بسم الله الرحمن الرحيم

Sudan University of Science & Technology

College of Graduate Studies

Measurement of Complete Blood Count (CBC) in alcohol consumer – Khartoum 2014

قياس إختبار الدم الكامل لدى متعاطي الكحول– الخرطوم

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الآية

بسم الله الرحمن الرحيم

قال تعالي:

يَا أَيُّهَا الَّذِينَ آمَنُوا إِنَّمَا الْخَمْرُ وَالْمَيْسِرُ وَالْأَنصَابُ
وَالْأَزْلَامُ رِجْسٌ مِّنْ عَمَلِ الشَّيْطَانِ فَاجْتَنِبُوهُ لَعَلَّكُمْ تُفْلِحُونَ

صدق الله العظيم
سورة المائدة الآية (90)

Dedication

To my mother.....

To my father.....

To my brothers...

To my sisters...

To my friends...

And my colleagues...
I dedicate this work with my best wishes to all.

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All my thanks are in the name of Allah, the most Gracious and the most Merciful.

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Abstract

This is a prospective case control study to investigate the effect of alcohol consumption on complete blood count (CBC) of alcohol consumers in Khartoum State from April to July 2014.The participants were 80 apparently healthy adult males; 50 of them are alcohol consumers and 30 are non-alcoholic (control).Their age was (41 ±7.3years).A questionnaire was constructed to obtain information about the participants after an informed verbal consent from all the participants. Ethical approval was obtained from the College of Medical Laboratory Sciences-SUST. The educational level of the subjects was 74% primary, 16% secondary and 10% had higher education. 84% of the alcoholic's participants use local drinks while the rest16% drink imported alcohol. The duration of alcohol consumption was 5-20years.

Blood was collected from all participants into EDTA containers then CBC was carried out immediately after collection; using an automated hematological analyzer (Sysmex KX-21N). Data obtained was analyzed using software program statistical package of social science (SPSS.V.11.5).The results obtained from alcoholic compared with the control were insignificantly different ($P \leq 0.05$) with regard to erythrocytes count (4.53±0.6),hemoglobin level (14.28±0.23) hematocrit(39.26±0.57), mean cell volume(86.75±1.54), mean cell hemoglobin (29.46±0.33),total leucocytes count (6.33±1.7), monocytes% (4.3±0.3), eosinophil%(1.88±0.1), basophil %(0.48±0.08) and platelet count(251.4±5.3). The alcohol consumers registered significantly higher values ($P<0.05$) than the control for RDWCV(13.65±0.17), absolute lymphocytes count (2.9±0.8), and significantly ($P \leq 0.05$)lower values than the control for absolute neutrophil count (2.96±1.45) and platelet count(P.V 0.06). Macrocytes, target cells, crenated cells, few fragmented cells were found in the peripheral blood of some alcoholic subjects.

ملخص الدراسة

الهدف من هذه الدراسة هوتقييم بعض المعايير الدموية بين مستهلكي الكحول في السودان ولاية الخرطوم .كماتم استخدام مجموعة ضابطة من عينة للدراسة .خلال الفترة مابين أبريل - يوليو٢٠١٤ . ونجد أن٥٠ من المشاركين يستخدمون الكحول، وكانوا ذكور، وكماتضمنت عينة الدراسة٣٠من الاصحاء جميعهم من الذكور.تم جمع٥.٢مل من على الفوربعد CBC ثم أجري اختبار EDTA الدم من المشاركين وضبطت في حاويات مؤشرات الخلايا، HCT،جمع العينات ونفذت فيها كرات الدم الحمراء،الهيموغلوبين وتعداد الكريات البيضاء التفاضلية المطلق،الصفائح الدموية،ومؤشرات TWBCs ،الحمرا تم تحليل البيانات التي تم (SYSMEX KX-21N) الصفائح الدموية (باستخدام محلل الدم .SPSS.V) .الحصول عليها بواسطة برنامج الحزمة الإحصائية للعلوم الاجتماعية11.5 والنتائج التي تم الحصول عليها من مستخدمي الكحول تم مقارنتها مع المجموعة الضابطة، PCV،أظهرت بشكل ملحوظ الاختلاف فيمايتعلق كرات الدم الحمراء،الهيموغلوبين ووحيدات الأحماض والقاعدة TWBCs .مؤشرات الخلايا الحمراء،الصفائح الدموية،والخلايا الليمفاوية المطلقة ويظهر زيادة اختلاف كبير في المواد الكحولية، RDWCV وبالمقارنة مع المجموعة الضابطة.ويظهر عدد الصفائح الدموية انخفاض كبير في المواد الكحولية بالمقارنة مع الضابطة .وصورة الدم الطرفية من المدمنين على الكحول وتظهر الاختلافات بالمقارنة مع الضابطة تغير كبير في كريات كبيرة ،الخلايا المستهدفة، وخلايا مفرض، خلايا قليلة مجزأة وجدت في بعض من المواد الكحولية.

Abbreviations

CD4: Cluster of differentiation

CML: ChronicmyeloidLeukemia

ESR: Erythrocyte Sedimentation Rate

HIV: Human Immunodeficiency Virus

HSCs: Hematopoietic Stem Cells

IFN: Interferon

IL1: Interleukins 1

IL2: Interleukins 2

RBC: redBloodcelles

Hb:hémoglobine

PCV:packedcell volume

MCV: MeanCell Volume

MCH:mean cell hemoglobin

MCHC:mean cell hemoglobin concentration

WBC:white blood cells

MPV: Mean Platelet Volume

CDT:carbohydrate-deficient transferrin

RNA: ribonucleic acid

DNA:deoxynucleic acid

ALD:alcoholic liver disease

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Chapter one

Introduction and literature review

1.1 INTRODUCTION

Alcoholism represents one of the most seriousworldwide socioeconomic and health problems. Alcohol, or ethyl alcohol (ethanol), refers to the intoxicating ingredient found in wine, beer and hard liquor. Alcohol arises naturally from carbohydrates when certain micro-organisms metabolize them in the absence of oxygen, called fermentation. Inchemistry, an alcohol is an organic compound in which the hydroxylfunctional group (-OH) is bound to a carbon atom. In particular, this carbon center should be saturated, having single bonds to three other atoms. (Room *etal.*,2005).

Alcoholic is a person who consumes an amount of alcohol capable of producing pathological changes .Risk factors for developing a drinking problem include low self-esteem, depression, anxiety or another mood problem, as well as having parents with alcoholism. In Sudan locally prepared alcohol used for drinking (Aragi andMarisa.) they are made from date and local seeds after fermentation ,Othersimported from out SudanThe amount of alcohol capable of producingdisease depends on a variety of factors, including geneticpredisposition malnutritionand concomitant viral infections ofthe liver ((Fuchs*etal.*,1995*)*.reported association ofheavy alcohol intake with a significant increase of all causeand non-cardiovascular mortality rates especiallyby cirrhosis, cancer and

Violent deaths. They alsoreported that all-cause mortality rates are lower for moderate drinkers than for non-drinkersmechanisms such as alcohol anti-aggregation properties. Heavy alcohol intake was reportedto

be associated with an increase in blood pressure by(Meade*etal.*,1998).Acute or chronic alcohol consumption causesdegeneration in different internal organs and systems of adults reported the effect of maternal alcohol consumption on different organs and systems of the developing fetus(Robert,2005). Alcohol has a variety of pathologic effects on hematopoiesis. It directly damages Erythroid precursors, thereby contributing to macrocytosis and the anemic state of chronic alcoholics. Megaloblastic anemia in chronic alcoholism results from a combination of nutritional deficiency and the effect of ethanol as a folate antagonist. Experimental studies suggest that alcohol may disturb hepatic folate metabolism. Ethanol induces sideroblastic anemia, perhaps by direct interference with heme synthesis (Roberts, 1998).chronic ingestion of alcohol can lead to various types of hemolytic anemia caused by alterations in the erythrocyte membrane lipids which occur in association with alcoholic liver disease. Alcohol directly suppresses platelet formation and decreases the platelet life span. These two mechanisms contribute to thrombocytopenia which is a common complication to chronic alcoholism. Since ethanol also interferes with platelet function, prolongation of bleeding time is common at all stages of alcoholism. Chronic ingestion of alcohol is associated with a diminished marrow granulocyte reserve and may lead to neutrocytopenia. Ethanol-Induced dysfunction of granulocytes, monocyte-macrophages and T-lymphocytes undoubtedly contributes to the predisposition to infection observedin alcoholic in contrast to alcohol-induced changes in liver, heart and central nervous system, hematopoietic disorders are reversible after alcohol withdrawal. (Lindenbaum, 2000).

1.2 literature review:

1.2.1 Blood Components and Functions:

Blood is a vital fluid of the body and such the life line of human body. It is a red coloredviscous fluid slightly salty in taste. Blood is alkaline in reaction pH = 7.4 and specific gravity range from 1.052 to 1.060. In adult human, blood volume ranges between 4.5 to6.0 litters and approximately about one thirteen of adult human body weight. Temperature of circulating blood is 37.7c. Blood has two main components cells and plasma. Cells consist of 40% to 45% of total amount of blood, and plasma consist of 55% to 60% of total amount of blood, cells are the formed elementsand are of three typesRed cells (erythrocytes), white blood cells (leukocytes) and platelet(thrombocyte), and each have its own characteristic (Talib, 1995).

1.2.2 Functions of blood:

Oxygen is carried from the lungs to thetissue; this function is performed by hemoglobin which is present in large amount in mature red cell. Transport absorbed nutrients from digestive tract e.g. monosaccharide especially glucose, amino acid and fatty acids to the cell of the body for use or storage. Transport hormones from endocrine glands to the organs where they are needed. Regulatory functions: The blood act as a buffer system in the plasma which maintains pH of the blood between 7.35 to 7.45. Blood assist in regulating the temperature of the body. Protective function when blood vessel is damaged, platelets
And blood coagulation factors interact to control blood loss by formation
Of clot. Leukocytes are involved in the body's immune defense (Monica Sheesbrough, 2006).

1.2.3 Hematopoiesis:

Is the process, by which blood cells produced, proliferated, differentiated, and maturated in, to different blood cellall cellular blood components are derived from hematopoieticstem cells. In a healthy adult person, approximately 10^{11}–10^{12}new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation. Hematopoietic stem cells (HSCs) reside in the medulla of the bone (bone marrow) andhave the unique ability to give rise to all of the different mature blood cell types and tissues. HSCs are self-renewing cells (Palis*etal*., 1998).

1.2.3.1Erythropoiesis:

Is the process by which red blood cells (erythrocytes) are produced Itis stimulated by decreased O2 in circulation, which is detected by the kidneys, whichthen secrete the hormone erythropoietin. In the early fetus, erythropoiesis takes place inthe mesodermal cells of the yolk sac. By the third or fourth month, erythropoiesis movesto the spleen and liver. After seven months, erythropoiesis occurs in the bone marrow. Increased level of physical activity can cause an increase in erythropoiesis. However, in humans with certain diseases and in some animals, erythropoiesis also occurs outside the bone marrow, within the spleen or liver. This is termedextra medullaryerythropoiesis. The size of the cell is reduced and the cytoplasmic matrix increases in amount, and the staining reaction of the cytoplasm changes from blue to pinkish red because of the decrease in the amount of RNA and DNA. Initially, the

Nucleus is large in size and contains open chromatin. But as red blood

Cells mature the size of the nucleus decreases and finally disappear with the condensation of the chromatin material (Palis and Segel, 1998)

1.2.3.2 Granulopoiesis:

The formation of granulocytes within the bone marrow. It is controlled by a number of substances including granulocyte colony stimulating factors.unipotentstem cell cannot be distinguished from other unipotent stem cells histologically. Myeloblast: large cell with blue-staining cytoplasm; large nucleus; often as in this example, a clear area near the nucleus can be seen - this is where the rather large Golgi is located. Promyelocyte: example not shown; still a rather large cell with azurophilic (not specifically stained) granules.Myelocyte: example not shown; overall cell still rather Large; nucleus still round without indentation; granules staining appropriately for the series, i.e., pink for eosinophilic, blue for basophilic, neutral for neutrophilic (Amazon *etal*.,2008)

1.2.3.3 Megakaryocytopoiesis:

Is the process by which bone marrow progenitor cells develop into mature megakaryocytes, which in turn produce platelets, required for normal hemostasis. Platelets are formed in the bone marrow from megakaryocytes (30-100 μmdiameter), very large cells with a polyploidy, multilobednucleus. Platelets are released from fragmenting megakaryocytes in at least two ways: extension of pseudopodia through the wall of the sinuses; pseudopodia contain "strings" of platelets that are pinched off and released into the circulation passage of mature megakaryocyte into circulation andfragmentation in the pulmonary vascular bed. (Amazon *et al.*, 2008).

1.2.4 Hematological abnormalities in alcoholism:

Alcohol abuse may produce reversible abnormalities of the peripheral blood. Hematological abnormalities have been associated with alcohol and due to bad malnutrition. Alcohol-induced structural abnormalities in red blood cell (RBC) structure. Normal RBC’s have a characteristic

dislike shape; the cell in the center is a neutrophil. Stomatocytes have a defect in their membranes that causes them to assume a mouth-, or stoma-, like shape when viewed under a microscope, Spur cells are characterized by spike like protrusions that result from the assimilation of excess cholesterol into the cell's membrane (Akani, 2010)

1.2.4.1 Red blood cells:

The red blood cell is a biconcave disc, 8 micrometer in diameter able to passrepeatedly through the microcirculation whose minimum diameter is 3.5 micrometer, to maintain hemoglobin in a reduced (ferrous) state and to maintain osmotic equilibrium despite the concentration of protein (hemoglobin) in the cell. The total journey through its 120 day life span.Has been estimated to be 480 km (300miles).Each red cell contains approximately 640 million hemoglobin molecules. Each molecule of normal adult hemoglobin (Hb A) which is the dominant hemoglobin in the blood after the age of 3- 6 months, consist of four polypeptide chains α2 and β2 , each with its own hem group. The molecular weight of HbA is 68000. Normal adult blood also contains small quantities oftwo other hemoglobin, HbF and HbA2. These also contain αchain, but with γ and δ chains respectively, instead of β. Hemoglobin synthesis: Occurs largely in a mitochondria by a series of biochemical reactions commencing with the condensation of glycine and Succinyl coenzyme A under the action of the key rate limiting enzyme δ amino laevulinic acid synthase .main Function of hemoglobin carrying

Oxygen from the lung to the tissue and returns in venus blood with carbon dioxide to the lungs (Hoffbrand and Petit, 2006) Alcohol-related abnormalities in RBC production manifest themselves not only in the bone marrow but also through the presence of defective RBC's in the blood. For example, grossly enlarged RBC's can occur in the blooda condition called macrocytosis aswell as oddly shaped RBC's that are

subject to premature or accelerated destruction(hemolysis) because of their structural abnormalities. As aresult, alcoholics frequently are diagnosed with anemia. (Rivara,2004).

1.2.4.2 Leukocytes:

The term leukocytes and white blood cells are used synonymously to refer to the colorless nucleated cells that circulate in peripheral blood and function as the main body's defense against foreign invaders such as bacteria, viruses, and other foreign antigens. The Extent of WBC count may vary depending on etiologic agent, severity of infection and Host factors. Most limited bacterial infection are associated with a WBC count of 12-14 x 10^9 / L. Massive infection may produce leukumoid reaction with elevation in WBC count 150-200x 10^9/L (Adelekan,1992).

Ethanol-induced dysfunction of granulocytes, monocyte macrophages and T-lymphocytes.an association between excessive alcohol ingestion and the development of infections. These observations suggest that alcohol interferes with the normal production and/or function of WBC's, which form the body's defense against microorganisms and other foreign substances. Because alcoholics commonly develop bacterial infections, much research has focused on alcohol's effects on neutrophils. Alcohol interferes with the function of the monocyte macrophage system, with clinically significant consequences. (Mfcarl*etal*. 2002)

1.2.4.4 Lymphocytes:

It's a granulocytic WBCsderive fromhemopoietic stem cell, Tand B lymphocytes, which is used in cellular immune-responseagainstviral, bacterial infection.(Hoffbrand ,2010) .in chronic alcohol abusethe recent development of more accurate immunological techniques for studying lymphocyte transformation, that use monoclonal antibodies directed against surface structures (CD3 and CD2) involved in antigen recognition, as well in adhesion functions, prompted us to study discrete

in vitro T-cell hypo-responsiveness in a series of alcoholic liver disease (ALD) patients with no evidence of malnutrition or hepatic cirrhosis. The results indicated that the CD2 pathway is markedly defective in ALD T lymphocytes, accompanied by reduced interleukin-2 (IL-2) receptor expression upon in vitro activation. This defect cannot be reversed by the addition of recombinant IL-2 (rIL-2) or rIL-1. Faulty intracellular signal transduction by protein kinase C (PKC) and defective intracellular Ca2+ mobilization may be responsible for the CD2 pathway impairment (Room,2005).

1.2.4.5 Monocytes:

These are usually larger than other peripheral blood leukocytes and possess a large Central oval or indented nucleus with clumped chromatin. Like neutrophils, constitutes an importantMonocytes and macrophages clear line of defense against infectionsForeign or defective proteins from the invading microorganisms as well asdestroying them. Alcohol interferes engulfing and subsequentlywith the function of the monocytecompared with healthy people alcoholics are less resistant to infections by microorganisms thatnormally are eradicated by monocytes and macrophages. Reducedelimination of bacteriaby the monocyte-Macrophagesystemthese effects generally appear to betemporary. Thus, in alcoholic patients whose monocyte-dependent eliminationof a defective form of albumin a protein normally present in the bloodis reduced at admission to a hospitalmonocyte function generally returnstonormal within one week of abstinencefrom alcohol. Further studies indicate that alcohol impairs monocyte/macrophagefunction rather than production Thus; the cells frequently remain at their normal locations in the tissuesrather than migrate to the sites of infections inadditionAlcoholinhibits the monocytes adhesion abilities(Ballard, 2010)

1.2.4.3 Neutrophils:

These cells have characteristic dense nucleus consistingof between two and five lobes, and pale cytoplasm with an irregular outline containing many fine pink blue (azurophilic) or grey - blue granules. Neutrophils are the primary white blood cells that respond to a bacterial. Chronic ingestion of alcohol is associated with a diminished marrow granulocyte reserve and may lead to neutrocytopenia. (Mitchell *etal*., 2006).

Alcoholics suffering from bacterial infections often exhibit a reduced number of neutrophils in the blood neutropenia For example, in a study of 10 alcoholics with severe bacterial pneumonia or other bacterial infections, neutropenia was present in 5 patients when they were admitted to the hospital and developed in the other 5 patients within 24 to 48 hours (Ballard, 2004). The neutropenia was transient, however, and in several patients a rebound leukocytosis occurred between 5 and 10 days after hospital admission. The observed neutropenia may berelated to impaired neutrophil development in the bone marrow. Thus, bone marrowanalysis of alcoholic patients during the neutropenia stagedemonstrated that virtually none of the neutrophil precursors had matured

Beyond an early developmental stage. Moreover, the neutrophil stores thatare maintained in the bone marrow to allow a quick response to a bacterialinfection were depleted more rapidly in active alcoholics than in healthycontrol subjects. Alcohol consumption also interferes with the neutrophils' ability to reach the site of an infection or inflammation (i.e., neutrophil delivery). When traveling to such a site, the neutrophils adhere to the walls of the blood vessels before migrating out ofthe blood vessels into the affected tissue. In tissue-culture experiments using nylon fibers to mimic this adherence, neutrophils could not adhere tothe fibers if the blood samples were incubated with alcohol. This effectwas more pronounced the higher the alcohol doses were. Neutrophils obtained from

intoxicated volunteers had the same defect. The degree and duration of this adherence defect correlated with the inhibition of neutrophil delivery observed in the body. Moreover, drugs that corrected the adherence defect in tissue-culture experiments also improved neutrophil delivery in humans. The function of neutrophils, including their adhesion ability, is regulated by hormone like substances called leukotriene. Thus, the impaired neutrophil functioning observed after alcohol treatment could be attributable to reduced leukotriene production or to the neutrophils' inability to respond to theleukotriene. (Ballard, 2010).

1.2.4.6 Eosinophil's:

These cells are similar to neutrophils, except that the cytoplasmic granules are coarser and more deeply red staining and there are rarely more than three nuclear lobes they enter inflammatory exudates and have special role in allergic response, defense against parasites and removal of fibrin formed during inflammation. (Monica Sheesbrough, 2006).

1.2.4.7 Basophils:

These are only occasionally seen in normal peripheral blood. They have many dark cytoplasmic granules which overlie the nucleus and contain heparin and histamine. In thetissues they become mast cell. They have immunoglobulin E (IgE) attachment sites and their granulation is associated with histamine release. (Monica Sheesbrough, 2006).

1.2.4.8 Thrombocytes:

The blood thrombocytes are fragments of the cytoplasm of megakaryocyte, the main function of platelet is the formation of mechanical plugs during the normal hemostatic response to vascular injury .the resent study on platelets mention that there is many other functions (Hoffbrand,2010).Thrombocytopenia is a frequent complicationof alcoholism, affecting 3 to43 percent of non-acutely ill, well-nourishedalcoholics and 14 to 81Percent of acutely ill,

hospitalizedalcoholics. Alcohol-related thrombocytopeniagenerally is transient, andplatelet counts usually return to normalwithinone week of abstinence. Therefore patients generally requireno therapeutic intervention other thanthat needed to ease alcohol withdrawal. Alcohol affects not only platelet production but also platelet function. Thus patients who consume excessiveamounts of alcohol can exhibit a widespectrum of platelet abnormalitiestheseabnormalities include impairedplatelet aggregation, decreased secretion or activity of platelet-derivedproteins involved in blood clotting, and prolongation of bleeding in theabsence of thrombocytopenia.(Duarte, 1995).

1.2.5.1 Anemia:

Anemia is defined as qualitative or quantitative deficiency of hemoglobin which normally carries oxygen from the lung to the tissue (Frank*etal*. 2002). some of the causes Of anemia reduce hemoglobin production, reduce DNA synthesis, and reduce stem cell Production, bone marrow infiltration, infection, increase red cell destruction and acute Or chronic Blood loss (Job*etal*., 2008) .Anemia can be classified as morphological approach by the size of red blood cells; this is done automatically or on microscopic examination of a peripheral blood smear. The size is reflected in the mean corpuscular volume (MCV). If the cells are smaller than Normal (under 80 fl), the anemia is said to be microcytic; if they are normal size (80–110 fl), normocytic; and if they are larger than normal (over 100 fl), the anemia is classified as macrocytic. (Halterman*etal*., 2001) Microcytic anemia is primarily a result of hemoglobin synthesis failure or insufficiency, which could be caused by several etiologies: Hem synthesis defect Iron deficiency anemia (microcytosis is not always present) and anemia of chronic disease (more commonly presenting as normocytic anemia). Globin synthesis defect: Alpha and beta-

thalassemia, HbEsyndrome, HbC syndrome and various other unstable hemoglobin diseases (Haltermanetal., 2001).

Macrocytic anemia: The most common cause of macrocytic anemia is due to a deficiency of either vitamin $_{B12}$, folic acid, or both. Deficiency in folate and/or vitamin B_{12} can be due either to inadequate intake or insufficient absorption. Folate deficiency normally does not produce neurological symptoms, while B_{12} deficiency does. Pernicious Anemia is caused by a lack of intrinsic factor, which is required to absorb vitamin B_{12} From food. A lack of intrinsic factor may arise from autoimmune condition targetingthe parietal cells (atrophic gastritis) that produce intrinsic factor or against intrinsic factor itself. These lead to poor absorption of vitamin B_{12} (Haltermanetal.,2001) alcohol abuse is also interferes with B_{12} and foliate metabolism Hemolysis can be an underlying causeof anemia, and several types of hemolytic anemia may be caused by chronicheavy alcohol consumption. Two ofThese disorders are characterized by thepresence of malformed RBC'sstomatocytes and Spurcells. Whereasone alcoholrelated hemolytic anemia caused by reduced phosphate levels in the blood In order to develop a diagnostic approach to the common problem of anemia associated with alcoholism, one hundred twenty one chronic alcoholics admitted to a general medical service with a low hematocrit were evaluated. Multiple contributing causes of anemia were present in most patients. Megaloblastic marrow change was found in 33.9% of patients, sideroblastic change in 23.1%, absent iron stores in 13.2%, aggregated macrophage iron in 81.0%, and acute blood loss in 24.8%. The MCV was of little value in predicting the presence of megaloblastic change unless markedly elevated (greater than 110 fl). In 15 of 41 patients with megaloblastic marrow morphology (36.6%) the MCV was normal or low. Among 40 patients with MCV values between 100 and 110 fl, megaloblastic change was not present in

the bone marrow smears of 24 (60.0%). Neutrophil hypersegmentation was 95% specific but only 78% sensitive for megaloblastic change; in contrast, the presence of macroovalocytosis was 90% sensitive. Normocytic anemia occurs when the overall hemoglobin levels are decreased, but the red blood cell size (mean corpuscular volume) remains normal. Causes include: Acute blood loss, Anemia of chronic disease, Aplastic anemia (bone marrow failure) andhemolytic anemia.Mild to moderate anemia that is often observed in alcoholism.(Halterman*etal.*, 2001).

1.2.5.2 Alcoholic disorder:

Alcohol-Induced disorders caused by excessive alcohol consumption. The symptoms are variable depending on the disorder involved. Some of the disorders are: alcohol abuse, alcohol dependence, alcohol intoxication, alcohol withdrawal, alcohol intoxication delirium, alcohol withdrawal delirium, alcohol-induced persisting dementia, alcohol-induced persisting amnestic disorder, alcohol-induced psychotic disorder, alcohol-induced mood disorder, alcohol-induced anxiety disorder, alcohol-induced sexual dysfunction, alcohol-induced sleep disorder, liver damage, liver cancer and esophageal cancer. More detailed information about the symptoms, causes, and treatments of alcohol-Induced disorders isavailablebelow.(Charalambous,2002).

1.2.5.3 Signs and symptoms:

Symptoms of alcohol-Induced disorders The list of signs and symptoms mentioned in various sources for alcohol-Induced disorders includes the 43 symptoms listed below psychotic, Sexualdysfunction, mood disordersSleeping problemsanxiety delirium, amnesia delusion psychoticsdisorders, Hallucinations, Alcohol dependence, Alcohol intoxication, Dizziness, Vomiting,AggressivenessConvulsions, Tremor,

Homicidal impulses,exaggerated,emotions,Coldsweater, aggregate demotions,Breathingdifficulty,Agitation,Anxietyitching,Musclespasms,Diarrhea Uninhibited behavior, Confusion, Shivering ,Slurred speech, Progressive lethargy,disorientation,Slowed verbal responseuncoordinated movements,Slowed verbal response, balance problems difficulty with fine motor skills,balance problems coordination difficulties,bloodshot eyes.(Charalambousmp,2002).

1.2.5.4HEMATOGICAL MARKERS OF ALCOHOLISM:

State markers that would permit the identification of heavydrinkers even when alcohol is nolonger present in the blood would beparticularly valuable diagnostic tools trait markers could help identify people at risk for alcoholism whocould benefit most from early, targeted preventionand intervention approaches prominently include first-degree these high-risk populations most relatives of alcoholics. Trait markersalso could provide important research tools for evaluating the genetic and environmental factors that may predispose person to alcoholism(Rivara*etal*., 2004)

1.2.5.5State Markers:

Chronic ingestion of large quantities of alcohol alters many physiological andbiological processes and compoundsincluding several blood-related (i.e.hematological) variables. Because bloodsamples are relatively easy to obtain structural and functional changes incirculating blood cells and plasma proteinspotentially can form the basis of hematological state markers commonly laboratory tests for screening, diagnosingand monitoring alcoholism. Twohematological state markers commonlyused for these purposes are the presenceof carbohydrate-deficient transferrin (CDT) in the blood, and an increase inthe size of red blood cells ismeasured by the mean corpuscularvolume(MCV). Carbohydrate-

deficient Transferrin (CDT) is one of the newest and perhaps the most promising of the hematological state markers. Transferrinisan iron-containing protein inthe plasma that transports iron, which isstored at various sites in the body; tothe developing RBC's in the bonemarrow for incorporation into hemoglobin transferrin molecules in the blood usually contain several carbohydrate components. In chronic heavydrinkers, however, the numbers of carbohydrate components in each transferrin molecule are reduced, resulting inCDT. The mechanism underlying thisalteration still is unclearbecause elevated CDT levels in the bloodAppear to be a specific consequenceof excessive alcohol consumptionrecent study investigated the utility of repeatedly monitoring serumCDT to detectrelapse among recoveringalcoholics. The study found that inmost ofthesubjects who relapsed, theelevation of CDT levels precededself-reportedalcohol consumption by at least 28 days. These findings suggest thatrepeated testing of alcoholicpatients for CDT permits early relapsedetection and thus may lead to early intervention. Early intervention, inturn, may decrease the need tore hospitalize patients for alcohol withdrawaland prevent some of the complicationsassociatedwith sustainedexcessive drinking, (Quaye, 1992).

1.2.5.6 Other Tools for Alcoholism check:-

Check symptoms: is a symptom caused by alcoholasking a question: ask patients like you a question about alcohol. Write a review: share your Alcohol experiencemore tools... (Klatsky,1992)

1.2.4 Previous study:

Blood cell precursors that cannot mature into functional cells. Alcoholics frequently have defective red blood cells that are destroyed prematurely, possibly resulting in anemia. Alcohol also interferes with the production and function of white blood cells, especially those that defend the body against invading bacteria. Consequently, alcoholics frequently suffer from bacterial infections. Finally, alcohol adversely affects the platelets and other components of the blood-clotting system. Heavy alcohol consumption thus may increase the drinker's risk of suffering a stroke.(Rivara,2004)

This study was conducted toevaluatehematological abnormality inalcohol consumer because to our best knowledge there is scary in a published data in Sudan. In order to develop a diagnostic approach to the common problem of anemia associated with alcoholism, 121 chronic alcoholics admitted to a general medical service with a low hematocrit were evaluated. Multiple contributing causes of anemia were present in most patients. Megaloblastic marrow change was found in 33.9% of patients, sideroblastic change in 23.1%, absent iron stores in 13.2%, aggregated macrophage iron in 81.0%, and acute blood loss in 24.8%. The MCV was of little value in predicting the presence of megaloblastic change unless markedly elevated (greater than 110 fl). In 15 of 41 patients with megaloblastic marrow morphology (36.6%) the MCV was normal or low. Among 40 patients with MCV values between 100 and 110 fl, megaloblastic change was not present in the bone marrow smears of 24 (60.0%). Neutrophil hypersegmentation was 95% specific but only 78% sensitive for megaloblastic change; in contrast, the presence of macroovalocytosis was 90% sensitive but only 68% specific. Serum lactic dehydrogenase, plasma folate, and erythrocyte folate levels had such low sensitivities and specificities for megaloblastic change as to be of little

predictive value. Hematologic responses to folic acid were often inadequate in patients with megaloblastic morphologic changes, apparently because of associated acute and chronic illness. (Rivara, 2004)
A study had been conducted on 200 adult males categorized into four groups: none drinkers, occasional drinkers, moderate drinkers and heavy drinkers. Fifty subjects were in each group, their age ranged between 20 and 57 (average 33.4) the values obtained for biochemical and hematological parameters in occasional and moderate drinkers showed no

Significant difference ($p > 0.05$) to those obtained for non-drinkers which served as control group. This may mean that occasional and moderate drinking has no effect on blood biochemistry and hematology. However, in heavy drinkers, there were significant differences ($p < 0.05$) in some of the biochemical and hematological results when compared to those of abstainers, occasional and moderate drinkers.(Oduola,2005)

A study done inusa shows that hematological examination of patient presenting with cytopenia and a history of hazardous drinking showed low incidence of anemia, abnormal platelet and leucocyte level were common inthe anemic alcoholic macrocytosis, reticulocytosis, thrombocytopenia, stomatocytosis and combined cytopenias are common in alcoholic patient compared with control non-alcoholic. (Latvala*etal*.,2004).

1.3Rational:

Alcohol is a major contributor to the global burden of disease, disability, and death in high, middle, and low-income countries. Harmful use of alcohol is one of the main factors contributing to premature deaths and avoidable disease burden worldwide and has a major impact on public health (Amazan, 2008). In Sudan there is scary in published data have been found about the study, so the aim of this study is to assess some hematological parameter among alcohol consumer in Khartoum state. The study can be help to avoid complication of the disease and reduce rate morbidity and mortality, and the study may be useful as indictor of disease progression. And increased the availability of information so the addict and other drinker to stop drinking.

1.4 Objectives:

1.4.1 General Objective:

To measure complete blood count inalcohol consumerIn Khartoum State, 2014.

1.4.2 Specific Objectives:

1_ toestimate hemoglobin, RBCs, PCV, red cell indices, leucocyte's count ,platelets count and platelets indices on alcohol consumer and compare it with healthy individuals.

2-Todetermine morphological abnormalities in blood cells on alcoholic comparedwith healthy individuals.

3_To determine the most common type and severity of anemia in alcoholics.

Chapter Two

Material and Methods

2.1 Study design:

This is analytical case control study, enrolled between Aprils to July 2014 to measure some hematological parameters onalcoholics in Khartoum state.

2.2 Study population:

Fifty cases participants (alcoholism) healthy volunteers selected as controls, these volunteers are non-alcoholic consumer and free from diseases or medication therapy in the last month before sample collection.

2.2.1 Inclusion Criteria:

Persons that drinks alcohol mild moderate or sever

2.2.2 Exclusion Criteria:

· Alcoholics participantswith disease

· Participantsunder treatment excluded from study population

2.3 Sample Size:

Fifty casesof volunteer were chosen by non-probability Sampling (50 male) and control group of (30) healthy individuals have been selected.

2.5 blood collection:

Eighty samples are collected from participants and control. 2.5 ml of venous blood was collected from each volunteer and controls via the antecubital vein using vacationer system; vacationer tube containsEthyleneDi-amine Tetra-acetic Acid (EDTA) as anticoagulant. Eachsample was mixed gently and thoroughly to prevent cell lysis and clotting of blood, then complete blood counts (CBC) was determined

within 2 hours after collection the remainder 2.5 ml of blood was used to prepare thin blood film for blood cells morphology. (full blood count analysis was done on the same day of collection) using Sysmex KN-21 XN, (manufactured by Sysmex corporation Kobe, Japan) a three- part auto analyzer able to run 19 parameters per sample including hemoglobin concentration, packed cell Volume, red blood cell concentration, mean corpuscular hemoglobin, mean cell volume, Mean corpuscular hemoglobin concentration, white blood cells and platelet values and the Reference value of these parameters see appendix-6 (Lewis, 2006).

2.5 Tools of Data Collection:

Data were collected using a personal interview questionnaire to the participants, included age, gender, duration, type of alcohol abuse, education level, and ethical consideration was conducted by interview discussion to get permission from the participants.

2.6 Principle of hematology analyzers Sysmex (KX2-1N):

This instrument perform blood count by DC detection method to measure WBC and differential count ,RBCs, HCT, MCV, MCH, MCHC, Hb and Platelets count,MPV, PDWSD, PDWCV. Blood sample is aspirated, measured to predetermined, then diluted at the specified ratio. Then fed into each transducer. The transducer chamber has a minute hole called.

The aperture.On both side of the aperture, there are the electrodes, between which flows direct current. Blood cells suspended in diluted sample pass through the aperture, causing direct current resistance tochange between the electrodes .As a direct current resistance Changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and histogram of blood cell size is blotted by determining the pulse sizes. Also analyzing histogram makes it possible to obtain various analysis data. Hemoglobin is measured by non-

cyanide hemoglobin analysis method which rapidly converts blood hemoglobin as oxy hemoglobin method and contains noPoisonous substance making it suitable for automated method. And the reference value of Hematological parameters shows in appendix-6 (Diamond, 1999).

2.6.1 Procedure of Sysmex KX-21N:

Measurement of blood cells (RBCs, WBCs, & Platelet), and hematological concentration Were obtained by aspiration of small volume of well mixed K_2 EDTA blood by sample Probe and mixed with isotonic diluents in nebulizer Diluted mixture aspiration was Delivered to RBCs aperture both for providing information about RBCs and Platelet Based on the cell size . Particles of 2to 20 fl counted as platelet. Above 36 fl was countedas reamed cell. Some portion of aspiration mixture induced into WBCs both in which Hemolytic reagent (Stromatolyzer) was added automatically to measure hemoglobin Concentration in build calorimeter, based on cyanomethemogolobin method (HICN). Blood cell were counted according to size information was generated in triplicate pulses According to electronic conductivity. Parameters were directly measured and displayed on (LCD) other values of red cell indices, platelets; leukocytes differential and absolute countwere calculated from given information and automated constructed histograms. Commercial close system reagent were provided by Sysmex KX- operator and Consists of cell pack, stromatolyser , detergent and cell cleaner and the referenceValue of Hematological parameters show in appendix-6 (Diamond,1999).

2.6.2 Reagents and Materials:

Commercial close system reagents were provided by Sysmex KX-21N operators and Consist of:

· Cell pack and Stromatolyser: diluents and lysing reagent for use in Sysmex.

· Detergent and Cell cleaner: use for cleaning solution to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex automated Hematology Analyzers, see appendix-5 (Dimond ,1999).

2.7 Preparation of thin Blood Film:

Clean labeled slide was placed on flat bench then one drop of well mixed EDTA blood Was added, spreader slide was possessed at angel 45 degree and moved back to touch Blood drop, then the spreader was bushed smooth with little pressure to make and ideal Thin blood film compose (head, body and feathered sharp tail) and let to air dry (Lewis, 2006).

2.7.1 Principle:

Manual rack method and Ralls 555 kit stain were used which compose of fixative, eosin, and methylene blue. The slide was dipped 5 seconds in Bottle (1) contain alcohol fixation and drained on filter paper and dipped 5 seconds in Bottle (2) contain eosin acidic dye, which gives color to a basic component and surface Solution drained in filter paper and dipped 5seconds in bottle (3) which contain methylene blue dye, a basic dye, which gives color to an acidic component then the slide was rinsed in distilled water and let to air dry then examined under microscope. These dyes differentiate the different components of blood cells as show in appendex-7

2.8 Ethical Consideration:

Ethical clearance was obtained in this study, and the sample collected after the consent of Participants whom were informed about the procedure of blood collection and the aim of the study.

2.9 Data Presentation:

Data obtained of was analyzed by Statistical Package of Social Science (SPSS.version -11.5) software program to obtain mean andp.values by Independent –sample T test. The results presented in form of tables for case and control.

Chapter Three

Results

3.1. Characteristics of the study population:

The study was conducted to assess some of hematological parameter in alcoholic in Khartoum state .The results were compared with apparently healthy subjects (controls). Thestudy included eighty subjects, 50participants were already known as alcoholic .with mean age (41±7.3 years) ,(100 % male) and30 healthy volunteers were selected as controls, these volunteers are known nonalcoholic and appear healthy and free from medication therapy in the last three months before sample collection.

- Fig (1) shows type of alcohol used by alcoholic.

- Fig (2) showduration of alcohol found in alcoholic

- Fig (3) shows types of education level found in alcoholic

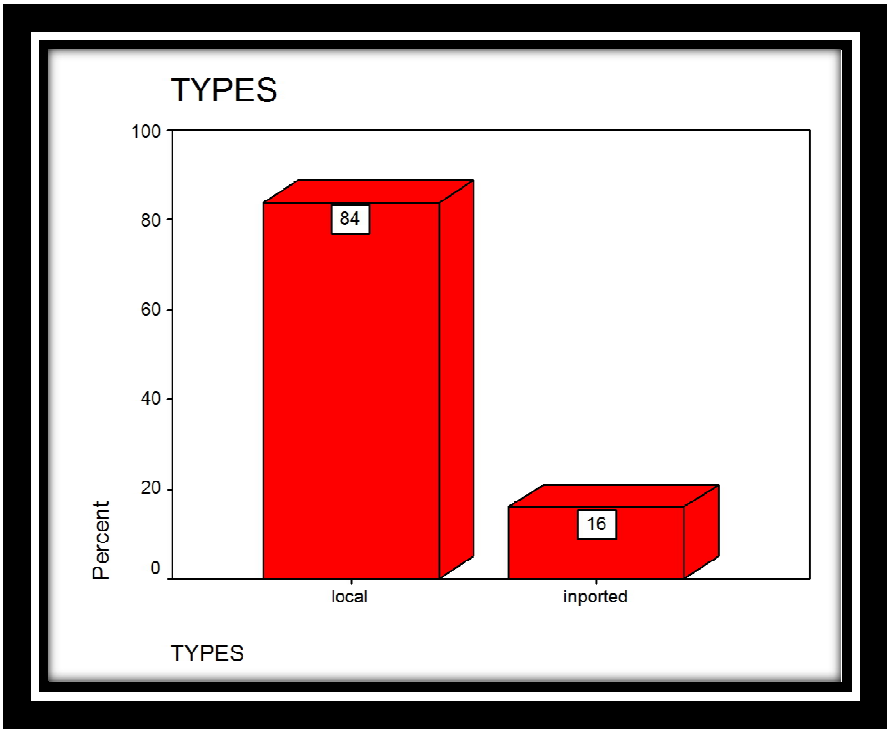


Fig3.1 Type of alcohol used byalcoholics:

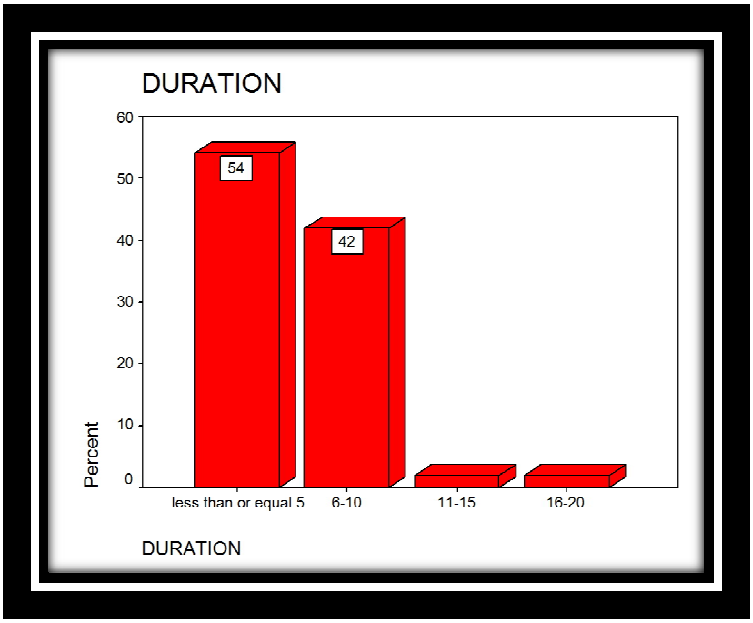


Fig 3.2 Duration(years) of alcohol abuse among the study group:

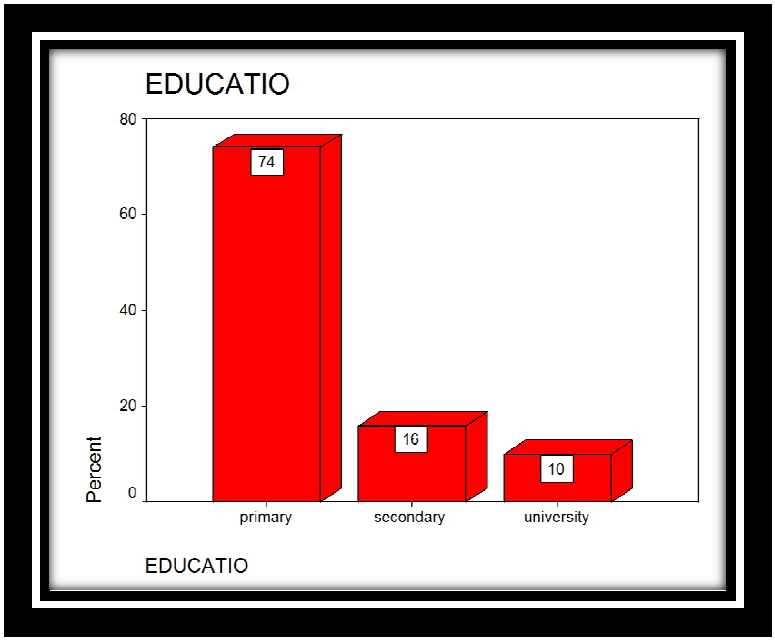


Fig 3.3 level of education among the study group:

3.2 Laboratory Data

Table 3-1 shows insignificantly (P<0.05) differences between alcoholics and control group with regard to
(, RBCs, Hb, PCV, MCV, MCH, MCHC, RDWSd,).
Significantly (P.0.06) difference in RDWCVbetween alcoholics and control group.

Table (3-1) the meanof erythrocytics series among study group:

Parameter	Variable	Number	Mean±SD	P.values (sig)
RBCsx10^{12}c/L	case	50	4.53±.06	0.43
	Control	30	4.55±.57	
Hb (g/dL)	case	50	14.28± .23	0.59
	Control	30	14.87±.23	
PCV (%)	case	50	39.26±.57	0.53
	Control	30	39.29±.68	
MCV (FL)	case	50	86.75±1.54	0.56
	Control	30	86.04±.78	
MCH (Pg.)	case	50	29.46±.33	0.63
	Control	30	29.48±.27	
MCHC (g/dL)	case	50	35.66±.25	0.62
	Control	30	35.47±.11	
RDWSD (f l)	Control	30	43.29±.54	0.14
	case	50	42.13±.52	
RDWCV (%)	Control	30	12.92±.16	0.0
	case	50	13.65±.17	

Significant at the **P.vale ≤ 0.05**.

Table 3-2 shows that alcohol consumption did not causeany significant effect on (TWBCs, monocytes, eosinophil, and basophil).The mean neutrophils count of alcoholicsshows significant (P.0.001) decreased when compared with controls .The mean lymphocytes count of alcoholics is significantly(P0.00)higher than that of the control.

Table (3-2) the mean leucocytes series of alcoholic and control group.

Parameter	variable	number	Mean±SD	p.value (sig)
Twbcsx10^9c/L	control	30	6.33±1.7	0.9
	case	50	6.34±1.9	
Neutrophils x10^9c/L	control	30	3.51±1.5	0.0
	Case	50	2.96±1.45	
Lymphocytesx10^9c/L	Control	30	2.3±0.6	0.0
	Case	50	2.9±0.8	
Monocytesx10^9c/L	Control	30	0.32±0.2	0.1
	Case	50	0.27±0.1	
Eosinophil's x10^9c/L	Control	30	0.11±0.11	0.0
	Case	50	0.11±0.14	
Basophilx10^9c/L	Control	30	0.02±0.2	0.2
	Case	50	0.03±0.1	

Significant at the **(≤ 0.05)**.

Table 3-3 shows the mean platelet count of alcoholics ($274.6\pm8.6x10^9$/L) and control group which is significantly (P.0.06) decreased when compared with controls group.

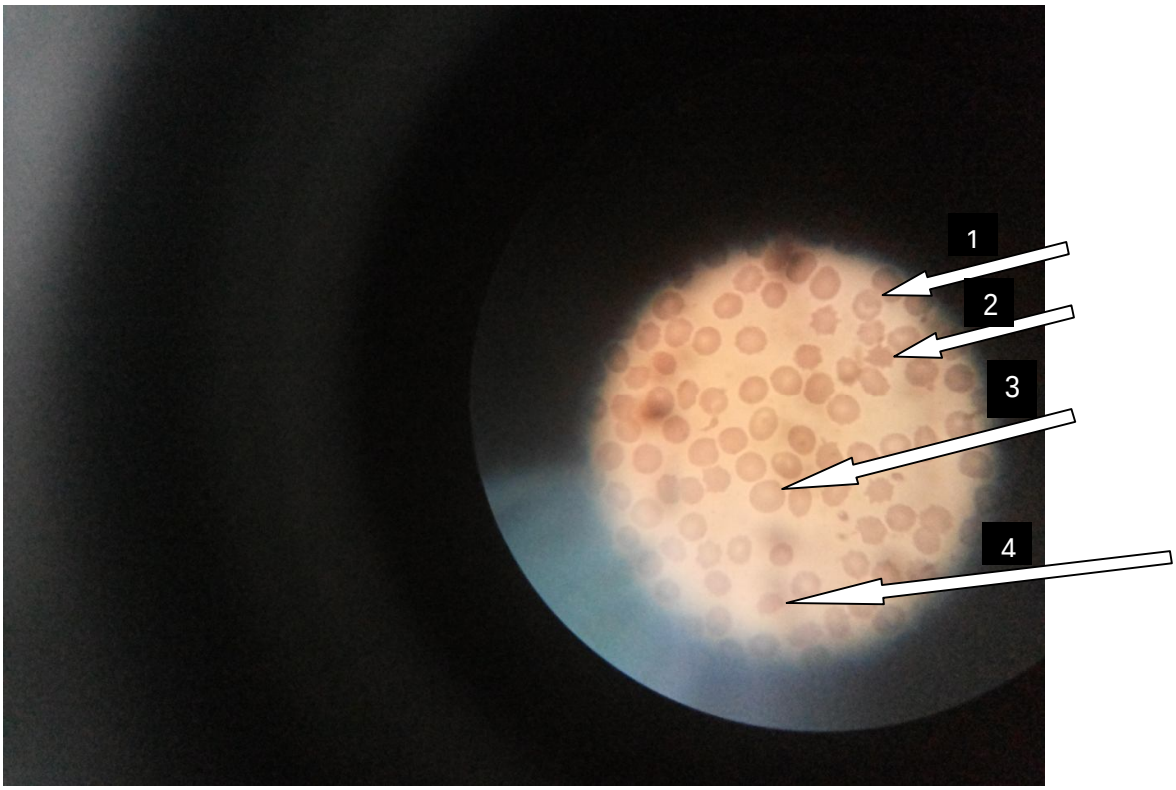
Shows insignificantly variations between alcoholic and control group with regard to (MPV, PDW, Plcr)

Table 3.3 shows mean Platelets Count and platelets indices of study groups:

Parameter	Variable	Mean±SD	P.values
plateletsx10^9/L	control	274.6 ± 8.6	0.6
	case	251.4 ± 5.3	
Mpvfl	control	9.53±.17	0.7
	case	8.57±.18	
PDW	control	11.76±.35	0.8
	case	11.89±.22	
Plcr %	control	21.8±1.36	0.6
	case	21.03±.78	

Significant at the **(≤ 0.05)**.

There is also significant change found in blood picture of alcoholic individual .target cell ,fragmented cell, crenated cell found in some of alcoholic blood picture. show below



1- Target cell.
2- Cernated cell.
3- Macrocyte cell.
4- Fragmented cell.

Chapter four

Discussion, conclusion and recommendation

4.1 Discussion:

Alcohol is a major contributor to the global burden of disease, disability, and death in high, middle, and low-income countries. Harmful use of alcohol is one of the main factors contributing to premature deaths and avoidable disease burden worldwide and has a major impact on public health. (Das, 2006)

This is case control study was conducted to assess some hematological parameters (CBC, RBC, Hb, PCV, and RBC indices, WBC and absolute differential leukocyte count, platelets count and its indices). The results obtained from the study carried on 50 casesknown as alcoholic in Khartoum state and 30 healthy volunteers (controls) who not received any kind of therapy or have any malignant or chronic disease throughoutthe last three months fromdateofinvestigation.

No affect was found on RBCs, Hb,PCV, and red cell indices due to alcohol drinking this result is same as that (occasional drinkers) done on 200 adult Nigerian males categorized into four groups: none drinkers, occasional drinkers, moderate drinkers and heavy drinkers(Oduola*etal* .,2005).

Neutropenia was documented in alcoholism decrease of absolute neutrophils count when compared with controls group show significantly decrease (P=0.001) the result agree with (Libre, 1999)study.

Lymphocytosis reported in alcoholicwas found significantly increase (P.values =0.0) compared to controls group. This increase is due to neutropenia.While there is no effect was found on monocyte, eosinophil, and basophil this result agree with (occasional drinkers) study (Oduola*etal.*,2005*.)*

Significantly (P.0.06) decrease in platelet count in participant was found when compared with control group this result agree with (Latvala*etal.*, 2004).Thrombocytopenia is a frequent complicationof alcoholics. The patients generally do not exhibitmanifestationsofexcessive bleeding. Moreover, alcohol-related thrombocytopeniagenerally is transient, and Platelet counts usually return to normalwithin one week of stop drinking patients generally do notrequiretherapeutic intervention other thanthat needed to use alcohol withdrawal. (Ballard, 2010.). While there is no affect found on MPV, PLCr.

There is also significant change found in blood picture of some alcoholic'sindividuals.

4.2. Conclusion:

This study concluded that:

Alcohol did not cause significant variation in the Hb, RBCs, PCV or red cell indices.

TWBCs count, monocytes, eosinophil and basophil were not affected by alcohol consumption.

A significant reduction in neutrophils, and platelet count was observed in alcoholic individuals compared with the control group.

Alcohol induced morphological changes in RBCs. show appendix no (8).

4.3. Recommendation:

1-The government should provide education for all the people.

2-Further studies should be done to diagnose alcohol induced diseases at early stages.

3-Clinical history of alcoholism during hematological investigation should be mandatory

4- NGOs should make a great effort in raising the public awareness of alcohol caused social and health problems.

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Appendix–1

Sudan University of Science and Technology

College of Graduate Studies

Department of Hematology

Measurement of Some Hematological Parameters among

Alcohol abuse persons - Khartoum State

Serial NO [ ]

الأسم:.....

الموضوع:_ جمع عينات لأجراء البحث التكميلي (سحب 2.5 مل دم).

	male	female
Sex		

	0	1	2	
Age	<20	21-40	>41	

	0	1
Types of alcohol	local	Exported

	0	1	2	3
Duration	<1year	2-5year	6-10 year	>10year

	0	1	2	3
Education	primary	secondary	university	H.degre

أمضاء الباحث .....

امضاء المتبرع .....

Appendix–2

بسم الله الرحمن الرحيم

جامعه السودان للعلوم والتكنولوجيا

كليه الدر اسات العليا برنامج ماجستير - محتبرات طبية

قسمأمراض الدم ومبحث المناعة

براءةأخلاقية

الاسم:.....

سوف يتم اخذ عينه من الدم (2.5 مل) من الوريد بواسطة حقنه وذلك بعد مسح منطقه اخذ العينه بواسطه المطهر كل الادوات المستخدمه لاخذ الينه معقمه ومتبع فيها مل وسائلالسلامة المعملية.

الامضاء:.....

التاريخ:.....

Appendix-3

RAL 555 stain

Appendix-4

Sysmex(KX-21N)

Appendix-5

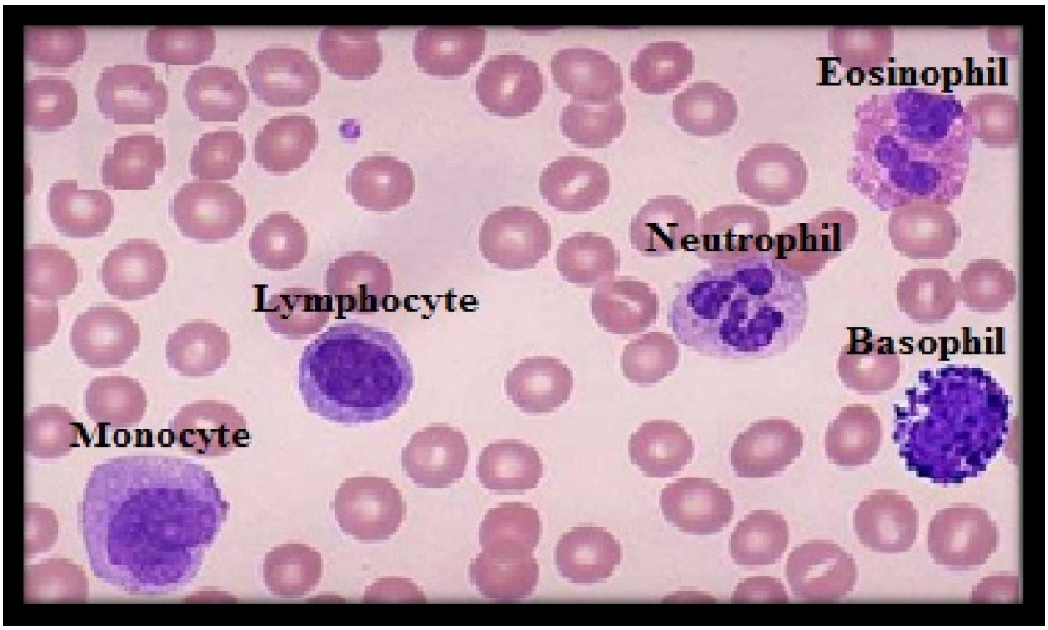
Reagents of Sysmex KX-21N

Appendix-6

Reference Value of some Hematological Parameters in adult:

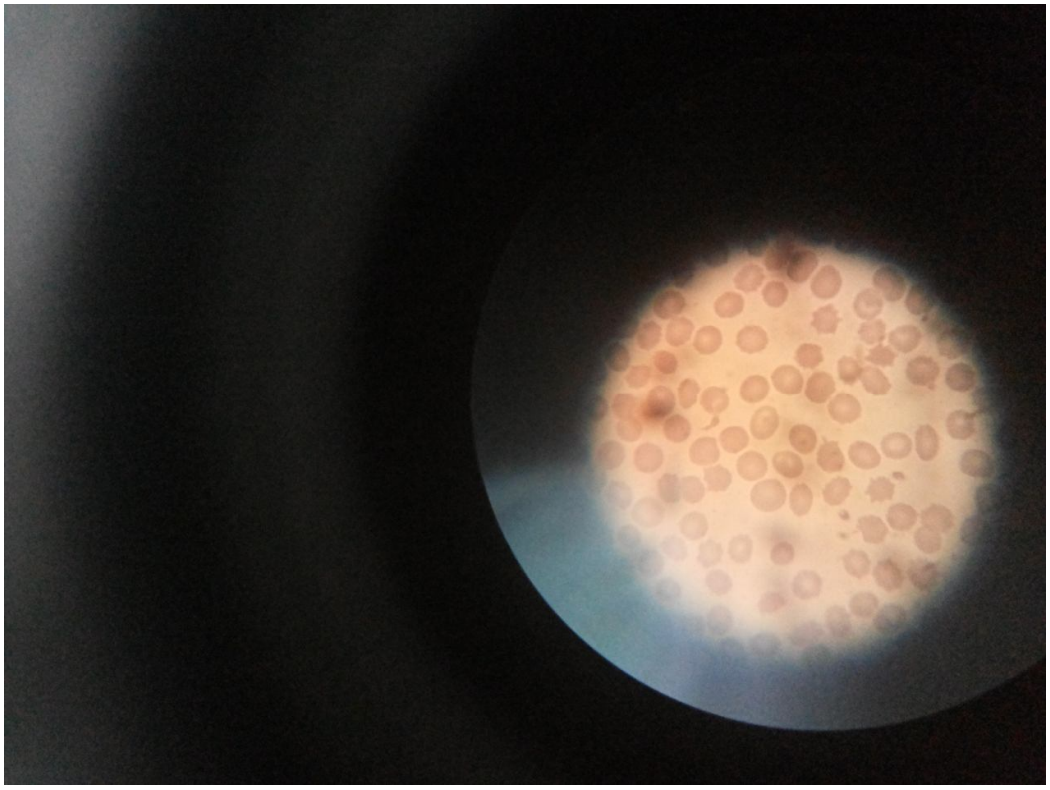
Parameters	Values
Hb	Male 15±2 g/L Female13.5±1.5 g/L
RBC	Male5 ± 0.5 $x10^6$/ul Female 3.3 ± 0.5 $x10^6$/u
PCV	0.45 ± 0.05 L/L
MCV	92 ± 9 fL
MCH	29.5 ± 2.5 Pg.
MCHC	330 ± 15 g/L
RDW SD	42.5 ± 3.5 fl
RDW CV	12.8 ±1.2%
WBC	4.0–10.0 × 10^9/l
Neutrophil	2 - 7x10^9/L(40–80%)
Lymphocyte	1 - 3 x10^9/L(20–40%)
Monocyte	0.2 - 1x10^9/L(2–10%)
Eosinophil	0.02 – 0.5 x10^9/L(1–6%)
Basophil	0.02 - 0.1 x10^9/L(<1–2%)
Platelet	150 - 450 x10^9/L

Appendix-7



Normal Blood Cells Morphology__

Appendix-8



Alcoholic blood picture.One of