

# **Chapter one**

## **Introduction and Literature Review**

### **1.1 Introduction :**

Since the discovery of blood groups in 1900, there have been efforts to discover a possible association between ABO and Rh blood groups and different diseases. The data obtained from studies on patients with gastric cancer, salivary gland tumors, duodenal ulcer, colorectal cancer, thyroid disorders, ovarian tumors, small cell carcinoma of lung and coronary heart disease have shown association with ABO blood groups (Waseem *et al.*, 2012) .

This information has led to the assumption that some other diseases might also be associated with ABO and Rh blood group .

Renal Failure occurs when the Kidneys partly or completely lose their ability to carry out normal functions . This is dangerous because water, waste and toxic substance build up that normally are removed from the body by the kidneys .It also causes other problems such as anemia , high blood pressure , acidosis , disorders of cholesterol , fatty acids and bone disease in the body by impairing hormone production by the Kidneys (Kathuria,2008)

According to previous informations above this work was conducted to determine distribution of ABO blood groups and Rhesus factor in patients with Chronic Renal Failure under hemodialysis in Khartoum state . The result of this study may add interpretation about that disease with unknown causes in which type of blood group may be the causative agent.

## **1.2 Literature Review :**

### **1.2.1Blood constituents:-**

Blood is a vital intravascular fluid circulates throughout heart and blood vessels, and classified as connective tissue. Blood is composed of two portions (can be observed after separation by centrifuge), solid portion constitutes (45%), consists of white blood cells, red blood cell and platelets. Fluid portion of plasma which constitutes about (55%) .(Hoffbrand,1981)

### **1.2.1Blood Group System**

A blood group could be defined as, ‘an inherited character of the red cell surface, detected by a specific alloantibody’. Do blood groups have to be present on red cells? This is the usual meaning, though platelet- and neutrophil-specific antigens might also be called blood groups. Blood group antigens may be:

- Proteins .
- Glycoproteins, with the antibody recognizing primarily the polypeptide backbone.
- Glycoproteins, with the antibody recognizing the carbohydrate moiety.
- Glycolipids, with the antibody recognizing the carbohydrate portion.

( Daniels and Bromilow, 2007)

### **1.2.2ABO Blood Group System**

#### **1.2.2.1Background:**

Karl Landsteiner discovered the ABO blood group system in 1900, which incited the beginning of modern blood banking and transfusion medicine. Landsteiner Performed a series of experiments demonstrating serological incompatibilities between individuals. In 1901, using his blood and the blood of his colleagues, he mixed the serum of some individuals with other people’s cells.

Inadvertently, he was the first person to perform forward and reverse grouping. This series of experiments led him to discover three of the four ABO groups: A, B, and O. Shortly after Landsteiner's initial discovery, his associates, Alfred von Decastello and Adriano Sturli, discovered the fourth blood group, AB. In later studies, Landsteiner correlated the presence of the ABO antigens on red cells and the reciprocal agglutinating antibodies in the serum of the same individual (e.g. A antigens on red blood cells and anti-B in the serum). This discovery was labeled Landsteiner's Law or Landsteiner's Rule. This rule is the basis for all transfusion therapy as well as a guideline for determining the compatibility of donor and recipients. ABO grouping is one of the primary tests performed in the blood bank. (Whitlock,2010)

Felix Bernstein discovered the group inheritance pattern of multiple alleles at one locus in 1924. This discovery explained the inheritance of ABO blood groups. Additionally, it was established that an individual inherits one ABO gene from each parent. These genes produce the antigens present on the surface of an individual's red cells. Like Landsteiner's discoveries, Bernstein's determination of inheritance patterns of the ABO group has played a major role in the knowledge base for all blood group systems. (Whitlock,2010)

In 1930, O. Thompson postulated a four-allele system of inheritance. This proposed system was based on the discovery of Emil Frieher von Dungern and Ludwig Hirtzfeld in 1911 that the group A antigen can be divided into two subgroups, A1 and A2. Thompson expanded this premise and proposed the four allelic genes: A1, A2, B, and O. His expansion of Landsteiner's original findings enhanced the ability to provide safe blood for transfusion. (Whitlock,2010)

#### **1.2.2.2 Biochemical nature of ABO antigens :**

A and B antigens are oligosaccharides. The most abundant structures on red cells carrying ABO activity are the *N*-linked oligosaccharides of red cell surface

glycoproteins, predominantly the red cell anion exchanger (AE1, the Diego blood group antigen, or band 3) and the glucose transporter (GLUT1), although some other glycoproteins are also involved. ABO-active oligosaccharides are also present on glycolipids. Oligosaccharides are chains of monosaccharide sugars: D-glucose (Glc); D-galactose (Gal); D-mannose (Man); *N*-acetyl-D-glucosamine (GlcNAc); *N*-acetyl-D-galactosamine (GalNAc); L-fucose (Fuc). An oligosaccharide is A-active when the terminal monosaccharide is GalNAc, in  $\alpha 1 \rightarrow 3$  linkage to a Gal residue that also has Fuc in  $\alpha 1 \rightarrow 2$  linkage, whereas an oligosaccharide is B-active when the terminal monosaccharide is Gal, in  $\alpha 1 \rightarrow 3$  linkage to the  $\alpha$  1,2-fucosylated Gal residue. GalNAc and Gal are the immunodominant sugars of A and B antigens, respectively. Group O red cells lack both GalNAc and Gal from the  $\alpha 1,2$ -fucosylated Gal residue, so express neither A nor B. The A and B trisaccharides may be attached to several different core oligosaccharide chains, but in red cells the fucosylated Gal residue is usually in  $\alpha 1 \rightarrow 4$  linkage to GlcNAc. This is called a type 2 core structure. Less abundant core structures, called type 3 and type 4, are only present on glycolipids and may also be involved A and B activity. Type 3 and type 4 structures express A antigen on A1 phenotype red cells, but not on A2 cells, which may account for the qualitative differences between A1 and A2. (Daniels and Bromilow, 2007)

### **1.2.2.3 Biosynthesis of ABO antigens and ABO molecular genetics :**

Oligosaccharides are built up by the stepwise addition of monosaccharides. The addition of each monosaccharide requires a specific transferase, an enzyme that catalyses the transfer of the monosaccharide from its donor substrate, a nucleotide molecule carrying the relevant monosaccharide, to its acceptor substrate, the non-reducing end of the growing oligosaccharide chain. A-transferase, the product of the A allele, is a GalNAc transferase, which catalyses the transfer of GalNAc from UDP-GalNAc (donor) to the fucosylated Gal residue (acceptor). B-transferase,

the product of the *B* allele, is a Gal-transferase, which catalyses the transfer of Gal from UDP-Gal to the fucosylated Gal residue of the acceptor. The *O* allele produces no active enzyme, and so the fucosylated Gal residue remains unsubstituted (and expresses H antigen). The genetic basis for oligosaccharide blood groups is fundamentally different from that of the protein blood groups. Protein antigens are encoded directly by the blood group genes, but the genes governing carbohydrate polymorphism encode the transferase enzymes that catalyse the biosynthesis of the blood group antigens. *A* and *B* alleles of the *ABO* gene. (Daniels and Bromilow, 2007)

#### **1.2.2.4 ABO Antigens :**

Agglutination tests are used to detect A and B antigens on red cells. Reagent antibodies frequently produce weaker reactions with red cells from newborns than with red cells from adults. Although A and B antigens can be detected on the red cells of 5- to 6-week-old embryos, A and B antigens are not fully developed at birth, presumably because the branching carbohydrate structures develop gradually. By 2 to 4 years of age, A and B antigen expression is fully developed and remains fairly constant throughout life. (Brecher, 2005)

#### **1.2.2.5 ABO Subgroups :**

ABO subgroups are phenotypes that differ in the amount of antigen carried on red cells and, for secretors, soluble antigen present in the saliva. Subgroups of A are more commonly encountered than subgroups of B.

The two principal subgroups of A are  $A_1$  and  $A_2$ . Red cells from  $A_1$  and  $A_2$  persons both react strongly with reagent anti-A in direct agglutination tests. The serologic distinction between  $A_1$  and  $A_2$  cells can be determined by testing with anti- $A_1$  lectin. There is both a qualitative and quantitative difference between  $A_1$  and  $A_2$ . The  $A_1$ -transferase is more efficient at converting H substance into A

antigen and is capable of making the repetitive Type 3A structures. There are about  $10.5 \times 10^5$  A antigen sites on adult  $A_1$  red cells, and about  $2.21 \times 10^5$  A antigen sites on adult  $A_2$  red cells. Approximately 80% of group A or group AB individuals have red cells that are agglutinated by anti- $A_1$  and thus are classified as  $A_1$  or  $A_1B$ . The remaining 20%, whose red cells are strongly agglutinated by anti A but not by anti- $A_1$ , are called  $A_2$  or  $A_2B$ . Routine testing with anti- $A_1$  is unnecessary for donors or recipients. Subgroups weaker than  $A_2$  occur infrequently and, in general, are characterized by decreasing numbers of A antigen sites on the red cells and a reciprocal increase in H antigen activity. Subgroups are most often recognized when there is a discrepancy between the red cell (forward) and serum (reverse) grouping. (Brecher, 2005)

Generally, classification of weak A subgroups ( $A_3$ ,  $A_x$ ,  $A_m$ ,  $A_{el}$ ) is based on the:

1. Degree of red cell agglutination by anti-A and anti- $A_1$ .
2. Degree of red cell agglutination by human and some monoclonal anti-A,B.
3. Degree of red cell agglutination by anti-H (*Ulex europaeus*).
4. Presence or absence of anti- $A_1$  in the serum.
5. Presence of A and H substances in the saliva of secretors.
6. Adsorption/elution studies.
7. Family (pedigree) studies.

The serologic classification of A (and B) subgroups was developed using human polyclonal anti-A, anti-B, and anti-A,B reagents. These reagents have been replaced by murine monoclonal reagents, and the reactivity is dependent upon which clone(s) is selected by the manufacturer. There are, however, some characteristics that should be noted.  $A_3$  red cells give a characteristic mixed-field pattern when tested with anti-A from group B or O donors.  $A_x$  red cells are characteristically not agglutinated by human anti-A from group B persons but are

agglutinated by anti-A,B from group O persons. A<sub>x</sub> red cells may react with some monoclonal anti-A reagents, depending on which monoclonal antibody is selected for the reagent. A<sub>el</sub> red cells are not agglutinated by anti-A or anti-A,B of any origin, and the presence of A antigen is demonstrable only by adsorption and elution studies. Subgroups of B are even less common than subgroups of A. Molecular studies have confirmed that A and B subgroups are heterogeneous, and the serologic classification does not consistently correlate with genomic analysis; multiple alleles yield the same weakened phenotype, and, in some instances, more than one phenotype has the same allele . (Brecher, 2005)

#### **1.2.2.6 ABO Antibodies :**

Antibodies directed against ABO antigens are the most important antibodies in transfusion medicine. This is a profound, but true statement. For this reason, ABO antibodies require detailed description.

The ABO blood group presents a unique situation in Immunohematology. It is the only example of a blood group where each individual produces antibodies to antigens not present on the red cells. These ABO antibodies were originally thought to be natural antibodies formed with no apparent antigenic stimulus.

Since the antibodies are not stimulated by exposure to red cells, they may also be considered non-red cell stimulated antibodies. However, some form of an antigenic stimulus must exist. The proposed mechanism is environmental. These “naturally occurring” substances resemble A and B antigens and stimulate the production of complementary antibodies to the antigens that are not present on the red cell surface. (Whitlock, 2010)

Newborns have no ABO antibodies. When newborns are tested, only a forward group is performed. Newborns may exhibit passive ABO antibodies that have crossed the placental barrier. Reverse grouping of a newborn or umbilical cord serum indicates the blood group of the mother. The child will begin antibody

production, and have a detectable titer, at three to six months of age. ABO antibody production peaks at age five to ten years of age and continues in immunocompetent individuals throughout life. (Whitlock, 2010)

#### **1.2.2.7 Clinical Significance of ABO Antibodies :**

ABO antibodies are capable of causing both Hemolytic Disease of the Fetus and Newborn (HDFN) and Hemolytic Transfusion Reactions (HTR). These issues explain the clinical significance of “naturally occurring” antibodies. HDFN usually presents itself with a maternal antibody of an IgG isotype that corresponds to an antigen on the surface of the baby’s red cells. The most common scenario is a group O mother and a group A baby. ABO hemolytic disease may affect a woman’s first pregnancy. This is in contrast to Rh HDFN where the antigenic stimulation usually occurs in the first pregnancy and subsequent antigen-positive newborns are affected. Hemolytic transfusion reaction occurs when a recipient is transfused with red cells that are an ABO group incompatible with the antibodies in his or her serum. Because of the complement-binding ability of the ABO antibodies, this is always a life-threatening situation. As the recipient antibodies react with the incompatible red cells, complement is activated and in vivo hemolysis, agglutination, and red blood cell destruction occurs. ABO compatibility is also significant in solid organ transplantation. For most organs, an ideal scenario for transplant is an ABO compatible solid organ. Post-transfusion antibody titer, and pheresis to reduce the titer of the incompatible antibody, will assist in achieving a positive outcome when an ABO incompatible organ is transplanted. (Whitlock,2010)



**Table 1.1: ABO antigens, antibodies, and genotypes.\***

<b>ABO group</b>	<b>Antigens on red cells</b>	<b>Antibodies in serum</b>	<b><i>Genotype</i></b>
O	None	Anti-A,B	<i>O\O</i>
A	A	Anti-B	<i>A/A or A/O</i>
B	B	Anti-A	<i>B/B or B/O</i>
AB	A and B	None	<i>A/B</i>

\*( Daniels and Bromilow, 2007)

## **1.2.3 Rh Blood Group System**

### **1.2.3.1 Background:**

The Rh blood group system was discovered in New York in 1939, with an antibody in the serum of a woman who had given birth to a stillborn baby and then suffered a haemolytic reaction as the result of transfusion with blood from her husband. Levine and Stetson found that the antibody agglutinated the red cells of her husband and those of 80% of ABO compatible blood donors. Regrettably, Levine and Stetson did not name the antibody. In 1940, Landsteiner and Wiener prepare antibodies by injecting rhesus monkey red cells into rabbits. These antibodies not only agglutinated rhesus monkey red cells, but also red cells from 85% of white New York Population and appeared to be the same as Levine and Stetson's antibody other human antibodies identified later. By 1962, however, it was clear that rabbit and guinea pig anti-rhesus reacted with a determinant that was genetically independent of that determined by the human antibodies, despite being serologically related. In consequence, the anti-Rhesus antibodies were renamed anti-LW, after Landsteiner and Wiener, and the human antibodies remained as anti-D of the Rh (not rhesus) blood group system. LW is expressed more strongly on D-than D- red cells, explaining the original error because weak antisera often fail to agglutinate D- red cells. As early as 1943 Rh started to become complex. From their work with four other antisera, anti-C, -c, -E, and -e, detecting two pairs of antithetical antigens, Fisher and Race postulated three closely linked loci producing D/d, C/c, and E/e. Anti-d has never been found and does not exist. Wiener, in New York, worked with antibodies of the same specificities, but came up with a different genetical theory involving only one gene locus. In 1986, Tippett provided another alternative theory: two loci; one producing D or no D, the other

producing C/c and E/e. Shortly after, Tippett's theory was validated by molecular genetic studies.( Daniels and Bromilow, 2007)

### **1.2.3.2 Biochemical Composition of Rh Antigens**

As with the ABO system, Rh antigens are located on the surface of red blood cells. In contrast to the ABO system, the major Rh antigens are found exclusively on red cells and not on tissue cells or in body fluids in soluble form. The biochemical nature of RhD and RhCE antigens is protein. Protein relies on lipids in the red cell membrane for physical support. Each of the antigens is constructed of 416 amino acids. The string of amino acids loops through the red cell membrane and displays short loops on the exterior .The active amino acids vary with an individual's genetic coding. Rh antigens are integral to the red cell membrane. This theory is supported by the fact that cells without any Rh antigens, Rh null, present an altered physical appearance and decreased red cell survival. Glycoproteins that are associated with the biochemical structure of the Rh system have been identified. These glycoproteins are not related to antigenic properties of any blood group system but rather are associated with the red cell membrane. These glycoproteins play a role in association of the RhD and RhCE with the red cell membrane. The glycoprotein associated with the red cell membrane is Rh Ag. Mutation or absence of these glycoproteins results in lack of expression of any Rh antigens (Rh null). There have been comparable glycoproteins identified in the brain, the liver, the kidney, and the skin. These glycoproteins have been labeled RhBG and RhCG. They have not been associated with any specific blood group antigens but research indicates involvement with ammonia transport. (Whitlock,2010)

### **1.2.3.3 Genetics of the Rh Blood Group System :**

The genes for the Rh system reside on Chromosome 1. The genetic composition of the Rh system includes two genes (*RhD* and *RhCE*) located in close proximity. These genes encode for the proteins RhD and RhCE. The RhD protein carries the D antigen while the latter carries C and E antigens. C and E can present in various combinations (e.g. CE, ce, Ce, cE). There is no antithetical component for the RhD antigen. Therefore, a “d” does not exist. If the D antigen is not present, there is a total absence or deletion in this location. This corresponds to the Rh negative or D negative phenotype. (Whitlock,2010)

The lack of any antigenic material is the result of absence of the RhD gene. The RhD and RhCE genes each have ten exons, are 97% identical, and most likely arose from gene duplication. RhD and RhCE differ by 32 to 35 of their 416 amino acid composition. The difference in antithetical antigens (e.g. C and c are antithetical) results from a difference of fewer amino acids than the comparison of antigens from alternate blood groups. This fact also explains the large degree of foreignness when the RhD antigen is introduced into an RhD negative individual. The highly antigenic nature of the RhD antigen is in contrast to other antigen systems. (Whitlock,201

### **1.2.3.4 D Antigen :**

The D antigen is the primary antigen in the Rh system. When present on red cells, the individual is designated as “Rh positive.” An individual may inherit one D gene from each parent. The inheritance of either one or two D genes will designate that person as “Rh positive.” The incidence of Rh positive individuals is 85% in the Caucasian population and 92% in the African-American population. (Whitlock,2010)

Conversely when no D gene is inherited from either parent, the individual is designated as “Rh negative.” Rh negative individuals comprise about 15% in the Caucasian population and 8% in the African-American population. The D antigen is very antigenic. More than 80% of Rh negative (D negative) individuals transfused with Rh positive blood will develop an anti-D on initial exposure. Rh positive individuals may be transfused with either Rh positive or Rh negative blood. Rh negative individuals, however, should always be transfused with Rh negative blood unless the situation is life threatening and only Rh positive blood is available. Exclusive administration of Rh negative blood is crucial for women of child-bearing age. Rh negative women who developed anti-D are likely to develop Hemolytic Disease of the Fetus and Newborn (HDFN) if an Rh positive infant is born to an Rh negative mother. The amount of D antigen present on the red cells varies with an individual’s genotype. (Whitlock,2010)

#### **1.2.3.5 Weakened Expression of D antigen :**

Testing for the presence of the D antigen on the red cell surface is performed using specific antisera. Detection of weak D antigens has been enhanced by the development of monoclonal antisera. However, when monoclonal antisera is present with a negative result in anti-D, it may be necessary to perform further testing using the indirect antiglobulin test (IAT). The weakened D antigen is produced through multiple genetic mechanisms. Most of these mechanisms are genetic point mutations. Any detection of D antigen, whether in initial testing or with IAT enhancement, classifies a donor unit as Rh positive. On the other hand, recipients (in particular, women of child bearing age) who type with a weakened D antigen may be classified as Rh negative for purposes of transfusion. (Whitlock, 2010)

#### **1.2.3.6 CcEe Antigens :**

Rh system contains numerous additional antigens. The most significant ones are the two pairs of alleles: Cc and Ee. Additional antigens may be present at this locus. They are less frequently encountered, but may produce typical antibodies when transfused into antigen-negative individuals. Some of these are less common antigens. (Whitlock,2010)

#### **1.2.3.7 Rh null :**

Rh null red cells carry no Rh system antigens. This is very rare, and no D, C, c, E, or e antigen is detectable when typing the RBCs. The cells also lack Rh29, often called “total Rh.” Two genetic pathways can lead to an Rhnull phenotype: Rh-negative person (lacking RhD) who also has an inactive RHCE gene, referred to as an Rhnull amorph, or, more often, inheritance of inactive RHAG gene, referred to as an Rh null regulator. RhAG protein is required for expression and trafficking of RhCE and RhD to the RBC membrane; so, although the Rh blood group proteins are made in the Rh null regulator individuals, they cannot reach the membrane due to mutation in RhAG. Rh null individuals who have been transfused or who are pregnant may form, among other Rh system antibodies, anti-Rh29. The serum of the people who form these antibodies agglutinates cells from all people except another Rhnull. Owing to the paucity of Rh null blood, it is recommended that people who have this rare blood type donate autologous blood and have it frozen for transfusion needs. (Daniels,2002)

#### **1.2.3.8 Rh Antibodies :**

Most Rh antibodies result from exposure to human red cells through pregnancy or transfusion. Occasionally, Rh antibodies (eg, anti-E, anti-Cw) are naturally-occurring. D is the most potent immunogen, followed by c and E. Although a few examples of Rh antibodies behave as saline agglutinins, most react best in high-

protein, antiglobulin, or enzyme test systems. Even sera containing potent saline-reactive anti-D are usually reactive at higher dilutions in antiglobulin testing. Some workers find enzyme techniques especially useful for detecting weak or developing Rh antibodies. (Brecher, 2005)

Antibody usually persists for many years. If serum antibody levels fall below detectable thresholds, subsequent exposure to the antigen characteristically produces a rapid secondary immune response. With exceedingly rare exceptions, Rh antibodies do not bind complement, at least to the extent recognizable by techniques currently used. As a result, primarily extravascular hemolysis, instead of intravascular hemolysis, occurs in transfusion reactions involving Rh antibodies. (Brecher, 2005)

#### **1.2.3.9 Clinical significance of anti-D :**

Anti-D is clinically the most important red cell antibody in transfusion medicine after anti-A and -B. It has the potential to cause severe HTRs and D+ blood must never be given to a patient with anti-D. As at least 30% of D-recipients of transfused D + red cells make anti-D, D-positive red cells are not routinely transfused to D-patients. ( Daniels and Bromilow, 2007)

Anti-D can cause severe HDFN. This occurs when IgG anti-D in an immunised mother crosses the placenta and facilitates destruction of D+ fetal red cells.

The effects of HDFN caused by anti-D at its most severe are fetal death at about the 17th week of pregnancy. If the infant is born alive, the disease can result in hydrops and jaundice. If the jaundice leads to kernicterus, this usually results in infant death or permanent cerebral damage. In most cases of HDFN, the mother was immunised to produce anti-D by fetal D+ red cells during a previous pregnancy. These D+ red cells leak into the maternal circulation via a transplacental haemorrhage, which generally occurs during delivery, but sometimes happens earlier in the pregnancy. Anti-D immunisation can be

prevented, in most cases, by administration of a dose of anti-D immunoglobulin to the D-mother immediately after delivery of a D+ baby. It is still not absolutely clear how the anti-D immunoglobulin prevents immunisation, but it is probably due to rapid removal of the D+ fetal cells from the maternal circulation. In 1970, at the very beginning of the anti-D prophylaxis programme there were 1.2 deaths per thousand births in England and Wales due to HDFN caused by anti-D; by 1989 this figure had been reduced to 0.02. In order to prevent the less common occurrence of the mother being immunised during the course of the pregnancy, anti-D immunoglobulin may be administered to D-pregnant women antenatally, and this has become the usual practice in many countries. It is imperative that D+ red cells are never transfused to D-girls or premenopausal women. If a D-young woman is given D+ blood products, then anti-D immunoglobulin should be given. Anti-D in women with a D variant antigen can cause severe HDFN in a fetus with a complete D antigen. If a woman known to have a D variant antigen gives birth to a D+ baby, she should be given anti-D immunoglobulin. ( Daniels and Bromilow, 2007)



### **1.2.4 The kidneys :-**

Play a central role in the homeostatic mechanisms of the human body. Reduced renal function strongly correlates with increasing morbidity and mortality. (Burtis,2008)

#### **1.2.4.1 Kidney Anatomy :-**

The kidneys are paired, bean-shaped organs located retroperitoneally on either side of the spinal column. Macroscopically, a fibrous capsule of connective tissue encloses each kidney. When dissected longitudinally, two regions can be clearly discerned : an outer region called the cortex and an inner region called the medulla. The pelvis can also be seen. It is a basin like cavity at the upper end of the ureter into which newly formed urine passes. The bilateral ureters are thick-walled canals, connecting the kidneys to the urinary bladder. Urine is temporarily stored in the bladder until voided from the body by way of the urethra. functional units of the kidney that can only be seen microscopically. Each kidney contains approximately 1 million nephrons. Each nephron is a complex apparatus comprised of five basic parts :

- The glomerulus— a capillary tuft surrounded by the expanded end of a renal tubule known as Bowman's capsule. Each glomerulus is supplied by an afferent arteriole carrying the blood in and an efferent arteriole carrying the blood out. The efferent arteriole branches into peritubular capillaries that supply the tubule.
- The proximal convoluted tubule—located in the cortex.
- The long loop of Henle—composed of the thin descending limb, which spans the medulla, and the ascending limb, which is located in both the medulla and the cortex, composed of a region that is thin and then thick.
- The distal convoluted tubule—located in the cortex.

■ The collecting duct—formed by two or more distal convoluted tubules as they pass back down through the cortex and the medulla to collect the urine that drains from each nephron. Collecting ducts eventually merge and empty their contents into the renal pelvis. (Bishop *et al.*, 2010)

#### **1.2.4.2 Renal Function :-**

The main biological functions of the kidneys are :

(1 excretion. (2) homeostatic regulation, and (3) endocrine.

##### **1.2.4.2.1 Excretion :-**

Urine is excreted by the kidneys, passed through the ureters, stored in the bladder, and discharged through the urethra. In health, it is sterile and clear. is of amber color . has a slightly acid pH (5.0 to 6.0); and has a characteristic odor, and specific gravity of about 1.024 g/mL. In addition to dissolved compounds, it contains a number of cellular fragments, complete cells, proteinaceous casts, and crystals (formed elements). Changes in these formed elements are studied using urine microscopy. (Burtis, 2008)

Urination, also termed micturition, is the discharge of urine. In normal adults, adequate homeostasis is maintained with a urine output of about 500 mL/day. Alterations in urinary output are described as anuria (less than 100 mL/day), oliguria (400 mL/day), orpolyuria (>3 L/day or 50 mL/kg body weight/day). The most common disorder of urination is altered frequency, which may be associated with increased urinary volume or with partial urinary tract obstruction (e.g., in prostatic hypertrophy). (Burtis, 2008)

The first step in urine formation is filtration of plasma water at the glomeruli. A net filtration pressure of about 17 mm Hg in the capillary bed of the tuft drives the filtrate through the glomerular membrane. The filtrate is called an ultrafiltrate

because its composition is essentially the same as that of plasma, but with a notable reduction in molecules of molecular weight exceeding 15 kDa. Each nephron produces about 100 pL of ultrafiltrate per day. Overall, approximately 170 to 200 L of ultrafiltrate pass through the glomeruli in 24 hours. In the passage of ultrafiltrate through the tubules, reabsorption of solutes and water in various regions of the tubules reduces the total urine volume, which typically ranges between 0.4 and 2 L/day. Transport of solutes and water occurs both across and between the epithelial cells that line the renal tubules. Transport is both active (energy requiring) and passive, but many of the so-called passive transport processes are dependent upon or secondary to active transport processes, particularly those involving sodium transport. All known transport processes involve receptor or mediator molecules, many of which have now been identified and characterized using molecular biological techniques. The activity of many of these molecules is regulated by phosphorylation facilitated by protein kinase C or A. Their renal distribution has been shown to correlate with the known regional functional activities. There are inherited disorders of specific tubular transporters and a well-known generalized disorder affecting all of the transport processes, causing Fanconi syndrome. Direct coupling of adenosine triphosphate (ATP) hydrolysis is an example of an active transport process. The most important of these in the nephron is Na<sup>+</sup>,K<sup>+</sup>-ATPase, which is located on the basolateral membranes of the tubuloepithelial cells. This enzymatic transporter accounts for much of renal oxygen consumption and drives more than 99% of renal sodium reabsorption. Renal epithelial cell membranes also contain proteins that act as ion channels. For example, there is one for sodium that is closed by amiloride and modulated by hormones such as atrial natriuretic peptide (ANP). Ion channels enable much faster rates of transport than ATPases, but are relatively fewer in number—approximately

100 sodium and chloride channels as against  $10^7$  Na<sup>+</sup>,K<sup>+</sup>-ATPase molecules per cell. (Burtis, 2008)

In the tubules, the solute composition of the ultrafiltrate is altered by the processes of reabsorption and secretion, so that the urine excreted may have a very different composition from that of the original filtrate. Different regions of the tubule have been shown to specialize in certain functions. In the proximal tubule, 60% to 80% of the ultrafiltrate is reabsorbed in an obligatory fashion, along with sodium, chloride, bicarbonate, calcium, phosphate, sulfate, and other ions. Glucose is virtually completely reabsorbed, predominantly in the proximal tubule by a passive but sodium-dependent process that is saturated at a blood glucose concentration of about 10 mmol/L. Uric acid is also reabsorbed in the proximal tubule by a passive sodium-dependent mechanism, but there is also an active secretory mechanism.

In the loops of Henle, chloride and more sodium without water are reabsorbed, generating dilute urine. Water reabsorption in the more distal tubules and collecting ducts is then regulated by Anti Diuretic Hormone (ADH is a non-peptide that binds to specific receptors on the basal membranes of renal collecting duct cells. It increases water permeability in the cortical cells, but increases both water and urea permeability in the medullary tubules). In the distal tubule, secretion is the prominent activity; organic ions, potassium ions, and hydrogen ions are transported from the blood in the efferent arteriole into the tubular fluid. It is also this region that secretes hydrogen ions and reabsorbs sodium and bicarbonate to aid in acid base regulation. Paracellular (between cell) movement is driven predominantly by concentration, osmotic, or electrical gradients. (Burtis, 2008)

#### **1.2.4.2.2 Regulatory Function :-**

The regulatory function of the kidneys has a major role in homeostasis. The mechanisms of differential reabsorption and secretion, located in the tubule of a nephron, are the effectors of regulation. The mechanisms operate under a complex

system of control in which both extrarenal and intrarenal humoral factors participate. (Burtis, 2008)

#### **1.2.4.2.2.1 Electrolyte Homeostasis :-**

The proximal convoluted tubule is predominantly concerned with reabsorption (Here about 75% of the sodium, chloride, and water of the ultrafiltrate is reabsorbed, as is most of the bicarbonate, phosphate, calcium, and potassium. Water reabsorption in the proximal convoluted tubule is termed "obligatory" because its volume is related to the heavy load of solutes being returned to the blood in the efferent arteriole. The amount of bicarbonate reabsorption is related to the glomerular filtration rate (GFR) and the hydrogen ion secretory rate. The amount of phosphate reabsorption is controlled in part by plasma calcium concentration and in part by the effect of parathyroid hormone on the tubular cells. Normally, the high-threshold substances-glucose and, to a great extent, amino acids-are reabsorbed here by means of specific intracellular active transport systems. Uric acid may be either reabsorbed or secreted in the proximal convoluted tubule by a two-way carrier-mediated process. In the ascending loop of Henle, 20% to 25% of filtered sodium is reabsorbed without concomitant reabsorption of water. This process generates dilute urine with an osmolality of 100 to 150 mOsm/kg of water and helps establish the corticomedullary osmotic gradient. The resulting hypertonicity of the interstitium is important in the pathogenesis of renal infections because the hypertonic environment interferes with leukocyte function. Subsequent water reabsorption is regulated by ADH. Although the reabsorption of  $\text{Na}^+$  in the loop of Henle is complex and incompletely understood, at least one mechanism consists of an active  $\text{Cl}^-$  pump with subsequent reabsorption of  $\text{Na}^+$  along an electrochemical gradient. This mechanism is apparently the one inhibited by the powerful loop diuretics. (Burtis, 2008)

The distal tubule is functionally the most active region of the nephron for the homeostatic regulation of plasma electrolytes and plasma acid-base concentrations. Here a combination of secretion and reabsorption takes place among  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{H}^+$ . Although excess plasma hydrogen ions are secreted all along the tubule, it is in the distal tubule that exchange of  $\text{H}^+$  for  $\text{Na}^+$  (which is reabsorbed) fine tunes the balance between  $\text{H}^+$  loss and retention. Potassium ions are also secreted in the distal tubule. Aldosterone is a potent modulator of  $\text{Na}^+$  reabsorption in the distal tubule, particularly when the need arises to conserve  $\text{Na}^+$ . Production of aldosterone in the adrenal cortex is stimulated by the renin-angiotensin system and by high plasma potassium concentration. Renal secretion of renin is complex, but is at least partly regulated by renal perfusion and plasma sodium concentration. Both inadequate perfusion and a low concentration of plasma sodium stimulate renin secretion. Organic anions, such as acetoacetate and Phydroxybutyrate, also consume  $\text{H}^+$  as they are eliminated in part in their non dissociated acid form. When  $\text{H}^+$  must be conserved to maintain plasma pH, distal tubule cells reduce the secretion of  $\text{H}^+$ , reduce  $\text{NH}_4^+$  generation, reduce  $\text{Na}^+$ - $\text{H}^+$  exchange, and increase bicarbonate excretion. The net effect is a reduction in plasma bicarbonate and restoration of normal plasma pH. (Burtis, 2008)

#### **1.2.4.2.2.2 Water Homeostasis :-**

Approximately 70% of the water content of the tubular fluid is reabsorbed in the proximal tubule, 5% in the loop of Henle, 10% in the distal tubule, and the remainder in the collecting ducts. Plasma membranes of all mammalian cells are water permeable but to variable degrees. Water homeostasis is intrinsically linked to renal urea processes. For example, urea transporter provides a very low affinity but high-capacity passive transport process linked to  $\text{Na}^+$  reabsorption in the proximal tubule. The importance of urea to water reabsorption is that the cortical

collecting ducts are impermeable to urea, as are the medullary collecting ducts, unless acted upon by ADH.. (Burtis, 2008)

### **1.2.3.2.3 Endocrine Function:-**

The endocrine functions of the kidneys may be regarded either as primary, because the kidneys are endocrine organs producing hormones, or secondary, because the kidneys are a site of action for hormones produced or activated elsewhere. In addition, the kidneys are a site of degradation for hormones such as insulin and aldosterone. In their primary endocrine function, the kidneys produce erythropoietin (EPO), prostaglandins and thromboxanes, renin, and 1,25 (OH<sub>2</sub>) vitamin D<sub>3</sub>. (Burtis,2008)

### **1.2.4.3 Renal dysfunction:**

#### **1.2.4.3.1 Glomerular Disease:**

Disorder that directly damage the renal glomeruli.

##### **1.2.4.3.1.1 Acute glomerulonephritis:-**

It is large inflamed glomeruli with diseased capillary lumen often related to recent infection by group a beta-hemolytic streptococcus. It is theorized that circulating immune complex trigger a strong inflammatory response in the glomerular basement membrane, resulting a direct injury to the glomerulus it self. Other causes include drug induced glomerulonephritis, Acute kidney infections other than bacteria (like viral infection), other systemic immune complex diseases, such as systemic lupus erythematosus( SLE), sub acute bacterial endocarditis SBE for unknown reasons.(Fishbach,2000)

##### **1.2.4.3.1.2 Chronic glomerulonephritis:-**

Lengthy glomerular inflammations, resulting from renal disease or from an idiopathic cause, lead to glomerular scarring and eventually loss of operational

nephrons. This process is often undetected for lengthy periods, because only minor decreases in renal functions occur at first. (Fishbach,2000)

#### **1.2.4.3.1.3 Nephritic syndrome:-**

Abnormally increased permeability of glomerular basement membrane. Primary causes are associated with glomerular disease state. Secondary causes are associated with infections (SBE or syphilis), renal vein thrombosis, generalized disease states (carcinoma, SLE, or amyloidosis), or transplant rejection. This defect yields such abnormal findings as massive proteinuria (more than 3.5 g/day and even up to 20 g/day), resultant hypoalbuminemia, and subsequent decreased plasma oncotic pressure, generalized edema, hyperlipidemia & lipiduria. Also, Disorders of several coagulation factors are observed. (Fishbach,2000)

#### **1.2.4.3.2 Tubular diseases:**

Tubular defect occurs in the progression of all renal disease as glomerular filtration rate falls (GFR). However, this aspect of the overall dysfunction becomes predominant. The result is decreased excretion/reabsorption of substances or reduced urinary concentrating capability. The most important defect is the primary tubular disorders affecting acid – base balance (renal tubular Acidosis (RTA)). Acute inflammation of the tubules and surrounding interstitium also may occur as result of analgesic drugs or radiation toxicity. (Fishbach,2000)

#### **1.2.4.3.3 Urinary tract abnormalitis :**

##### **1.2.4.3.3.1Infection:-**

By bacterial, viral, fungal organisms. The site of infection may be either in kidney themselves (pyelonephritis), or in urinary bladder (cystitis). In general, a microbiologic colony count of local bacteriuria, hematuria & puria. In particular, the presence of WBCs casts in the urine is considered diagnostic for pyelonephritis. (Fishbach,2000)



#### **1.2.4.3.3.2 Obstruction:-**

Either raise the intratubular pressure until nephrons necrosis and chronic renal failure ensues, or predispose urinary tract to repeated infections. Obstruction located in either the proximal or distal urinary tract. Blockages in the upper tract lead to constricting lesion below distal collecting duct. Obstructions of the lower tract lead to presence of residual urine in bladder after cessation of micturation. A cause of obstructions is quite varied. They can include neoplasia as such as prostate/bladder carcinoma or lymph node tumors constricting ureters. (Fishbach,2000)

#### **1.2.4.3.4 Renal calculi:-**

Commonly termed as renal stones, formed by combination of variety of crystallized substances (calcium oxalate, magnesium ammonium phosphate, calcium phosphate, uric acid & cystein). Calcium oxalate stones are most common in tropics and subtropics areas. (Fishbach,2000)

#### **1.2.4.3.5 Renal Failure**

##### **1.2.4.3.5.1 Acute Renal Failure :-**

Acute renal failure is a sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys, defined as occurring when the GFR is reduced to less than 10 mL/minute. This syndrome is subdivided into three types, depending on the location of the precipitating defect.

Prerenal failure: The defect lies in the blood supply before it reaches the kidney. Causes can include cardiovascular system failure and consequent hypovolemia.

Primary renal failure: The defect involves the kidney. The most common cause is acute tubular necrosis; other causes include vascular obstructions/inflammations

Postrenal failure: The defect lies in the urinary tract after it exits the kidney.

Generally, acute renal failure occurs as a consequence of lower urinary tract obstruction or rupture of the urinary bladder. (Bishop *etal.*, 2010)

Toxic insults to the kidney that are severe enough to initiate acute renal failure include hemolytic transfusion reactions, myoglobinuria due to rhabdomyolysis, heavy metal/solvent poisonings, antifreeze ingestion, and analgesic and aminoglycoside toxicities. These conditions directly damage the renal tubules. Hypoxic insults include conditions that severely compromise renal blood flow, such as septic/hemorrhagic shock, burns, and cardiac failure. The most commonly observed symptoms of acute renal failure are oliguria and anuria (400mL/day). The diminished ability to excrete electrolytes and water results in a significant increase in extracellular fluid volume, leading to peripheral edema, hypertension, and congestive heart failure. Most prominent, however, is the onset of the uremic syndrome or ESRD, in which increased Blood Urea Nitrogen and serum creatinine values are observed along with the preceding symptoms. The outcome of this disease is either recovery or, in the case of irreversible renal damage, progression to chronic renal failure. (Bishop *etal.*, 2010)

#### **1.2.4.3.5.2 Chronic Renal Failure (Chronic Kidney Disease) :-**

Chronic kidney disease (CKD) is a clinical syndrome that occurs when there is a gradual decline in renal function over time . According to the 2007 U.S. Renal Data System (USRDS) Annual Data Report, one in nine U.S. adults has CKD and 20 million more are at risk. Early detection and treatment are needed to prevent progression to ESRD and complications such as coronary vascular disease. The National Kidney Foundation has formulated guidelines for earlier diagnosis, treatment, and prevention of further disease progression . GFR and evidence of kidney damage based on measurement of proteinuria or other markers form the basis of the classifications. The conditions that can precipitate acute renal failure also may lead to chronic renal failure. (Bishop *etal.*, 2010)

**Table 1.3: Etiology of Chronic Renal Failure.\***

<b>Etiology</b>	<b>Examples</b>
Renal circulatory diseases	Renal vein thrombosis, malignant hypertension
Primary glomerular diseases	Systemic lupus Erythmatosis (SLE), chronic glomerulonephritis
Renal sequelae to metabolic disease	Gout, diabetes mellitus, amyloidosis
Inflammatory diseases	Tuberculosis, chronic pyelonephritis
Renal obstructions	Prostatic enlargement, calculi
Congenital renal deformity	Polycystic kidneys, renal hypoplasia
Miscellaneous conditions	Radiation nephritis

\*(Bishop *et al.*, 2010)

**Table 1.4: Stages of chronic renal failure.\***

<b>Stage Description</b>	<b>Glomerular Filtration Rate (GFR) ml/m</b>
I- Kidney damage (e.g., protein in the urine) with normal GFR	90 or above
II- Kidney damage with mild decrease in GFR	60 to 89
III- Moderate decrease in GFR	30 to 59
IV- Severe reduction in GFR	15 to 29
V- Kidney failure	Less than 15

\*(Bishop *et al.*, 2010)

#### **1.2.4.3.5.2.1 Increasing Incidence of Chronic Kidney Disease :-**

There is an increasing incidence of CKD in the United States due to the increase in diabetes, the aging population, obesity, and metabolic syndrome. Diabetes mellitus can have profound effects on the renal system. Patients with type 1 diabetes have an insulin deficit. Approximately 45% of patients with type 1 diabetes will develop progressive deterioration of kidney function (diabetic nephropathy) within 15–20 years after diagnosis. (Bishop *et al.*, 2010)

A smaller percentage of patients with type 2 diabetes will also develop this condition. The effects are primarily glomerular, but they may affect all kidney structures as well and are theorized to be caused by the abnormally hyperglycemic environment that constantly bathes the vascular system. Typically, diabetes affects the kidneys by causing them to become glucosuric, polyuric, and nocturic. These states are caused by the heavy demands made on the kidneys to diurese hyperosmotic urine. In addition, a mild proteinuria (microalbuminuria) often

develops between 10 and 15 years after the original diagnosis . Eventually, chronic renal insufficiency or nephrotic syndrome may evolve, and each may be identified by their characteristic symptoms and laboratory findings. Early treatment of diabetes that focuses on good control of blood glucose and prevention of high blood pressure may prolong the onset of chronic renal failure. Aside from hypertension and diabetes, age is the key predictor of CKD. Due to the decline in fertility and increase in the average life span, the percentage of the population aged 65 years or older is subjected to increase from 12.4% in 2000 to 19.6% in 2030, according to the U.S. Census Bureau. This continual rise, over the previous decade and those to come, contributes significantly to the increasing incidence of CKD. Epidemiologic evidence links obesity to CKD and ESRD. However, diabetes mellitus and hypertension have potential confounding roles because obesity is a risk factor for diabetes and hypertension, the two most common causes of CKD and ESRD. Previous studies showed that obesity itself increased the risk of kidney injury. (Bishop *et al.*, 2010)

As an individual gains weight, the nephron number remains the same; however, the GFR increases to meet the higher metabolic demands, which results in damage to the kidney. The metabolic syndrome, characterized by the presence of at least three of the following risk factors : abdominal obesity, hypertension, low high-density lipoprotein cholesterol level, hypertriglyceridemia, or hyperglycemia—is a prevalent disorder in the United States. In a population study of a representative sample of the U.S. general population, the risk of CKD and microalbuminuria increased progressively with a greater number of components of metabolic syndrome. Individuals with metabolic syndrome had a 2.6-fold increased risk for developing CKD compared with individual without metabolic syndrome. Interventions that target biochemical components of metabolic syndrome may reduce the risk of CKD. (Bishop *et al.*, 2010)

### **1.2.4.3.5.3 Dialysis :-**

Dialysis is the process of separating macromolecules from ions and low molecular weight compounds in solution by the difference in their rates of diffusion through a semipermeable membrane. Crystalloids pass readily through this membrane, but colloids pass very slowly or not at all. Two distinct physical processes are involved: diffusion and convection. Dialysis procedures include hemodialysis (HD), hemodiafiltration (HDF), and peritoneal dialysis (PD). (Burtis, 2008)

#### **1.2.4.3.5.3.1 Hemodialysis :-**

Hemodialysis is the most common method used to treat advanced and permanent kidney failure. Operationally, it involves connecting the patient to a hemodialyzer into which their blood flows. After filtration to remove the wastes and extra fluids, the cleansed blood is returned to the patient. It is a complicated and inconvenient therapy requiring a coordinated effort from a healthcare team . The most important functional part is the dialyzer membrane. A variety of membranes are available with different surface areas and filtration characteristics. The oldest type of membrane was made from cuprophane and cellulose acetate; however, these have been replaced by more biocompatible synthetic membranes made from polysulfone and polyacrylonitrile. Patients are dialyzed in home-based or hospital-based units, with dialysis usually performed three times a week for sessions lasting between 3 and 5 hours. (Burtis , 2008)

#### **1.2.4.3.5.2 Hemodiafiltration :-**

HFD is a method of dialysis that combines HD and hemofiltration (HF). It offers the advantages of both HD and HF in a single therapy. The replacement fluid, previously supplied in autoclaved bags, is now generated "online" from concentrated bicarbonate and uses 20 to 30 L of water per session. The result

is that HDF provides 10% to 15% increase in urea clearance compared with HD as well as increased middle molecule clearances. (Burtis, 2008)

### **1.2.4.3.5.3 Peritoneal dialysis :-**

Peritoneal dialysis is a type of dialysis in which dialysate is instilled into the patient's peritoneal cavity with the peritoneum then employed as the dialysis membrane . Continuous ambulatory peritoneal dialysis (CAPD) is now available that is performed in ambulatory patients during normal activities. Operationally, CAPD uses the patient's own peritoneal membrane (surface area approximately 2 m<sup>2</sup>) across which fluid and solutes are exchanged between the peritoneal capillary blood and the dialysis solution placed in the peritoneal cavity. Fluid removal (UF) is achieved by using dialysis fluids containing high concentrations of dextrose acting as an osmotic agent; as the dextrose passes across the peritoneal membrane, the rate of fluid removal decreases. Conventional therapies use four daily exchanges of approximately 2L of fluid with approximately 10 L of spent dialysate generated, including UF. (Burtis, 2008)

### 1.3 Previous Study:-

A study by Hasson *et al.*, (2013) in Iraq showed the frequency and the relationship between Hemodialysis patients and their ABO blood grouping , they concluded that there was a relation between renal failure and blood group O which was the highest distribution group than other blood group (Hasson *et al.*, 2013) .

Roberts (1957) in London conducted a study in Blood groups and susceptibility to diseases which included many types of cancer as duodenal cancer also common in patients of Group O than those of the other blood groups but cancer of the stomach and pernicious anemia have a higher relative incidence in individuals of Group A (Roberts, 1957) .

Wazirali *et al.*, (2005) in Pakistan conducted study to show association between coronary heart diseases with ABO and Rh blood groups they concluded that blood group phenotype A is associated with a substantially increased risk for CHD (Wazirali *et al.*, 2005)

Waseem *et al.*, (2011 ) in Pakistan , conducted a study to find out the association of diabetes mellitus with ABO and Rh blood groups , their results showed a lower percentage of Blood group A and B and a higher percentage of blood group AB in the diabetic group when compared to control group There is also a positive association between Rh negative blood groups and Diabetes (Wasseem *et al.*, 2011) .



#### **1.4 Rationale:-**

Chronic renal failure is a major health problem and greatly affects the economic and social status of such patients. About 70-140 cases per million every years is the incidence of chronic renal failure in Sudan and the problem is expected to continue unless greater efforts will be directed towards this disease .(Ali *et al.*,2008)

Due to the incidence of Renal failure in Sudan this study aimed to determine distribution of ABO blood group system and Rhesus factor among chronic renal failure patients under hemodialysis .

## **1.5 Objectives**

### **1.5.1 General Objective :-**

To determine distribution of ABO blood group system and Rhesus factor among patients with chronic Renal failure under hemodialysis in Khartoum state .

### **1.5.2 Specific Objectives :-**

- To detect A and B Antigens in red cells of chronic renal failure patients under hemodialysis and compare the results of patients group with control group .
- To detect Rhesus Antigen (D factor) in red cells of chronic renal failure patients and compare the results of patients group with control group .
- To correlate distribution of ABO blood group system and Rhesus factor with gender and age .

## **Chapter Two**

### **Materials and Methods**

#### **2.1 Study Design**

This is an observational, analytical case control study.

#### **2.2 Study Area**

This study was conducted in Khartoum Teaching Hospital and Ibn Sina Specialized Hospital\_ Khartoum State .

#### **2.3 Study Population**

Population in this study were chronic renal failure patients under Hemodialysis , both sexes and all age groups were included .

#### **2.4 Inclusion Criteria**

All chronic renal failure patients under Hemodialysis presented to these two hospitals over a period of (March\_April 2014) .

#### **2.5 Exclusion Criteria**

Any individual without Renal failure disease or not under hemodialysis were excluded .

#### **2.6 Sampling and Sample Size**

Total of 100 Sudanese patients with chronic renal failure attended to Khartoum Teaching Hospital and Ibn Sina Specialized Hospital. While control group was 100 individuals from co-patients and hospital staff .

#### **2.7 Data Collection Tools**

All data about participants collected by using questionnaire .

## **2.8 Characteristic of the participants**

From 100 Sudanese patients suffering from chronic renal failure under Hemodialysis some factors were obtained such as ( gender \_ age \_history of disease ) while in control group (gender\_age) .

## **2.9 Ethical Consideration**

Consent of selected individuals to the study was taken after being informed with all detailed objectives of the study.

## **2.10 Laboratory Investigations Methods :**

### **2.10.1 Sample collection**

Two ml venous blood were collected after sterilization by 70% alcohol with limited occlusion of the arm by the tourniquet . The blood was added to evacuated EDTA tube and was mixed gently . (Boorman, *et al.*,1988)

### **2.10.2 ABO Blood Grouping by slide technique**

#### **Principle :-**

When red cells were mixed with various reagents of antisera (soluble antibody), agglutination occur on the slides containing cells positive (possessing the antigen) for the corresponding antigen. No agglutination occur in the red cells did not contain the corresponding antigen .

#### **Reagents :-**

Monoclonal anti-A and anti-B reagents .

#### **Procedure :-**

- 1- On the section of slide labeled anti-A one drop of antisera A was placed .
- 2- On the section of slide labeled anti-B one drop of antisera B was placed .
- 3- One drop of cells was placed in each antisera containing circle.
- 4- Mentioned solution was mixed carefully with a separate applicator stick.
- 5-The slide was slowly tilted for one minute, then agglutination was observed .

**Interpretation:-**

- A positive reaction was indicated by clumping of RBCs due to presence of antigen A or antigen B or antigen A and antigen B (agglutination).
- While in a negative reaction there was no clumping , RBCs appeared free and the preparation was homogenous due to absence of antigen A and antigen B (no agglutination) .

**2.10.3 Rhesus (D factor) grouping by slide technique :****Principle :-**

When red cells were mixed with antisera D reagent (soluble antibody), agglutination occur on the slide containing cells positive (possessing the antigen) for the corresponding antigen. No agglutination occur when red cells did not contain the corresponding antigen

**Reagents :-**

Monoclonal anti-D reagent .

**Procedure :-**

- 1- On new slide labeled anti-D one drop of antisera D was added .
- 3- One drop of cells was added to antisera .
- 4- Mentioned solution carefully was mixed with a separate applicator stick.
- 5-The Slide slowly tilted for one minute, then agglutination was observed .

**Interpretation:-**

- A Positive reaction was indicated by clumping of RBCs due to presence of D antigen (agglutination).
- While in a negative reaction there was no clumping , RBC's appeared free and the preparation was homogenous due to absence of D antigen (no agglutination) .

#### **2.10.4 D<sup>u</sup> technique**

##### **Principle :-**

Red blood cells containing D<sup>u</sup> antigen was sensitized when anti – D was added , sensitized red cells converted to visible agglutination when coomb's sera is added .

##### **Reagents:-**

Monoclonal anti-D reagent .

Coomb's sera .

##### **Procedure:-**

1. In clean and dry small test tube 2 drops of anti -D were placed .
2. two drops of 5% red cell suspension were added and mixed well .
3. Mentioned solution was Incubated at 37c° for 15-30 minutes and Centrifuged at 1000 RPM for 1 minute .
4. The tube was mixed well and read macro, in case of no agglutination was washed 3 times by normal saline .
5. One drop of Coomb's sera was added to the final packed red cell and mixed well .
6. Centrifuged at 1000RPM for 1 minute
7. The tube was mixed well and read macroscopically and microscopically .

##### **Interpretation:-**

Agglutination indicated for a positive result .

No agglutination indicated for a negative result .

#### **2.11Data Analysis**

The Data was analyzed by using SPSS computer program . Frequencies and Chi\_ square test values were calculated.

## **Chapter Three**

### **Results**

This study aimed to determine distribution of ABO blood groups and Rhesus factor in 100 chronic renal failure patients under hemodialysis and compared the results with 100 control group. Mean age in patients group was 46 years and 47 years of control group. Males In patients group comprised 75% and 25% were females while in control group 56% were males and 44% were females .

#### **Distribution of study group according to gender :**

Among patients group males were the highest distribution ( 75%) . Also in control group males were the highest distribution (56%) . Table (3-1)

#### **Distribution of study group according to age groups:**

The highest distribution in patients group was found between (20-40) year while the lowest distribution was found between (81-100) year .In control group the highest distribution between (41-60) year while the lowest distribution between (81-100) year also . Table (3-2).

#### **Distributin of gender among age groups in study population .**

In patients group the highest distribution was males found in age group between 41-60 year (30 males) while the lowest distribution were females in age group between 81-100 year.Also the highest distribution in control group was males found in age group between 41-60 year (25 males) and the lowest distribution were females in age group between 81-100 year. Table (3-3)

**Distribution of ABO Blood groups among study group :**

In patients group the highest distribution of ABO blood group system was O (60%) while the lowest distribution was AB (4%). in control group the highest distribution was O (47%) ) while the lowest distribution also was AB(8%).

Table (3-4)

**Table (3-5) Distribution of Rhesus factor among study group:**

The highest distribution in cases group was D+ve (86%). In control group (94%) were D+ve . Table (3-5)



**Table (3-1) Distribution of study group according to gender :**

<b>Sex</b>	<b>Patients</b>		<b>Control</b>	
	<b>Number</b>	<b>Percent</b>	<b>Number</b>	<b>Percent</b>
Male	75	75.0	56	56.0
Female	25	25.0	44	44.0
Total	<b>100</b>	100.0	<b>100</b>	100.0

**Table (3-2) Distribution of study group according to age groups:**

<b>Age group</b>	<b>Patients</b>		<b>Control</b>	
	<b>Number</b>	<b>Percent</b>	<b>Number</b>	<b>Percent</b>
<b>20-40</b>	43	43%	36	36%
<b>41-60</b>	35	35%	42	42%
<b>61-80</b>	20	20%	20	20%
<b>81-100</b>	2	2.0%	2	2.0%
<b>Total</b>	<b>100</b>	100.0	<b>100</b>	100.0

**Table (3-3) Distributin of gender among age groups in study population :**

<b>Age Group</b>	<b>Patients</b>		<b>Control</b>	
	<b>Male Number</b>	<b>Female Number</b>	<b>Male Number</b>	<b>Female Number</b>
<b>20-40</b>	25	18	19	17
<b>41-60</b>	30	5	25	17
<b>61-80</b>	18	2	10	10
<b>81-100</b>	2	0	2	0
<b>Total</b>	<b>75</b>	<b>25</b>	<b>56</b>	<b>44</b>

**Table (3-4) : Distribution of ABO Blood groups among study group :**

<b>ABO Blood Group</b>	<b>Patients</b>		<b>Control</b>		<b>p.value</b>
	<b>Number</b>	<b>Percent</b>	<b>Number</b>	<b>Percent</b>	
A	21	21.0	32	32.0	.149
B	15	15.0	13	13.0	
AB	4	04.0	8	08.0	
O	60	60.0	47	47.0	
Total	<b>100</b>	100.0	<b>100</b>	100	

**Table (3-5) Distribution of Rhesus factor among study group :**

<b>Rhesus (D factor)</b>	<b>Patients</b>		<b>Control</b>		<b>p.value</b>
	<b>Nuumber</b>	<b>Percent</b>	<b>Number</b>	<b>Percent</b>	<b>.059</b>
+ve	86	86.0	94	94.0	
-ve	14	14.0	6	06.0	
<b>Total</b>	<b>100</b>	<b>100.0</b>	<b>100</b>	<b>100.0</b>	

## **Chapter Four**

## Discussion, Conclusion and Recommendations

### 4.1 Discussion:

This study was carried out on 200 subjects (100 renal failure patients under hemodialysis and 100 control subjects) who attended to Khartoum Teaching Hospital and Ibn Sina specialized Hospital during the period between March to May 2014 .

The aim of the study is to determine the distribution of ABO blood group system and Rhesus factor among chronic renal failure patients under hemodialysis .

The results of this study showed that : blood group O was the highest distribution then blood group A, blood group B and blood group AB was the lowest distribution. While in control group Blood group O was the highest distribution followed by blood group A, then blood group B and blood group AB with p.value (.149) . Table (3-4)

A previous study was conducted by Hasson *et al.*, (2013) in Iraq to study the relationship between hemodialysis patients and ABO blood group among 60 patients in which blood group O also found with the highest distribution then followed by blood group B , blood group A and blood group AB .

Other previous studies showed the association between ABO blood groups system and many diseases rather than renal failure

Roberts (1957) conducted study in London on Blood groups and susceptibility to disease included many types of cancer as duodenal cancer which is common in patients of Group O while cancer of the stomach and pernicious anemia have a higher relative incidence in persons of Group A . (Roberts,1957)

Wazirali *et al* (2005) in Pakistan conducted study to show association between coronary heart disease with ABO and Rh blood groups they concluded to blood

group phenotype A is associated with a substantially increased risk for CHD ,then followed by B blood group , O blood group and AB blood group. (Wazirali *et al.*, 2005)

Waseem *et al* (2011 ), in Pakistan conducted a study to determine the association of diabetes mellitus with ABO , their results showed a lower percentage of Blood group A and B and a higher percentage of blood group AB in the diabetic group when compared to control group .

While distribution of Rhesus factor in this study showed that : D +ve higher than D-ve .In control group also D+ve was higher than D-ve with p.value (.059) .Table (3-5)

In previous study of Hassoon D+ve was higher than D-ve. (Hassoon *et al.*, 2013 ) According to the results, it was concluded that blood group O and Rh D+ve were most common among both patients and control subjects .This indicate to there is no association between ABO blood groups , Rhesus factor and renal failure .

## **4.2 Conclusion:**

1. Blood group O found with the highest distribution among chronic Renal failure patients under hemodialysis then blood group A , blood group B where as blood group AB was the least common .
2. Rhesus factor D +ve is most common than D –ve in patients group.
3. There is no significant association between renal failure, ABO blood groups and Rhesus factor.

### **4.3 Recommendations :**

1. More advanced technique such as Gell system, PCR and molecular techniques should be performed to obtain more specific result .
2. More studies are needed to look for Blood grouping systems in Sudanese patients with most common diseases .
3. Researches on normal distribution of ABO blood group system and Rhesus factor among Sudanese people should be done to establish data base program .

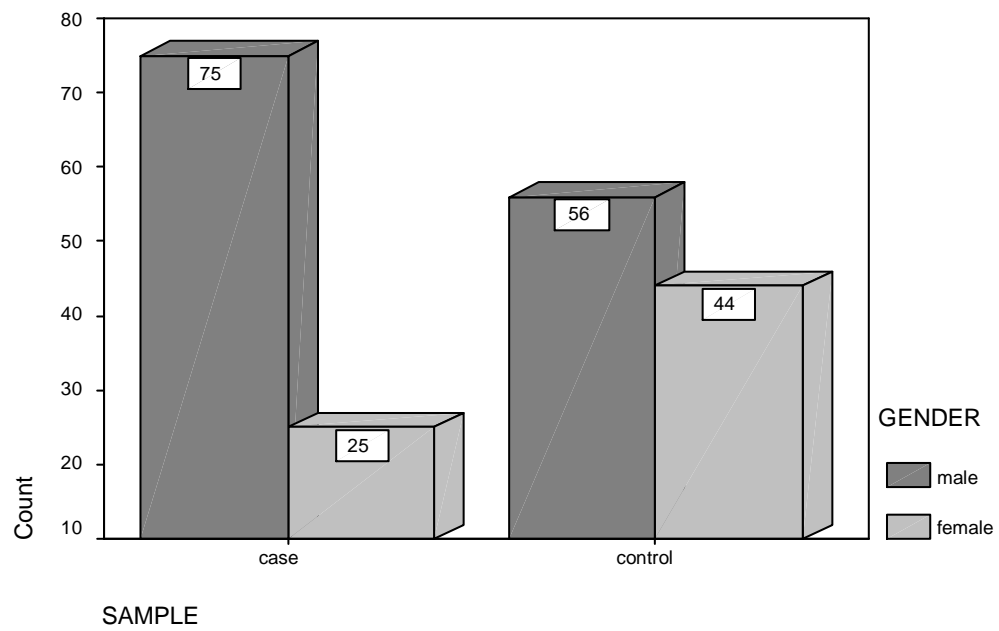
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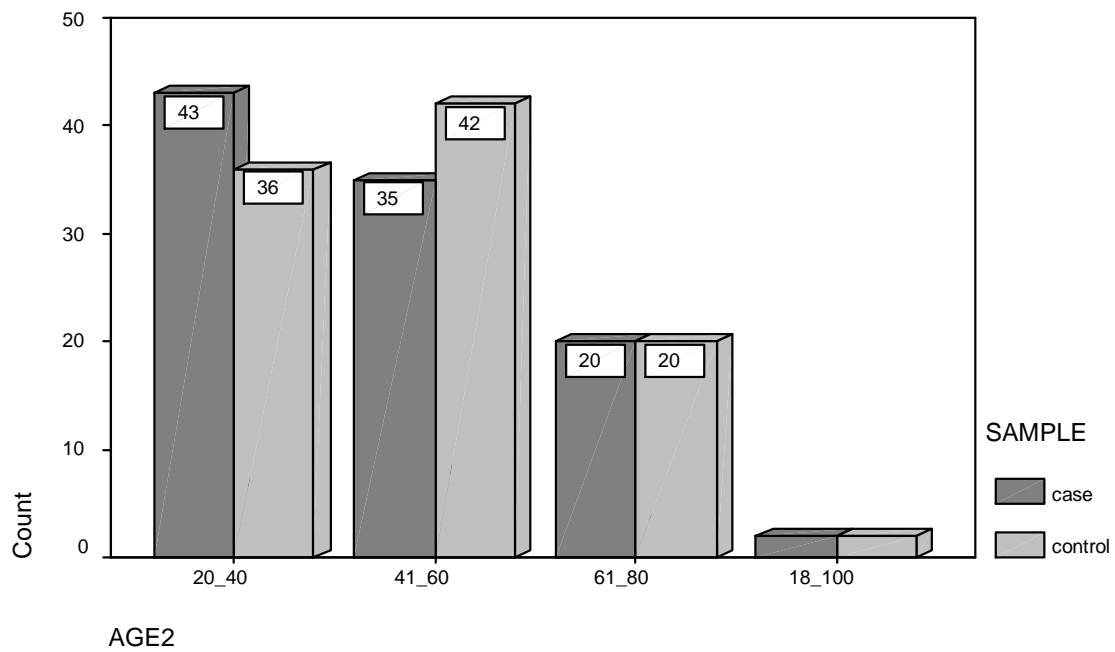
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## Appendix (1)



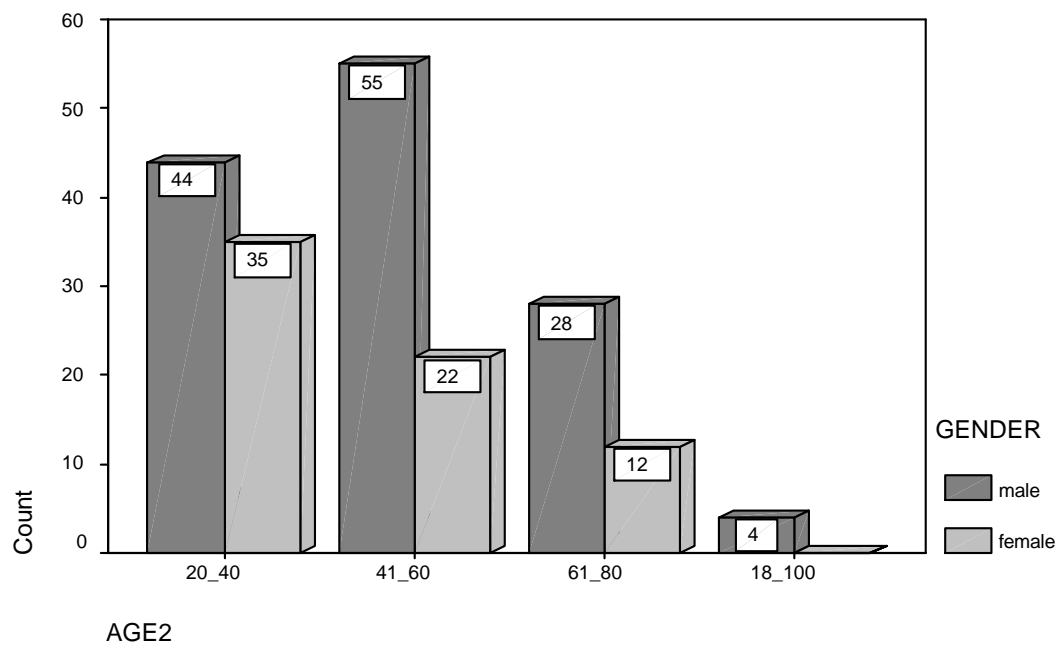
**Figure(3.1) Distribution of study group according to gender**

## Appendix (2)



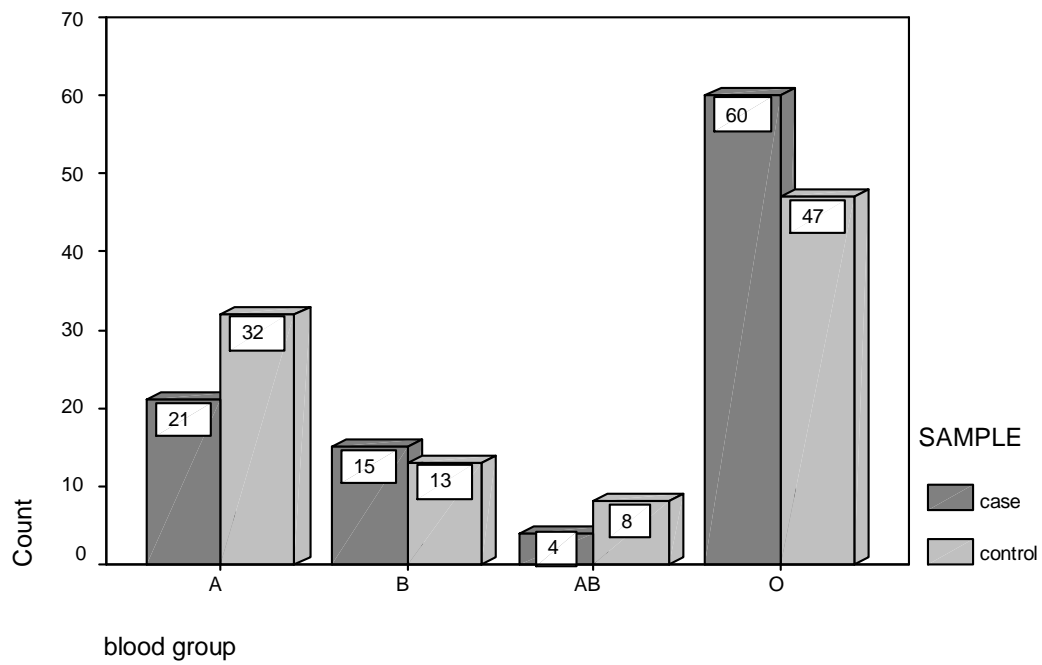
**Figure(3.2) Distribution of study group according to age**

### Appendix (3)



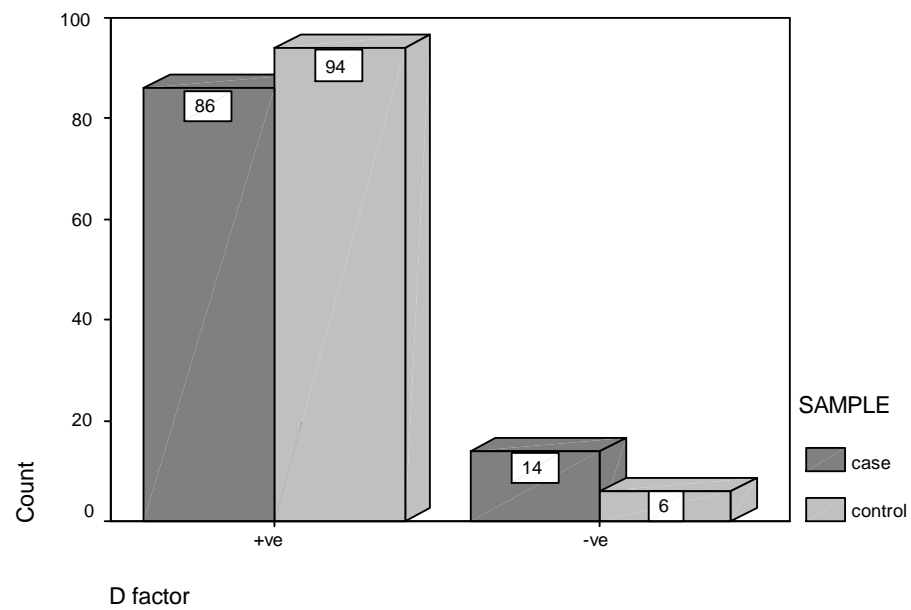
**Figure(3.3) Distribution of gender among age groups in study population .**

## Appendix (4)



**Figure(3.4) Distribution of ABO Blood group among study group**

## Appendix (5)



**Figure(3.5)Distribution of Rhesus factr among study group**

**Appendix (6)**  
**Sudan University of Science and Technology**  
**College of Graduate studies**  
**Hematology Department**  
**Questionnaire about ABO blood grouping and Rhesus factor in**  
**patients with chronic renal failure**

**Sex :.....**

**Age :.....**

**Residence :.....**

**History of Disease :.....**

**others :.....**

.....

.....

**Lab investigation:-**

**Blood Group .....**

**Rh Factor .....**

**Signature : .....**

**Date : .....**

**Appendix (7)**  
**جامعة السودان للعلوم و التكنولوجيا**  
**كلية الدراسات العليا**  
**دراسه لنيل الماجستير**  
**اقرار بالموافقه و المشاركه**

الاسم .....

سوف يتم أخذ عينة من الدم الوريدي بواسطة حقنه و ذلك بعد تعقيم منطقة أخذ العينه بواسطة مطهر . جميع الادوات المستخدمه معقمه و متبع فيها وسائل السلامة المعملية و ليس هناك آثار جانبية للعملية . قد يحدث تورم بسيط في منطقة أخذ العينه و سوف يزول بعد فتره قصيره . الغرض من أخذ العينه هو البحث العلمي و سوف يتم تسليمكم نسخه من النتائج و سوف تحفظ النتائج بسريه تامه .

وافق انا المذكور اعلاه علي أخذ عينة الدم

التوقيع .....

الاسم .....





