a group A person lacks the B antigen, the serum contains anti-B agglutinins. Similarly, a group B person lacks the A antigen and the serum contains anti-A, while the serum of a group O person, who lacks both A and B antigens, contains anti-A and anti-B and a group AB persons have neither antibody in their serum (Hosoi, 2008).

Table (2) The antigens (Ag), antibodies (Ab) and genotypes of ABO blood group system (Laura, 2005)

Genotypes	Ab present on blood cells	Ag present on blood cells	Blood group
AA or AO	Anti-B	A antigen	A
BB or BO	Anti-A	B antigen	B
AB	None	AB antigen	AB
OO	Anti-A & Anti-B	None	O

The Rhesus (Rh) blood groups system was first described 60 years ago.It is seconed only to ABO in importance in blood transfution. The principle antigen is D, and the terms Rh positive and Rh negative refer to the presence or absence of D antigen (Neil *et al*., 2000).

2.4. Previous studies

The study was conducted in Saudi Arabia in (2012), to investigate the changes in various hematological parameters that occur in renal failure patients before and after HD compared to the control group, showed that, patients with renal disease on regular haemodialysis display various degrees of changes in haematological

parameters. The RBCs count, Hb and PCV levels in healthy subjects, while the mean of MCV, MCHC level were not affected (Alghythan and Alsaeed, 2012).

A group of researcher in Pakistan performed observational study, which was focused on the double comparison of hematological disturbances in the patients suffering from chronic kidney disease (CKD) & in kidney transplant (KT) patients, they found that, changes in the hematological profile , was low RBCs count, Hb, PCV, MCH, MCHC and increased WBCs. While PLTs and MCV were normal (Afshan *et al*., 2013).

A Study conducted in india, was performed to observe the hematological changes like RBC count, Hb concentration, hematocrit, platelet count and TLC in patients suffering from chronic renal failure. The researcher were reported that, significant reduction in RBCs, Hb conc, PCV and PLTs count and in significant reduction in total leucocyte count. They attributed this to impaired production of EPO and other factors like increased haemolysis suppression of bone marrow erythropoiesis, hematuria and gastrointestinal blood loss (Suresh *et al*., 2013).

A study in Iraq, aimed to determine the relationship between hemodialysis patients and ABO blood grouping, revealed that (55, 25, 10, 10%) of the HD patients were belonging to (O, B, A and AB) respectively (Hasson *et al*., 2013).

CHAPTER THREE
MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a case control study carried out in chronic renal failure patients, in Omdurman Military Hospital.

3.1.2. Study duration

The study was conducted in the period from March to June 2015.

3.1.3. Study population

Hundred adults patients (males and females) with chronic renal failure and under-going haemodyalysis were enrolled in this study, and thirty healthy subjects were enrolled as a control group.

3.2. Inclusion criteria

Participants of this study include chronic renal failure patients undergoing haemodyalysis.

3.3. Exclusion Criteria

Patients on peritoneal dialysis (PD) and continuous ambulatory dialysis (CAPD). Chronic renal failure patients with other disease which affected the result.

3.4. Collection of samples

Blood samples of 2,5 ml were collected from the venous blood through clean venepuncture from each patient via the antecubital vein, using sterile disposable plastic syringes and were dispensed into commercially prepared concentration of Ethylene Di-amine Tetra-acetate (EDTA) bottles. Each sample was mixed gently and thoroughly to prevent cell lysis and ensure anticoagulation. Complete blood count (CBC) was done within 2 hours of collection.

3.5. Sysmex KX-21N (Japan)

The sysmex KX-21N is an automatic multi-parameter blood cell counter for in vitro diagnosis used in clinical laboratory. It processes approximately 60 samples an hour and display on LCD screen the particle distribution carve of WBCs and platelets count, along the data of parameters, as analysis result.

3.5.1. Principle of Sysmexautoanalyzer

The KX-21N employs three detector blockes and two kinds of reagents for blood analysis. WBC count was measured by WBC detector block using detection method. RBC and platelets counts were taken from RBC detector block, also using the DC detection method. The hemoglobin detector block measures hemoglobin concentration using the Non-cyanide hemoglobin method.

A blood sample was aspirated, measured to a predetermined volume, diluted at the specific ratio, then fed into each transducer. The transducer chamber has a minute hold called the aperature. One both side of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell size is plottedby determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data. For hemoglobin determination the Non-cyanide hemoglobin method was used. Blood hemoglobin was converted rapidly to oxyhemoglobin. The later absorbance was measured in the Hb flow cell at 555 nm wavelength against diluents and the concentration of Hb was calculated.

Sysmex calculated RBC indices (mean corpuscular volume MCV, mean corpuscular Hb MCH, mean corpuscular Hb concenteration MCHC) from red cell count (RBC), hemoglobin concenteration (Hb) and backed cell volume (PCV).

3.6. Blood grouping

3.6.1. Methodology of Blood grouping

Agglutination of red blood cells with a specific anti-sera indicates the presence of the corresponding Ag on the RBC and is interpreted as a positive test. Absence of agglutination indicates the corresponding Ag is not present.

3.6.1.1. Equipment

Glass test tube size 12x75 mm, test tube rack, Pasture pipettes, Centrifuge and Cotton.

3.6.1.2. Reagent

Isotonic buffered saline with a pH of 6.9, Monoclonal anti-A, anti-B, anti-AB.

3.6.1.3. Procedures

1-Four glass tubes were labeled A, B, AB and D.

2- In each glass tube one drop of specific anti-sera reagent was dispensed according to tubes lable then one drop of 2% cell suspension were added to each glass tube.

3- The tubes were mixed gently and incubated at room temperature for five minutes then the tubes were centrifuged at 1000 rpm for 15 seconds.

4- The cells were resuspended gently agitation and then examined macroscopically for agglutination.

5-The result were immediately recorded in the appropriate spaces on the work sheet.

3.7. Data collection

Basic information of the participant were collected through a questionnaire.

3.8. Ethical consideration

The study was approved by the medical ethical committee of Medical Laboratories Collage- Sudan University of Science and Technology. A written consent was obtained from the participants after they had been informed with the objective, benefits and expected outcome of the study. The participants were insured that the collected information will be kept confidential and will not be used for any other purpose than this study.

3.9. Data analysis

Collected data were analyzed using the application of Statistical Package for Social Sciences (SPSS).

CHAPTER FOUR

RESULTS

4.1. Characteristic of haemodialysis patients

Table (3) Shows the distribution of the study group according to gender. The majority were males (63%). The majority of the participants were in the age group (40-59year) (46%). (49%) of the participants were on hemodialysis for more than three years. Frequency of hemodialysis was twice per week in (93%). All the patients have been on treatment with EPO, iron therapy, folic acid and vitamine B_{12} .

4.2. Mean of haematological parameters in chronic renal failure patients under HD and the control group

Chronic renal failure patients exhibited significant ($p<0.05$) decrease in the following parameters compared with the control group: Hb, RBC,PCV, and Platelets count . CRF patients showed significantly higher MCV value than the control subject. While the leucocyte count, MCH and MCHC did not vary between the chronic renal failure patients and the control group **Table (4).**

4.3. Mean of haematological parameters in chronic renal failure patients under HD according to gender

Table (5) Shows a statistically significant decrease ($P<0.05$) in MCV in the males compared with the females (92.2 ± 6.1 vs 94.6 ± 5.4 fl). However, all the other measured indices did not vary significantly with the gender.

4.4. Distribution of ABO blood group among the patients and the control group was it in the following order

Table (6) The results of case group was O(37%)>A (31%)>B (28 %) >AB (4 %). While in the control group was O (60%) > B (23.4%) > A (13.3%) > AB (3.3), which showed insignificant difference ($P > 0.05$).

Table 3.Characteristic of heamodiaylysis patients

Characteristic		Percent %
Sex	Male	63%
	Female	37%
Age group	20-39 years	35%
	40-59 years	46%
	>60 years	29%
Duration of being on dialysis	1 years	22%
	3years	29%
	>3 years	49%
Frequency of dialysis per week	Twice	93%
	Three	7%
Treatment with EPO ,iron therapy, folic acid and vitB_{12}		100%

Table 4.Mean hematological parameters of chronic renal failure patients under HD and the control group

Hematological Parameter	Sample	No	Mean ± SD	P-value
Hb g/dL	Case	100	10.2 ± 2.1	* 0.00
	Control	30	12.7± 1.7	
RBC $\times 10^6/\mu L$	Case	100	3.5 ± 0.8	* 0.00
	Control	30	4.5± 0.6	
PCV %	Case	100	33.5 ± 8.8	* 0.00
	Control	30	39.6 ± 5.5	
MCV fL	Case	100	93.1 ± 6.0	* 0.01
	Control	30	90.0 ± 5.4	
MCH pg	Case	100	28.9 ± 2.2	0.06
	Control	30	28.1± 1.9	
MCHC g/dL	Case	100	31.1± 1.2	0.64
	Control	30	31.2 ± 1.2	
PLT $\times 10^3/\mu L$	Case	100	197.3 ± 88.1	* 0.00
	Control	30	270.2 ± 63.6	
WBC $\times 10^3/\mu L$	Case	100	5.1 ± 1.7	0.43
	Control	30	5.4 ± 1.6	

(*) Significance level at $P \leq 0.05$

Table 5. Mean of hematological parameters in chronic renal failure patients under HD according to gender

Hematological Parameters	Sex	No	Mean ± SD	P-value
Hb g/dl	Male	63	10.4 ± 2.1	0.25
	Female	37	9.9 ± 2.1	
RBC $\times 10^6/\mu L$	Male	63	3.6 ± 0.7	0.23
	Female	37	3.4 ± 0.8	
PCV %	Male	63	34.3. ± 9.7	0.23
	Female	37	32.2 ± 6.1	
MCV fL	Male	63	92.2 ± 6.1	*0.05
	Female	37	94.6 ± 5.4	
MCH pg	Male	63	28.8 ± 2.2	0.58
	Female	37	29.**1** ± 2.0	
MCHC g/dL	Male	63	31.2 ± 1.3	0.07
	Female	37	30.8 ± 1.1	
PLT $\times 10^3/\mu L$	Male	63	196.4 ± 81.5	0.56
	Female	37	188.2 ± 57.2	
WBC $\times 10^3/\mu L$	Male	63	5.4 ±1.8	0.97
	Female	37	5.1 ± 1.6	

(*) Significance level at $P \leq 0.05$

Table 6. Distribution of ABO blood group among the patients and the control group it was in the following order

		Samples		P-value
		Case	Control	
Blood Group	A	31 (31%)	4 (13.3%)	0.321
	B	28 (28%)	7 (23.4%)	0.321
	AB	4 (4%)	1 (3.3%)	0.123
	O	37 (37%)	18 (60%)	0.345
Total		100 (100%)	30 (100%)	

(*) Significance level at $P \leq 0.05$

CHAPTER FIVE

DISCUSSION

The Hb, RBCs count, PCV, MCV values and platelets count of chronic kidney disease patients were significantly lower when compared with the values in healthy subjects. Similar results were reported by Alghythan *et al., (*2012),that is Hb,RBCs count, HCT levels in chronic kidney failure patients were significantly lower on comparison with the control subject.

This study showed that MCV level was significantly increased in chronic renal failure patients, similar results were reported by Afshan *et al., (*2013). MCH and MCHC in the present study did not any changes was observed,which is on line with the finding of Afshan *et al.,(*2013),who did not find any variation in MCH, MCHC values between the control and chronic renal failure patients.

Total leucocyte count is decreased numerically in chronic renal failure patients, which agree with the results of Suresh *et al.,* (2012), who observed insignificant decrease in WBCs count with chronic renal failure.

The study found that platelets counts level is decreased in chronic renal failure, which is on line with the results reported by Suresh *et al.,* (2012), that the platelets count is significantly decreased in CRF patients.

The results according to the distribution of ABO blood group in chronic kidney failure patients showed that O blood group was more frequent (37%), followed by A blood group was (31%), B blood group (28%) and AB blood group was (4%),this is consistent with the result reported by Hasson *et al.,* (2013) that O

blood group of the highest frequency(55%). There is no relationship between chronic renal failure and ABO blood group.

CONCLUSION

Males are more affected with chronic renal failure than females.

Hb, RBCs count, PCV, MCV and PLTs counts were significantly affected by CRF, mean values of WBCs count, MCH and MCHC were not affected. Chronic renal failure patients have lower haematological indices in spite of all the offered medication that is EPO, iron therapy, folic acid and vit B^{12}.

No correlation was found between CRF and ABO blood group.

RECOMMENDATION

1. Further studies should be done to determine the type and severity of anemia among chronic renal failure patients.

2. Good follow up for taking medications.

3. It is necessary to determine the blood group for patients with renal failure for use in blood transfusion.

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بسم الله الرحمن الرحيم

QUESTIONNAIRE

Sudan University of Sciences and Technology

Collage of Graduate Studies

Evaluation of haematological parameters changes and relationship of ABO blood group with Chronic Renal Failure patients under haemodialysis attending Omdurman Military Hospital

Demographic data:

Name:……

Sex: female ( )……..male ( )……..

Age:……..

Duration of being on dialysis:

one:…….( ) three:……( ) more than…….( )

Time of dialysis per week: Twice:……( ) three…….( )

Medication:

Iron supplement:……..( ) Folic acid……( )

$VitB_{12}$:……( ) Erythropoietin:……..( )

Blood group: A……..( ) B……..( )

AB……( )O……..( )