1.1 Introduction:-

Blood is a two-phase suspension of formed elements which are cells and plasma. These cells are red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs) which are suspended in an aqueous solution of organic molecules, proteins, and salts called plasma (Baskurt and Meiselman, 2003).

The production, development, differentiation, and maturation of all blood cells occurring through process called hematopoiesis which is occuring within the bone marrow which has ability to produce 3 billion red blood cells, 1.5 billion white blood cells, and 2.5 billion platelets per day per body weight (Caldwell, 2012).

When the hemoglobin concentration (Hb), RBCs count and hematocrit or packed cell volume are decreased below normal range that condition is called anemia which is characterized by decreased oxygen carrying capacity of blood and shows decreased Hb and PCV. The criteria of anemia according to WHO for Hb in adult males is below than 13mg/dl and for females below than 12mg/dl. The grading of anemia according to WHO into mild (Hb 9.1–10.5 g/dL), moderate (Hb 6.0–9.0 g/dL) and severe (Hb < 6.0 g/dL) (Naya, 2014).

The common global health and medical problems conditions are seen everyday in clinical practice are iron deficiency and iron-deficiency anemia which are going to be the top-ranking cause of the worldwide type of anemia which are effecting on young children and premenopausal women in both low-income and developed countries (Camaschella, 2015).

The reduction of iron stores refers to iron deficiency that proceeds overt iron-deficiency anemia or persists without progression. While the iron-deficiency anemia is a more severe condition in which low levels of iron are associated with anemia and the presence of microcytic hypochromic red cells (Goodnough *et al*., 2010).

Iron is so important for the biologic functions including respiration, energy production, DNA synthesis, and cell proliferation (Hentze *et al*., 2010).

Human body can conserve the iron in several ways that including the recycling of iron after breakdown of red blood cells and retention of iron in the absence of an excretion mechanism. However, the absorption of iron is limited to 1 to 2 mg daily and the most of the iron that needed daily is about 25 mg that is provided through recycling by macrophages which phagocytosed the erythrocytes. They are controlled by the

hepcidin, which is a hormone that maintains total-body iron within normal ranges, avoiding both iron deficiency and excess (Weiss and Goodnough, 2005).

The mechanisms of iron acquisition are tightly regulated by hepcidin which is a peptide hormone that is synthesized primarily by the liver. It has a role as an acute-phase reactant that adjusts fluctuations in plasma iron levels caused by absorptive enterocytes and the macrophages in the spleen by binding to and inducing the degradation of ferroportin, which exports iron from cells (Nemeth *et al*., 2004).

Hepcidin expression increases in response to high circulating and tissue levels of iron and in persons with systemic inflammation or infection. While it is inhibited by the expansion of erythropoiesis, iron deficiency, and tissue hypoxia in response to signals that originating in the bone marrow, liver, and probably muscle tissue and adipocytes (Camaschella, 2013).

In iron deficiency, transcription of hepcidin is suppressed so facilitates the absorption of iron and release of the iron from body stores. As well as the intestinal iron uptake from the gut lumen through divalent metal transporter 1 (DMT1) is increased by the activation of hypoxia (Mastrogiannaki *et al*., 2013).

Divalent metal transporter-1 is known to be able to transport iron, zinc, copper, manganese and other divalent metals from endosomal vesicles into the cytoplasmic space that functionality it's reflecting as a proton-divalent metal countertransport protein (Beard, 2001).

Iron is required by the host for mounting an effective immune response. In humans with intact immune systems the nonspecific and humoral immunity are affected by iron deficiency in several ways. In nonspecific immunity the macrophage phagocytosis is generally unaffected by iron deficiency, but bactericidal activity of these macrophages is attenuated. Neutrophils have reduced the activity of the iron-containing enzyme myeloperoxidase which produces reactive oxygen intermediates responsible for intracellular killing of pathogens. There are also decreasing in both T-lymphocytes number and T-lymphocytes blastogenesis and mitogenesis in iron deficiency in response to a number of different mitogens. This alteration is largely correctable with iron repletion. In the humoral immunity appears to be less affected by iron deficiency than cellular immunity. In iron-deficient humans, antibody production in response to immunization with most antigens is preserved (Beard, 2001).

The immune system plays a critical role in the development of co-infections, promoting or preventing establishment of multiple infections and shaping the outcome of pathogen-host interactions (Mastrogiannaki *et al*., 2013).

During infection, the production of IL-10 is increased in primary macrophages and the availability of iron to erythroid developing cells ultimately depends on macrophages, thus the high concenteration of IL10 plays a key regulatory role which has both direct anti-inflammatory effect and as well as indirect effect on iron restriction through the up-regulation of hepicidin which is the major inhibitor of iron absorption and it's occurring through IL10 that induced hepcidin secretion through effects which were mediated signal transducer and activator of transcription (STAT) 3-phosphorylation and completely abrogated by a specific STAT3 inhibitor (Huang *etal*., 2014).

1.2 Rational:-

Iron deficiency anemia is a major health problem that arises when the balance of iron intake, iron stores, and the body's loss of iron are insufficient to fully support production of erythrocytes (Miller, 2013).

Iron losses can result from many mechanisms of a host that occurs during hemolysis, hematuria, sweating and gastrointestinal bleeding which allowed to anemia. In chronic states of disease that can lead to inflammation which leading to production and elevation of cytokines as acute-phase protein response which affect on the liver that producing hepcidin hormone leading to hepcidin up-regulation which has a negative impact on the iron transport and absorption channels within the body that literally may explain a potential of new mechanism behind iron deficiency (Peeling *et al*., 2008).

IL 10 has a direct role as pro-inflammatory cytokine and indirect effect role on hepcidin induction which was mediated via STAT3-phosphorylation that was inhibited with a specific inhibitor of STAT3 phosphorylation (Drakesmith *et al*., 2005).

In Sudan, there are no published data that focusing and discussing the effectiveness of iron deficiency on the function of immune system that may be leading later on to functional immunodeficiency.

1.3 Objectives:-

1.3.1 General objective:-

To evaluate level of IL10 in Sudanese among the iron deficiency anemic adults.

1.3.2 Specific objectives:-

1. To measure level of IL10 among IDA Patients.

2. To find the possible correlation effects of IL-10 on hematological variables and other risk factors.

2.1 Hematopoiesis:-

Hematopoiesis is a tightly controlled process that requiring the ordered of gene expression regulation to be mature and fully differentiated cells throughout the development from hematopoietic stem cells (HSCs) (Sandberg *et al*., 2005).

2.2 Hematopoietic stem cells (HSCs):-

Hematopoietic stem cells (HSCs) are rare and multipotent cells that generate via progenitor and precursor cells of all blood lineages (Riether *et al*., 2015).

They are defined by their ability to undergo self-renewal and maintain the capacity to generate to all mature haematopoietic cell types within the blood and immune system (Ramalingam *et al*., 2017).

All cellular blood components are derived from HSCs which are reside in the medulla of the bone marrow and have the unique ability to give rise to all different mature blood cell types and tissues (Rieger and Schroder, 2012).

As the function, the HSC is the cell that isolated from the bone marrow. It can renew itself and as well as proliferate and differentiate to a variety of specialized cells. As well as, it can mobilize out of the bone marrow into circulating blood, and can undergo with programmed cell death which is called apoptosis (Bryder *et al*., 2006).

HSC pool maintenance and concomitant lineage differentiation are facilitated either by asymmetric self-renewal in which specific cell fate determinants are redistributed unequally to the two daughter cells; or via environmental symmetry, in which one daughter cell leaves the niche that sustains HSC self-renewal and is then exposed to an environment that promotes lineage of differentiation (Wilson and Trump, 2006).

Pluripotent hematopoietic stem cells are cells have the ability to differentiate into myeloid or lymphoid stem cells. Myeloid stem cells give rise to a second level of lineage-specific CFU cells that go on to produce neutrophils, monocytes, eosinophils, basophils, mast cells, megakaryocytes, and erythrocytes. While the lymphoid stem cells give rise into B-lymhocytes and T-lymphocytes (Weiskopf *et al*., 2016).

The myeloid and lymphoid progenitor cells can differentiate and produce one or more specific types of blood cells but they cannot renew themselves (Kondo, 2010).

Eventually, the production of blood cells relies on the ability of HSC to self-renewal and differentiated into all blood cells lineage. The adult stem cells and cord blood stem cells are considered as multipotent cells which can develop into more than one cell type, but are more limited than pluripotent cells (Prashad *et al*., 2015).

2.3 Locations of hematopoietic stem cells:-

Blood is normally formed early in the process of embryogenesis which is originating within the haemopoietic stem cells in the para-aortic mesoderm of the embryo. The primitive red blood cells, platelet and macrophages are initially formed in the vasculature of the extra-embryonic yolk sac which is the principal site of haemopoiesis then it shifts to the fetal liver at around five to eight weeks of gestation and remains the main source of blood until shortly before birth, although the bone marrow starts to develop haemopoietic activity from as early as 10 weeks of gestation. Normally after birth, the marrow is the major site of haemopoiesis and within the first few years of life. Nearly, all the marrow cavities contain red haemopoietic marrow, but this recedes such that by adulthood haemopoiesis is limited to marrow in the vertebrae, pelvis, sternum and the proximal ends of the femora and humeri, with minor contributions from the skull bones, ribs and scapulae (Hatton, 2013).

During postnatal life, also the HSC pool size is maintained through the regulation of HSC self-renewal and differentiation because of the bone marrow contains specialized niches in which the multipotency of HSCs is conserved through cell divisions, while their progeny are directed towards lineage differentiation (Hoffman and Calvi, 2014).

In humans, the bone marrow is the major site for hematopoiesis and called intramedullary hemopiesis however in certain pathological conditions called extramedullary erythropoiesis are observed where occur in several organs including the liver (Lenox *et al*., 2009).

2.4 Haematopoietic growth factors (HGFs):-

HGFs known as colony-stimulating factors which are the family of glycoproteins that are required for the survival of hematopoietic progenitors. They regulate the production of all the cellular elements of blood cells through interactions with specific receptors on primitive stem cells by stimulating their proliferation and differentiation toward committed lineages. Also they regulate the functional activation of mature myeloid elements (Metcalf, 2010).

The mature blood cells are produced more than one million cells per second in the adult human. They are derived from the hematopoietic stem cells (HSCs) through the cycle which is very infrequently and primarily reside in the G_0 phase of the cell cycle under homeostatic conditions (Seita and Weissman, 2010).

HGFs of the hematopoietic system include interleukins (ILs), colony-stimulating factors (CSFs), interferons, erythropoietin (EPO) and thrombopoietin (TPO) (Robb, 2007).

Also, they are glycoproteins which are present normally in the circulation such as SCF, G-CSF, EPO and TPO in very low doses, or are stimulated to be appear in response to infection or inflammation as GM-CSF, IL-3, IL-5, IL-6, and IL-11 (Bhattacharya, 2015)

2.5 Hemoglobin:-

Hemoglobin (Hb) is a tetrameric noncovalent complex consisting of two α- and two β-globin chains each each of them is attached with a heme group (Scarff *et al*., 2009).

2.5.1 Heme synthesis:-

Erythropoiesis requires the proper biosynthesis of heme for erythroblasts maturation and their demand for iron and heme are increasing dramatically (Chung *et al*., 2012).

Heme biosynthesis occurs through a series of enzymatic reactions that take place in the cytosol, mitochondria and the proper intercellular and intracellular trafficking of iron. As well as, the heme can also be acquired by intestinal absorption and intercellular transport (Chung *et al*., 2012).

Heme is synthesized through an eight-step enzymatic cascade that occurs in the cytosol and in the mitochondria (Schultz *et al*., 2010).

The first step takes place in the mitochondrial and involves the condensation of glycine with succinyl-coenzyme A by δ-aminolevulinic acid synthase 2 (ALAS2) in erythroid cells that resulting the δ-aminolevulinic acid (ALA) which is subsequently exported to the cytosol and converted through four enzymatic reactions to coproporphyrinogen III .Then the coproporphyrinogen III is transported into the mitochondrial intermembrane space and converted to protoporphyrin IX by two enzymes. Finally, ferrochelatase catalyzes the insertion of ferrous iron (Fe^{2+}) to form heme (Chiabrando *et al*., 2014).

Most of cells are acquiring the iron via transferrin receptor-mediated endocytosis of circulating transferrin-iron (III) (Fe^{3+}) (Jensen *et al*., 2011).

2.5.2 Globin synthesis:-

The structure of the globin chains is coded by genes of chromosome 16 and chromosome 11 (Petrakos *et al*., 2016).

The chromosome 16 is the α-gene cluster which is composed from the α- and ζ-globin chains. While the chromosome 11 is the β-gene cluster that comprises the globin chains γ, ε, β, and δ **(**Petrakos *et al*., 2016).

2.6 Functions of hemoglobin:-

Erythrocytes regulate vascular function through the modulation of oxygen delivery and the scavenging and the generation of nitric oxide (NO) (Helms *et al*., 2018).

The hemoglobin of the red blood cell binds with oxygen in the lungs and delivers it to the tissues throughout the body in an allosterically regulated process that modulated by oxygen, carbon dioxide and as well as proton concentrations. (Helms *et al*., 2018)

Nitric oxide and carbon monoxide are also able to bind with hemoglobin. With carbon monoxide, the binding is much more readily than oxygen, so for this reason the carbon monoxide is so seriously poisoning (Omaye, 2002).

2.7 Types of hemoglobin:-

There are seven types of haemoglobin molecules throughout a human's life. Four when he is an embryo, one once he develops into a fetus and then as an adult, he has two (Manning *et al*., 2007).

Embryonic hemoglobin presents in four forms which are Hb gower I, Hb Gower II ($\alpha_2\varepsilon_2$), Hb Portland I ($\zeta_2\gamma_2$) and Portland II ($\zeta_2\beta_2$). Then fetal hemoglobin is ($\alpha_2\gamma_2$) and remains in the child's blood until it is around six months then it is replaced with adult haemoglobins which are HbA ($\alpha_2\beta_2$), HbA_2 ($\alpha_2\delta_2$) and a small amount of Haemoglobin F is remaining (Manning *et al*., 2007).

2.8 Variants of hemoglobin:-

There are several hundred variants form of haemoglobin such as hemoglobin S (HbS) which is a structural variant of normal adult hemoglobin (HbA) caused by an amino acid substitution at position 6 of the β-globin gene. The heterozygous Individuals (AS) who have inherited HbS are usually asymptomatic, whereas the homozygous individuals (SS) with HbS suffer from sickle cell anemia (SCA). (Piel *et al*., 2016)

HbC is another structural variant of HbA caused by a different amino acid substitution at the same position of the β-globin gene. The heterozygous individuals (AC) are asymptomatic, whereas the homozygosity individuals (CC) cause mild hemolytic anemia due to the reduced solubility of the red blood cells that can lead to crystal formation (Piel *et al*., 2016).

2.9 Lifecycle of erythrocytes:-

The production of RBCs needs a number of raw materials must be present in adequate amounts. These include the same nutrients that are essential to the production and maintenance of any cell, such as glucose, lipids, and amino acids. However, erythrocyte production also requires several trace elements such as iron, Vitamins, Zinc and Copper (Berger *et al*., 2019).

Iron presents as many forms either as heme or nonheme form. Heme iron which presents in animal foods is absorbed more efficiently than non-heme iron form from plant foods. The iron transports across the ferroportin in the intestinal cell plasma membranes and it binds with transferring then stores in the bone marrow, liver and spleen as protein compounds of ferritin and hemosiderrin. Copper is a component of two plasma proteins which are hephaestin and ceruloplasmin. Hephaestin where Located in intestinal villi and it enables iron to be absorbed by intestinal cells, either the Ceruloplasmin transports the copper. Without of these components, the hemoglobin could not be adequately produced because they enable the oxidation of iron from Fe^{2+} to Fe^{3+} form to bind it with transferrin then it transports iron to body cells. In a state of copper deficiency, the transport of iron for heme synthesis decreases and iron can accumulate in tissues where it can eventually lead to organ damage (Berger *et al*., 2019).

Zinc acts as a co-enzyme that facilitates the synthesis of the heme portion of hemoglobin.Vitamins such as the folate (vitamin B6) and vitamin $B_{12;}$ they act as co-enzymes that facilitate DNA synthesis. Thus, both are critical for the synthesis of new cells, including erythrocytes (Berger *et al*., 2019).

Human mature red cells have a lifespan of 120 days before they become senescent and again come into contact with macrophages which play a critical role in iron homeostasis via their intimate association with developing and dying red cells. Phagocytosis of red blood cells is the main source of iron flux in the body, because heme must be recycled from approximately 270 billion hemoglobin molecules in each red cell, and roughly 2 million senescent red cells are recycled each second (Korolnek and Hamza, 2015).

The RBC is removed by mononuclear phagocyte system (MPS) in which the membrane disrupted and the Hb is broken-down within macrophage into globin and Hb portions (De Back *et al*., 2014).

The globin portion is the protein part of hemoglobin and it is broken down into amino acids which can be sent back to the bone marrow to be used in the production of new erythrocytes (De Back *et al*., 2014).

The Heme portion is broken down into iron and non iron portions. The iron contained in the heme portion of hemoglobin may be stored in the liver or spleen, or carried through the bloodstream by transferrin to the red bone marrow for recycling into new erythrocytes. The non-iron portion of heme is degraded into the waste product called biliverdin which is a green pigment, and then into another waste product called bilirubin which is a yellow pigment. Bilirubin binds to albumin and travels in the blood to the liver, which uses it in the manufacture of bile. Then that compound is released into the intestines to help emulsify dietary fats. In the large intestine, the bacteria breaks the bilirubin apart from the bile and converts it to urobilinogen and then into stercobilin which is then eliminated from the body in the feces and alters the color of feces. The kidneys also remove any circulating of bilirubin and other related metabolic byproducts such as urobilins and secrete them into the urine (De Back *et al*., 2014).

In human, iron is strictly conserved in large part by recycling about 20 mg/day of the iron from hemoglobin of senescent red blood cells to provide iron for new red blood cells. There are smaller amounts of iron from myoglobin and various enzymes are also recycled (Ganz, 2005).

In most circumstances, the human diets are containing more iron that is necessary to replace the small daily losses and it is about 1–2 mg/day (Ganz, 2005).

2.10 Iron:-

Iron is an essential element for numerous fundamental biologic processes. It is a transition metal that can readily donate and accept electrons to participate in oxidation–reduction reactions that are essential for a number of fundamental biologic processes (Daugirdas, 2017).

In human, iron is incorporated into proteins as a component of heme (e.g., hemoglobin, myoglobin, cytochrome proteins, myeloperoxidase, nitric oxide synthetases), iron sulfur clusters (e.g., respiratory complexes I-III, coenzyme Q_{10}, mitochondrial aconitase, DNA primase), or other functional groups (e.g., hypoxia inducible factor prolyl hydroxylases). These iron-containing proteins are required for vital cellular and organismal functions including oxygen transport, mitochondrial

respiration, intermediary and xenobiotic metabolism, nucleic acid replication and repair, host defense, and cell signaling (Daugirdas, 2017).

2.11 Absorption of iron:-

The body iron is distributed in two compartments. The first one is a functional compartment which is formed of a number of compounds including hemoglobin, myoglobin, transferrin and enzymes which are requiring iron as a cofactor or a prosthetic (ion or haem) group. The second compartment is the storage of ferritin and hemosiderin (Barragán-Ibañez *et al*., 2016).

The body iron content of a normal 70 kg individual is around 3.5–4 g in women and 4–5 g in men. Most of the iron is distributed as follows: 65% in hemoglobin (2300 mg), 15% in myoglobin and enzymes, 20% in iron stores, and only 0.1–0.2% is bound to transferrin (Barragán-Ibañez *et al*., 2016).

Iron is predominantly absorbed from the duodenum and less well from the first part of jejunum due to increasingly the alkaline environment which leads to the formation of insoluble ferric hydroxide complexes (Hurrell and Egli, 2010).

In physiological conditions, the iron loss and excretion is about 1-2 mg daily which occurs mainly when epithelial cells are sloughed from the lining of the gastrointestinal tract and from the skin. It is also excreted in the urine, in menstrual blood and breast milk, or lost through perspiration. Normal blood loss during menstruation is around 30 ml, although it can be as high as 118 ml in some women (Barragán-Ibañez *et al*., 2016).

2.12 Dietary and luminal factors:-

There are 2 types of dietary iron which are nonheme iron and heme iron. The nonheme iron is present in both plant foods and animal tissues, while the heme iron is liberated from hemoglobin and myoglobin in animal source foods. Nonheme iron is usually absorbed much less well than heme iron because of all nonheme food iron that enter the common iron pool in the digestive tract is absorbed to the same extent and it depends on the balance between inhibitors and enhancers of iron absorption and the iron status of the individual.Heme iron is estimated to contribute 10–15% of total iron intake, but because of its higher and more uniform absorption, it is estimated at 15–35% of total absorbed iron (Hurrell and Egli., 2010).

The acidic pH, vitamin C and some low-molecular-weight chelates such as sugars and amino acids enhance iron absorption and it is reduced as a result of the affect of

dietary non-haem iron such as phytates and tannates in tea.Then the dietary iron is absorbed by duodenal enterocytes and circulates in plasma where bound to transferrin which literally incorporated into hemoglobin in erythroid precursors and mature red cells, myoglobin and other tissues as enzymes and cytochromes or stored in parenchymal cells of the liver and reticuloendothelial macrophages (Hurrell and Egli, 2010).

2.13 Iron metabolism:-

Iron metabolism is regulated in cells to ensure that iron supplies are adequate and nontoxic because of the Iron is a hazard bio-metal, so it is delivered to tissues by circulating transferring which is a transporter that captures iron released into the plasma mainly from intestinal enterocytes or reticuloendothelial macrophages. The binding of iron-laden transferrin to the cell-surface transferrin receptor 1 results in endocytosis and uptake of the metal. Internalized iron is transported to mitochondria for the synthesis of haem or iron–sulfur clusters, which are integral parts of several metalloproteins, and excess iron is stored and detoxified in cytosolic as ferritin (Wang and Pantopoulos, 2011)

Iron metabolism is balanced by two regulatory systems which are depending on the hormonal of hepcidin and the iron exporter ferroportin (Hentze *et al*., 2010).

2.13.1 Iron exporter ferroportin:-

Bioavailable iron in the diet is mostly present either in its ferric (Fe^{3+}) form or as heme. The uptake of ferric iron is mediated by a combination of ferric reductase (duodenal cytochrome *b*), and a ferrous iron transporter (divalent metal transporter 1 (DMT1)). The ferric reductase reduces the ferric iron to its ferrous (Fe^{2+}) form, while the DMT1 moves the iron across the cell membrane (Gulec *et al*., 2014).

Depending on the cell type, iron can be taken up by several distinct pathways. The macrophages that recycle iron from senescent erythrocytes are phagocytes the erythrocytes firstly, then lyse them, and eventually extract the iron from hemoglobin using heme oxygenase. Other cells import iron using transferrin receptors (TfRs) that capture and endocytose diferric transferrin and then use low vacuolar pH to strip the iron from the transferrin-TfR complex. The transport of iron across vacuolar membranes of macrophages probably involves DMT1. In the cytoplasm, iron is stored bound to ferritin. Ferroportin is the sole known as exporter of iron in all of these cell types, and it requires a ferroxidase which is hephaestin in enterocytes and

ceruloplasmin in macrophages to deliver ferric iron to transferring (Korolnek and Hamza, 2015).

2.13.2 Hepicidin:-

Hepcidin is a 25-amino acid small peptide hormone synthesised in the liver. It is the principal regulator of systemic iron homeostasis. It controls the plasma iron concentration and tissue distribution of iron by inhibiting intestinal iron absorption, iron recycling by macrophages, and iron mobilization from hepatic stores. It acts by inhibiting cellular iron efflux through binding to and inducing the degradation of ferroportin (Nemeth and Ganz, 2006).

Synthesis of hepcidin is homeostatically increased by iron loading and decreased by anemia and hypoxia. Hepcidin is also elevated during infections and inflammation, causing a decrease in serum iron levels and contributing to the development of anemia of inflammation. At the opposite side of the hepcidin deficiency appears to be the ultimate cause of most forms of hemochromatosis either due to mutations in the hepcidin gene itself or due to mutations in the regulators of hepcidin synthesis (Nemeth and Ganz, 2006).

The hepicidin also regulates the expression of ferroportin on the cell membranes. When iron stores are adequate or high, the liver produces hepcidin which circulates to the small intestine where causes for the ferroportin to be internalized, blocking the sole pathway for the transfer of iron from enterocytes to plasma. When iron stores are low, hepcidin production is suppressed and ferroportin molecules are displayed on basolateral membranes of enterocytes, and there they transport iron from the enterocyte cytoplasm to plasma transferring. Similarly, the hepcidin-ferroportin interaction also explains how macrophage recycling of iron is regulated so in the presence of hepcidin, the ferroportin is internalized, iron export is blocked, and iron is trapped within macrophages (Ganz and Nemeth, 2006).

2.14 Iron storage:-

Mostly of iron is stored in the liver as ferritin or hemosiderin. Ferritins are major classical iron storage and mineralization proteins that have capacity of about 4500 ferric irons ions per protein molecule in which the iron is stored within the protein shell of ferritin as a hydrous ferric oxide nanoparticle with a structure similar to that of the mineral “ferrihydrite”. The eight hydrophilic channels that traverse the protein shell are thought to be the primary avenues by which iron gains entry to the interior of

eukaryotic ferritins. Twenty-four subunits constitute the protein shell are two types H and L which have complementary functions in iron uptake. The H chain contains a dinuclear ferroxidase site that is located within the four-helix bundle of the subunit; it catalyzes the oxidation of ferrous iron by O_2, producing H_2O_2. The L subunit lacks this site but contains additional glutamate residues on the interior surface of the protein shell which produce a microenvironment that facilitates mineralization and the turnover of iron (III) at the H subunit ferroxidase site. If the capacity for storage of iron in ferritin is exceeded, a complex of iron with phosphate and hydroxide forms formed and it is called hemosiderin (Wang et al., 2014)

Ferritin is considered the major iron storage protein which maintains a large iron core in its cavity (Arosio *et al*., 2017).

Excess of the body iron beyond the normal levels that can lead to excess of hemosiderin deposition and stored in many organs especially the liver, heart and pancreas then can lead to impair the functions of these organs and eventually death (Gujja *et al*., 2010).

2.15 Anemia and its causes:-

Anemia describes as a condition in which the blood cells have lower amount of Hb than the normal. It is usually classified as either chronic or acute. Chronic anemia occurs over a long period of time while the acute anemia occurs quickly which assists doctors in finding the cause. This also helps to predict how severe the symptoms of anemia may be. In chronic anemia, symptoms typically begin slowly and progress gradually; whereas in acute anemia symptoms can be abrupt and more distressing. World Health Organization (WHO) estimates that two billion people are anemic worldwide and attribute approximately 50% of all anemias to iron deficiency. It occurs at all stages of the life cycle but is more prevalent in pregnant women and young children (Abbaspour *et al*., 2014).

Anemia is the result of a wide variety of causes that can be isolated, but more often coexist. Some of these causes include the following:-

2.15.1 Iron deficiency anemia:-

It is the most significant and common cause of anemia (Abbaspour *et al*, 2014). When iron intake is limited or inadequate due to poor dietary intake that may be causing anemia. Iron deficiency anemia can also occur when there are stomach ulcers or other

sources of slow and chronic bleeding such as colon cancer, uterine cancer, intestinal polyps and hemorrhoids (Johnson-Wimbley, 2011).

2.15.2 Anemia of chronic disease:-

This type of anemia is the second most prevalent after anemia caused by iron deficiency and develops in patients with acute or chronic systemic illness or inflammation (Zarychanski and Houston, 2008)

This condition is termed anemia of inflammation due to elevated hepcidin which blocks both the recycling of iron from the macrophages and iron absorption (Weiss and Goodnough, 2005).

2.15.3 Anemia from active bleeding:-

This anemia is resulting from loss of blood through heavy menstrual cycle and from gastrointestinal ulcers or cancers such as cancer of the colon (Weiss and Goodnough, 2005).

2.15.4 Anemia related to kidney disease:-

The kidneys release a hormone called erythropoietin that helps the bone marrow to make RBCs. In people with chronic kidney disease, the production of this hormone is diminished leading to turn diminishes the production of RBCs causing anemia (O'Mara, 2008).

So the deficiency of erythropoietin is the primary cause of anemia in chronic renal failure. (Nurko, 2006)

2.15.5 Anemia related to pregnancy:-

They account for 75% of all anemias as Iron deficiency anemia in pregnancy due to gain in plasma volume during pregnancy that dilutes the RBCs and may be reflected as anemia (Sifakis and Pharmakids, 2000).

2.15.6 Anemia related to poor nutrition:-

Vitamins and minerals are required to make RBCs. In addition to iron, vitamin B12, viamin A, folate, riboflavin, and copper are required for the proper production of hemoglobin. Deficiency in any of these micronutrients may cause anemia because of inadequate production of RBCs. Poor dietary intake is an important cause of low vitamin levels and therefore anemia (Abbaspour *et al*., 2014).

2.15.7 Obesity and anemia:-

Obesity is characterized by chronic, low-grade, systemic inflammation, elevated hepcidin which in turn has been associated with anemia of chronic disease (Ausk and Loannou, 2008).

2.15.8 Alcoholism:-

Alcohol has numerous adverse effects on the various types of blood cells and their functions. Alcoholics frequently have defective RBCs that are destroyed prematurely. Alcohol itself may also be toxic to the bone marrow and may slow down the RBCs production (Niemelä and Parkkila, 2014)

2.15.9 Sickle cell anemia:-

Sickle cell anemia is one of the most common inherited diseases. It is a blood-related disorder that affects the hemoglobin molecule and causes the entire blood cell to change shape under stressed conditions. In this condition, the hemoglobin problem is qualitative or functional. Abnormal hemoglobin molecules may cause problems in the integrity of the RBC structure and they may become crescent-shaped (sickle cells). There are different types of sickle cell anemia with different severity levels. It is particularly common in African, Middle Eastern, and Mediterranean ancestry (Cox *et al*., 2012).

2.15.10 Thalassemia:-

It is the most common-inherited single-gene disorders in the world related to Hb group disorders. It is resulting from the absence of or errors in genes responsible for production of hemoglobin synthesis which are α and β structures. There are many types of thalassemia, which vary in severity from mild (thalassemia minor) to severe (thalassemia major). These are also hereditary, but they cause quantitative hemoglobin abnormalities, meaning an insufficient amount of the correct hemoglobin type molecules (Ganz and Nemeth, 2006).

2.15.11 Aplastic anemia:-

Aplastic anemia is a disease in which the bone marrow is destructed and the production of blood cells is diminished (Segel *et al*., 2010).

Aplastic anemia causes a deficiency of all three types of blood cells (pancytopenia) including RBCs (anemia), white blood cells (leukopenia), and platelets (thrombocytopenia) (Young *et al*., 2006).

2.15.12 Hemolytic anemia:-

Hemolytic anemia is a type of anemia characterized by the rupture of RBCs which known as hemolysis and in which the RBCs are destroyed faster than the bone marrow that can replace them. It is often categorized as acquired or hereditary. Common acquired causes of hemolytic anemia are autoimmunity, microangiopathy, and infection. Common hereditary causes are disorders of RBC enzymes, membranes, and hemoglobin (Dhaliwal *et al*., 2004).

2.16 Iron deficiency anemia:-

Iron deficiency is defined as a condition in which there are no mobilizablation of iron stores (WHO, 2001).

2.16.1 Causes of iron deficiency:-

The primary causes of iron deficiency include low intake of bioavailable iron, increased iron requirements as a result of rapid growth, pregnancy, menstruation, and excess blood loss caused by pathologic infections, such as hook worm and whipworm causing gastrointestinal blood loss and impaired absorption of iron (Larocque *et al*., 2005).

The frequency of iron deficiency rises in female adolescents because menstrual iron losses are superimposed with needs for rapid growth (Harvey *et al*., 2005).

Other risk factors for iron deficiency in young women are high parity, use of an intrauterine device, and vegetarian diets (Abbaspour *et al*., 2014).

Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorption from the diet. Dietary iron bioavailability is low in populations consuming monotonous plant-based diets with little meat (Larocque *et al*., 2005).

2.16.2 Clinical finding:-

Iron-deficiency anemia is frequently asymptomatic and thus may often go undiagnosed. Weakness, fatigue, difficulty in concentrating, and poor work productivity are nonspecific symptoms. Iron deficiency has been making to decrease cognitive performance and to delay mental and motor development in children. In pregnancy, the iron deficiency is associated with an increased risk of preterm labor, low neonatal weight, and increased newborn and maternal mortality. Iron deficiency may predispose a person to infections, precipitate heart failure, and cause restless leg syndrome (Anker *et al*., 2009).

2.16.3 Laboratory diagnosis of IDA:-

The evaluation of the cause of anemia includes a complete blood cell count, peripheral smear, reticulocyte count, serum iron studies and bone marrow biopsy (Bermejo and Garcia-Lopez, 2009).

The complete blood cell count shows low Hb values than normal range which is less than 7.7 mmol/l (13 g/dl) in men and 7.4 mmol/l (12 g/dl) in women according to The World Health Organization. Also, it shows low in the mean corpuscular volume that measures the average of red blood cell volume and the mean corpuscular hemoglobin concentration which measures the concentration of hemoglobin in a given volume of packed red blood cells than the normal range. The normal reference ranges for mean corpuscular volume is 80–100 fL and for the mean corpuscular hemoglobin concentration is 320–360 g/l. The reticulocyte is useful test because it reflects of the iron available in the bone marrow for erythropoiesis and the normal range less than 2% in blood circulation Iron deficiency anemia is characterized by microcytic, hypochromic erythrocytes peripheral blood picture and low iron stores in the bone marrow (Bermejo *et al*., 2009).

Iron studies is the diagnostic for iron deficiency anemia that consist of a low serum iron (<7.1 µg/l), a low serum ferritin (storage form of iron) (<30 ng/l), a low transferrin saturation (<15%), and a high total iron-binding capacity (>13.1 µmol/l) (Bermejo *et al*., 2009).

2.17 Immunity:-

Immunity referred to the protection from disease and specifically from infectious disease. The cells and molecules that responsible for immunity constitute the immune system, and their collective and coordinated response to the introduction of foreign substances is called the immune response. Immunology is the study of immune responses in this broader sense and of the cellular and molecular events that occur after an organism encounters microbes and other foreign macromolecules.The physiologic function of the immune system is defense against infectious microbes (aboabbas, 2015).

The immune system has two major pillars which are the innate and adaptive immune system. Innate immune system is the generalized defense while the adaptive is specialized defense. Both systems work closely together and take on different tasks (Medzhitov and Janeway, 2000).

The first line of immune defense is innate immune system which is based on detection of pathogen-associated molecular patterns (PAMPs) that evoke toxic and inflammatory response which recognizes the antimicrobial peptides through the Pattern recognition receptors constituting the bulwark of host defenses in invertebrates. The germline genome encodes the mediators of innate immunity, obviating the need for a complicated somatic differentiation process. Constitutive expression by many cell types of the rather limited set of pattern recognition receptors obviates the need for clonal expansion of specific cells. Then, many of the effector responses elicited by innate detection mechanisms are mediated via a single signal transduction mechanism (Medzhitov and Janeway, 2000).

Adaptive immunity recognizes specific molecular structures and depends on the generation of large numbers of antigen receptors, ie, T-cell receptors (TCRs) and immunoglobulins, by somatic rearrangement processes in blast cells. Once T cells recognize foreign antigens presented to them, they initiate adaptive immune responses against precisely these antigens. These responses include direct attack of antigen-bearing cells by cytotoxic T lymphocytes, stimulation of B cells to produce antibodies against the antigens, and induction of inflammation, with enhanced innate responses, in the area where the antigen is present. All these responses cooperate during host defenses to eliminate the foreign particle or microorganism. When expressed inappropriately, they can give rise to autoimmune diseases or allograft rejection (Hansson *et al*., 2002).

Adaptive immunity comprises defense mechanisms mediated by immune cells known as lymphocytes (T, B, and natural killer cells) and the specialized molecules required for their function. The term *adaptive* is applied because lymphocytes rapidly adapt to the situation at hand (*e.g.*, a specific type of microbial infection) generating specialized cells, cytokines, and antibodies as well as long-lasting immunologic memory (Yatim and Lakkis, 2015).

2.18 Cytokines:-

Cytokines are small secreted proteins released by cells have a specific effect on the interactions and communications between cells. They have numerous names include the lymphokines that made by lymphocytes, monokines which made by monocytes, chemokine which means that cytokines with chemotactic activities, and the

interleukins which are cytokines made by one leukocyte and acting on other leukocytes (Zhang and Jianxiong, 2007).

Cytokines are made by many cell populations, but the predominant producers are helper T cells (Th) and macrophages (Xie *et al*., 2006).

Cytokines may act on the cells that secrete them called autocrine action, or oaan nearby cells named paracrine action, or in some instances on distant cells which called endocrine action (Zhang and Janxiong, 2007).

2.18.1 Pro-inflammatory cytokines:-

Proinflammatory cytokines are produced predominantly by activated macrophages. They are involved in the up-regulation of inflammatory reactions such as IL-1β, IL-6, and TNF-α (Copray *et al*., 2001).

IL-1β is released primarily by monocytes and macrophages as well as by nonimmune cells, such as fibroblasts and endothelial cells, during cell injury, infection, invasion, and inflammation (Copray *et al*., 2001).

IL-6 has been shown to play a central role in the neuronal reaction to nerve injury (Niemelä *et al*., 2012).

TNF-α, also known as cachectin, is another inflammatory cytokine that plays a well-established, key role in some pain models. TNF acts on several different signaling pathways through two cell surface receptors, TNFR1 and TNFR2 to regulate apoptotic pathways (Zhang and Janxiong, 2007).

2.18.2 Chemokines:-

These factors represent a family of low molecular weight secreted proteins that primarily function in the activation and migration of leukocytes although some of them also possess a variety of other functions. For example MIP-1α and MCP-1 are up-regulated not only in models of neuroinflammatory and in injured peripheral nerve (White *et al*., 2005).

2.18.3 Anti-inflammatory cytokines:-

They are series of immunoregulatory molecules that control the pro-inflammatory cytokine response. They act in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response. Also, there is a physiologic role in inflammation and pathologic role in systemic inflammatory states (Wieseler Frank *et al*., 2004).

Major anti-inflammatory cytokines include interleukin (IL)-1 receptor antagonist, IL-4, IL-10, IL-11, and IL-13. Leukemia inhibitory factor, interferon-alpha, IL-6, and transforming growth factor (TGF)-β are categorized as either anti-inflammatory or pro-inflammatory cytokines, under various circumstances. Specific cytokine receptors for IL-1, TNF-α, and IL-18 also function as inhibitors for pro-inflammatory cytokines (Wieseler Frank *et al*., 2004).

Among all the anti-inflammatory cytokines, IL-10 is a cytokine with potent anti-inflammatory properties, repressing the expression of inflammatory cytokines such as TNF-α, IL-6 and IL-1 by activated macrophages. In addition, IL-10 can up-regulate endogenous anti-cytokines and down-regulate pro-inflammatory cytokine receptors. Thus, it can counter-regulate production and function of pro-inflammatory cytokines at multiple levels (Wieseler Frank *et al*., 2004).

The family of TGF-β comprises 5 different isoforms (TGF-β1 to -β5) (Dobolyi *et al*., 2012) inhibiting macrophage and Th1 cell activity; counteracts IL-1, IL-2, IL-6, and TNF; and induces IL-1ra 6 (Dinarello, 2018).

2.19 Interleukins:-

Interleukins (ILs) are a type of cytokine produced by many body cells. They play essential roles in the activation and differentiation of immune cells, as well as proliferation, maturation, migration, and adhesion. They also have pro-inflammatory and anti-inflammatory properties. The primary function of interleukins is to modulate growth, differentiation and activation during inflammatory and immune responses (Zhu *et al*., 2017),

Interleukins consist of a large group of proteins that can elicit many reactions in cells and tissues by binding to high-affinity receptors in cell surfaces. They have both paracrine and autocrine function (Zhu *et al*., 2017).

Table (2-1):-Interleukins that are made by many cell populations and producing many functions: (Akdis et al., 2016)

ILs	Principal Source	Primary Activity
IL-1α IL-β	Antigen presenting cells (APCs)	Co stimulation of APCs and T cells. Inflammation and fever. Acute phase response. Hematopoietic.
IL-2	Activated Th1 cells, NK cells	Proliferation of Bcells and activated T cells, NK functions.

ILs	Principal Source	Primary Activity
IL-3	Activated T cells	Growth of hematopoietic progenitor cells.
IL-4	Activated T cells	B cell proliferation. eosinophil and mast cell growth and function. IgE and class II MHC expression on B cells. Inhibition of monokine production
IL-5	Th2 and mast cells	Eosinophil growth and function
IL-6	Activated Th2 cells and APCs	Acute phase response, B cell proliferation, thrombopoiesis, synergistic with IL-1and TNF on T cells.
IL-7	Thymic and marrow stromal cells	T and B lymphopoiesis
IL-8	macrophages, somatic cells	Chemoattractant for neutrophils and T cells
IL-9	T cells	Hematopoietic and thymopoietic effects
IL-10	Activated Th2 cells, CD8+ T and B cells, macrophages	Inhibits cytokine production. Promotes B cell proliferation and antibody production. Suppresses cellular immunity, mast cell growth
IL-11	Atromal cells	Synergistic hematopoietic and thrombopoietic effects
IL-12	B cells, macrophages	Proliferation of NK cells, IFN production, promotes cell-mediated immune functions
IL-13	Th2 cells	IL-4-like activities
IL-18	Macrophages	potent inducer of interferon by T cells and NK cells
IFN-α IFN-β	Macrophages, neutrophils and some somatic cells	Antiviral effects, induction of class I MHC on all somatic cells, activation of NK cells and macrophages

ILs	Principal Source	Primary Activity
IFN-γ	Activated Th1 and NK cells	Induces of class I MHC on all somatic cells, induces class II MHC on APCs and somatic cells. activates macrophages, ,NK cells neutrophils, promotes cell-mediated immunity, antiviral effects
MIP-1α	Macrophages	Chemotaxis
MIP-1β	Lymphocytes	Chemotaxis
TGF-β	T cells, monocytes	Chemotaxis, IL-1 synthesis, IgA synthesis, inhibit proliferation
TNF-α	macrophages, mast cells, NK cells, sensory neurons	Cell death, inflammation, pain
TNF-β	Th1 and Tc cells	phagocytosis, NO production, cell death
GM-CSF	Th cells	Growth and differentiation of monocytes and dendritic cells

2.20 Interleukin 10:-

Interleukin-10 (IL-10) is a Type II cytokine and the 'founding' member of a family of cytokines that include IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. All of these cytokines have similar intron–exon genomic organization, bind to receptors with similar structures and in some cases shared components, and all activate Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathways (David and Zhang, 2008).

Interleukin 10 (IL-10) is a potent anti-inflammatory cytokine that plays a crucial, and often essential, role in preventing inflammatory and autoimmune pathologies (Sabat *et al*., 2010).

In vivo, major sources of IL-10 include T helper cells, B cells, cytotoxic T cells, macrophages, monocytes, dendritic cells, NK cells, mast cells, and granulocytes like neutrophils and eosinophils (Jung *et al*., 2004).

The main biological function of IL-10 appears to be exerted on DCs and macrophages because it inhibits the differentiation of DCs from monocyte precursors, and it also inhibits DC maturation. IL-10 is a potent inhibitor of antigen presentation which inhibits major histocompatibility complex class II expression as well as the upregulation of costimulatory molecules CD80 and CD86 to prevent the production of the Th1-associated cytokines IL-2 and interferon-γ (IFN-γ), and also the Th2-associated IL-4 and IL-5. The other profound effect of IL-10 is to inhibit the production of proinflammatory cytokines and mediators from macrophages and DCs. The major inflammatory cytokines are IL-1, IL-6, IL-12, and tumor necrosis factor (TNF) which are all dramatically repressed following exposure to IL-10 (Akdis and Blaser, 2001).

Interleukin-10 (IL-10) is an inflammatory inhibitor, but with an unclear function in the regulation of hepcidin expression (Huang *et al*., 2017). The effect of IL-10 on hepcidin induction was mediated via STAT3-phosphorylation that was inhibited with a specific inhibitor of STAT3 phosphorylation (Drakesmith *et al*., 2005).

3.1 study design:

This is prospective, analyttical case control study.

3.2 Study area, setting and duration:

Study was conducted in Military Hospital in Khartoum state during the period from March to November (2019).

3.3 Study population:

Study population consisted of 88 Sudanese individuals of age between 18-45 years, divided into 2 groups as follow IDA made up of 44 patients and 44 control group.

3.4 Inclusion cretaria:-

The anemic patients who are suffering from iron deficiency with abnormal Hb, HCT and RBCs indicies. As well as the peripheral blood picture that was diagnosed by clinician of hematology pathologist shows the RBCs are microcytic hypochromic with pencil cells to exclude the other causes of microcytic hypochromia. The control group apparently healthy individuals and normal included.

3.5 Exclusion criteria:-

Generally, any disease that can affect on cytokine level including physiological factors such as smoking and alcoholism. As well as the other diseases such as autoimmune diseases, allergy, infectious diseases and hypersensitivity were excluded.

3.6 Ethical consideration:-

Permission and written consent were taken from participants after being informed with all details of objectives and benefits of the study with simple dialog.

3.7 Sampling:-

Under sterile condition with 70% ethanol, 3ml of venous blood was collected from superficial vein of volunteers by using sterile disposable plastic syringes in Ethylene diamine tetra acetic acid (EDTA) containers. The plasma of samples is separated in cryo-tube containers and preserved in the refrigerator under -20^{o}c.

3.8 Sample size:-

The sample size was set as 88 (44 of them diagnosed as IDA and the rest of them as normal).

3.9 Hematological techniques:

3.9.1 sysmex automated Principle:-

The cells suspended in an electerically diluents increase the resistance between two electrodes when passing through a sensing aperture. The impedence of the direct current (DC) creates measurable vltage pulses. The size of pulse generated by the cell proportion to it is volume. The cell cunt is determinrd by number of pulses generated. (anne *et al*.,1998)

3.9.2 Procedure:

The blood sample was mixed, then by machine the accurate volume was absorbed into machine and the result was out printed in paper (Bain *et al*., 2017)

3.9.3 Reagents:

The regents that was used by analyzer was cetrimide, 10% formaldehyde, glacial aceticacid, NaCl and water (Bain *et al*., 2017).

3.9.4 Quality Control:

Quality control is intended to ensure that measurements are sufficiently precise day by day or batch by batch within established limit. Result on patient sample should not be issued until it is clear from the control data that there has been not significant problem in the analytic procedure (Bain *et al*., 2017).

The best known method is to test a control sample at intervals a alongside the routine specimens, and to plot the results on a levey- jenning control chart. This linear graph showing the mean and limits of standard deviations (SD) at 1SD and 2SD. The result of sequential (daily) measurements are plotted on the graph, when system is in good control, not more than 1 in 20 measurement should fall outside 2SD. When two or more consecutive measurement are outside this limits there is likely to have been a random error, whereas several consecutive values within 2SD, but all on one side of the mean, indicate a consistent bias. A wildly deviant result outside 3SD may occur as a result of a gross error ('blunder') (Bain *et al*., 2017).

3.10 Principles and procedures of ELISA:-

3.10.1 Principle of ELISA:-

Biolegend's ELISA MAX^{TM} Deluxe Set is a sandwich Enzyme-linkedimmunosorbent Assay (ELISA). A human IL-10 specific monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and IL10 binds to

immobilized capture antibody. Next, a biotinylated anti-human IL10 detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horse reddish peroxidase is subsequently added, followed by TMB substrate solution, producing a blue color in proportion to the concentation of IL10 present in the sample. Finally, the stop solution changes the reaction color from blue to yellow and the microwell absorbance is read at 450 nm with a microplate reader (biolegend USA, 2013)

3.10.2 ELISA Washer Principle:-

First the wash solutiion is pump from the wash bottle, the solution is dispense to the cuvette by short pins, and then the wash liquid is aspirate from the cuvette by the long pins, at the end the waste liquid was pumped into the waste bottle by the vaccum pump. (www.diasource.be, 2019)

3.10.3 ELISA Reader Procedue:-

White light produced by the lamp is focused into a beam by the lens and passes through the sample. Part of the light is absorbed by the sample and the remaining light is transmitted. It is filtered by interference filters and foucsed onto the photodiodes. The photodiode converts the received light into an electerical signal which is transformed into a digtal form, from which the microprocessor calculates the absorbance, taking in account of the blank and dichromatic selection. (www.wordpress.com, 2015)

3.10.4 ELISA procedure:-

100 ML of diluted capture antibody solution was added to all wells of a 96-well plate. The plate sealed and incubated overnight (16-18hrs) between 2^0-8^0C. then plate washed 4times with at least 300ML. wash buffer per well and residual buffer was blotted by fimly tapping plate upside down on absorbent paper. To block non-specific binding and reduce background, 200ML 1X assay diluent was added per well, plate sealed abd incubated at room temperature fot 1 hour with shaking at 200 rpm on a plate shaker. Plate washed 4 times with wash buffer. 50Ml of matrix diluent was added to the standard wells. 50ML of assay diluent A was added to the sample wells. 50ML/well of standards were added to the standard wells. 50ML/well of serum samples were added to the sample wells. Plate sealed and incubated at RT for 2 hours with shaking. Plate washed 4 times with wash buffer. 100ML of diluted detection antibody solution was added to each well, plate sealed and incubated at RT for 1 hour

with shaking. Plate washed 4 times with wash buffer. 100ML of diluted avidin-HRP solution was added to each well, plate sealed and incubated at RT for 30minutes with shaking. Plate washed 5 times with wash buffer for the final wash. Wells weresoaked in wash buffer for 30 seconds in 1 minute for each wash. To minimize background, 100ML of TMB substrate solution was added and incubated in the dark for 15 minutes. Positive wells were turned blue in color. Reaction was stopped by addition of 100ML of stop solution to each well. Positive wells were turned from blue to yellow, absorbance was read at 450nm within 30 minutes. For results calculation the standard curve was plotted on log-log axis graph paper with analyte concentration on the x-axis and absorbance on the y-axis. A best fit line was drawn through the standard points. To determine the unknown analyte concentrations in the samples, the absorbance value of the unknown on the y-axis was found and a horizental line was drawn to the standard curve. At the point of intersection, vertical line was drawn to the x-axis and the corresponding analyte concentration was read. (biolegend USA, 2013)

3.11 Data analysis:-

The data was entered, checked and analyzed by Statistical Package for the Social Sciences (SPSS) version 20 using mean±SD for age, sex, Hb, MCV,MCH MCHC and IL10, T-independent test and probability value ≤ 0.05 was considered significant.

4. Results:-

88 subjects of age between 18-45 years old were enrolled in this study, 44 subjects were IDA patients with mean of age 31.16±7.7 year with percentage of gender equivelent 50% for each male and female. While similar in gender frequency with mean of age 28.30±6.8 for 44 control group, while gender were 1.5±.5 and 1.5±.5 respectivelly for both.

Mean level of Hb(g/dl) was 9.3±1.3 and 13.6in patients with IDA when compared with 13.6±1.3 in healthy one. Mean of HCT (%) was 31.1±4.0 in caes compared to 40.2±4.4 in control. Mean level of MCV (pg),MCH(FL) and MCHC(L/L) in case study group were 70.6±3.4, 21.4±3.4 and 29.1±4.6 respectivelly; while for control group were 86.2±4.8, 29.3±2.2 and 33.6±1.7 respectivelly.

Mean level of IL10(pg/ml) was 3.5±3.9 in case compared 1.90±1.92 in control , with *p.value* was .02.

The *p.value* for correlation of IL10 with Hb, HCT, MCV, MCH MCHC, age and gender were 0.02,0.01, 0.25, 0.16, 0.13, 0.04 and 0.1 respectivelly.

Table (3-1):- mean±SD and p.value of IL10 level in both case study and case control groups:-

Subject	Case study group	Case control group	*P.value*
Mean±SD of IL10	3.5±3.9	1.90±1.92	0.02

p.value ≤0.05=significant

Table (3-2): Correlation between IL10 level with other risk factors:-

Parameters	*p.value*
Hb	0.02
HCT	0.01
MCV	0.25
MCH	0.16
MCHC	0.13
Age	0.04
Gender	0.13

p.value ≤0.05=significant

5.1 Discussion:

In the present study plasma level of IL10 (pg/ml) is significantly higher in IDA patients compared to healthy control group (*p.value* 0.02), this finding was also supporting by Nussenblat and his colleagues who demonstrate the statistical significantly *p.value* < 0.0001 of IL-10 concentration that shows there was a clear association of IL10 to anemia (Nussenblat *et al*., 2001), Ludwiczek *et al* were finding the anti-inflammatory cytokine IL-10 stimulates TfR-mediated iron uptake into activated monocytes which effects on TfR mRNA levels, surface expression, and iron uptake, thus stimulating TfR-mediated iron acquisition with statistical significantly p.value < 0.05 (Ludwiczek *et al*., 2003), the finding also was supported by Bergman *et al* who demonstrated statistical significantly *p.value* < 0.05 that showed the in-vitro cytokine IL10 is higher in production in patients with IDA than controls (Bergman *et al*., 2004), while Kuvibidila and warrier were finding the iron deficiency has a generalized deleterious effect on cells that secrete IL10 cytokine with *p.value* lesser than 0.05 (Kuvibidila and warrier, 2004), as well as Lee *et al* were finding the IL-10 has little or no stimulatory effect (p.value < 0.05) (Lee *et al*., 2005), Bel'mer *et al* showed the significant increases of in-vitro pro-inflammatory cytokine of IL-10 from patients with IDA than control group (p.value < 0.05) (Bel'mer *et al*., 2014), Chaung et al were enrolled the study results that raise the possibility of IL-10 with *P.value* < 0.0001which may play a role in iron homeostasis (Chaung *et al*., 2014) and Huang and his colleagues who demonstrate that IL-10 with *p.value* lesser than 0.05 by ELISA and concluded the IL10 can improve iron metabolism and alleviate anemia via suppressing inflammatory factor, modulating STAT3 signal pathway, down-regulating hepcidin expression and inhibiting TfR expression (Huang *et al*., 2017).

I agree with the all previous studies that showed the alteration of iron deficiency anemia on the plasma level of IL10.

Effects of IL-10 on hematological laboratory variables and other risk factors revealed IL-10 has statistical strong correlation with Hb, HCT and age. While, neither RBCs indices nor gender showed there were negatively correlation changed with plasma IL 10 in patients.

Kuvibidila and his colleagues were finding the negative correlation of IL 10 with indicators of iron status (Kuvibidila *et al*., 2003), as well as Kuvibidila and warrier

were finding there were positively correlated of IL 10 level with indicators of iron status (Kuvibidila and warrier, 2004).

I disagree in the correlation result of IL10 that showed by previous researchers neither in negatively nor positively with iron status, that may be due to collection of sample, storage of sample, environmental situations, or in machines that used.

The correlation of IL 10 with age and gender did not show by any previous studies and the present study is first study showed that.

5.2 Conclusions:-

- Plasma level of IL10 was compared in IDA patients group and healthy subjects group with statistically significant difference (*P.value* 0.016)
- There was no correlation between IL10 level with gender and RBCs indices, but there were strong correlation between IL10 with Hb, HCT and age.
- IL10 can be considered as risk factor for IDA patients.

5.3 Recommendations:-

- The alteration of iron deficiency anemia on the serum level of IL10 should be determined.
- The effectiveness of iron deficiency on the functional immune system should be focusing by doing other measurable tests of functional immune system.
- Large sample size should be used.

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Appendix (1):- Questionnare

Sudan university of sciences and technology

Evaluation of plasma IL10 level in Sudanese Iron Deficiency Anemia Patients, Khartoum State

Date/2019

ID Number…….

Age:……

Sex:…….

Education:……

Occupation:……..

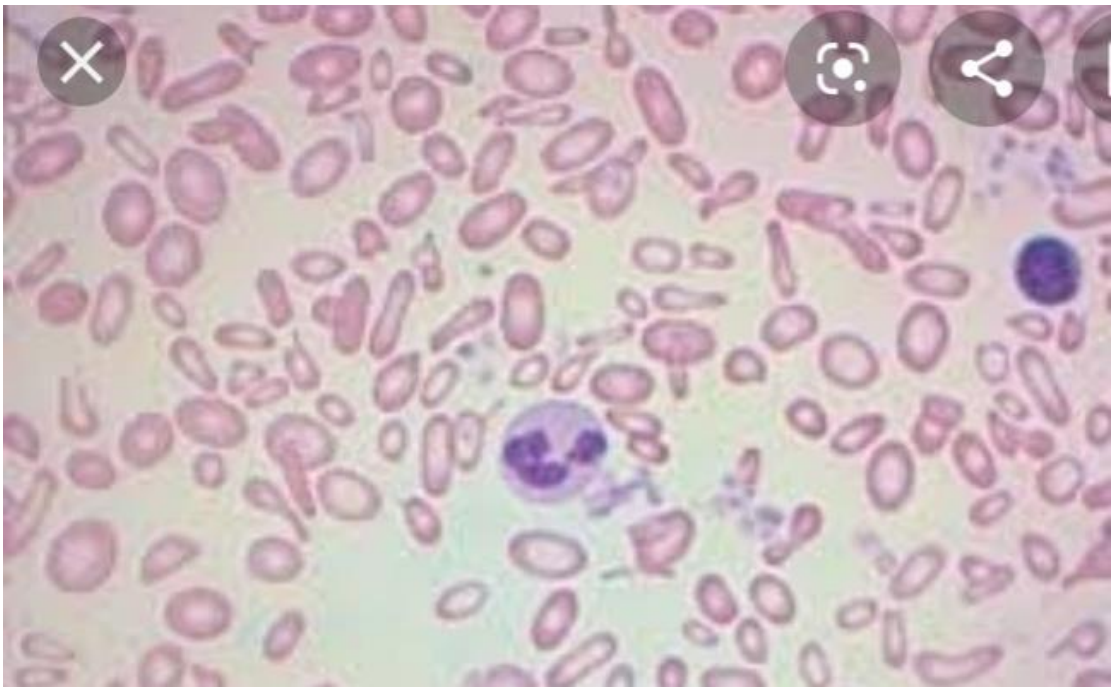
Locality:……

Clinical disease: yes……..(Identify……) No……..

Appendix 2:

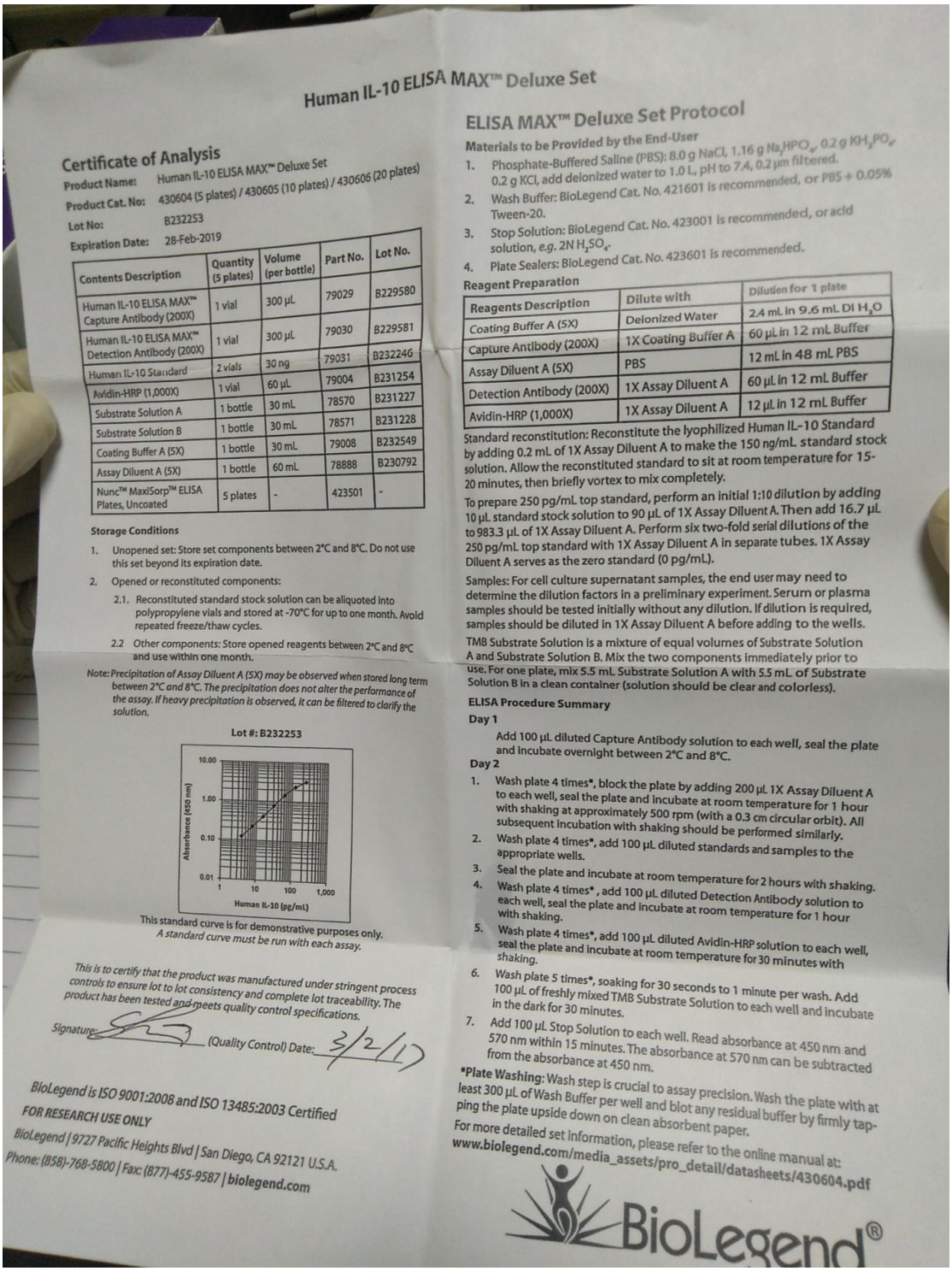
sysmex

Appendix (3):



PBP of IDA

Appendix (4):



Human IL-10 ELISA MAX™ Deluxe Set

Certificate of Analysis

Product Name: Human IL-10 ELISA MAX™ Deluxe Set
Product Cat. No: 430604 (5 plates) / 430605 (10 plates) / 430606 (20 plates)
Lot No: B232253
Expiration Date: 28-Feb-2019

Contents Description	Quantity (5 plates)	Volume (per bottle)	Part No.	Lot No.
Human IL-10 ELISA MAX™ Capture Antibody (200X)	1 vial	300 µL	79029	B229580
Human IL-10 ELISA MAX™ Detection Antibody (200X)	1 vial	300 µL	79030	B229581
Human IL-10 Standard	2 vials	30 ng	79031	B232246
Avidin-HRP (1,000X)	1 vial	60 µL	79004	B231254
Substrate Solution A	1 bottle	30 mL	78570	B231227
Substrate Solution B	1 bottle	30 mL	78571	B231228
Coating Buffer A (5X)	1 bottle	30 mL	79008	B232549
Assay Diluent A (5X)	1 bottle	60 mL	78888	B230792
Nunc™ MaxiSorp™ ELISA Plates, Uncoated	5 plates	-	423501	-

Storage Conditions

1. Unopened set: Store set components between 2°C and 8°C. Do not use this set beyond its expiration date.
2. Opened or reconstituted components:
   2.1. Reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
   2.2 Other components: Store opened reagents between 2°C and 8°C and use within one month.

Note: Precipitation of Assay Diluent A (5X) may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the assay. If heavy precipitation is observed, it can be filtered to clarify the solution.

Lot #: B232253

This standard curve is for demonstrative purposes only.
A standard curve must be run with each assay.

This is to certify that the product was manufactured under stringent process controls to ensure lot to lot consistency and complete lot traceability. The product has been tested and meets quality control specifications.

Signature: _____ *(Quality Control) Date:* 3/2/17

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ELISA MAX™ Deluxe Set Protocol

Materials to be Provided by the End-User

1. Phosphate-Buffered Saline (PBS): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1.0 L, pH to 7.4, 0.2 µm filtered.
2. Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20.
3. Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, *e.g.* 2N H_2SO_4.
4. Plate Sealers: BioLegend Cat. No. 423601 is recommended.

Reagent Preparation

Reagents Description	Dilute with	Dilution for 1 plate
Coating Buffer A (5X)	Deionized Water	2.4 mL in 9.6 mL DI H_2O
Capture Antibody (200X)	1X Coating Buffer A	60 µL in 12 mL Buffer
Assay Diluent A (5X)	PBS	12 mL in 48 mL PBS
Detection Antibody (200X)	1X Assay Diluent A	60 µL in 12 mL Buffer
Avidin-HRP (1,000X)	1X Assay Diluent A	12 µL in 12 mL Buffer

Standard reconstitution: Reconstitute the lyophilized Human IL-10 Standard by adding 0.2 mL of 1X Assay Diluent A to make the 150 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.

To prepare 250 pg/mL top standard, perform an initial 1:10 dilution by adding 10 µL standard stock solution to 90 µL of 1X Assay Diluent A. Then add 16.7 µL to 983.3 µL of 1X Assay Diluent A. Perform six two-fold serial dilutions of the 250 pg/mL top standard with 1X Assay Diluent A in separate tubes. 1X Assay Diluent A serves as the zero standard (0 pg/mL).

Samples: For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. Serum or plasma samples should be tested initially without any dilution. If dilution is required, samples should be diluted in 1X Assay Diluent A before adding to the wells.

TMB Substrate Solution is a mixture of equal volumes of Substrate Solution A and Substrate Solution B. Mix the two components immediately prior to use. For one plate, mix 5.5 mL Substrate Solution A with 5.5 mL of Substrate Solution B in a clean container (solution should be clear and colorless).

ELISA Procedure Summary

Day 1

Add 100 µL diluted Capture Antibody solution to each well, seal the plate and incubate overnight between 2°C and 8°C.

Day 2

1. Wash plate 4 times*, block the plate by adding 200 µL 1X Assay Diluent A to each well, seal the plate and incubate at room temperature for 1 hour with shaking at approximately 500 rpm (with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.
2. Wash plate 4 times*, add 100 µL diluted standards and samples to the appropriate wells.
3. Seal the plate and incubate at room temperature for 2 hours with shaking.
4. Wash plate 4 times* , add 100 µL diluted Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour with shaking.
5. Wash plate 4 times*, add 100 µL diluted Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes with shaking.
6. Wash plate 5 times*, soaking for 30 seconds to 1 minute per wash. Add 100 µL of freshly mixed TMB Substrate Solution to each well and incubate in the dark for 30 minutes.
7. Add 100 µL Stop Solution to each well. Read absorbance at 450 nm and 570 nm within 15 minutes. The absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

***Plate Washing**: Wash step is crucial to assay precision. Wash the plate with at least 300 µL of Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on clean absorbent paper.

For more detailed set information, please refer to the online manual at:
www.biolegend.com/media_assets/pro_detail/datasheets/430604.pdf

BioLegend®

Leflet-working

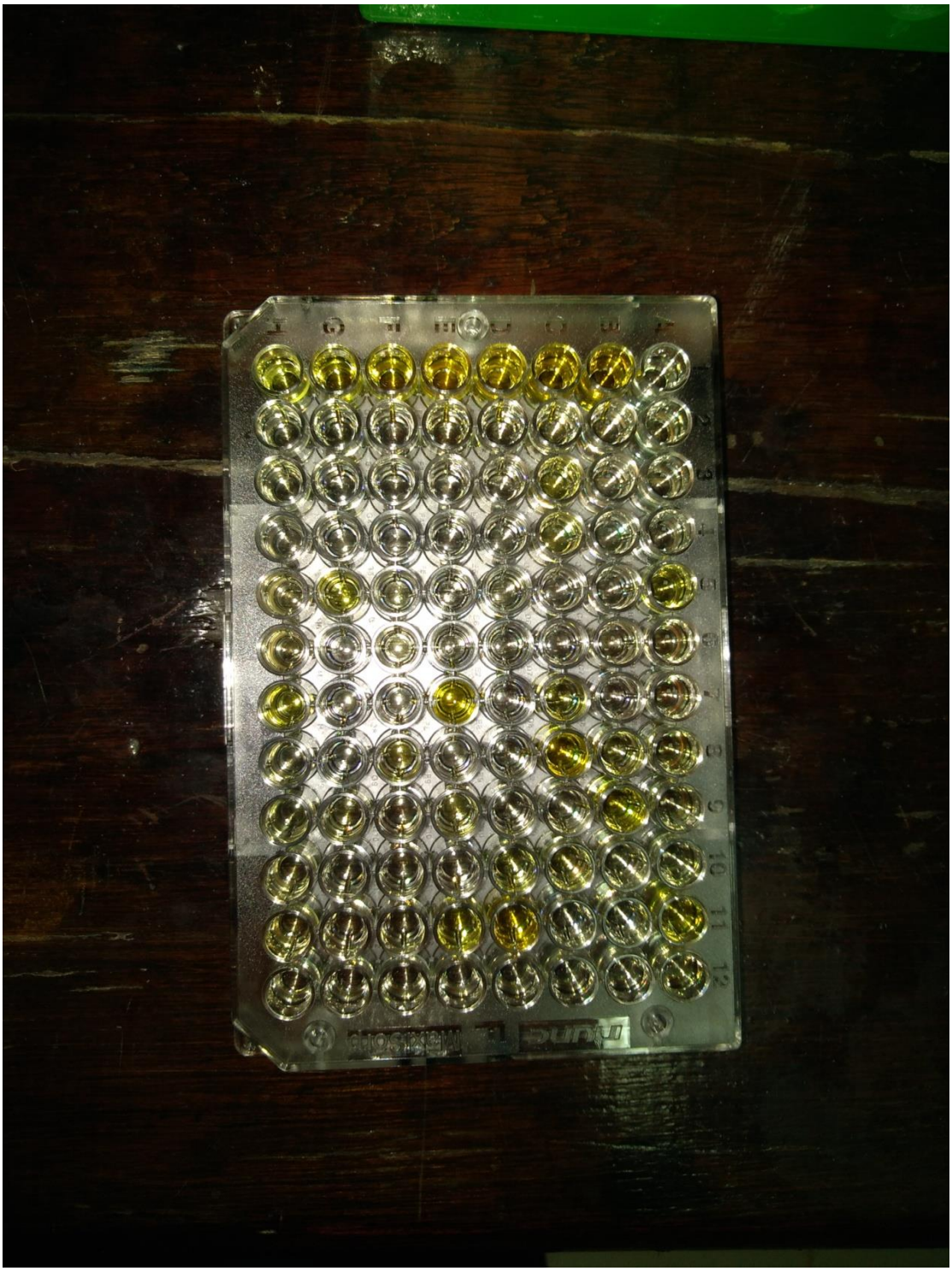
Appendix (5a):

ELISA Washer

Appendix (5b):

ELISA Reader

Appendix (6):



plate