



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



**Republic of Sudan**  
**Ministry of Higher Education and scientific Research**  
**Shendi University**  
***Faculty of Graduate Studies and Scientific Research***

**ASSESSMENT OF VITAMIN B<sub>12</sub> LEVEL AMONG  
SUDANESE NEURO PSYCHIATRIC PATIENTS  
DURING MARCH TO APRIL IN KHARTOUM STATE**

*A thesis submitted for partial fulfillment for the requirement of the MSc degree  
in Hematology*

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2018

## إستهلال

الاية :



اية الكرسي-سورة البقرة 256

## *Dedication*

*To my parents the reason of existence*

*To my siblings..... Rocks in my life*

*To my friends wherever they are*

*To my home school Elimam ALmahdi university.*

## **Acknowledgement**

Thanks to Allah for giving me strength to achieve this work with a lot of help from my supervisor Dr Mohammed Mubark, who did not save an effort to help and support. University of Shandi- faculty of graduate study's membership an honor to be carried though Master program. Thanks to assistant members of psychological hospitals around Khartoum state for great aid provided.

Thanks to everyone involved or help and not intentionally miss-mentioned.

## **ABSTRACT**

### **Background:**

Vitamin B<sub>12</sub> levels was measured among psychiatric diagnosed subjects and mentally healthy individuals in order to find a relationship between disorders and the low level of vitamin B<sub>12</sub>, which is known with its role in central nervous system and various organs in human body . Psychological disorders have been bound to low level of vitamin B<sub>12</sub> as it known to be particularly important in the function of central nervous system. Psychological disorders are sorted to several types according to many criteria, but all of them share symptoms of behavior abnormalities and emotion disturbance .

### **Objectives:**

The aim of this study is to assess vitamin B<sub>12</sub> level among psychotic patients and to compare the data with control group contained healthy individuals .

### **Material and method :**

This is cross sectional study involved 60 professionally diagnosed psychiatric patients were enrolled in this study beside 20 healthy individuals collected blood samples were analyzed by enzyme linked immunsorbantoassay (ELISA), (Biosystem analyzer BTS350) at Algialy Khalid Musa medical laboratory, Omdurman- Khartoum State , during March and April 2018.

## **Result :**

The results produced low level of vitamin B<sub>12</sub> among 8% of patients , 5% of them were dementia patients and 3% were depression patients .

## **Mean of B<sub>12</sub> comparing :**

1. case and control.
2. male and female.
- 3.dementia and depression.

## **Correlation between age and B<sub>12</sub> level :**

Negetive Correlation.

## **Conclusion :**

Vitamin B<sub>12</sub> level has been found low level among some of psychotic patients involved in this study, so it recommended to be tested for patients whose develop symptoms of dementia or depression .

## الخلاصة

### خلفية:

تم قياس مستويات فيتامين ب 12 بين المرضى الذين تم تشخيصهم نفسيًا والأفراد الأصحاء عقليًا من أجل إيجاد علاقة بين الاضطرابات وانخفاض مستوى فيتامين ب 12 ، والذي يعرف بدوره في الجهاز العصبي المركزي والأعضاء المختلفة في جسم الإنسان .وقد ادت الاضطرابات النفسية إلى مستوى منخفض من فيتامين B12 لأنه من المعروف أنه مهم بشكل خاص في وظيفة الجهاز العصبي المركزي . يتم تصنيف الاضطرابات النفسية إلى عدة أنواع وفقا لمعايير كثيرة ، ولكن كلهم يشتركون في أعراض اضطرابات السلوك واضطراب العاطفة.

### الأهداف:

الهدف من هذه الدراسة هو تقييم مستوى فيتامين ب 12 بين مرضى الذهان ومقارنة البيانات مع مجموعة المراقبة التي تحتوي على أفراد أصحاء.

### المواد وطريقة:

هذه دراسة مقطعية متداخلة شملت 60 مريض نفسي تم تشخيصهم بشكل مهني تم تسجيلهم في هذه الدراسة إلى جانب 20 شخص سليم جمعوا عينات دم تم تحليلها عن طريق إنزيم محلل (ELISA) immunsorbantoassay ، بجهاز (Biosystem BTS350) في

مختبر الجيلي خالد موسى الطبي ، أم درمان - ولاية الخرطوم ، خلال شهري مارس وأبريل  
عام 2018.

### نتيجة:

أدت النتائج الى مستوى منخفض من فيتامين B12 بين 8 % من المرضى ، وكان 5 %  
منهم من مرضى الخرف و 3 % من مرضى الاكتئاب.

### متوسط مقارنة B12 بين:

1. اشخاص اصحاء ومرضى نفسيين .

2. الذكور والإناث.

3. الخرف والاكتئاب.

### الارتباط بين العمر ومستوى B12 :

لا يوجد ارتباط .

### استنتاج:

تم العثور على مستوى فيتامين ب 12 بمستوى منخفض بين بعض مرضى الذهان  
المشاركين في هذه الدراسة ، لذلك أوصى بالفحص للمرضى الذين تظهر لديهم أعراض  
الخرف أو الاكتئاب.



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### List of Abbreviation

<b>DNA</b>	<b>deoxyribonucleic acid</b>
<b>EDTA</b>	<b>Ethylene diamin tetra acetic acid</b>
<b>ELISA</b>	<b>Enzyme linked immunsorbentoassay</b>
<b>HoloTC</b>	<b>Holo-transcobalamin</b>
<b>IF</b>	<b>Intrinsic factor</b>
<b>MMA</b>	<b>methylmalonic acid</b>
<b>WHO</b>	<b>World health organization</b>

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# CHAPTER ONE

## INTRODUCTION

### 1.1 General introduction:

Psychiatric symptoms attributable to vitamin B<sub>12</sub> deficiency have been described for decades. The earlier reports are for the most part in accordance with more recent ones, despite being diagnostically less specific in psychiatric and hematological terms. These symptoms seem to fall into several clinically separate categories: slow cerebration; confusion; memory changes; delirium, with or without hallucinations and/or delusions; depression; acute psychotic states; and (more rarely) reversible manic and schizophrenic form states (1). Apart from these conditions, some cases of dementia, violent behavior and fatigue have also been documented to be caused by vitamin B<sub>12</sub> deficiency (2). There are a few case reports of catatonia, attributed to vitamin B<sub>12</sub> deficiency (3). The psychiatric symptoms can develop long before anemia or spinal cord symptoms (4\_6). The most common neurological manifestations are sensory impairment in the form of paresthesia and decreased vibratory and touch sensations and ataxia (7,8). In various studies, the neuropsychiatric abnormalities were present in up to 28% of patients in the absence of any change in the hematocrit values or increase in the red cell mean corpuscular volume (MCV) (7,8). However, we could not come across a presentation as predominant negative symptoms associated with vitamin B<sub>12</sub> deficiency in literature. A case of vitamin B<sub>12</sub> deficiency is reported which presented with negative symptoms followed by neurological symptoms.

In Africa the study was done in Uganda since 2014 neuropsychiatric patients showed 28.6 % they have depression with vitamin B<sub>12</sub> deficiency.

In Sudan there is two studies were done in Khartoum, one of them since Sep2015 showed 11%. Other since 2013 was show the effect of Vit B<sub>12</sub> and folic acid in neuropsychiatric patient that revealed 6% of patient has Vit B<sub>12</sub>deficiency with normal folic acid in all patients under study. The aim of this study to assess the effect and impact of vitamin B<sub>12</sub> deficiency in neuropsychiatric patients ,so as to prevent the complication with early intervention.

### **1.2 Study hypothesis:**

Psychotic disorders may be related to low level of vitamin B<sub>12</sub>, as it related to CNS composition and this study assumed that.

### **1.3 Problem statement:**

May be low B<sub>12</sub> level bind to sort of disorders not differentially diagnosed as it should link to generalized psychosis.

## **1.4 Rational:**

A wake up call needed for getting psychiatric disorders enough tension, as they have economically, socially and genetic causes, and regardless the causative agent, supportive and supplementary drugs should be available, B<sub>12</sub> has effect on central nervous system and therefore can lead to some kind of psychological disorders as depression, so this study aimed to focus on its deficiency among Sudanese mentally ill subjects and find relationship with kind of definitive disorder and may lead to further concern and make it obvious to be set in the program of treatment.



## **1.5 Objectives:**

### **1.5.1 General Objectives:**

The aim of this study is to assess vitamin B<sub>12</sub> level among the study population.

### **1.5.2 Specific objectives:**

- To evaluate vitaminB<sub>12</sub>level in different neuropsychotic cases.
- To correlate the deficiency of vitamin B<sub>12</sub> with type of psychotic disorders
- To correlate deficiency with age, duration of the disease and genders of patients involved in this study.

## **CHAPTER TWO**

### **LITRETURE REVIEW**

#### **2.1 vitamin B<sub>12</sub> :**

Vitamin B<sub>12</sub>, also called cobalamin, is a water soluble vitamin that plays a fundamental role in the normal functioning of the brain and nervous system, and for the formation of blood. Dietary vitamin B<sub>12</sub>, obtained from animal food, is bound to animal proteins. The acid and pepsin in the stomach breakdown these proteins and release vitamin B<sub>12</sub>. The free vitamin B<sub>12</sub> then binds a protein called haptocorrin (previously known as Transcobalamin I or R Factor or R-protein), which is produced by the salivary glands and parietal cells of the stomach whose essential function is to protect vitamin B<sub>12</sub> from degradation from the acidic environment of the stomach. In the duodenum, the pancreatic enzymes degrade the haptocorrin, and vitamin B<sub>12</sub> is released again which then binds to the intrinsic factor (IF) produced by parietal cells. Absorption of vitamin B<sub>12</sub> occurs in the terminal ileum (i.e. most distal part of the small intestine) and is aided by binding the complex to the IF receptor on the mucosal surface. In addition to this method of absorption, evidence supports the existence of an alternate pathway that is independent of the IF. This pathway is important in relation to oral supplementation (approximately 1% of a large oral dose of vitamin B<sub>12</sub> is absorbed by this second mechanism) (9). Once absorbed, vitamin B<sub>12</sub> is bound to two carrier proteins in blood: haptocorrin and transcobalamin. The majority of vitamin B<sub>12</sub> (70-80%) is bound to haptocorrin (named as holo-haptocorrin) and is not biologically active. Only less than 30% of the B<sub>12</sub> is bound to transcobalamin II (named as holo-transcobalamin (HoloTC) which is the active fraction that enters cells for metabolic reactions. The interruption of one or any combination of these

steps places a person at risk of developing vitamin B<sub>12</sub> deficiencies (10). Vitamin B<sub>12</sub> is required as a cofactor in both reactions, whereas folate is required in only one of the reactions. In the first reaction, vitamin B<sub>12</sub> is required for the conversion of methylmalonic acid (MMA) to succinyl-CoA. MMA is a substance produced when proteins in the body are broken down<sup>4</sup>. Folate does not play any role in this reaction. Deficiency in vitamin B<sub>12</sub> can lead to increased levels of serum MMA<sup>2</sup>. In the second reaction, both vitamin B<sub>12</sub> (in the form of methylcobalamin) and folic acid act as cofactors in the conversion of the substrate homocysteine (a homologue of the amino acids cysteine and methionine) to methionine (an amino acid and one of the 20 building blocks of proteins) by the enzyme methionine synthase (10,11). More importantly, this pathway is closely linked to the generation of thymidine which is vital for deoxyribonucleic acid (DNA, i.e. the building block of the human body which carries genetic information) synthesis. A deficiency in either vitamin B<sub>12</sub> or folic acid or both can lead to increased homocysteine levels in plasma<sup>2</sup>. In addition, deficiency of either vitamin can result in perturbation of these two key pathways with consequent disruption of DNA synthesis caused by thymidine lack and resulting in megaloblastic anemia, as well as other adverse effects on the nervous system and other organs<sup>2</sup>. It is this metabolic reaction that clearly links the two vitamins and is responsible for the common or shared neuropsychiatric and hematologic disorders (12).

## **2.2 Vitamin B<sub>12</sub> Resources:**

Vitamin B<sub>12</sub> is present in animal products such as meat, poultry, fish (including shell fish), and to a lesser extent milk, cheese and eggs, and it is not present in plant products<sup>6</sup>. The recommended dietary allowance for vitamin B<sub>12</sub> is 2.4 µg/day (13) and most individuals can meet this level through dietary intake (14). Individuals over the age of 50 who have reduced protease secretions in the stomach (as well as strict vegetarians) (15). Food fortification is defined as the process of adding micronutrients (such as vitamins and minerals) to food (16). The risk of toxicity from vitamin B<sub>12</sub> intake from supplements and/or fortified foods is low (17). Vegetarians and the elderly are at higher risk of vitamin B<sub>12</sub> deficiency. Among the elderly, vitamin B<sub>12</sub> deficiency occurs in about 20% of the population; more than 60% of these deficiencies are due to food-cobalamin malabsorption syndrome (18) caused by gastrointestinal problems. Deficiency related to a lack of intrinsic factor results in severe neurologic damage and life-threatening anemia; therefore, such individuals require medical treatment including vitamin B<sub>12</sub> injections. In strict vegans, vitamin B<sub>12</sub> deficiency is mainly caused by avoidance of foods derived from animal origin, which are the only good dietary sources of vitamin B<sub>12</sub>. In the United States, the estimated average daily intake of vitamin B<sub>12</sub> is about 5 µg/d for men and 3.5 µg/d for women. The recommended intake for vitamin B<sub>12</sub> is 2.4 µg/d. Between 10% and 30% of older people are unable to absorb vitamin B<sub>12</sub> from foods; therefore, the Institute of Medicine of the National Academies in the United States recommends that individuals older than 50 years of age consume foods fortified with B<sub>12</sub> or supplements containing B<sub>12</sub>. This is because higher doses of vitamin B<sub>12</sub> can result in better absorption despite gastrointestinal difficulties (19).

### **2.3 Psychological disorders:**

In 1948, the World Health Organization defined health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity (20). Psychology is the Science of Mental Life, both of its phenomena and of their conditions. The phenomena are such things as we call feelings, desires, cognitions, reasoning's, decisions, and the like (21). The nomenclature of neuropsychiatric disorders has a contentious history, with periodic attempts to bring cohesion to this diverse and disparate field. The WHO and national surveys report that there is no single consensus on the definition of mental disorder/illness, and that the phrasing used depends on the social, cultural, economic and legal context in different societies (22). The WHO reports that there is intense debate about which conditions should be included under the concept of mental disorder; a broad definition can cover mental illness, mental retardation, personality disorder and substance dependence, but inclusion varies by country and is reported to be a complex and debated issue(23), Also the World Health Report focuses on a number of common disorders. These include depression, substance use disorders, schizophrenia, epilepsy, Alzheimer's disease, mental retardation and disorders of childhood and adolescence. One in every four people, or 25% of individuals, develops one or more mental or behavioral disorders at some stage in life, both in developed and developing countries. These disorders can now be diagnosed as reliably and accurately as most of the common physical disorders (24). Individual with psychotic disorders are distinguished from each other based on the duration, dysfunction, type of delusions and hallucinations, presence of depression and mania, and associated substance use or medical condition (25).

Previous researches have shown nutritional deficiencies that correlate with some mental disorders (26,27). Generally, a psychiatric diagnosis is considered to be

descriptive (28). The clinician will describe a syndrome (its nature, timespan, and severity) within the framework of a disorder, and often include a differential diagnosis, predisposing protective or vulnerability factors, and provoking or maintaining factors (29).

#### **2.4 Psychological disorders caused by B2 deficiency:**

Some of psychological disorders can be caused by vitamin B<sub>12</sub> deficiency are Irritability, personality change, Mild memory impairment, dementia, depression, psychosis and Alzheimer's disease (30). Researchers have postulated that biochemical factors such as homocysteine might be involved not only in heart disease, but also in brain function. Therefore, the relationship of B vitamins and homocysteine to cognitive function is under investigation. Homocysteine concentrations, but not vitamin B<sub>12</sub>, were associated with a decline in cognitive performance in a normal aging population (31). In another study, B vitamins (2 mg folic acid plus 1 mg vitamin B<sub>12</sub>) lowered plasma homocysteine concentration by 30% in those with dementia or mild cognitive impairment, but no effect on cognitive function was observed (32). Some studies have shown B<sub>12</sub> supplementation has been shown to improve symptoms indicative of delirium (33). It had been found that patients with Alzheimer disease who had lower than normal vitamin B<sub>12</sub> levels showed more frequent behavioral and psychological symptoms of dementia than patients with normal values (34). Vitamin B<sub>12</sub> deficiency rises with age, but only about 10% of those with low vitamin B<sub>12</sub> also have low folate levels (35). Therefore, given the prevalence of both vitamin B<sub>12</sub> deficiency and mental disability among the elderly, supplementation with vitamin B<sub>12</sub> might reduce the risk of age-associated mental disability or improve the quality of life among those with dementia (36, 37).

## 2.5 Previous studies:

Many studies concerned about psychotic subjects and related causative possibilities; one of them was B<sub>12</sub> as it has a major role in human central nervous system and other vital functions in human body. One of these studies a review of psychiatric manifestations obtained by B<sub>12</sub> deficiency since 1986 to the present. Specific clinical entities reviewed include depression, organic psychosis, obsessive-compulsive disorder, childhood manifestations and dementia. It observed that B<sub>12</sub> deficiency as an etiological factor in some cases of depression, organic psychosis, certain childhood disorders and dementia. Routine screening of people over age 65 years was also addressed. Vitamin B<sub>12</sub> deficiency was high 5% in populations over age 50 and up to 10% in patients over age 65. It suggested that the deficiency can result from inadequate intake e (e.g., chronic unbalanced diet, breast -fed babies of vegan mothers), impaired absorption (e.g., gastric lesions, pernicious anemia, gastrostomy, small bowel disease), vitamin B<sub>12</sub> degradation (e.g. nitrous oxide anesthesia), increased requirement (e.g., pancreatic insufficiency, hyperthyroidism), or can be multifactorial (e .g, chronic alcoholism) (38). While other study found that higher vitamin B<sub>12</sub> levels were significantly associated with a better outcome for treatment of major depression, suggesting that vitamin B<sub>12</sub> supplementation could be used to augment antidepressant treatments (39). A Sudanese study concerned about the same manner, measured vitamin B<sub>12</sub> among psychiatric subjects in Khartoum state, it found that low level of vitamin B<sub>12</sub> was found with high frequency in dementia subjects (40).

# **CHAPTER THREE**

## **MATERIAL AND METHOD**

### **3.1 Study design:**

It is cross sectional study was conducted among psychotic patients and healthy people during April and May 2018.

### **3.2 Study population :**

Sudanese patients diagnosed with psychotic disorders.

### **3.3 Study area :**

Khartoum state – EltiganiElmahi neuro and psychological hospitals.

### **3.4 Sample size :**

60 case group contained psychotic patients and control group contained 20 healthy subjects.

### **3.5 Inclusion criteria:**

Diagnosed any adult psychiatric patient:

- Male and female.
- More than 18 years and healthy individual as control .



### **3.6 Exclusion criteria:**

- Vegan diet deficiency.
- Conditions affecting the stomach like gastrectomy.
- Condition affecting the intestine.
- patients under B12 drug administration.

### **3.7 Ethical consideration:**

This study is approved by the ethical committee of Shendi University faculty of medical laboratory science.

Hospital administration and patients legal guardians gave formal consents for this study was obtained before sample collection.

### **3.8 Data collection:**

Data were collected by questionnaire used to obtain data of the patients, contained age, gender, diagnosis of the disease and its duration (appendix I). And also the data obtained by observation of laboratory result .

### **3.9 blood samples :**

Five ml of blood samples were collected in plain blood containers under a septic conditions and using standard materials from each patient and control individual.

### **3.10 Methodology :**

According to the manufacturer procedure, vitamin B<sub>12</sub> levels were measured in serum of case group and control group using an enzyme linked immunosorbent assay device (BTS350-Germany) (appendix II) and kit AccuDiag, China (appendix III).

### **3.10.1 Principle of B<sub>12</sub>analysis:**

The ELISA is based on the competitive binding enzyme immunoassay technique. The micro titer plate provided in this kit has been pre-coated with an antibody specific to Vitamin B<sub>12</sub>. During the reaction, Vitamin B<sub>12</sub> in the sample or standard competes with a fixed amount of biotin-labeled Vitamin B<sub>12</sub> for sites on a pre-coated Monoclonal antibody specific to Vitamin B<sub>12</sub>. Excess conjugate and unbound sample or standard are washed from the plate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each micro plate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Vitamin B<sub>12</sub> in the samples is then determined by comparing the O.D. of the samples to the standard curve (appendix IV).

### **3.10.2 Data analysis:**

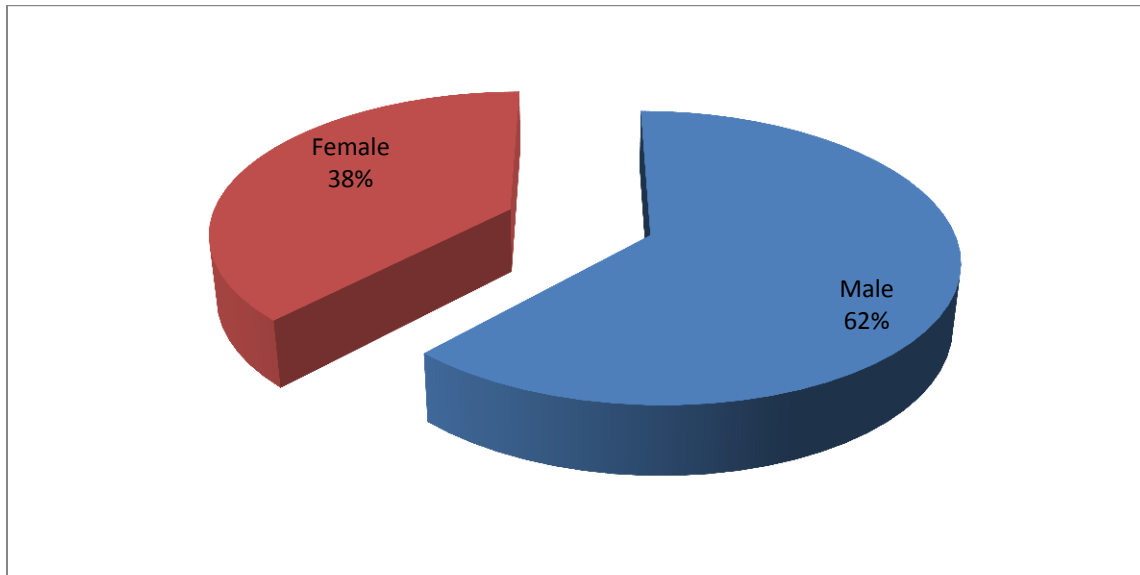
Data were analyzed by using statistically package of social science (SPSS) program version 21.

## CHAPTER FOUR

### RESULTS

#### 4.1 Distribution of psychiatric patients according to gender:

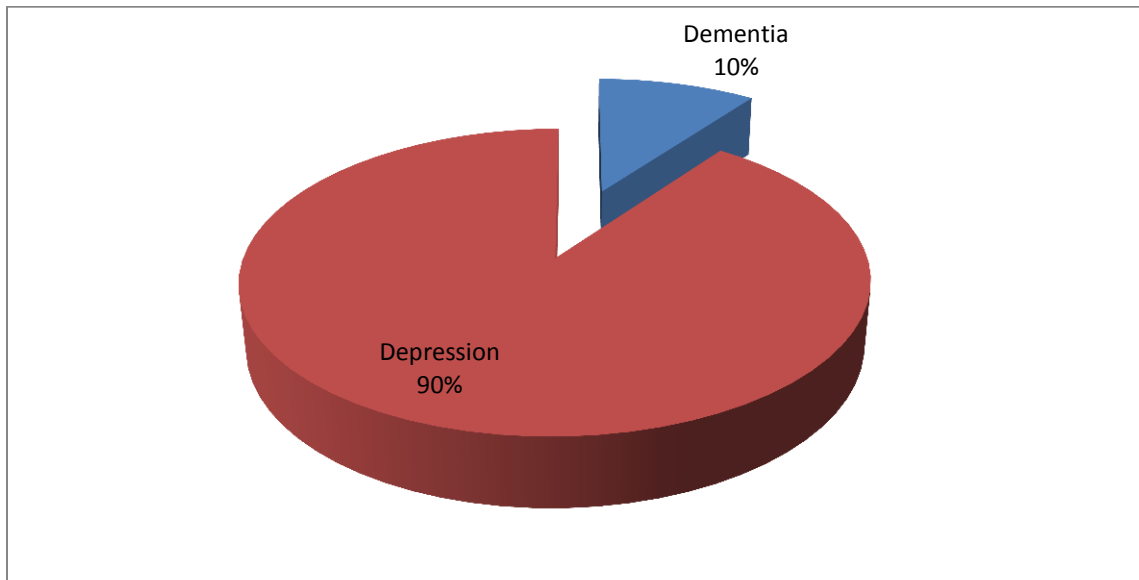
This cross sectional study enrolled among psychiatric patients 60 patients attended to neuro-psychiatric clinics in Khartoum state (Eltigani Elmahi neuro and psychological teaching hospital), and 20 healthy individuals as control group. In cases 23 (38%) were females and 37 (62%) were males as in figure 4-1



**Figure (4-1) gender distribution in cases.**

## 4.2 Classification of clinical diagnosis in cases :

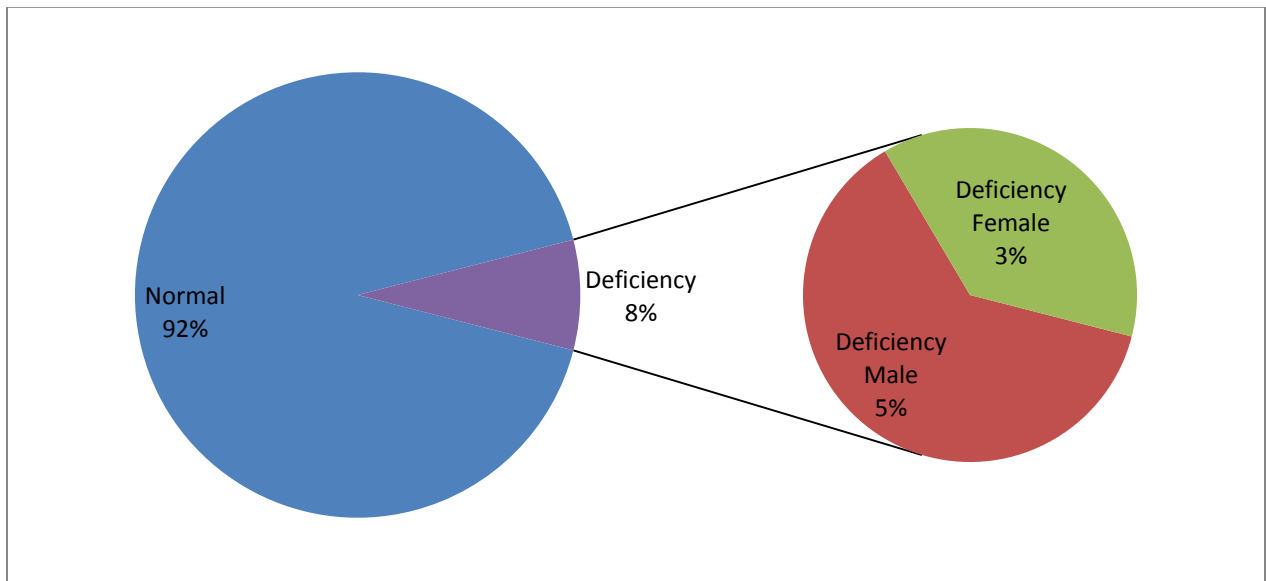
Considering the clinical diagnosis, the result readied 6 (10%) of cases were diagnosed with dementia and 54 (90%) were diagnosed with depression as in figure (4-20).



**Figure (4-2) Classification of clinical diagnosis in cases**

### 4.3 Vitamin B<sub>12</sub> Deficiency according to gender :

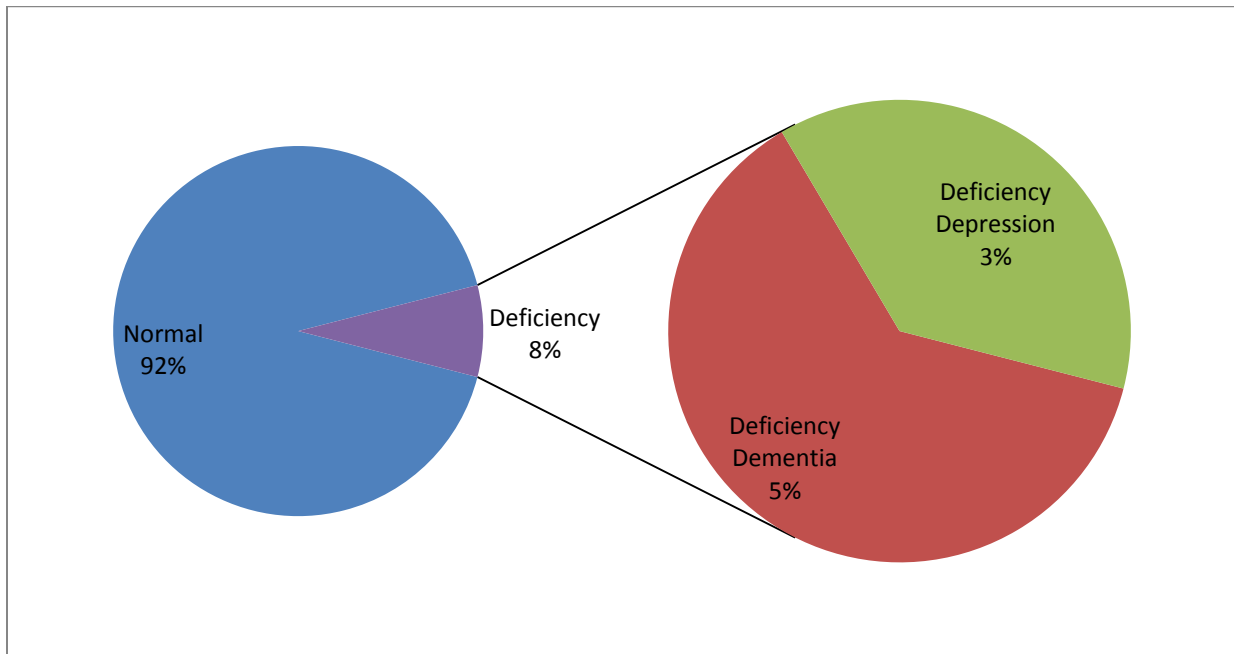
Measurement of vitamin B<sub>12</sub> level among patients revealed that its deficiency among 5 (8%) of patients, 3% of them were males and 5% were females as in figure 4-3.



**Figure (4-3) B12 Deficiency according to gender**

#### 4.4 Vitamin B<sub>12</sub> Deficiency according to the clinical diagnosis :

The results showed 8% of cases were vit B<sub>12</sub> deficient, 5% of them were the previously diagnosed as dementia, while 3% were from the depression patients in figure( 4-4).



**Figure (4-4) B<sub>12</sub> Deficiency according to diagnosis**

**Table (4-1) Mean concentration of B<sub>12</sub> comparison in case versus the control group**

S.population	B <sub>12</sub> level mean±SD(pg/L)	P. value
Cases	604 ± 246.13	0.009
Control	779.63 ± 119.54	

**Table (4-2) Mean concentration of B<sub>12</sub> comparison cross the gender**

Type of gender	B <sub>12</sub> level mean±SD(pg/L)	P. value
Male	599.81 ± 256.62	0.872
Female	612.3 ± 310.86	

**Table (4-3) Mean concentration of B<sub>12</sub> comparison cross the diagnosis**

Clinical case	B <sub>12</sub> level mean±SD(pg/L)	P. value
Dementia	461 ± 202.53	0.016
Depression	620.48 ± 258.89	

#### 4.5 Correlation between age and B<sub>12</sub> level :

Duration of the disease and level of B<sub>12</sub> did not bring significant difference and no correlation obtained.

Pearson correlation for vitamin B<sub>12</sub> level and age, gave a negative correlation as with increasing of age B<sub>12</sub> level goes lower as in figure 4-1.

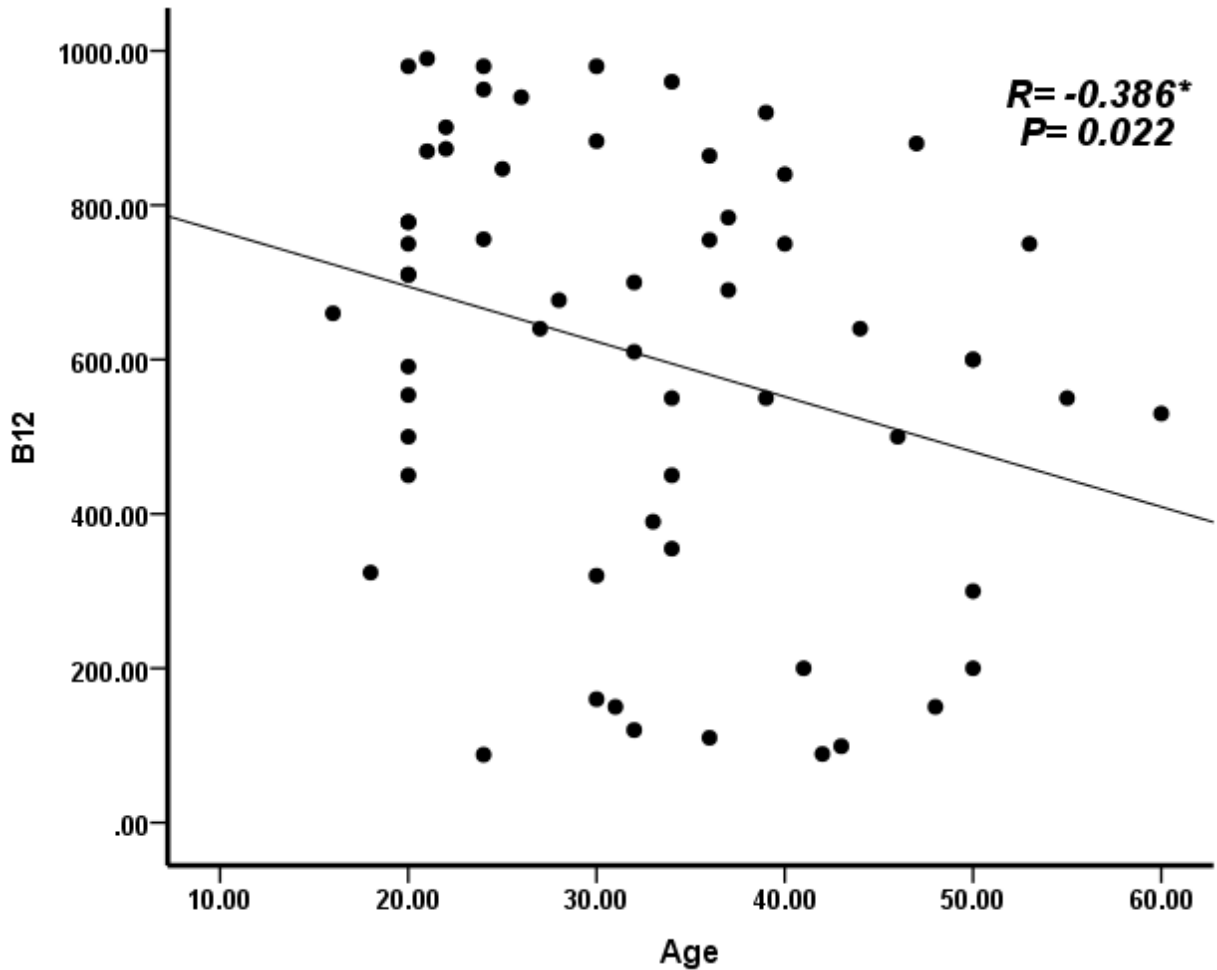


Figure (4\_5) correlation between age and B<sub>12</sub> level



## CHAPTER FIVE

### DISCUSSION

Vitamin B<sub>12</sub> is one of the most common vitamin deficiencies(41). Additionally, cobalamin pseudo-deficiency (low cobalamin serum levels but no true deficiency) can rarely be caused by a transcobalamin deficiency (42). Findings of decreased serum cobalamin (43). A very common cause of cobalamin deficiency is the widespread use of gastric acid-blocking agents, especially in the aging population (44). The normal variation in cobalamin and folate levels has been tied to various genetic loci in different populations. The gene products are involved in the pathways of cobalamin uptake and metabolism (45). Genetic defects in the intracellular processing of cobalamin have been classified into nine complementation groups (46). The most recently described mutation involves the adenosine triphosphate (ATP)-binding cassette transporter ABCD4, which is involved in the release of cobalamin from lysosomes into the cytoplasm (47). While the other mutations result in severe phenotypes with little appreciable skin findings, this mutation has been reported to produce a phenotype of skin pigmentation(48). A very rare cause of cobalamin deficiency is the recreational abuse of nitric oxide gas (49). In this study vitamin B<sub>12</sub> level was measured among psychiatric patients, whom were dementia and depression diagnosed, and a group of healthy subjects involved as well. Comparison between psychiatric patients and health subjects, B<sub>12</sub> showed decreased level among patients (8%) than normal healthy individuals, dementia patients with low levels of B<sub>12</sub> were more than depression patients, this is agreed with the findings of two studies conducted among Sudanese psychiatric patients, both of them revealed that deficiency of vitamin B<sub>12</sub> was more likely among psychiatric patients more than control group of

healthy individuals (40,50). Also another study conducted in Uganda, considered low level of B<sub>12</sub> and psychotic manifestations, it partially in agreement with this study as it revealed that a prevalence of low serum B<sub>12</sub> in 28.6% of the participants (51). The finding in the current study may reflect the life style of study population, which can stop B<sub>12</sub> deficiency, other studies had vegetarians participants' or elder more than population and in this study may be other health factor contribute in this issue. Also agreement obtained with this finding of another study, which assessed series of cases and showed how vitamin B<sub>12</sub> had been implicated in the pathogenesis of various neuropsychiatric disorders. A review of 19 patients (14 vegetarians) with demonstrable vitamin B<sub>12</sub> deficiency showed varied psychiatric symptomatology, with the majority not having significant hematological or neurological manifestations that indicate diet pattern can be an indirect cause of psychiatric disorders, as vegetarian diet program (52). An update of B<sub>12</sub> assessment though psychological disorders, a review showed that B<sub>12</sub> deficiency considered an etiological factor for some case of depression, dementia, and other psychological disorders(53), which agrees with our finding of this study. Other study enrolled among dementia, elderly people, observed the pattern of their behavior though administration of B<sub>12</sub> therapy, and it suggested that cobalamin deficiency may cause a reversible dementia in elderly patients. This dementia may be differentiated from that of Alzheimer's disease by a thorough neuropsychological evaluation (54). Depression diagnosed patients also assessed for B<sub>12</sub> deficiency in lacunar stroke patient's vitamin B<sub>12</sub> deficiency was found and also depression after stroke (55). Vitamin B<sub>12</sub> deficiency may increase the risk of dementia via elevated levels of homocysteine (HCY). B<sub>12</sub> is necessary for the conversion of HCY to methionine and the accumulation of HCY may lead to neurotoxic effects (56).

Our study revealed no significant difference in the frequency of low level of vitamin B<sub>12</sub> between patients and controls; this finding is in agreement with previous reports (57,58).

# CHAPTER SIX

## CONCLUSION AND RECOMMENDATION

### 6.1 Conclusion :

It is obviously that B<sub>12</sub> low levels presented with some disorders, dementia and depression, even low percentage of patients got the deficiency of vitamin B<sub>12</sub>, and high effect could be showed up.

## **6.2 Recommendation :**

1. No enough education and knowledge about psychiatric disorder in Sudan.
2. Health promotion about psychiatric disorder and attention to consider all factors leading and elimination of them to track reverse damage as soon as possible.
3. There should be are enough awareness about important of vit B<sub>12</sub> to health status.
4. Vit B<sub>12</sub> supplementary drug should be provided with in therapeutic protocol for many disorders including anti-psychiatric drugs protocol.
5. I recommended in the future work up and study should be conducted in the largest sample size and cover more geographical area .

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**Appendix I:**  
**Questionnaire**

Name: .....

Age: .....

Gender:.....

Duration of disease:.....

Differential

diagnose:.....

.....

B12 level: .....

**Appendix II:**  
**BTS 350 DEVICE**



**Appendix III:**  
**ELISA KIT**



# Appendix IV:

## PRINCIPLE OF VIT B12 MEASUREMENT



### AccuDiag™ Vitamin B12 ELISA

Cat# 3125-15

IVD See external Label 2-8°C Σ=96 Tests

Test	Vitamin B12
Method	Delayed Linked Immunosorbent ELISA
Principle	Delayed Competitive ELISA
Sample	50 µl serum or plasma
Total Time	~ 95 min.
Shelf Life	12-14 Months
Sensitivity	70.13 pg/ml

### INTRODUCTION

The Diagnostic Automation, Inc. Vitamin B12 ELISA Kit is an enzyme immunoassay (ELISA) for the quantitative detection of Vitamin B12 concentration in human serum. The Vitamin B12 ELISA test is for in-vitro diagnostic use only.

### SUMMARY AND EXPLANATION

Vitamin B12 plays an important role in the functioning of a healthy body. It is essential to the production of red blood cells that carry oxygen through the body's tissue. This in turn helps in the development of myelination for proper functioning of the nervous system. The effects of Vitamin B12 deficiency can range from fatigue, weakness, depression, and memory problems to more severe symptoms such as anemia. Vitamin B12 deficiencies progress slowly, and thus it may take several years for initial signs to develop.

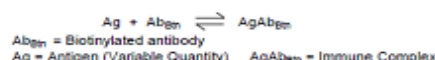
Two groups of individuals that are most prone to Vitamin B12 deficiency are the elderly and vegans. As a person ages, the ability to absorb and digest water soluble vitamins (like Vitamin B12) decreases, and if there is not an efficient source of Vitamin B12 in the diet, they are at risk for a deficiency. Unless vegans take a regular supplement of Vitamin B12, they are prone to deficiency because their diet is void of one of the major sources of Vitamin B12 - animal meat.

The absorption of Vitamin B12 occurs through saliva at the ingestion stage, and then during digestion, the B12 from food proteins is released with the help of acids in the system. The absorption of B12 depends on a protein called intrinsic factor (IF). Once combined with IF, Vitamin B12 can be absorbed in the intestines.

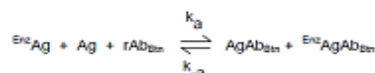
Vitamin B12 and Folate deficiencies have similar symptoms, so it is important to have accurate tests that can detect the differences. Even though the symptoms of both deficiencies show increased levels of homocysteine, a Vitamin B12 deficiency is the only one of the two that causes an additional increase in methylmalonyl CoA. Severe diseases that affect the vascular system, like Parkinson's Disease, atherosclerosis, or coronary heart disease, are caused by increased levels of methylmalonyl CoA and homocysteines. This increase also causes stress at the cellular level, eventually leading to apoptosis.

### TEST PRINCIPLE

Delayed Competitive Enzyme Immunoassay (TYPE 9): The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equation:

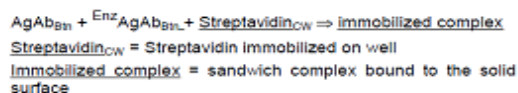


After a short incubation, the enzyme conjugate is added (This delayed addition permits and increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binding sites (not consumed in the first incubation).



EnzAg = Enzyme-antigen Conjugate (Constant Quantity)  
EnzAgAb<sub>B12</sub> = Enzyme-antigen Conjugate-Antibody Complex  
rAb<sub>B12</sub> = Biotinylated antibody not reacted in first incubation.  
K<sub>a</sub> = Rate Constant of Association  
K<sub>d</sub> = Rate Constant of Disassociation  
K = k<sub>a</sub> / k<sub>d</sub> = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.



The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

### MATERIALS AND COMPONENTS

#### Materials provided with the test kits

- Vitamin B-12 Calibrators - 1ml/vial - Icons A-F**  
Six (6) vials of human serum albumin reference for Vitamin B-12 at concentrations of 0 (A), 100 (B), 200 (C), 400 (D), 1000 (E), and 2000 (F) in pg/ml. Store at 2 – 8 °C. A preservative has been added. The calibrators can be expressed in molar concentrations (pM/L) by multiplying by 0.738. For example: 100 pg/ml x 0.738 = 73.8 pM/L.
- Vitamin B -12 Enzyme Reagent - 6.0 ml/vial**  
One (1) vial of Vitamin B-12 (Analog) – horseradish peroxidases (HRP) conjugate in a protein-stabilizing matrix. Store at 2 – 8 °C.
- Vitamin B-12 Biotin Reagent - 6.0 - Icon**  
One (1) bottle of reagent contains anti-Vitamin B-12 biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2 – 8 °C.
- Streptavidin Coated Plate - 96 wells - Icon**  
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent.  
Store at 2 – 8 °C.

Diagnostic Automation/Cortes Diagnostics, Inc.  
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