Sudan University of Science and Technology

College of Graduate Studies

Assessment of Complete Blood Count among Sudanese Patients using Antiepileptic Drugs in Khartoum- Sudan

تقييم تعداد الدم الكامل للمرضى السودانيين الذين يستخدمون الأدوية المضادة للصرع في الخرطوم – السودان

A Thesis Submitted for Partial Fulfilment of the Requirements for the M.Sc. Degree in Medical Laboratory Science (Hematology and Immunohematology).

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قَالَ تَعَالَىٰ: أَعُوذُ بِٱللَّهِ مِنَ ٱلشَّيۡطَٰنِ ٱلرَّجِيمِ ﴿ ٱقۡرَأۡ بِٱسۡمِ رَبِّكَ ٱلَّذِي خَلَقَ ﴿١﴾ خَلَقَ ٱلۡإِنسَٰنَ مِنۡ عَلَقٍ ﴿٢﴾ ٱقۡرَأۡ وَرَبُّكَ ٱلۡأَكۡرَمُ ﴿٣﴾ ٱلَّذِي عَلَّمَ بِٱلۡقَلَمِ ﴿٤﴾ عَلَّمَ ٱلۡإِنسَٰنَ مَا لَمۡ يَعۡلَمۡ ﴿٥﴾ كَلَّآ إِنَّ ٱلۡإِنسَٰنَ لَيَطۡغَىٰٓ ﴿٦﴾ أَن رَّءَاهُ ٱسۡتَغۡنَىٰٓ ﴿٧﴾ إِنَّ إِلَىٰ رَبِّكَ ٱلرُّجۡعَىٰٓ ﴿٨﴾ ﴾

صدق الله العظيم

سورة العلق الآيات(1-8)

Dedication

This research is lovingly dedicated to my parents who have been my constant source of inspiration. They have given me the drive and discipline to tackle a task with enthusiast and determination. Without their love and support this project would not have been made possible.

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My grateful thank for ALLAH who guided me to the straightway in my life.

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Abstract

This is a case- control study aimed to evaluate some hematological parameters in patients under treatment with antiepileptic drugs and in normal control subjects in Khartoum state during period from March to December (2019).

Hundred subjects, selected randomly in this study, with age varies from 10-60 years, 50 subjects were epileptic patients (26 males and 24 female) as case group and 50 subject (25 males and 25 female) were age and sex matched healthy control group. The age grouped in three age groups and age group (10-25 year) high frequent in both case and control 25(50%), 31(62%). Followed by age group (26-50 year) in case and control 19(38%), 15(30%), followed by age group (>50year) low frequent in both case and control 6(12%), 4(8%) respectively. Venous blood (3ml) was collected in ETDTA container from each subject. Hematological analyzer (Sysmex KX 21N) was used for complete blood count. Statistical analysis was performed using Statistical Package for Social Science (SPSS) program version 22. The data display as mean ± SD, frequency, person correlation, one-way ANOVA test and *P. value* ≤ 0.05.

There was no statistical correlation between levels of Hb and MCHC in both case and control *p.* value 0.055 and 0.82 respectively. Statistically there was significant correlation in MCV and MCH level between case and control *p.* value was 0.03 and 0.011 respectively.

There was insignificant relationship between Hb, MCHC and type of treatment *p. value* 0.992 and 0.66. but there was significant relation between MCV, MCH and type of treatment *p. value* 0.027 and 0.042 respectively. Post-hoc using LSD test was conducted, showed significant correlation with Na valproate and phenytoin *p. value* 0.11 and 0.26 respectively. There was no statistical correlation between Hb level and dose and duration of treatment *p. value* 0.413 and 0.911 respectively.

The study concluded that estimation of hematological parameters, vitamin B12 level and folic acid level be strictly monitored regularly in individuals administered antiepileptic drugs for long period.

مستخلص البحث

هذه دراسة حالة ضابطة هدفت إلى تقييم بعض المتغيرات الدموية لدى مرضى الصرع الذين يخضعون للعلاج بمضادات الصرع مقارنة مع الأفراد الطبيعيين بولاية الخرطوم في الفترة من مارس إلى ديسمبر (2019).

اختير مائة شخص بشكل عشوائي في هذه الدراسة وكانت أعمارهم تتراوح من 60-10عاما، 50 من مرضى الصرع (26رجل24امرأة) و50 من الأفراد الطبيعيين (25 رجل و25 امرأة). تم تقسيم الافراد تحت الدراسة إلى ثلاثة فئات عمرية وكانت الفئة العمرية (25-10 عاما) هي الأكثر شيوعا 25 (50%) ،(62%) 31. تليها الفئة العمرية (50-26 عاما) 19(38%)، (30%) 15. وكانت الفئة العمرية > 50عاما الأقل شيوعا 4(8%)،6(12%) في كل من مرضى الصرع والافراد الطبيعيين على الترتيب. سحبت 3 مل عينة وريدية من كل مشارك في انبوبة تحتوي على مانعة التجلط EDTA. استخدم محلل الدم Sysmex KX21N. حللت البيانات باستخدام الحزمة الإحصائية للمجتمع (نسخة 22) استخدم انوفا (ANOVA) وPerson للارتباط لمقارنة المتوسطات وكانت القيمة المطلقة متوافقة عند اقل من 0.05.

ليس هنالك أي اختلاف ذو دلالة إحصائية في قياس الهيموغلوبين ومتوسط كمية الهيموغلوبين داخل خلية الدم الحمراء الواحدة فيما بين المرضى الذين يستخدمون مضادات الصرع والأشخاص الطبيعيين (القيم الاحتمالية 0.82 ,0.055على الترتيب). هنالك اختلاف ذو دلالة إحصائية في قياس متوسط حجم كرية الدم الحمراء ومتوسط كتلة الهيموغلوبين في كرية الدم الحمراء فيما بين المرضى الذين يستخدمون مضادات الصرع والأشخاص الطبيعيين (القيم الاحتمالية 0.011 ,0.03 على الترتيب).

لا توجد دلالة وصفية حسابية لقياس الهيموغلوبين ومتوسط كمية الهيموغلوبين داخل خلية الدم الحمراء فيما بين أنواع الادوية المستخدمة (القيم الاحتمالية 0.66 ,0.0992 على الترتيب). لكن توجد دلالة وصفية حسابية لمتوسط حجم كرية الدم الحمراء ومتوسط كتلة الهيموغلوبين في كرية الدم الحمراء فيما بين أنواع الادوية المضادة للصرع (القيم الاحتمالية 0.042 ,0.027 على الترتيب).

لا توجد دلالة وصفية حسابية لمتوسط الهيموغلوبين في المرضى الذين يخضعون للعلاج بمضادات الصرع والمتغيرات المختارة (جرعة العلاج ومدة استخدام العلاج) وكانت (القيم الاحتمالية0.911 ,0.413 على الترتيب).

لقد توصلت الدراسة الى ان قياس المتغيرات الدموية، مستوى فيتامين ب 12 وحمض الفوليك يجب ان يكون بصورة دورية ومنتظمة لمرضى الصرع الذين يستخدمون مضادات الصرع لفترة طويلة.

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List of Abbreviation

AED	Anti-epileptic drugs
CBZ	Carbamazepine
CNS	Central nervous system
DNA	Deoxyribo nucleic acid
DUMP	Deoxyuridine monophosphate
EDTA	Ethylene- diamine- tetra- acetic acid
FL	Femtoliter
FBP	Folate binding protein
GABA	Gamma- aminobutyric acid
GGT	Gamma-glutamyl-transferase
Hb	Hemoglobin
HICN	Cyanomet hemoglobin
HoloTC	Holotranscobalamin
IF	Intrinsic factor
ILAE	International league against epilepsy
ID-LC-MS	Isotope dilution-liquid chromatography-tandem mass spectrometry
LCD	Liquid crystal display
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean corpuscular volume
MMA	Methylmalonic acid
PA	Pernicious anemia
Pg	Pico gram
PHT	Phenytoin
P. value	Probability value
RBCs	Red blood cells
SAM	S- adenosylmethionine
SD	Stander deviation
SPSS	Statistical package for social science
tHcy	Total homocysteine
THF	Tetrahydrofolic acid

WBCs	White blood cells
WHO	World health organization

CHAPTER I

INTRODUCTION

Chapter I
Introduction

1.1. Introduction

Since it was first described in 1849 by Thomas Addison, megaloblastic anemia has been attributed to both congenital (uncommon) and acquired (common) problems. It is most frequently related to vitamin B12 deficiency due to defective absorption, folic acid deficiency due to malnutrition, or both. However, because of the correction of most of the dietary causes of vitamin B12 and folate deficiency, drug-induced megaloblastic anemia has become a more prominent cause of megaloblastic anemia. The drugs that may cause condition are commonly used in clinical practice (Charles *et al*., 2015).

One of drugs that interfere with absorption or proper distribution of folic acid is anti-epileptic drugs (AED) (Shipton, 2015). Epilepsy is a chronic neurological disorder caused by transient cerebral dysfunction due to disordered electrical activity of human brain. It is a common neurological disease with world-wide prevalence of 7.0 % (Hong-Li Huang *et al*., 2016). Approximately 50 million people worldwide are affected by epilepsy, one of the most common serious neurological disorders that has potentially deadly consequences (WHO, 2020).

Men and women receiving some AEDs are at risk for low levels of serum and red blood cell folic acid. Phenytoin and other anticonvulsant agents have been implicated in drug-induced megaloblastic anemia. These drugs cause megaloblastosis through increasing folate catabolism or inhibiting folate absorption (Al Qahtani, 2018). However, most antiepileptic medications increase hepatic microsomal enzyme activity, and it is believed that this increase in activity may result in an increase in the use of folic acid, thus leading to a decrease in serum folate levels. Similarly, these drugs may enhance hepatic detoxification enzymes, thus causing an increased breakdown of folic acid (Linebank *et al*., 2011).

The hallmark of megaloblastic anemia is nuclear-cytoplasmic dissociation; the nucleus remains immature in appearance while the cytoplasm matures more normally. This dissociation is manifested in the marrow and other proliferating tissues in the

body by large cells containing a large nucleus with a diffuse and immature-appearing chromatin content, surrounding by a normal appearing cytoplasm (Turgeon, 2012).

1.2. Rationale

Epilepsy is a chronic noncommunicable disease of brain that affects all people of all ages, more than 50 million people worldwide have epilepsy making it one of the most common neurological disease globally (WHO, 2019).

Antiepileptic medicines, such as valproic acid may play a crucial role in enhancing folate metabolism and enzyme induction, thus inducing megaloblastic anemia (Barik, 2016). Phenytoin is an anticonvulsant widely used, being effective against various forms of partial and generalized seizures, one of its unwanted effects is megaloblastic anemia (Rang *et al.*, 2017).

This study is very important because there are no published previous studies about megaloblastic anemia associated with antiepileptic therapy in Sudan.

1.3. Objectives

1.3.1. General objective

To assess complete blood count among Sudanese patients using antiepileptic drugs in Khartoum- Sudan.

1.3.2. Specific objectives

1. To measure complete blood count parameters, which include Hb level, MCV, MCH, MCHC, WBCs and PLTs among study group.

2. To compare the results of Hb level, MCV, MCH, MCHC, WBCs and PLTs between epileptic patients and normal control.

3. To correlate complete blood count parameters with possible risk factors e.g.: type, dose and duration of treatment.

CHAPTER II

LITERATURE REVIEW

Chapter II

Literature Review

2.1. Anemia

Anemia is a condition in which the number and size of red blood cells, or the hemoglobin concentration, falls below an established cut-off value, consequently impairing the capacity of the blood to transport oxygen around the body. It is an indicator of both poor nutrition and poor health (WHO, 2014). The symptoms of anemia depend on the degree of reduction in the oxygen- carrying capacity of the blood, change in the total blood volume, the rate at which these changes occur, the degree of severity of the under lying disease contributing to anemia, and the power of the cardiovascular and hematopoietic system to recuperate and compensate (Makaron and Josieph, 2016).

2.2. Causes of anemia

Anemia has the number of etiologies including iron deficiency, deficiency of other micronutrients particularly folate and vitamins B12, genetic disorders, high demands for iron in conditions like pregnancy, during menstruation, time of rapid growth and worm infestation (Ciesla, 2013).

2.3. Classification of anemia

Anemia can be classified from three points of view: pathogenesis, red cell morphology, and clinical presentation. Pathogenic mechanisms involved in the production of anemia are very simple: inadequate production and loss of erythrocytes as a result of bleeding or hemolysis. Based on pathogenic mechanisms (Makaron and Josieph, 2016). anemia can be divided into two types:

2.3.1. Hypo-regenerative anemia

When bone marrow production is decrease as a result of impaired function, decreased number of precursor cells, reduced bone marrow infiltration, or lack of nutrients (Moreno, 2011).

2.3.2. Regenerative anemia

When bone marrow responds appropriately to a low erythrocyte mass by increasing production of erythrocytes (Turgeon, 2012).

In practice, classification based on basic parameters of red cell morphology such as mean corpuscular volume (MCV), allows for a quicker diagnostic approach. Anemia also can be classified according to the form of clinical presentation as acute (usually bleeding or hemolysis) or chronic (Stabler, 2013).

The main causes of anemia can be usefully classified according to associated red cell change:

- Hypochromic, microcytic- including iron deficiency and thalassemia.
- Normochromic, macrocytic- including B12 and folate deficiency, alcohol.
- Poly chromatic, macrocytic- hemolysis.
- Normocytic, normochromic- chronic disorders, renal failure, diseases of bone marrow.
- Leucoerythroblastic- myelofibrosis, leukemia, metastatic carcinoma (Lanzkowsky, 2016).

2.4. Megaloblastic anemia

Megaloblastic anaemia is defined as a highly characteristic set of morphological changes which affect cells of the erythroid, myeloid and megakaryocytic lineages in the peripheral blood and bone marrow (Provan, 2015). Macrocytic anaemia refers to a blood condition in which the red cells are abnormally large (mean corpuscular volume, MCV >95 femtoliter fl). There are several causes but they can be broadly subdivided into megaloblastic and non-megaloblastic, based on the appearance of developing erythroblasts in the bone marrow (Hoffbrand, 2016).

The megaloblastic anemias are a group of disorders characterized by the presence of distinctive morphological appearances of the developing red cells in the bone marrow. The cause is usually deficiency of either cobalamin (vitamin B 12) or folate, also may arise because of inherited or acquired abnormalities affecting the metabolism of these vitamins or because of defects in DNA synthesis not related to cobalamin or folate (Hoffbrand and Moss, 2016).

Two vitamins, cobalamin and folic acid are essential for DNA biosynthesis. Thymidine is a component of DNA but not RNA, and it is present in cells in rate-limiting amounts. The other nucleotides tend to be present in excess (Jeffrey, 2015). Thymidine can be salvaged from the turnover of DNA, but the main source is the addition of a methyl group to the 5-position of the pyrimidine ring to convert deoxyuridylate to deoxythymidylate. This methylation process depends crucially on folate and vitamin B12 (Khanduri, 2010).

Deficiency of either vitamin results in asynchrony in the maturation of the nucleus and cytoplasm of rapidly regenerating cells (Carmel, 2014). In hematopoietic system this asynchrony results in abnormal nuclear maturation with normal cytoplasmic maturation, apoptosis, ineffective erythropoiesis, intramedullary hemolysis, pancytopenia and typical morphological abnormalities in the blood and marrow cells (Jeffrey, 2015).

2.5. Vitamin B12

2.5.1. Chemistry of vitamin B12

The chemical structure of vitamin B12 (cobalamin) is shown in Fig.1. it has 1 cobalt atom and 4 pyrole rings in the center of the corrin ring. Cobalamin is given different names, depending on the radical to which it is bound. When it binds to a cyanoradical, it is called cyanocobalamin or vitamin B12, a highly stable compound. Other functional forms of cobalamin include adenosylcobalamin (adenosylradical) and methylcobalamin (methyl) (Giedyk *et al*., 2015).

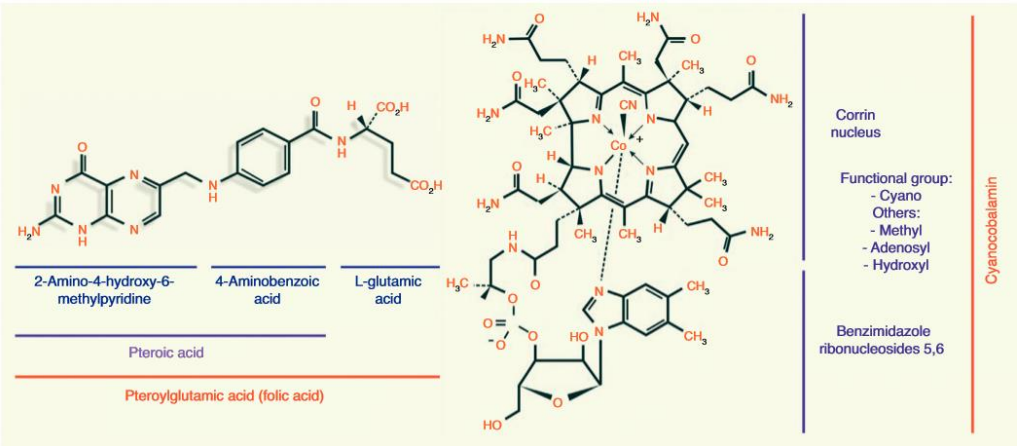

Figure (1.1): Chemical structure of vitamin B12 and folic acid (Castellanos- Sinco *et al*., 2015).

2.5.2 Sources of vitamin B12

Vitamin B12 is a water soluble vitamin obtained through the ingestion of fish, meat, and dairy products, as well as fortified cereal and supplements. It is coabsorbed with intrinsic factor (IF), a product of the stomach's parietal cells, in the terminal ileum after being extracted by gastric acid. Vitamin B_{12} is crucial for neurological function, red blood cell production, and DNA synthesis (Robert and Andrew, 2017).

The recommended dietary allowance of vitamin B12 is shown in Table 1. The body stores are between 2 to 5 mg of vitamin B12 for between 3 and 4 months. It is mainly stored in the liver (Brown, 2013).

Table 1.1: Recommended dietary allowance of folic acid and cobalamin (Castellanos- Sinco *et al*., 2015).

Patient population	Folic acid (mcg)	Vitamin B12 (mcg)
Adults, including women of child bearing age	400	2
Pregnant women	600	2.6
Breastfeeding women	500	2.6
Children and adolescents	50-200	0.4-1.8

2.5.3. Absorption of vitamin B12

After ingestion, vitamin B12 is bound in the mouth to haptocorrin (transcobalamin II), from which it becomes disassociated in the stomach because of the presence of gastric enzymes and acid. The haptocorrin is replaced by intrinsic factor secreted by stomach parietal cells. The vitamin B12- intrinsic factor complex attaches to receptor cubilin, which present on surface of epithelial cells of terminal ileum and facilitates the absorption of vitamin B12- intrinsic factor complex. Intrinsic factor is degraded within the ileal cells, and vitamin B12 is absorbed into blood stream, where it become bound to transcobalamin II, which transport it for various organs for DNA synthesis (Charles, 2015).

2.5.4. Physiological role of vitamin B12

Vitamin B12 is used by the body in two forms, either as methylcobalamin or 5-deoxyadenosyl cobalamin. The enzyme methionine synthase need methylcobalamin as a cofactor. This enzyme is normally involved in the conversion of amino acid homocysteine into methionine, while methionine, in turn, is required for DNA methylation (Fiona *et al*., 2010).

5-Deoxyadenosyl cobalamin is a cofactor needed by the enzyme that converts 1-methylmalonyl CoA to succinyl CoA. This conversion is an important step in the extraction of energy from proteins and fats. In addition, succinyl CoA is necessary for the production of hemoglobin which is the substance that carries oxygen in red blood cells (Mahmood, 2014).

2.5.5. Causes of cobalamin (B12) deficiency

1.A strict vegetarian (vegan) diet contain very little cobalamin

2.Gastrectomy: loss of IF production and gastric acid.

3.Disorders of alimentary tract: produce lack of intrinsic factor, impairment of the absorptive capacity of the intestinal mucosa, and interference with normal absorption by bacteria or parasites (Wolffenbuttel *et al.*, 2019).

4. pernicious anemia in which the atrophy of the gastric parietal cells results in lack of secretion of both IF and chlorydic acid.

5. Some drugs such as H2 antagonists, omerprazole, colchicine, neomycin and biguanides may reduce cobalamin absorption through different mechanisms: inhibition of IF and acid secretion and transenterocytic transport of cobalamin (Chiara *et al*., 2013).

6. Increase utilization of vitamin B12 because of parasitic infections such as fish tape worm and pathogenic bacteria (Turgeon, 2012).

The most common cause of cobalamin deficiency is pernicious anemia which is an autoimmune chronic gastritis, resulting in destruction of the parietal cell and loss of IF production. It occurs in all ethnic group, although the highest incidence appears to be in person of Scandinavian, English, Scottish, and Irish descent. In Caucasians the

average age onset is about 60 years, although it can be seen at all ages including children (Kern, 2012).

2.5.6. Pernicious anemia:

This is caused by autoimmune attack on the gastric mucosa leading to atrophy of the stomach. The wall of the stomach becomes thin with a plasma cell and lymphoid infiltrate of the lamina propria. Intestinal metaplasia may occur. There is achlorhydria and secretion of IF is absent or almost absent. Serum gastrin levels are raised. Helicobacter pylori infection may initiate an autoimmune gastritis which presents in younger subject as iron deficiency and in the elderly as pernicious anemia (Hoffbrand and Moss, 2016).

The standard method to diagnose pernicious anemia, once cobalamin deficiency is confirmed, is the schilling test. Radiolabeled cobalamin is given orally, a large dose of un labeled B12 is given intramuscularly, and urine is collected for 24 hours. The amount of radioactivity in the urine indicates how much B12 was absorbed orally. Typically, recovery of < 6% in the urine indicates malabsorption of B12. If the initial value is abnormal, a second stage is performed in which intrinsic factor is given together with the labeled B12. An increase in the amount of B12 absorbed during the second stage of the schilling test indicates pernicious anemia. (the purpose of the intramuscular B12 is saturate the B12-binding sites in the serum, and there by flush all of the orally absorbed B12 in to the urine, where it can be measured. (Kern, 2012).

2.6 Folic acid

2.6.1. Chemistry of folic acid

Folic acid, also known as pteroyl-glutamate or pteroylglutamic acid. Is made up of: (1) pteroic acid; and (2) l-glutamic acid (one or more strands (see Fig.1.) The functional form folate is tetrahydrofolic acid (Castellanos-Sinco *et al*., 2015).

2.6.2. Sources of folic acid

Folate is present in nearly all foods but is destroyed by 10 to 15 minutes of cooking. The main dietary sources of folic acid are green vegetables such as asparagus, broccoli, spinach and lettuce (Eunah, 2012). It is also found in fruits, such as lemons, oranges, bananas, and melons, and in cereals, grains, nuts, beans, beef, fish, liver and

kidneys. The principal site of intestinal absorption is upper third of small intestine (Kumar *et al*., 2013).

Dietary folates (5 methyltetrahydrofolate) are readily transported across the intestinal membranes. It is required for the conversion of methionine to S- adenosylmethionine (SAM). Thus, when folate levels are low, SAM is depleted, resulting in a reduction in the methylation of cytosine in DNA (Jeffrey, 2015).

2.6.3. Physiological role of folic acid

Folate is required in one of its coenzyme forms, 5, 10- S- adenosylmethionine (THF) polyglutamate, in the synthesis of thymidine monophosphate from its precursor deoxyuridine monophosphate (dUMP) (Bailey, 2010). The human body needs folate to synthesize, repair, and methylate DNA as well as to act as a cofactor in certain biological reactions (Weinstein, 2013). It is especially important in aiding rapid cell division and growth, such as in infancy and pregnancy. Children and adults both require folate to produce healthy red blood cells and prevent anaemia. Vitamin B12 is needed to convert methyl THF, which enters the cells from plasma, to THF, from which polyglutamate forms of folate are synthesized (Barua, 2014).

2.6.4. Causes of folate deficiency

1. Dietary folate deficiency is common. Indeed, in most patients with folate deficiency a nutritional element is present. Certain individuals are particularly likely to have diets containing inadequate amounts of folate, including the old, edentulous, poor, alcoholic and psychiatrically disturbed, and patients after gastric operations (Hoffbrand, 2016).
2. Antifolate drugs large number of epileptics who are receiving long-term therapy with phenytoin (Dilantin) or primidone (Mysoline), with or without barbiturates, develop low serum and red cell folate levels (Hoffbrand *et al*., 2016).
3. Tropical sprue is one of the most common malabsorption syndromes contributing to folic acid deficiency. In which the villi that line the digestive tract are flattened leading to low absorption activity (Kawathalkar, 2013).

2.7. Clinical features of megaloblastic anemia

The onset of megaloblastic anemia is usually insidious with typical anemic symptoms of lethargy, weakness, and a yellow or waxy pallor. Glossitis with a beefy red tongue or more commonly a smooth pale tongue is characteristic. Loss of weight and loss of appetite are common complaints. Atrophy of the gastric parietal cells causes decreased secretion of intrinsic factor and hydrochloric acid (Antony, 2012).

Neurologic disturbances occur only in cobalamin deficiency, not in folic acid deficiency. These are the most serious and dangerous clinical signs because neurological damage may be permanent if deficiency is not treated promptly (Bain, 2017).

2.8. Tests to diagnose cobalamin deficiency

2.8.1. Mean cell volume and blood film examination

Identification of hypersegmented neutrophils, defined as >5% of neutrophils with five or more lobes and the presence of oval macrocytes, may suggest either cobalamin or folate deficiency, but they are not sensitive in early cobalamin deficiency and are not specific for it. Oval macrocytes, hypersegmented neutrophils and circulating megaloblasts in the blood film and megaloblastic change in the bone marrow are the typical features of clinical cobalamin deficiency. However, an elevated mean cell volume is not a specific indicator of cobalamin deficiency (Galloway & Hamilton, 2007).

2.8.2 Serum cobalamin

A serum cobalamin assay is currently the standard initial routine diagnostic test. It quantitates both the ‘inactive’ forms (transcobalamin I- and transcobalamin III-bound, now referred to as holohaptocorrin) and the ‘active’ form (transcobalamin II-bound, now referred to as holotranscobalamin) of cobalamin in serum (Vinod *et al.*, 2014).

2.8.3. Plasma total homocysteine (tHcy)

Deficiency of cobalamin results in elevation of plasma total homocysteine (tHcy). Plasma tHcy is a sensitive biomarker of cobalamin deficiency and increases early in the course of deficiency, sometimes preceding symptoms, and progresses as the

deficiency worsens (Wile, 2010). However, tHcy is not specific to cobalamin deficiency as concentrations of tHcy are also elevated in folate deficiencyB6 deficiency and in patients with renal failure, hypothyroidism and as a result of certain genetic polymorphisms (Mazokopakis, 2012)

2.8.4. Plasma methylmalonic acid (MMA)

Plasma MMA is raised in cobalamin deficiency. However, it also may be falsely elevated in subjects with renal disease, small bowel bacterial overgrowth and haemoconcentration (Carmel, 2011).

2.8.5. Holotranscobalamin

Holotranscobalamin (HoloTC), the active fraction of plasma cobalamin, may be more specific than serum cobalamin levels, and an immunoassay for this fraction is now available (Nexo, 2011). In clinical research studies, the HoloTC assay performs better than the serum cobalamin assay in assessing deficiency based on MMA levels (Hardlei, 2010).

2.8.6. Bone marrow examination

Bone marrow examination was historically recommended in situations where the clinical picture is unclear based on laboratory tests alone.

However, some cobalamin deficient patients have no overt haematological abnormalities and the value of a bone marrow examination, in this context, is unknown (Heil *et al.*, 2012).

2.9. Tests to diagnose folate deficiency

2.9.1. Serum folate

The serum folate concentration reflects recent folate status and intake. Most clinical laboratories today measure serum folate by competitive folate binding protein (FBP) assays using chemiluminescence or fluorescence detection systems. Despite considerable variation in performance between assays, using the isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) international reference methods, there was close correlation of the consensus mean in the UK NEQAS surveys (Black *et al.*, 2014). There is no clear consensus on the level of serum folate that indicates deficiency. Conventionally, clinicians have used serum

folate lower than 7 nmol/l as a guideline because the risk of megaloblastic anaemia greatly increases below this level (Yetley, 2011).

2.9.2. Red cell folate

The red cell folate level gives an assessment of the tissue folate status over the lifetime of the red cells and is therefore regarded as an indicator of longer term folate status than the serum folate assay. A red cell folate level below 340 nmol/l has been regarded as consistent with clinical folate deficiency in the absence of cobalamin deficiency (Breu *et al*., 2015).

2.9.3. Homocysteine

Elevated plasma tHcy is a sensitive indicator of folate status and is strongly correlated with serum folate levels in the low physiological range [i.e. serum folate levels below about 10 nmol/l. tHcy arises as a by-product of methionine metabolism and is normally present in plasma at concentrations below 12 µmol/l, depending on age, gender, renal function, genetic factors and the nutritional status of several other vitamins. It is not, therefore, a specific marker of folate status (Ma *et al*., 2017).

2.10. Macrocytic anemia without megaloblastosis

Large circulating erythrocytes are not always associated with a pathologic process or condition. Normally Red blood cells (RBCs) of newborns and infants tend to be larger than normal adult RBCs, and large erythrocytes can be seen during pregnancy in the absence of an obvious etiology, macrocytosis is frequently linked to alcoholism, with or without liver disease (Florence, 2006).

Of other possible etiologies, hypothyroidism, liver disease and primary bone marrow dysplasias, including myelodysplasia and myeloproliferative disorders are some of the more common causes (Joyce and Cheryl, 2009).

2.11. Epilepsy

Epilepsy is defined as the repeated occurrence of sudden, excessive and/or synchronous discharges in central cortical neurons resulting in disruption of consciousness, disturbance of sensation, movements, impairment of mental function, or some combination of these signs. Because of their sudden nature, seizures are

called ictal evens, from the Latin ictus meaning "to strike" (James Bowman *et al*., 2001).

The term epilepsy, seizure and convulsion are not synonymous. A seizure always is a symptom of abnormal function in central nervous system (CNS) rather than disease itself. A seizure discharge may be initiated in an entirely normal cerebral cortex by a variety of acute insults, such as withdrawal from alcohol, low blood sodium, or certain toxins. Seizures are to be distinguished from epilepsy, which is a chronic condition in which seizures occur repeatedly due to an underlying brain abnormality which persist between seizures. A convulsion is a forceful involuntary contraction of skeletal muscles. It is a physical manifestation of a seizure, but the term is inappropriate as a synonym for epilepsy when epilepsy may consist only of a temporary alteration of consciousness or sensation (James Bowman *et al.*, 2011).

2.12. Classification of epilepsy

Epilepsy is clearly not a uniform condition and comprises many different syndromes and seizure types. It traditionally subdivided into two main forms: partial and generalized epilepsies and each can be further classified according to etiology into symptomatic, cryptogenic (I.e. presumed symptomatic) or idiopathic. In 2010, the international league against epilepsy (ILAE) proposed a new classification which focuses more on the underlying etiology (genetic or structural/ metabolic) and uses a more flexible multidimensional design than the ILAE 1989 classification (Berg, 2010).

Table 1.2: Classification of epileptic seizures (Berg, 2010)**:**

ILAE Classification of Epileptic Seizures
i. Partial (focal, Local) seizures A. Simple partial seizures (consciousness not impaired) 1. With motor symptoms 2. With somatosensory or special symptoms 3. With autonomic symptoms 4. With psychic symptoms B. Complex partial seizures (with impairment of consciousness) 1. With simple partial onset followed by impairment of consciousness 2. With impairment of consciousness at onset C. Partial seizures evolving to secondary generalized seizures 1. Simple partial seizures evolving to generalized seizures 2. Complex partial seizures evolving to generalized seizures 3. Simple partial seizures partial seizures evolving to complex partial seizures evolving to generalized seizures ii. Generalized Seizures (convulsive or Nonconvulsive) A. Absence seizures 1. Typical absence seizures 2. Atypical absence seizures B. Myoclonic seizures C. Clonic seizures D. Tonic seizures E. Tonic-clonic seizures F. Atonic seizures iii. Unclassified Epileptic Seizures

2.13. Treatment of epilepsy

The mainstay of treatment is with anti-epileptic medication, or anti-epileptic drugs(AEDs). Approximately 70% of people stop having seizures at some point after introducing treatment. These types of antiepileptic medication do not stop a seizure

once it has started and they are not viewed as cures for epilepsy, nor are they cures for the cause of epilepsy. The goal of treatment is to try to prevent a seizure occurring (Marvin, 2010). Brown (2016) identified three ways by which AEDs exert their effects:

- By mainly targeting sodium and calcium channels to modulate the intrinsic membrane conducting activities to inhibit excessive firing neurons.
- Inhibit GABA metabolism or block GABA transport.
- Inhibit the excretory mechanism.

Most antiseizure medications increase hepatic microsomal enzyme activity, which increase the use of folic acid, thus leading to a decrease in serum folate levels. It is also may enhance hepatic detoxification enzymes thus causing an increased breakdown of folic acid. They are also associated with a considerable decrease in intestinal absorption of folic acid. Phenytoin and other anticonvulsants bear structural resemblances to folate may cause a decrease in serum folate levels by reducing the transport of folate. (Charles *et al.*, 2015). Nearly 14 antiepileptic drugs have been licensed for use in the past 20 years for common epilepsies and a range of more unusual syndromes. They are classified into first, second and third generation drugs according to the year of entry

into market (Table1.3) (Morrell, 2012).

Table 1.3: Classification of antiepileptic drugs.

First generation	**Second generation**	**Third generation**
Phenytoin	Lamotrigine	Lacosamide
Carbamazepine	Levetiracetam	Rufinamide
Phenobarbitone	Topiramate	Retigabine
Sodium valproate	Oxcarbazepine	Brivaracetam
Benzodiazepine	Tiagabine	Perampanel
Ethosuximide	Felbamate	Ganoxolone
	Vigabatrin	Carbabersat

2.14. Previous studies

Yubin and his colleagues based on previous articles performed in India. Showed that phenytoin (PHT) monotherapy is associated with the increase serum homocysteine and decreased levels of folate and vitamin B12 (Yubin *et al*., 2019).

Sharma and his colleagues evaluate homocysteine metabolism and hematological parameters in early stage of phenytoin treated epileptic children, they found that hematological parameters did not show any significant differences after phenytoin monotherapy as compared to before therapy. But a highly significant decrease was observed in serum folate and vitamin B12 levels after phenytoin monotherapy as compared to before therapy (Sharma *et al.*, 2017).

In china a case reported by Chen and his colleagues show development of anemia in the setting of a short term sodium valproate therapy for prevention of postoperative seizures in a 79-year-old man after 3 weeks' standard dose sodium valproate (Chen *et al.*, 2018).

In India Nayyar and his colleagues estimate serum folic acid level in 25 epileptic patients on long term phenytoin therapy the result showed a significant drop in serum folate level after 6 month of phenytoin treatment (Nayyar *et al*., 2013).

In china Huang and his colleagues estimate folic acid and B12 level of 68 patients diagnosed with epilepsy showed that various AED decreased the serum levels of folate and B12 (Huang *et al*., 2016).

In Iran Ghamari and her colleagues found that Hb level was significantly lower in 70.6% of women and 68.4% of study population (Ghamari *et al*., 2013).

In Germany Linnebank and his colleagues study interaction of antiepileptic drugs with folate and B12 serum levels showed significant increase in mean level of MCV IN case group compared to control group (Linnebank *et al*., 2011).

CHAPTER III

MATERIALS AND METHODS

Chapter III

Materials and Methods

3.1. Study design

This study is analytical prospective case control study.

3.2. Study area

This study was conducted in Bashaier and Ibrahim Malik Hospitals in Khartoum State.

3.3. study duration

The study conducted during the period from March to December (2019)

3.4. Study population

Fifty Sudanese patients using anticonvulsant were matched to fifty apparently healthy individuals were included in this study.

3.5. Inclusion criteria

Patients using anticonvulsant therapy, different ages from both sex were enrolled.

3.6. Exclusion criteria

Patients previously diagnosed with megaloblastic anemia due to another cause (any cause of folic acid or vitamin B12 deficiency), patients on chemotherapy or immunocompromised patients was excluded.

3.7. Ethical consideration

Research was approved from Scientific Ethical Committee of Medical Laboratory Science, Sudan University of Science and Technology. And participants were informed verbally with simple language about the research, it benefits and method of sample collection, then their approval will be taken.

3.8. Sample size

A total of 100 subjects were enrolled in this study. 50 samples were collected from epileptic patients and 50 samples were collected from healthy volunteer.

3.9. Data collection

The data were collected using a direct interviewing questionnaire. Medical information was collected from patients with help of the physician. Structured questionnaire used to collect demographic and clinical data.

3.10. Sampling techniques

Three ml of venous blood were collected from patients and control in EDTA container.

3.11. Procedure of sample collection

1. Patients were either sat or lid down on an examination table.

2. The arm was positioned on the armrest so that the vein identified become under some tension and its mobility was reduced.

3. The skin was cleaned with 70% ethanol and allowed to dry.

4. Personal details were checked up on the forms and on blood vials.

5. Tourniquet was applied to the arm, tight sufficiently to distend the vein, but not rightly to cause discomfort.

6. 3 ml of blood were taken from the superficial vein of the forearm.

7. Blood was collected in K2EDTA, blood sample was analyzed by sysmex (Bain, 2017).

3.12. Complete blood count

3.12.1. Principle of Sysmex® KX-21N hematological analyzer

Measurement of blood cells (RBC's, WBC's, and platelet) and hemoglobin concentration obtained by aspiration of small volume of well mixed (K2EDTA) blood by sample probe and mixed with isotonic diluents in nebulizer. Diluent aspiration delivered to RBC's aperture bath for providing information about RBC's and platelet. Other portion of aspirated sample induced in to WBC's bath in which hemolytic reagent (stromatolyzer) added to break down RBC's and release of hemoglobin which measured in build colorimeter based on cyanomet hemoglobin method (HICN). The through three sensing apertures for each cell type, cells counted and size information

generated in triplicate pulses acting to electronic conductivity. Mentioned pulses converts in to digital number using in bulid calculator programmed and designed for RBC's and WBC's counts. Some portion of diluted sample delivered to in bulid hemoglobin meter at the same time, hence three values directly measured (RBC's, TWBC's, Hb) And displayed on (LCD). Other values of red cell indices, leukocyte differential and absolute count calculated from given information, the result printed out aced to the setting mode. On the other hand, platelet count and histogram determined from pulses acting to the platelet (Bain, 2017). Reagents and materials provided by sysmex manufacture and contain:

1. Sample: well mixed K2EDTA blood.

2. Cell back.

3. Stromatolyzer.

4. Detergent.

5. Cell cleaner.

3.12.2. Procedure of Sysmex® KX-21N

The reagent needed was checked and the power switch was turned. Self-auto rinse, and back ground check was automatically performed. Whole blood mode was selected. Sample number was entered. Sample was mixed sufficiently. The tube was set to the sample probe, and in that condition the start switch was pressed. When the sucking of the sample was done, the tube was removed. After that automatic analysis was done and the result was displayed in the screen.

3.13. Data analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) program version 22. The data display as mean ± SD, frequency, person correlation, one-way ANOVA test and *P. value* ≤ 0.05.

CHAPTER IV

RESULTS

Chapter IV

Results

4.1: Results

Hundred volunteers of age between 10-60 years were enrolled in this study, 50 were epileptic patients with mean of age 26.3 ± 12.8, 26/50(52%) were male and 24/50(48% were female). Fifty were apparently healthy subjects with mean of age 28.84 ± 12.66, 25/50(50%) were male and 25/50(25%) were female (Table 4.1).

The age grouped in three age groups and age group (10-25 year) high frequent in both case and control 25(50%), 31(62%). Followed by age group (26-50 year) in case and control 19(38%), 15(30%), followed by age group (>50year) low frequent in both case and control 6(12%), 4(8%) respectively (Table4.2).

Among case 6/50(12%) volunteers are taking folic acid and 44/50(88%) not taking folic acid (table 4.3). Four types of epilepsy treatment are enrolled in this study.16/50(32%) volunteers use Na valproate, 11/50(22%) use phenytoin, 4/50(8%) use lamotrigine and 19/50(38%) use carbamazepine (Figure4.1).

The mean level of Hb (g/dl) were 12.84 ± 1.88, 12.37 ± 1.59 in the case group and control group respectively. The mean level of MCHC (g/dl) were 34.14 ±2.03, 40.44 ± 43.67 in case group and control group respectively. There was no statistical difference between levels of Hb and MCHC in both case and control *p*. value 0.055 and 0.82 respectively. The mean level of MCV (fl) were 85.13 ± 5.8, 82.03 ± 7.19 in case group and control group respectively. The mean level of MCH (pg) were 29 ± 28.2, 28.2 ± 2.94 in case group and control group respectively. Statistically there was significant difference in MCV and MCH level between case and control *p*. value was 0.03 and 0.011 respectively. The mean of WBCs was 6.97 ± 2.10 ,7.80 ± 2.59 in case group and control group respectively. The mean of platelets was 286.32 ± 114.11 ,330.99 ± 124.3 in case group and control group. There was no significant difference between WBCs and PLTs count in both case and control group *p. value* 0.778 and 0.541 respectively (Table 4.4).

There was no statistical correlation between Hb level and dose and duration of treatment *p. value* 0.413 and 0.911 respectively (Figures 4.2 and 4.3).

One-way ANOVA test showed insignificant relationship between Hb, MCHC and type of treatment *p. value* 0.992 and 0.66 respectively. Also showed significant relation between MCV, MCH and type of treatment *p. value* 0.027 and 0.042 respectively(Table4.5). Post-hoc using LSD test was conducted, showed significant correlation with Na valproate and phenytoin *p. value* 0.11 and 0.26 respectively (Table 4.6).

Table (4.1): Mean ± SD of age and gender distribution among the study groups:

Study group	**Mean ±SD of age**	**Male**	**Female**	**Total**
Case group	26.3 ± 12.8	26(52%)	24(48%)	50(100%)
Control group	28.84 ± 12.66	25(50%)	25(50%)	50(100%)
Total		51	49	100(100%)

Table (4.2): Age group distribution:

Age group	**Case**	**Control**
(10-25)	25(50%)	31(62%)
(26-50)	19(38%)	15(30%)
>50	6(12%)	4(8%)
Total	50(100%)	50(100%)

Table (4.3): Folic acid administration among study population:

Folic acid	**Case**			**Control**		
	No. of Patients	Male	Female	No. of Patients	Male	Female
Yes	6(12%)	5	1	0(0%)	0	0
No	44(88%)	21	22	50(100%)	25	25

Table (4.4): Comparison of Hb, MCV, MCH and MCHC level between case group and control group:

Variable	Cases	Control	*P-Value*
	Mean ± SD	**Mean ± SD**	
Hb	12.84 ± 1.88	12.37 ± 1.59	0.055
MCV	85.13 ± 5.8	82.03 ± 7.19	0.030
MCH	29 ± 28.2	28.2 ± 2.94	0.011
MCHC	34.14 ±2.03	40.44 ± 43.67	0.82
TWBC	6.97 ± 2.10	7.80 ± 2.59	0.778
PLT	286.32 ± 114.11	330.99 ± 124.3	0.541

Table (4.5): Correlation between Hb, MCV, MCH and MCHC and type of treatment in case group:

Variable	Mean ± SD	*P-value*
Hb	12.85 ± 2.33	0.992
MCV	82.70 ± 5.49	0.027
MCH	28.24 ± 2.73	0.042
MCHC	34.11 ± 3.36	0.66

Table (4.6): post hoc test:

Dependent Variable	**Treatment I**	**Treatment J**	**Mean Difference (I-J)**	***P. Value***
MCV	Na Valproate	Phenytoin	-5.64830*	.011
		Lamotrigine	1.65625	.587
		Carbamazepine	-3.47270	.065
	Phenytoin	Lamotrigine	7.30455*	.026
		Carbamazepine	2.17560	.295
	Lamotrigine	Carbamazepine	5.12895	.092
MCH	Na Valproate	Phenytoin	-2.44716*	.013
		Lamotrigine	.89375	.509
		Carbamazepine	-.77204	.349
	Phenytoin	Lamotrigine	3.34091*	.022
		Carbamazepine	1.67512	.072
	Lamotrigine	Carbamazepine	1.66579	.214

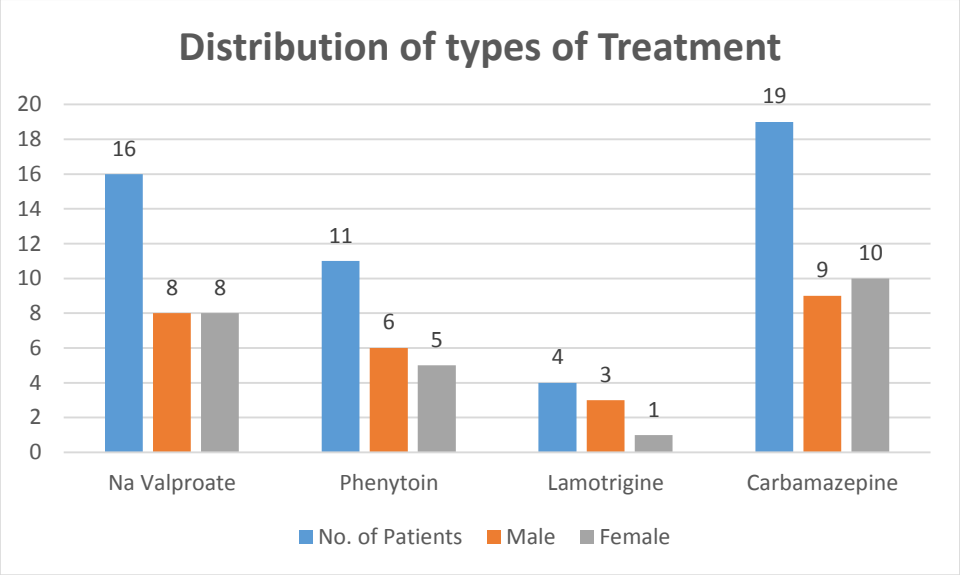

Figure (4.2): Distribution of study population according to the type of treatment.

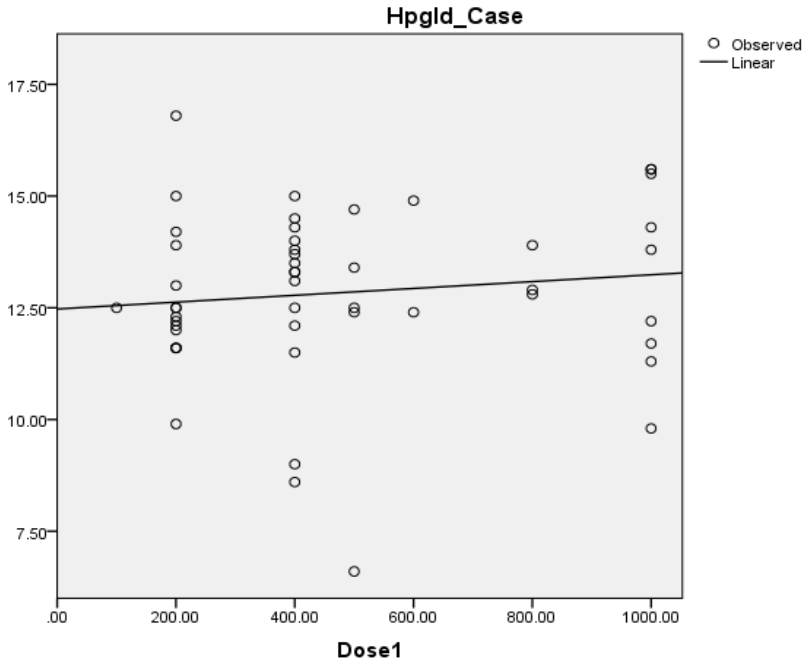

Figure (4.3): Correlation between Hb and dose of treatment for case group.

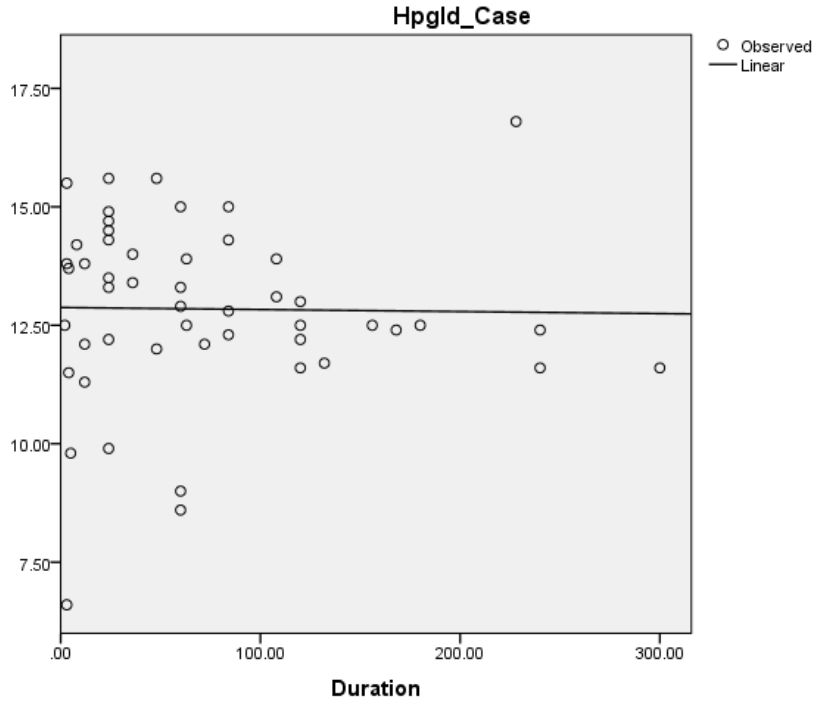

Figure (4.4): Correlation between Hb level and duration of treatment for case group.

CHAPTER V

DISSCUSSION, CONCLUSION AND RECOMMENDATIONS

Chapter V

Discussion, Conclusion and Recommendations

5.1. Discussion

The prolonged usage of antiepileptic drugs has necessitated the need to study their toxicological effects on some biochemical and hematological parameters. Hematological parameters include hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) (Akunne *et al*., 2017).

In the present study Hb and red cell indices was measured in 50 epileptic patients and 50 apparently healthy control. The result relieves that the mean level of MCH in case group was higher than control and the difference was significant (*p. value* 0.011). this result was supported by Linnebank *et al* who demonstrate significant elevation in level of MCH in case than control (*p. value* was less than 0.05) (Linnebank *et al*., 2011).

Mean level of MCV in case group was higher than control group and the difference was significant (*p. value* 0.03). Similar result found by Linnebank *et al* whom measure MCV of epileptic patients under treatment (*p. value* less than 0.05) (Linnebank *et al*., 2011) which support my study.

In the present study the mean level of Hb and MCHC had insignificant difference between case group and control group (*p. value* 0.055 and 0.82 respectively). These results were supported by Sharma *et al* whom demonstrated insignificant difference in hematological parameters in phenytoin treated epileptic children (Sharma *et al*., 2017).

In present study there was no correlation between Hb concentration and selected variables (duration *p. value* 0.413 and dose *p. value* 0.911). Similar result was accomplished by Akunne and his colleagues found that AEDs decrease hemoglobin concentration and the decreasing of Hb concentration has not linked to dosage and duration of administration treatment (Akunne *et al*., 2017).

5.2. Conclusion

From the results of our study we conclude that epileptic patients on long term antiepileptic therapy showed higher MCV and MCH compared to normal healthy individuals with statistically significant difference (*p. value* 0.03 and o.011 respectively).

There was no statistical difference in Hb level and MCHC between epileptic patients and normal healthy individuals (*p. value* 0.055 and 0.82 respectively).

There was no correlation between Hb concentration and dose and duration of treatment.

5.3. Recommendations

1. A larger sample size and estimation of folic acid and vitamin B12 are needed to confirm diagnosis of folic acid and vitamin B12 deficiency.
2. This study recommends baseline estimation of vitamin B12 level and folic acid level in epileptic patients before starting treatment.
3. It also recommends annual screening of vitamin B12 folic acid level if treatment is advised for prolonged period.

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Appendix (1)

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Complete Blood Count of Sudanese Patients using antiepileptic Drugs in Khartoum – Sudan

تقييم تعداد الدم الكامل للمرضى السودانيين الذين يستخدمون الأدوية المضادة للصرع في الخرطوم – السودان

Date: / /2019

ID: ……

Age: ……. Years

Gender: Male ……. Female ……

Education level: …….

Treatment: …….

Dose: ……...

Duration of administration treatment: ……

Chronic diseases: Yes: ……No: ...….

Family history for convulsions: Yes: ……. No: ……

History for anemia: Yes: …… No: …….

Investigations:

Hb: …….....RBCs count: …….

PLT count: ……. MCV: …….……...

MCH: ……. MCHC: …….

Appendix (2)

Sysmex KX-21N Hematology Analyzer