Introduction and literature review.

1-1-General introduction;
Malaria remains the most serious and widespread protozoal infection of humans. Over 40% of the world’s population is at risk of contracting malaria, which is endemic in 91 countries, mostly developing. Malaria was defined as a “typical blood disease” characterized by fever, anemia and splenomegaly. \(^1\)

It is currently considered a typical example of a hemolytic anemia in more recent hematology textbooks, due to an acquired extra-corpuscular cause. As parasites of the blood for the majority of their complex life cycle, they expectedly induce hematological alterations. The hematological abnormalities that have been reported to invariably accompany infection with malaria include anemia, thrombocytopenia, splenomegaly, and mild-to-moderate atypical lymphocytosis and rarely disseminated intravascular coagulation (DIC). \(^2\)

There have also been reports of leucopenia and leucocytosis. Other hematological reactions to malaria that have been reported include neutropenia, eosinophilia, neutrophilia and monocytosis. \(^3\)

Some controversies appear to exist; however, many of the studies on the hematological abnormalities have been conducted in endemic countries, some only in children and some only in severe malaria patients. \(^4\)

Relatively few studies have been done among non-immune or semi-immune travelers returning from endemic areas or patients returning from their endemic countries. \(^3(5)\)
1-2- Literature review.

1-2-1- Blood constituent and function:

Blood is specialized body fluid that delivers substances to the body cells such as nutrients and oxygen and transports waste products away from those some cells.\(^{(6)}\)

It is composed of blood cells suspended in liquid called plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains dissipated proteins, glucose, mineral iron, hormones, carbon dioxide. The blood cells present in blood are mainly red blood cells (also called erythrocyte), white blood cells, and platelets. The most abundant cells in blood are red blood cells. These certain haemoglobin, an iron containing protein, which facilitates transportation of oxygen by reversibly binding to respiratory gas and greatly necessary it is solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellularly dissolved in plasma as bicarbonate iron.\(^{(6)}\)

The average person has approximately 70ml blood per kilogram body weight (70mm/kg) or 5L total for 70-kg. Approximately 50-60% of the blood volume is liquid body weight, the remainder is the cells. The liquid component called plasma is nearly 90% water. The remaining 10% includes iron, glucose, amino acid and other metabolites, hormone and various proteins.\(^{(6)}\)

1-2-1-1- Erythrocyte (Red Blood Cell):

1-2-1-1-1- production:

Bone marrow

Normoblast terminology refers to nucleated erythrocyte (NRBC) and is more descriptive. The rubriblast nomenclature is the previous terminology which does not describe the cell.\(^{(7)}\)
1-2-1-1-2-Maturation:
- Pronormblast (rubriblast)- large, with large nucleus containing nucleoli; minimal dark blue cytoplasm; nuclear to cytoplasm (N:C) ratio of 6:1
- Basophilic normoblast (proruriblast)-slightly smaller than the previous cell; large nucleus with no visible nucleoli; more dark blue cytoplasm; N:C of 1:4.
- Polychromatophilic normoblast (rubricyte)-medium sized cell: a smaller, more condensed nucleus; first identifiable haemoglobin (Hb) synthesis that result in a light blue–gray abundant cytoplasm.
- Orthochromatophilic normoblast (metarubticyte)-smaller size, small pycnotic(degenerated) nucleus ; and abundant cytoplasm that is slightly blue – gray. This is the last nucleated stage of erythrocyte maturation.
- Reticulocyte- slightly larger than the mature red blood cell may be blue; polychromasia, due to remnant of RNA and less Hb; no nucleus; confirm as a reticulocyte only if stained with a reticulocyte stain.
- Mature erythrocyte 6 to 8 micro meter in diameter; 80-95 fl volume; biconcave; no nucleus; most dense of all blood cells due to hemoglobin content. (7)

1-2-1-1-3-Function:
Transport oxygen from the lung to the body tissues.

1-2-1-1-4-Major regulatory factor:
Erythropoietin (EOP).

1-2-1-1-5-Reference range:
4.2 to 6.2 x 10^6 mm or 4.0-5.0 x 10^{12} /l . (7)

1-2-1-2- Leukocytes (WBC):
The white blood cells (leucocytes) may be divided into two broad groups: the phagocytes and the immunocytes. Granulocyte, which include three types of cell-neutrophils (polymorphs) , eosinophils and basophils- together monocytes comprise the phagocytes. (7)
1-2-1-2-1-WBC functions:
1- Rid the body of invading organisms.
2- Immune response.
3- Mediate hypersensitivity reactions protein regulators: leukopoiesis.
Sophisticated haematology analyzers produce the total WBCs count and five part WBCs differential (percentage and absolute numbers of each cell type, but the absolute numbers is really more relevant than percent. (7)

1-2-1-2-2-Reference range:
4500 to 11,000 WBC/mm$^3$ (4.5-11 x 10$^9$/l). (7)

1-2-1-3-Thrombocyte (platelets):
1-2-1-3-1-production:
bone marrow
1-2-1-3-2-Maturation:
Megakaryoblast: medium to large, with a larger round nucleus containing nucleoli and light blue cytoplasm. The morphology is similar to that of myeloblast of leukocytes.
Promegakaryocyte: larger than the blast, multilobed nucleus, gray cytoplasm with purple granules.
Megakaryocyte- larger than the promegakaryocyte with multilobed nucleus and an increase amount Of DNA (polyploid). The cytoplasm is gray with purple granules, especially around the periphery of the cell where the mature thrombocytes are developing.
Mature thrombocyte- 2-4 micro meter in diameter .9 fl volumes, a nucleated disk gray irregular cytoplasmic fragment with purple granules. (7)

1-2-1-3-3-Function:
Respond to a break in the vasculature (blood vessels) by adhering to the site, releasing regulatory factors such as phospholipid (PF3), which provides a surface for coagulation pathway reactions to enhance the
formation of thrombus (clot) m aggregating to form an initial plug and secondary retraction to prevent blood from escaping from the injured blood vessel. 

1-2-1-3-4-Platelet structure:
Platelets are extremely small and discoid, 3.0 x0.5 µm in diameter, with a mean volume 7-11 fL. The ultrastructure of platelets is represented in the granules that discharged into the open canalicular system. Platelets are also rich in signalling and cytoskeletal proteins which support the rapid switch from quiescent to activation that follows vessel damage. 

![Platelet structure diagram]

**Figure 1-1: platelet structure**

1-2-1-3-5-Major regulatory factors:
GM-CSF, mega-CSF, and thrombopoietin (TPO)

1-2-1-3-6-Reference range:
Count= 150-400 x 10^{12}/mm^3 or 150-40 x 10^9/l. 

(8)
1-2-2- Thrombocytopenia:
Thrombocytopenia is defined as a platelet count below the reference range for a particular laboratory less than 150,000 / µl. the consequence of thrombocytopenia is risk of hemorrhage, in general, assuming normal platelet function, the risk of hemorrhage correlate with the platelet count. a platelet count more than 100,000 / Ml is associated with no risk of hemorrhage, even with surgery. A platelet count 10,000-20,000/Ml is associated with a significant risk of spontaneous severe hemorrhage. The relationships between platelet count and risk of hemorrhage may not be valid if the patient has a primary platelet disorder, on medication that interferes with platelet function, or has other risk factors for bleeding. Diagnosis of thrombocytopenia is made from the clinical history, peripheral blood count, the blood film and bone marrow examination.\(^6\)
Thrombocytopenia can occur via a three general mechanism:
- Impaired platelet production.
- Increased platelet utilization or destruction.
- Platelet sequestration in the spleen.
Causes of thrombocytopenia can be divided into inherited thrombocytopenic syndromes, congenital but not inherited causes, acquired immune-related cause, and acquired non-immune causes (infection).\(^6\)
1-2-3- platelet indices:
Circulation platelets are very different in size, metabolism and functional activity. The largest are more reactive and produce a greater quantity of thrombogenic factor. Automated counters provide platelet count and generate the MPV (mean platelet volume) and measure of their size variability PDW (platelet distribution width) and measure P-LCR (platelet large cell ratio) the percentage of large platelets with a volume >12 fl (The great dispersion of platelet volumes depends on the process
of platelet production by fragment of cytoplasm of megakaryocyte and pro platelet formation.\(^{(9)}\)

In healthy subjects, there is an non linear inverse correlation between MPV and platelet concentration, MPV tended to decrease in subjects with higher platelet counts. The MPV reference intervals should, therefore, be expressed as a function of platelet concentration. This wide dispersion of normal values limit the usefulness of MPV as a screening tend to clinical conditions characterized by extreme values such as some hereditary thrombocytopenia (e.g., Wiskott-Aldrich syndrome. In which there are decreased values and Bernard soulier syndrome, in which values are increased).\(^{(9)}\)

In healthy population, there is direct relation between MPV and PDW, this relationship is maintained in idiopathic thrombocytopenic purpura and chronic myeloid leukemia, in which both are increased. This does not occur in hypoplastic anemias or megaloplastic anemia or during chemotherapy, in which the MPV decreases with an increasing PDW. The PDW can also be useful in differentiating reactive thrombocytosis from the essential type, especially when it is combined mathematically with the MPV and platelet count to obtain a discriminant function.\(^{(9)}\)

**1-2-3-1- PDW (Platelet distribution width):**

PDW is the distribution width on 20% frequency level with the peak taken as 100%. The unit applied is fl (femto-liter).

**1-2-3-2-MPV (Mean platelet volume):**

MPV is calculated by the following formula:

\[
= \text{MPV (fl)} \times \frac{\text{PCT} \text{(%)} \times 1000}{\text{PLT} \text{(1000/\muL)}}
\]
Where PCT (%) represent the value weighted with PLT frequency and is called platelet - crit or platelet volume ratio. It was calculated by PLT pulse height detection method.

1-2-3-3-Normal Values of Platelet Indices:
Reference intervals (normal population reference range) were developed for the KX-21N using normal individuals the ranges for each parameter (PDW, PMV and P-LCR) were determined and displayed.

Table (1-1) Normal values of platelet indices. (9)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range for females</th>
<th>Range for males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age of 33.4</td>
<td>Mean age of 42.2</td>
</tr>
<tr>
<td>PDW</td>
<td>9.4 - 18.1</td>
<td>9.8 - 18.0</td>
</tr>
<tr>
<td>MPV</td>
<td>8.5 - 12.4</td>
<td>8.1 - 12.4</td>
</tr>
<tr>
<td>P-LCR</td>
<td>15-35%</td>
<td>15-35%</td>
</tr>
</tbody>
</table>

1-2-4- Malaria definition and historical background:
Malaria is acute febrile disease caused by malaria parasite. (10)
The term malaria is derived from two Italian words, mal (bad) and aria which mean (air). Malaria is the most important parasitic disease of man. (11)

Malaria is a complex multi –disease. Erythrocytes, the brain, kidney and placenta are the main organs affected.
Malaria is caused by four species of genus plasmodium, P.falciparum, which causes malignant tertian malaria P.vivax which causes benign tertian malaria P.malariae which causes quartan malaria and P.ovale, (12) which causes ovale tertian malaria. The four species of malaria parasites known to be infective to man coexist in sudan, whith P.falciparum being the predominant species malaria disease in tropical countries. P.malariae distribution extends over both tropical and sub –tropical areas ,in west
and est Africa and parts of India, but its presence in various zones tend to be patchy. P. vivax occurs throughout most of the temperate zones and also in large areas of tropics, but it is less prevalent in tropical Africa, especially, in its western part. P. ovale has been recorded chiefly from western region of tropical Africa. It was also reported from the west pacific regions, southern China, Burma and south east Asia. (13)

1-2-5- Classification of Plasmodium:

Kingdom = Animalia
Subkingdom = Protozoa
Phylum = Apicomplexa
Class = Sporozoa
Subclass = Coocidia
Order = Eucoccidiida
Suborder = Haemosporina
Family = Plasmodiidae
Genus = Plasmodium
Species:

P. Falciparum
P. malariae
P. ovale
P. vivax. (13)

1-2-6- Plasmodium species:

1-2-6-1- Plasmodium falciparum:

Causes falciparum malaria, also called tropical malaria, subtertian malaria and malignant tertian malaria, which is the most severe form of malaria. (14)

It is characterized by cycles of most dominant species in tropical countries and responsible for 80-90% of all malaria infections. It may infect 20-40 of the host RBCs. (14)
1-2-6-2- *Plasmodium vivax*:
Causes benign tertian malaria, in which the cycle of fever recurs every 48 hours. It is the second most important species. Older erythrocytes are preferentially infected, in contrast to *P. falciparum* infection; the percentage of cells infected is much lower.

1-2-6-3- *Plasmodium ovale*:
Is generally less common than *P. falciparum* and *P. vivax*, however, it is more common in West Africa. Fever cycle occurs every 48 hours.

1-2-6-4- *Plasmodium malariae*:
Causes quartan malaria which is prevalent throughout the tropics. It produces a fever cycle of 72 hours. Incubation period varies between 29-40 days but can extend to several months depending on the number of parasites inoculated.\(^{14}\)

1-2-7- Epidemiology of malaria:
More than 500 million people are exposed to malaria in about 100 countries lying between latitude 60 N and 40 S. Malaria is absent at height 7,000 feet above sea level.\(^{15}\)

In tropical Africa, south of the Sahara, malaria shows a high endemicity, but has low endemic potential. Exceptions to this statement will be found on the slopes of mountains in Kenya, Zimbabwe, and Republic of South Africa in which epidemics were well known. In Africa south of the Sahara, it was estimated that between 27 and 480 millions clinical malaria cases occur every year. 74% of the population living in highly endemic areas and about 30% of febrile patients were attributed to malaria. About one million deaths occurred in children under five years. Malaria transmission depends on different factors; anopheles mosquito is the most important factor. A mosquito must live enough period of time for sporogony to be completed. The transmission is directly proportional to
the density of the vector. Hence, the disease coincides with increased mosquito numbers.\(^{(15)}\)

Man act as the main reservoir host and plays an important role in the epidemiology of malaria for the transmission of infection. There must be gametocytes carriers among the population. Gametocyaemia and high parasitic density are detected more easily in young children than in old children and adults. Thus the younger age groups probably represent the main reservoir and are the main recipient of the infection.\(^{(16)}\)

1-2-8 Life cycle:

The female anopheline mosquito becomes infected when it feed on human blood containing gametocyte, the sexual form of malaria parasite. The development in the mosquito takes from 7-20 days. Sporozoites (the infective stage passed in the saliva of the mosquito and formed inside an oocyst by the process of sporogony inoculated by infected mosquito disappear from the human blood within half an hour and enter the liver. After some days merozoites (the product of division by schizogony\(^{(17)}\) leave the liver and invade red blood cells, where further asexual cycles of multiplication take place, producing schizonts) the stage undergoing asexual division by multiple fission or segmentation. Schizont may be found in the liver cell (pre-erythrocytic schizonts) or in the erythrocyte (erythrocytic schizont). A rupture of the schizont releases more merozoites into the blood and causes fever, whose periodicity depends on the species of parasites. \textit{P. vivax} and \textit{P. ovale} may persist in the liver cell as dominant forms capable of developing into merozoite months or years. Thus the first attack of clinical malaria may occur long after the patient has left the endemic area, and the disease may relapse or recurrence due to the invasion of blood by merozoites from the late pre-erythrocytic stage previously known as secondary exoerythrocytic stage\(^{(17)}\) after treatment with drugs that kill only the erythrocytic stage of parasite. \textit{P. falciparum}
and *P. malariae* have no persistent exoerythrocytic phase but recrudescence is due to the survival of erythrocytic form so fever may result from multiplication in red cells of parasites which have not been eliminated by treatment and immune processes. (18)

Figure (1.2) Life cycle of malaria parasite
1-2-9- **Pathogenesis:**
All of the pathogenicity of malaria is related to the erythrocytic infection. The inoculated sporozoites promptly leave the blood stream without having caused apparent harm, and they lodge in the hepatic parenchymal cells where exo–erythrocytic schizogony take place. Destruction of invaded liver cells occurs, but without noticeable host reaction or clinically evident ill effect. The exo–erythrocytic merozoites invade red blood cells and pathogenic effect then become significant.

The disease caused by a malaria infection is dynamic interplay between the mechanisms of multiplication and the survival of the mechanisms vary from species to species and even from strain to strain and with race, age, and immune status of the host the nature of the disease, malaria is quite protean. (18)

1-2-9-1- **Effect on red cells and capillaries:**
Malaria is always accompanied by haemolysis and in severe or prolonged attack anemia may be profound. The causes of anemia in malaria are:

- Haemolysis of infected erythrocytes
- Haemolysis of uninfected erythrocytes
- Dyserythropoiesis
- Splenomegaly causing erythrocytes sequestration and haemodilution.
- Depletion of folate stores.

Haemolysis is more sever with *P.falciparum*, which invade red cells of all ages but specially young cells. *P.vivax* and *P.ovale* Invade reticulocytes, and *P.malariae* normoblast, so that infections remain lighter.

In *P.falciparum* malaria, red cell containing schizonts adhere to the lining of capillaries in brain, kidney, liver, lung and gut. The vessels become
congested and organ anoxic. Rubtore of schizonts liberates toxic and antigenic substances that cause further damage. Thus the main effects of malaria are haemolytic anemia, and with *P. falciparum* wide spread organ damage.\(^{(18)}\)

1-2-9-2- Renal failure

There is a cortical vasoconstriction and consequent hypoperfusion in severe malaria with renal impairment. Acute tubular necrosis presumably results from renal microvascular obstruction as a consequent of sequestration in the kidney. Significant glomerulonephritis is rare. The role of local cytokines release and altered regulation of renal microvascular flow is uncertain. Massive haemolysis compounds the insult in black water fever complicating severe malaria.\(^{(11)}\)

1-2-9-3- Anemia:

The pathogenesis of anemia is multifactorial. There is obligatory desrtruction of red cells containing parasites of merogony. There is also accelerated destruction of non parasitized red cells that parallel disease severity. In severe malaria anemia develops rapidly, the rapid haemolysis of unparasitized red cells is a major in haematocrit. The haemolytic anemia is compounded by bone marrow dysfunction. Dyserythropoiesis persists for days or weeks following acute malaria and reticulocyte counts are usually low in acute phase of disease. Red cells survival is shortened in malaria, and this is unaffected by corticosteroids.\(^{(11)}\)

1-2-9-4- Coagulopathy and thrombocytopenia:

there is accelerated coagulation cascade activity with accelerated fibrinogen turnover, consumption of antithrombin III and inceased concentration of fibrin degradation products. In severe infections the prothrombin and partial thromboplastine times may be prolonged and in 5% of patients bleeding may be significant. Thrombocytopenia is caused by increased splenic clearance. Erythrocytes containing mature parasites
may activate the coagulation cascade directly, and cytokine release acts as a pro coagulant. It has been suggested that disseminated intravascular coagulation (DIC) (Is a syndrome characterized by the formation of multiple fibrin thrombi in small blood vessels). \(^{(17)}\)

Is important in the pathogenesis of sever malaria, but detailed prospective clinical and pathogenesis studies have refuted this. Coagulation cascade activity is directly proportional to the disease severity, but hypofibrinoginaemia resulting from DIC is significant in 5% of patients with severe malaria, and lethal hemorrhage is unusual. Intravascular thrombus is observed rarely at autopsy in fatal cases. \(^{(11)}\)

**1-2-9-5- Black water fever:**

This condition is defined as sever malaria with intravascular haemolysis, haemoglobinuria and renal failure. Clinical findings include oliguria, jaundice, dark brown or red urine, haemoglobin casts and proteinuria. This is poorly understood condition, in which there is massive intravascular haemolysis and passage of (Coca-Cola) coloured urine. Black water (urine) occurs in three circumstances:

- Occasionally when patients' with G6PD deficiencies have malaria and receive quinine treatment.
- In some patients with severe quinine treated falciparum malaria that has normal erythrocyte G6PD levels.
- When patient with G6PD deficiency take oxidant irrespective of whether they have malaria or not
- Who quinine cause black water fever in these two last situations is not known as it is not an oxidant drug. \(^{(11)}\)

**1-2-10-Laboratory diagnosis:**

The diagnosis of malaria should be suspected in patients presenting with a febrile illness (or history of malaria) in a malaria- endemics area, and elsewhere in febrile individuals who have travelled in an endemic area
(particulary during the last 12 months). Laboratory test abnormalities that may heighten the clinical suspicion of malaria include thrombocytopenia associated with a normal white cell count malaria pigment in macrophages and other white blood cell, (16) abnormal liver function tests and an elevated lactate dehydrogenase, or haemoglobinuria. Anemia is uncommon in non-immune adults who present early but is a common finding in children living in endemic areas, importantly, the cerebrospinal fluid is normal in cerebral malaria. Malaria should be notified to the relevant health authorities and blood slides sent to a reference laboratory for confirmation. (16)
1-3- Rationale:
Malaria remains today one of the major health problem in the tropical and subtropical countries particularly Africa and Asia, with increased morbidity and mortality. More than 40% of the world populations reside in malaria-endemic area and it is predictable that 300-500 million cases and 1.5 - 2.7 million deaths occur each year. Hematological changes, which are the most common complication, play a significant role in these serious complications. The hematological abnormalities that have been reported to consistently companion which comprise are anemia and thrombocytopenia. Today the presence of hematological changes becomes a highly sensitive clinical marker for malaria diagnosis. (22)

The overall goal of the project is to reduce the malaria burden to the extent that it is no longer a public health problem in Khartoum state. And according to WHO malaria programs in Sudan, the malaria one of cause morbidity and mortality. (19)
1-4- Objectives:

1.4.1 General objective:
- To determine complete blood count and platelet indices among Sudanese patient with malaria.

1.4.2 Specific objective:
- To determine haemoglobin level, RBCs count in Sudanese patients with malaria.
- To determine WBCs count in Sudanese patients with malaria.
- To determine platelets count, mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) in Sudanese patients with malaria.
Materials and methods

2-1-Study design:
Cross sectional descriptive study aim to determine CBC and platelet indices from November 2014 to December 2014.

2-2-Study population:
Individual infected with malaria parasite in Khartoum and Senga state.

2-3-Study area:
Sample collected from several areas in Khartoum and Senga state.

2-4-Sample collection:
2.5ml of venous blood collected using sterile disposable plastic needle with EDTA vacationer and aseptic standard non traumatic vein puncture technique and using immediately.

2-5- Laboratory requirements:
- Automated haematological analyzer sysmex KX-21N for determination of complete blood count and platelet indices.
- Microscope.
- Leishman stains for preparation of thin film.
- Slides cover glass and oil.
- Test tube.
- EDTA vacotainer
- 70% alcohol (ethanol) and cotton

2-6-The blood film:

2-6-1-Preparation of thin film:
clean slide wiped immediately before use small drop of fresh anti coagulant blood placed in the center line of slide about 1-2 cm at an angle of 45c to slide and move back to make contact with the drop, the drop was spread quickly along the line of contact of spreader with slide. The film lifted to air dry. (20)
2-6-2-Staining film:
The slide filled with lieshmans stain on the staining rack, after 3 minutes double volume of buffer added for 7 minutes and then washed with tape water and left to air dry. \(^{(20)}\)

2-6-3-Examination of blood film:
Blood film is examined for cell size, shape, haemoglobin, distributed, eukocyte deferential, abnormality in staining properties, and inclusion bodies. The films were examined by X10 of eye lens for staining quality and X40 and X100 for blood cells differentiation. \(^{(20)}\)

2-7-Complete blood count (CBC):
CBC and blood film examination usually medicate wherever there is any abnormalities in blood cells, sysmex KX2IN (automated haemoglobin analyzer) is use.

2-7-1-Principle of sysmex:
Principle of automated haematological analyzer system (sysmex):
The counting of cellular elements in blood is done with impedancemetry technique. Thus technique based on the modification of the impedance of a calibrate aperture soaked in an electrolyte and going through a constant course deliver by two small aperture on the wall is immersed into a breaker that contains particle suspend in a low concentration electrolyte two electrodes, one inside the aperture tube and one outside the aperture tube but inside the breaker, are place and a current path is provide by the electrolyte when an electrodes is then measure. The aperture creates what is called (a sensing zone) particles in low concentration. Suspended in the electrolyte can be count by passing them through the aperture. As a particle passes through the aperture a volume of electrolyte equivalent to the immersed volume of the particle is displaced from the sensing zone. This causes a short term change in the impedance across the aperture. This change can be measure as a voltage pulse or current pulse. The pulse
height is proportional to the volume of sense particle. If constant particle density is assume, the pulse height is also proportional to the participle mass. This technology thus also called aperture technology. Using count and pulse height analyzer or circuits, the number of particle and volume of each particle passing through sensing zone can be measure. If the volume of liquid passing through the aperture can be precisely control and measure, the concentration of the sample can be determined. \(^{(20)}\)

2-7-2-Procedure:

1- The reagent needed was checked for expiry date before use
2- The power switch was turned and background check will be automatically performed and the vend (vend for analysis) will appear .Sample number inputed by pressing simple number then number of sample was entered. The enter key was pressed . Sample was mixed sufficiently. The tube was sited to the sample probe, and in that condition the start switch was pressed. When the LCD screen display analyzing the tube was removed. After that the unit executes automatic analysis and the result was display in the LCD screen. The result was point out.

2-8-WBCs, RBCs and Platelets count:

The counting of the cellular elements in blood sample was done with impedancemetry technique. This technique was based on the modification of the impedance of calibrated aperture soaked in an electrolyte and going through constant course delivered by two electrodes located on both side of the aperture. Avacuum applied on aside of aperture allows the cells passage, they oppose their physical volume to the coarse passage voltage impulse was registered at the electrode terminal. The height of the impulse is proportional to the cell volume.

2-9-Hemoglobin:

Hemoglobin is intensely colored and this property has been utilized in the methods for estimation its concentration in the blood. Erythrocytes
contain a mixture of hemoglobin, oxyhemoglobin, carboxyhemoglobin, methemoglobin and minor amount of other form of hemoglobin. To determine hemoglobin concentration in the peripheral blood, red cells were lysed and hemoglobin variants were converted to stable component cyan methemoglobin for quartation by absorption at 450 nm. Hemoglobin measurement was directly done in the WBCs chamber, by spectrophotometer by formation of chromogen cyano methemoglobin for lytic solution without cyanide measurement of the blank Hb was done for each analytical cycle and during the startup rising steps.

2-10-Platelets (PLTS) analysis:
Platelets analysis was made by empedancemetry in the RBCs count chamber at the same time with the red blood cells.

2-11-Statistical analysis:
SPSS software program, to obtain mean, standard deviation and P. Value by T. Test and one way anova. Data is presented in form of tables.
Results

Ninty five malaria patient's blood samples tested against fifty control blood sample to evaluate the hematological parameters, and platelet indices of them.

Table (3-1) The mean value of WBCs, RBCs, hemoglobin and hematocrit of patients with malaria parasite and control.
- Show significant differences in hemoglobin and hematocrite in comparison with control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample</th>
<th>N</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs x 10^9/L</td>
<td>Case</td>
<td>95</td>
<td>6.3 ± 2.1</td>
<td>0.163</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>6.8 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>RBCs x 10^{12}/L</td>
<td>Case</td>
<td>95</td>
<td>4.9 ± 0.7</td>
<td>0.498</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>4.8 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>Case</td>
<td>95</td>
<td>13.2 ± 2</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>14.2 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>Case</td>
<td>95</td>
<td>39.4 ± 5.6</td>
<td>0.041</td>
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<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>41.3 ± 4.6</td>
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</tr>
</tbody>
</table>
Table (3-2) The mean value of platelet count and platelet indices of patients with malaria parasite and control.

- Show significant differences in platelet count and platelet indices except (MPV) in comparison with control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample</th>
<th>N</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet x 10^9/L</td>
<td>Case</td>
<td>95</td>
<td>190.1 ± 102</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>269.1 ± 63.7</td>
<td></td>
</tr>
<tr>
<td>PDW (fl)</td>
<td>Case</td>
<td>95</td>
<td>13.5 ± 2.4</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>11.9 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>Case</td>
<td>95</td>
<td>10.0 ± 1.2</td>
<td>0.296</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>9.8 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>P. LCR (%)</td>
<td>Case</td>
<td>95</td>
<td>26.9 ± 7.6</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>50</td>
<td>23.7 ± 6.6</td>
<td></td>
</tr>
</tbody>
</table>
Table (3-3) *The mean value of hematological parameters of patients with malaria parasite and age groups.*

- show no significant differences in comparison with age groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age group</th>
<th>N</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wbc x 10^9/L</td>
<td>&lt;40</td>
<td>42</td>
<td>6.3 ± 2.2</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>53</td>
<td>6.3 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Rbc x 10^12/L</td>
<td>&lt;40</td>
<td>42</td>
<td>4.8 ± 0.87</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>53</td>
<td>4.9 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>&lt;40</td>
<td>42</td>
<td>13.0 ± 2.4</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>53</td>
<td>13.3 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>&lt;40</td>
<td>42</td>
<td>38.6 ± 6.6</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>53</td>
<td>39.9 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>Platelete x 10^9/L</td>
<td>&lt;40</td>
<td>42</td>
<td>191.1 ± 109.7</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>53</td>
<td>189.2 ± 96.6</td>
<td></td>
</tr>
<tr>
<td>PDW (fl)</td>
<td>&lt;40</td>
<td>42</td>
<td>13.2 ± 2.7</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>53</td>
<td>13.7 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>&lt;40</td>
<td>42</td>
<td>9.9 ± 1.2</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>53</td>
<td>10.1 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>P.LCR (%)</td>
<td>&lt;40</td>
<td>42</td>
<td>25.7 ± 7.6</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>53</td>
<td>27.7 ± 7.5</td>
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</table>
Table (3-4): The mean value of hematological parameters of patients with malaria parasite and gender.
Show no significant differences in comparison with gender.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sex</th>
<th>N</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wbcs x 10$^9$/L</td>
<td>Male</td>
<td>49</td>
<td>6.3 ± 2.2</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>6.3 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Rbcsx 10$^{12}$/L</td>
<td>Male</td>
<td>49</td>
<td>4.9 ± 0.85</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>4.8 ± 0.58</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>Male</td>
<td>49</td>
<td>13.5 ± 2.3</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>12.9 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>Male</td>
<td>49</td>
<td>40.0 ± 6.4</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>38.6 ± 4.6</td>
<td></td>
</tr>
<tr>
<td>Platelete x 10$^9$/L</td>
<td>Male</td>
<td>49</td>
<td>182.1 ± 97</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>198.5 ±107.5</td>
<td></td>
</tr>
<tr>
<td>PDW (fl)</td>
<td>Male</td>
<td>49</td>
<td>13.3 ±2.3</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>13.7 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>Male</td>
<td>49</td>
<td>9.9 ± 1.2</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>10.1 ±1.3</td>
<td></td>
</tr>
<tr>
<td>P.LCR (%)</td>
<td>Male</td>
<td>49</td>
<td>26.3 ± 7.9</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>46</td>
<td>27.5 ± 7.2</td>
<td></td>
</tr>
</tbody>
</table>
Discussion, Conclusions and Recommendations

4.1 – Discussion:

Malaria is a major cause of morbidity and mortality in tropical countries. There is a strong association between change in hematological variables and outcome in malaria. \(^{21}\)

In this study ninety five malaria patient's blood samples tested against fifty control blood sample to evaluate the hematological variables and platelet indices of them.

This study showed that there was significant differences in hemoglobin and hematocrit between malaria patient (Hb 13.2g/dl ± 2.1, HCT 39.4% ± 5.6) and control (Hb14.2 ± 1.8, HCT 41.3 ± 4.6), (P value of Hb = 0.005) (P value of HCT= 0.041) which agreed with the study done in India by Pradhan et al showed (Hb level and HCT low in 62.5%). Also agreed with study done in india by Shamim et al showed Hb level low in 84.6% of patient with malaria .\(^{22}\)

Also the study show there was no statistical significant differences in RBCs count between malaria patient(4.9x 10\(^{12}\)/L ± 0.7) and control(9.8±0.6), (P.value = 0.498).

Our study showed there was no statistical significant differences in WBCs count between malaria patient(6.3 x 10\(^{9}\)/L ± 2.1) and control (6.79 ± 1.9), (P value = 0.163) Which agreed with the study done in Saudi Arabia by Layla et al showed normal WBCs count in 78.3% in malaria patient. Also agreed with study done India by Shamim Akhtar showed normal WBCs count in 81.1%.

It showed there was significant differences in platelet count and platelet indices between malaria patient (platelet count 190.7 x 10\(^{9}\)/L ± 102, PDW 13.5fl ± 2.4, P.LCR 26.8% ± 6.6)and control (platelet count 269.1 ± 63.7, PDW 11.9 ± 1.7, P.LCR 23.7 ± 6.6), ( P value of platelet count = 0.000 , P value of PDW = 0.000, P value of P.LCR=0.012,). Which
agreed with study by Shamim Akhtar in which showed platelet low in 71.6% and also agreed with Layla A.M. Bashawri showed platelet low in 85% and MPV high in 25%.

This study show no significant differences in MPV between malaria patient (10.0 fl ± 1.2) and control (9.8 ± 0.9), (P value= 0.296).

The study showed no significant different in all parameter in comparison with age groups and gender, it also showed no significant different in severity of infection except in platelet count and platelet indices it was highly statistically significant.

Finally the study showed the main type of anemia in patient with malaria was normocytic normochromic anemia. This result agreed with Layla A.M. Bashawri showed normocytic normochromic anemia in 59.2% and microcytic hypochromic anemia in 17.7 %. (23)
4.2- Conclusions:

This study was concluded the following:

1. Some hematological variables affected with malaria parasite. There was decreased in hemoglobin and hematocrit confirmed with peripheral blood film indicated to normocytic normochromic anemia.

2. The red blood cells and white blood cells show a normal value, compared to control and normal range.

3. This study shows a thrombocytopenia and increased in platelet distribution width (PDW) and platelet large cell ratio (P-LCR).
4.3- Recommendations:

1- Increase the education health problem about the high risk of anemia and thrombocytopenia in individuals infected with malaria to minimize number of cases.

2- Increase sample size to give more reliability and accuracy by further study.

3- Involve more hematological parameters in this study to give more accuracy results.
References


13- Gilles H.m, Warrel. D. A, Arnold. E. Bruce- Chwatt's essential
Appendices

Appendix-1

-1,2 Show normocytic and normochromic RBCs with malaria parasitic infection
Appendix-2

show ideal blood film
Appendix-3

Show sysmex KX21N (automated haemoglobin analyzer)
Appendix-4

Table (3-5) The mean value of WBCs, RBCs, hemoglobin and hematocrit of patients of malaria parasite and severity of infection.
-show no significant differences in comparison with severity of infection.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(I) (Severity)</th>
<th>(J)</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wbcx10^9/L</td>
<td>mild moderate</td>
<td>Hyper</td>
<td>-0.6889</td>
<td>0.4988</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>Mild Hyper</td>
<td></td>
<td>-0.0777</td>
<td>0.5287</td>
<td>0.884</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>Mild Hyper</td>
<td>0.6889</td>
<td>0.4988</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>0.7666</td>
<td>0.5455</td>
<td>0.163</td>
</tr>
<tr>
<td></td>
<td>Hyper Mild</td>
<td>Moderate</td>
<td>-0.0777</td>
<td>0.5287</td>
<td>0.884</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.7666</td>
<td>0.5455</td>
<td>0.163</td>
</tr>
<tr>
<td>Rbcx10^12/L</td>
<td>mild moderate</td>
<td>Hyper</td>
<td>-0.15052</td>
<td>0.17510</td>
<td>0.392</td>
</tr>
<tr>
<td></td>
<td>Mild Hyper</td>
<td></td>
<td>-0.31655</td>
<td>0.18561</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>Mild Hyper</td>
<td>0.15052</td>
<td>0.17510</td>
<td>0.392</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>-0.16603</td>
<td>0.19150</td>
<td>0.388</td>
</tr>
<tr>
<td></td>
<td>Hyper Mild</td>
<td>Moderate</td>
<td>0.31655</td>
<td>0.18561</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.16603</td>
<td>0.19150</td>
<td>0.388</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>mild moderate</td>
<td>Hyper</td>
<td>-0.5236</td>
<td>0.4886</td>
<td>0.287</td>
</tr>
<tr>
<td></td>
<td>Mild Hyper</td>
<td></td>
<td>-0.8469</td>
<td>0.5180</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>Mild Hyper</td>
<td>0.5236</td>
<td>0.4886</td>
<td>0.287</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>-0.3233</td>
<td>0.5344</td>
<td>0.547</td>
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<tr>
<td></td>
<td>Hyper Mild</td>
<td>Moderate</td>
<td>0.8469</td>
<td>0.5180</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.3233</td>
<td>0.5344</td>
<td>0.547</td>
</tr>
<tr>
<td>Hct(%)</td>
<td>mild moderate</td>
<td>Hyper</td>
<td>-1.1254</td>
<td>1.3614</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>Mild Hyper</td>
<td></td>
<td>-2.0973</td>
<td>1.4432</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>Mild Hyper</td>
<td>1.1254</td>
<td>1.3614</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
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<td>1.4890</td>
<td>0.516</td>
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<td>Moderate</td>
<td>2.0973</td>
<td>1.4432</td>
<td>0.150</td>
</tr>
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<td></td>
<td></td>
<td>0.9719</td>
<td>1.4890</td>
<td>0.516</td>
</tr>
</tbody>
</table>
## Appendix-5

**Table (3-6): The mean value of platelet count and platelet indices of patients of malaria parasite and severity of infection.**

- Show significant differences between severity of infection.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>(I)</th>
<th>Severity (J)</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets $x10^9$/L</td>
<td>Mild</td>
<td>Moderate</td>
<td>56.558*</td>
<td>22.257</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>112.912*</td>
<td>23.594</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>Mild</td>
<td>-56.558*</td>
<td>22.257</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>56.353*</td>
<td>24.343</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>hyper</td>
<td>Mild</td>
<td>-112.912*</td>
<td>23.594</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>-56.353*</td>
<td>24.343</td>
<td>0.023</td>
</tr>
<tr>
<td>PDW (fl)</td>
<td>Mild</td>
<td>Moderate</td>
<td>-0.7876-1.7941*</td>
<td>0.5526</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>-1.0065-</td>
<td>0.5858</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>Mild</td>
<td>0.7876</td>
<td>0.5526</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>-1.0065-</td>
<td>0.6044</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>hyper</td>
<td>Mild</td>
<td>1.7941*</td>
<td>0.5858</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>1.0065</td>
<td>0.6044</td>
<td>0.099</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>Mild</td>
<td>Moderate</td>
<td>-0.4855-0.8268*</td>
<td>0.2907</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>-0.8268*</td>
<td>0.3082</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>Mild</td>
<td>0.4855</td>
<td>0.2907</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper</td>
<td>-0.3413-</td>
<td>0.3179</td>
<td>0.286</td>
</tr>
<tr>
<td></td>
<td>hyper</td>
<td>Mild</td>
<td>0.8268*</td>
<td>0.3082</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>0.3413</td>
<td>0.3179</td>
<td>0.286</td>
</tr>
<tr>
<td>P. LCR (%)</td>
<td>Mild</td>
<td>Moderate</td>
<td>-2.9246-5.3873*</td>
<td>1.7690</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hyper</td>
<td>-5.3873*</td>
<td>1.8752</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>mild</td>
<td>2.9246</td>
<td>1.7690</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hyper</td>
<td>-2.4627-</td>
<td>1.9348</td>
<td>0.206</td>
</tr>
<tr>
<td></td>
<td>hyper</td>
<td>mild</td>
<td>5.3873*</td>
<td>1.8752</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>moderate</td>
<td>2.4627</td>
<td>1.9348</td>
<td>0.206</td>
</tr>
</tbody>
</table>
Sudan University of Science and Technology
College of Medical Laboratory Science
Department of Hematology
Questioner

- Name: ………………………………………………………………
- Sex: ………………………………………………………………
- Age: ………………………………………………………………
- Residence: ………………………………………………………
- Parasitemia: ……………………………………………………

- **Result:**
  - WBbc count: ………………………………………… x10^9/L
  - RBbc count: ………………………………………… x10^{12}/L
  - Hb: ……………………………………………… g/dl
  - Hct: ……………………………………………… %
  - Platelet count: …………………………………… x10^9/L
  - Platelet indices
    - PDW: ………………………………………… fl
    - MPV: ………………………………………… fl
    - P.LCR: ……………………………………… %

- Date: ……………………………………………………………
- Signature: …………………………………………………