بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

# Evaluation of Serum Folate Level and its Effects on Red Cells Parametersin Celiac Disease Patients

تقييم مستوى حمض الفوليك واثره على قياسات كريات الدم الحمراء في مرضى الداء البطني

A dissertation submitted in partial fulfillment for the requirement of the M.Sc. degree in Hematology and Immunohematology


**Submitted by:**

**Isra Altag Osman Abdelkreem**

B.Sc (Honor) in Medical Laboratory Sciences

(Hematolog and Immunohmatology)

(Khartoum University 2010)

**Supervisor:**

**Dr.Elshazali Widaa Ali**


December 2016

# بسم الله الرحمن الرحيم

قال تعالى :

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

سورة البقرة الآية 32

# Dedication

*I dedicate this work:*

*To soul of my beloved mother,,*

*To my supportive and kindest father,,*

*To my lovely brothers & sisters,,*

*To my wonderful friends*

*Isra*

# Acknowledgement

Praise to Allah who gave me the health, strength and patience to conduct this study.

Great thanks for my supervisor Dr. Elshazali Widaa Ali for his good supervision and orientation.

I’m grateful to the workers at Ibn Seina Hospital in unit of celiac disease for their kind help, and special thanks to Dr. Fathia for her corporation .

My gratitude to my best friends for their encouragement and advice.

Many thanks to my kindest colleagues at Aljawda Hospital for their support and help.

# Abstract

Celiac disease is a genetically based auto-immune disorder that leads to malabsorption from the small intestine, it occurs in children as well as adults who are susceptible when they eat gluten which is a protein found in cereals such as wheat, rye, barely and possibly oat(Green and Cellier,2007).

This is descriptive cross-sectional study, conducted on 40 patients with celiac diseasereferred to Ibn Sinaa Hospital; Khartoum, Sudan. At the period from April to August 2016; their age ranged from 2 to 43 years old (16.5 $\pm$10.2); 30(75%) were females and 10 (25%) were males.

The study aimed to assess serum folate level in patients with celiac disease, and to correlate the finding with patient's gender, age, red cells parameters, dietary program commitment, and duration of disease.

Three milliliter of blood were collected from each patient in plain container; serum is prepared and then serum folate measured by immunoassay using cobas e411.

The result showed that there were 11(27.5%) of the patients were with low serum folate level {reference values for adults: 2-20 ng/ml; children 5-20 ng/ml}, all of them were children, 6 (66%) of them were males and 5 (44%) were females.

The study found that except for MCV & MCH there was no significant correlation between folate level and red cells parameters, duration of disease and diet; but there is significant difference with age and gender (p.value <0.05).

The study concluded that there were 11(27.5%) patients with folate deficiency and all of them were children, a significant correlation was found between folate

deficiency and gender, except for MCV and MCH no statistically significant difference was found in RBCs parameters, diet and duration of disease.

# الخلاصه


الداء البطني هو واحد من امراض اضطراب المناعة يؤدي إلى سوء الامتصاص من الأمعاء الدقيقة، يحدث في الأطفال وكذلك الكبار الذين هم عرضة عندما يأكلون الغلوتين وهو البروتين الموجود في الحبوب مثل القمح ، الشعيرو الشوفان.

هذه دراسه وصفيه مستعرضه, اجريت في اربعين مريض من مرضى الداء البطني في مستشفى ابن سينا,الخرطوم- السودان. في الفتره من ابريل الى اغسطس 2016,حيث كانتاعمارهم بين 2 الى 43 سنه 16.5 +10.2:30(75%) كانوا اناث 10 (25%) كانوا ذكور).

هدفت هذه الدراسه لتقييم مستوى حمض الفوليك في الدم في مرضى الداء البطني, وربط مستوى حمض الفوليك مع عمر وجنس المرضى, مع مدة المرض, الالتزام بالحميه الغذائيه ومع معلمات خلايا الدم الحمراء.

ثلاث مل من الدم اخذت من المرضى لتحضير السيروم, لقياس مستوى حمض الفوليك **cobas. e411** باستخدام تقنية التحليل المناعي بواسطة جهاز

اظهرت هذه الدراسه وجود مرضى لديهم نقص في حمض الفوليك وكان عددهم 11 (27.5%) كانوا جميعا اطفال 6 (66%) كانوا ذكور و 5 (34%) كانوا اناث.مريض

لم تظهرهذه الدراسه وجود علاقه ذات اهميه بين حمض الفوليك والحميه الغذائيه ومدة المرض, ايضا لم تكن هناك علاقه ذات اهميه مع معلمات خلايا الدم الحمراء عدا العلاقه مع متوسط حجم الخليه ومتوسط الهيموجلوبين في الخليه كات ذات اهميه.

## List of contents

## List of figures

## List of tables

## Abbreviations

| | |
|---|---|
| CD | Celiac disease |
| Hb | Haemoglobin |
| HLA | Human leukocyte antigen |
| MCH | Mean cell hemogolobin |
| MCHC | Mean cell haemoglobin concentration |
| MCV | Mean cell volume |
| PCV | Packed cell volume |
| RBCs | Red blood cells |
| TGS | Tissue trans-glutaminase |

# Chapter One
# Introduction and Literature Review

## 1.1 Introduction

Celiac disease(CD) is a genetically based auto-immune disorder that leads to malabsorption from the small intestine, itoccurs in children as well as adults who are susceptible when they eat gluten which is aprotein found in cereals such as wheat, rye, barely and possibly oat (Ciclitira , 1999).

Celiac disease is unique from other autoimmune diseases in that there is a clearly identified environmental trigger (gluten), a dominant HLA contribution required for disease to occur (DQ2 or DQ8), and auto antibodies against TG tissue transglutaminase are detectable in over 95% of individuals with celiac disease. The clinical presentation of celiac disease is remarkably varied and depends on age. The classic presentation with failure to thrive, malnutrition, diarrhea, abdominal pain and distension within the first couple of years of life represents the tip of what is commonly referred to as the “celiac disease iceberg”,(Fsano *et al.*, 2003).

Diagnosis of celiac disease is usually first suggested by the presence of transglutaminase autoantibodies, but established by biopsy of the small intestine by upper intestinal endoscopy. Histology will show some degree of villous atrophy and crypt hyperplasiya(Green and Cellier , 2007).

Folic acid is water soluble, heat stable compound, destroyed by light & acid medium. Rich Sources: Yeast, liver, green leafy vegetables, cereals, pulses, oil seeds. Folic acid is absorbed from upper part of

jejunum as polyglutamate forms which are first cleaved into monoglutamate form by the intestinal enzymes before absorption; it is transported in blood bound to β – globulins. Found relatively in higher concentrations in the liver. The main functions of folic acid are: synthesize of DNA and RNA , aiding rapid cell division and growth, and to produce healthy red blood cells; so deficiency of folic acid result in anaemia (Bailey and Lynn, 2009).

## 1.2 Literature review

CD is a chronic immune-mediated disease that specifically affects the proximal small intestines due to intolerance to gluten, a protein found in wheat andto lesser extent in barley, rye and oat.The disorder is sometimes called celiac sprue or gluten sensitive enteropathy. It was previously termed non-tropical sprue, celiac syndrome, idiopathic steatorrhea and primary mal-absorption. The present name of celiac disease was introduced by Rubin *et al* (1970); the term sprue itself is of obscure Dutch or Flemish origin, and means to sprinkle(Ciclitira, 1999).

### 1.2.1 Epidemiology

Its prevalence has been underestimated, but it is now considered one of the most common genetic disorders in the West with a prevalence of 1 - 2.6%, previously described mainly in children but is now increasingly being diagnosed in persons of all ages (Setty *et al.,* 2008). The first report of CD in Sudan was by Dr.Gaafar Ibn Aof (1978), who diagnosed and reported seven Sudanese celiac children, the diagnosis based on clinical and histological improvement after gluten free/ sorghum free diet (Sulieman ,1978).

### 1.2.2 Pathophysiology of the disease

It involves autoimmune mechanisms playing a role in intestinal mucosal injuries due to hypersensitivity against gluten in genetically predisposed individuals resulting in malabsorption.Gluten and other proline-rich proteins are poorly digested in the normal human small intestinal tract due to a lack of prolyl- endopeptidases. This results in the

generation of gluten peptides that can be as large as 10–50 amino acids in length. Gluten is also rich in the amino acid glutamine; some of the glutamines in the peptides generated in the small intestine can be deamidated by the enzyme tissue transglutaminase (tTG). This results in their conversion to negatively charged glutamic acid residues. Large peptides with a specific spacing of proline and glutamic acid may contain one or more sequences that are uniquely able to bind to the human HLA class II DQ2 molecules DQ2 and DQ8 on antigen-presenting cells with the ensuing activation of pathogenic populations of $CD4^+$ T cells in the intestinal mucosa. The humoral immune response in celiac disease is directed both against the exogenous antigen gluten and against the autoantigen tTG. The full role of tTG remains to be fully elucidated, and it may be involved in multiple levels of the immune response. (Green and Jabri, 2006).

Disease diagnosed in about 10% of first degree relatives of an individual with celiac disease (Fine, 1996). Although hereditary factors play a significantrole, genetic factors alone do not explain the development of the disease because the disease is concordant in only 60 to 70% of identical twins. Additional factors such as hormones and infectious agents may also be putative in triggering aberrant interactions between ingested gluten and the host's mucosal immune system (Logan, 1992).

**1.2.3 Clinical manifestations**

Clinical manifestations of disease are manifestation of malabsorption and include symptoms of diarrhea, steatorrhea, nutritional and vitamin deficiencies.Secondary immunologic illnesses, such as atopic dermatitis, dermatitis herpetiformis, and alopecia may be the primary presentation. However, patient presentations with extraintestinal signs are becoming more

common in CD (Jones *et al*., 2006).

### 1.2.4 Diagnosis of disease

#### 1.2.4.1 Serologic test

The initial detection of possible celiac disease is probably best obtained by the use of a simple and accurate serologic test; the immunoglobulin (IgA) AtTGA. Serologic testing is based on identifying IgA antibodies against gliadin, endomysium, and tissue transglutaminase. Antiendomysium antibodies (EMAs) and antitTGAs have been shown to be highly sensitive and specific(Hadithi *et al.,* 2007).

Anti-tTGAs are now widely used for the diagnosis of CD and for monitoring gluten-free diet adherence (Reeves *et al.,* 2006).

#### 1.2.4.2 Endoscopic duodenal biopsy specimen

Also used for diagnosis of the disease; demonstrating characteristic histological changes in the small intestinal mucosa, it is considered the gold standard method for CD diagnosis (Hopper *et al.,* 2008).

### 1.2.5 Complications

Most complications of celiac disease are:

- Malignancy:the classic malignancy associated with CD is a lymphoma of small intestine, esophagus and pharynx.
- Osteoporosis: is develop10 times more likely in patients with celiac disease.
- Neurological complications in celiac patients with established diagnosis is uncommon, it is about 8%. It is more common in asymptomatic celiac disease (Feighery, 2001).

### 1.2.6 Treatment

Treatment of celiac disease requires a strict, lifelong adherence to a

gluten free diet. Clinicians need to ensure that patients have adequate education, motivation, and support to achieve this diet. Consultation with an experienced dietician, referral to a support [group, and clinical follow-ups for compliance are recommended. Treatment of nutritional deficiency states (e.g., iron, folate, vitamin $B_{12}$) is essential, and a determination of bone mineral density to assess for osteoporosis is recommended (Alaa *et al.*, 2006).

### 1.2.7 Hematological manifestations in celiac disease

The hematologic system is one of the most affected extra intestinal systems, in CD patients; with anemia being the most presented sign (Farrel *et al*., 2002).The anemia of CD is usually due to malabsorption of micronutrients such as iron, folic acid, and vitamin B12. CD is also frequently implicated in the etiology of other blood-count abnormalities, splenic hypofunction, and intestinal lymphomas. Gluten-free diets, the main treatment of the disease, reduce ailments, prevent complications, and also improve hematologic signs(Halfdanrson *et al.,*2007).

## 1.3 Blood

Blood is a circulating tissue composed of fluid plasma and cells (Robinson and Reich, 2013).

### 1.3.1 Functions of blood

Blood has important transport, regulatory, and protective functions in the body(Robinson and Reich, 2013).

#### 1.3.1.1 Transportation

Blood transport oxygen form the lungs to the cells of the body and carbon dioxide from the cells to the lungs. It also carries nutrients

from the gastrointestinal tract to the cells, heat and waste products away from cells and hormones form endocrine glands to other body cells (Robinson and Reich, 2013).

#### 1.3.1.2 Regulation

Blood regulates pH through buffers. It also adjusts body temperature through the heat-absorbing and coolant properties of its water content and its variable rate of flow through the skin, where excess heat can be lost to the environment. Blood osmotic pressure also influences the water content of cells, principally through dissolved ions and proteins (Robert *et al.*, 2006).

#### 1.3.1.3 Protection

The clotting mechanism protects against blood loss, and certain phagocytic white blood cells or specialized plasma proteins such as antibodies, interferon, and complement protect against foreign microbes and toxins (Robert *et al.*, 2006).

### 1.3.2 Constituents of blood

Blood constitute about 45% formed elements (cells) consist of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets), and 55% fluid which is the plasma (Robert *et al.*, 2006).

#### 1.3.2.1 Plasma

A fluid that is blood liquid medium, which by itself is a straw yellow in color. The blood plasma volume totals of 2.7–3.0 liters (2.8–3.2 quarts) in an average represent 55% of the blood. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), andremoves waste products, such as carbon dioxide. Other

important components include: Serum albumin, Blood clotting factors, Immunoglobulin lipoprotein particles, and other proteins, various electrolytes (Purves*et al.,* 2004).

**1.3.2.2 Formed elements (cells)**

**1.3.2.2.1 Leukocytes**

Colorless cells of the immune system that circulate mainly in the blood and lymph and participate in reactions to invading microorganisms or foreign particles, comprising the B cells, T cells, macrophages, monocytes, and granulocytes, usually life spans from hours two years

according to type of the WBCs. Normal range4 - $11x10^9$ /l (Purves*et al.*, 2004).

**1.3.2.2.2 Thrombocytes**

Small, round cell fragments 2–3 μm in greatest diameter that functions in the clotting of blood. Platelets contain no nuclei and are formed in the BM from precursor cells called megakaryocytes. Life span 5-10 days, normal range 150 - 450 $x10^9$ /l(Purves*et al.,* 2004).

**1.3.2.2.3 Erythrocytes**

Is the mature red blood cell, a biconcave disc in shape measure 7-8 micrometer in diameter that contains haemoglobin confined within a lipodmembrane. It is the major cellular element of the circulating blood and transport oxygen as its principal function. The number of red cells per microliter of blood is 4.5-5.5 million for male and 4.2-4.8 million for female(Alberts and Bruce, 2012).

**1.3.2.2.3.1 Production**

Erythrocyte formation takes place in red marrow in adults, and in spleen, liver and bone marrow in fetus. Are arise from large nucleated stem cell (Promegaloblast) which give rise to Pronormoblast in which haemoglobin

appear, this give rise to Normoblast which extrude their nucleus. Erythrocyte at this stage possesses a reticular fine network known as Reticulocyte. This reticular structure usually lost before mature cell enter the circulation as mature erythrocyte. The proper formation of erythrocyte depends primarily in nutrition with protein, iron, folic acid, B12, copper and other essential vitamins (Alberts and Bruce, 2012).

**1.3.2.2.3.2 Functions**

Include transportation of oxygen and carbon dioxide, also they are important in maintaining the acid-base balance, since they help determine the viscosity of blood they influence the specific gravity also.

Their average life span is 120 days; they are subjected to much wear and tear in the circulation and eventually removed from circulation by reticulo-endothelial system(Alberts and Bruce, 2012).

## 1.4 Anemia

Is a reduction in the Hb concentration of the blood below the normal for age and sex. Normal values vary between laboratories, typical values would be less than 13.5 g/dl in adult males and less than 11.5 g/dl in females. From age of 2 years to puberty less than 11.0 g/dl indicates anaemia (Hoffbrand and Moss,2011**).**

### 1.4.1 Causes

- Dietary deficiencies: Iron, Vitamin B12 and Folic Acid are essential elements for formation of red cells.
- Blood loss: may be due to injury, donating blood, ulcers, etc.
- Increased RBCs destruction: as in haemolytic anaemias.
- Decrease in RBCs production: as in bone marrow failure, chronic disease (renal failure)(Hoffbrand and Moss,2011**).**

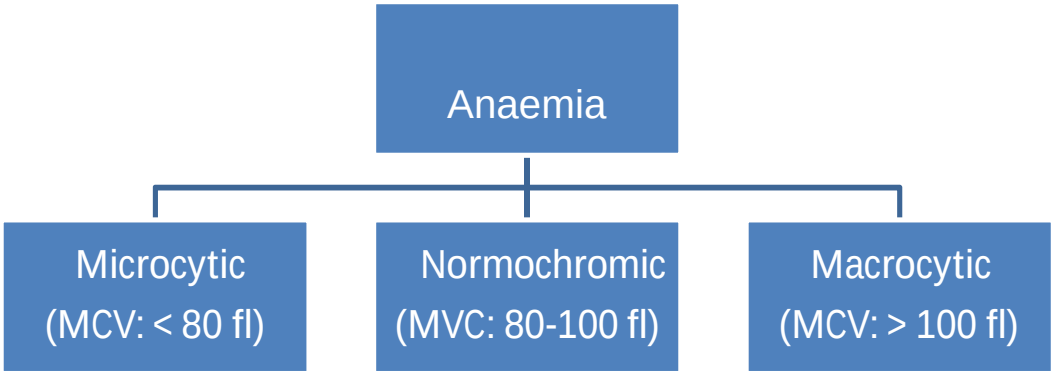
### 1.4.2 Classification

Anemiasbest classification is that based on morphology; based on the average size of the cells(MCV)and the hemoglobin concentration:

- Macrocytic
- Normocytic Normochromic
- Microcytic Hypochromic, figure 1.1(Cotran *et al.*,2005).

Also may be classified functionally into:

- Hypoproliferative (when there is proliferation defect)
- Ineffective (when there is a maturation defect)
- Hemolytic (when there is survival defect),figure 1.2




Iron deficiency anaemia Survival defect (haemolysis, Megaloblastic:
Chronic disease anemia Hemorrhge) $B_{12}$ deficiency
Thalassemia Stem cell transplant Folate deficiency
SidroblasticanemiaBM replacement - Non megaloblastic
Lead poisoing liver disease
Alcoholism

**Figure 1.1:** Morphological classifications of anaemia(Cotran *et al.*,2005)

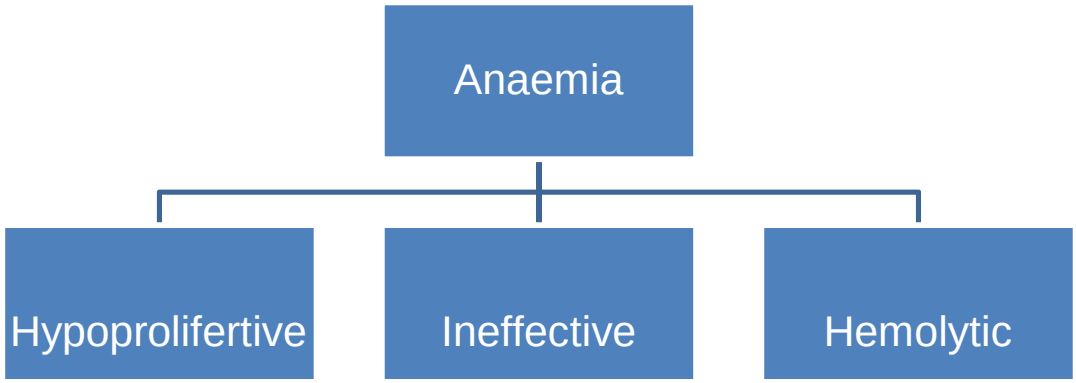



**Figure 1.2:** Functional classification of anaemia (Cotran *et al.*,2005)

### 1.4.3 Clinical features

The presence or absence of clinical features can be considered under four major headings:

1- Speed of onset: rapidly progressive anaemia causes more symptoms than anaemia with slow onset because there is less time for adaptation in cardiovascular system.

2-Severity: mild anaemia often produces no symptoms or signs {Hb 9-10 g/dl}. Even sever anaemia {Hb less than 6 g/dl} may produce few symptoms.

3- Age: The elderly tolerate anaemia less well than young because the status of cardiovascular symptoms.

4-Haemoglobin $O_2$ dissociation curve: anaemia in general associated with shift the curve to the right so $O_2$ is given up more readily to tissues, but this depend on type of anaemia eg: in sickle cell anaemia the curve shift to left

due to low affinity Hb.(Hoffbrand and Moss,2011**).**

* Clinical features of anemia are variable, but may include:

- Fatigue: One of the most common and debilitating symptoms of anemia is fatigue (lack of energy), particularly with exercise.
- Increased heart rate: The body increases heart rate to compensate for the low oxygen carrying capacity of the blood.
- Shortness of breath: This is a compensation for the poor delivery of oxygen to the tissues.
- Pale Skin.
- Low blood pressure: viscosity of blood drops as the hematocrit decreases, which leads to lowering the mean arterial blood pressure (MAP)(Hoffbrand and Moss,2011**).**

### 1.4.4 Laboratory diagnosis

- Complete blood count (CBC): The first step in diagnosis, it's a part from reporting the number of red blood cells and the hemoglobin level, also red cell indices, which are important tool in distinguishing between the causes of anemia. According to the result of CBC confirmatory tests are choosed.

- Blood smear examination: Blood is smeared on a glass slide for microscopic examination of RBCs, which can sometimes indicate the cause of the anemia.

- Reticulocytes count: A measure of young RBCs, this helps to determine if RBC production is at normal levels.

- Confirmatory tests: Special tests used to confirm the suspected type of anaemia, e.g.; iron profile for microcytic anaemias, Hb electrophoresis for haemoglobinopathies.

- Bone marrow aspiration and biopsy: This test can help determine whether cell production is happening normally in the bone marrow. It's the only way to diagnose aplastic anemia definitively and is also used for evaluating iron status(Dacie *et al.*,2006).

**1.5 Folate**

Folic acid (pteroylglutamic acid) is a yellow, crystalline, water soluble substance (molecular weight 441). It is the parent compound of a large family of folate compounds. Pteroylglutamic acid consists of three parts: pteridine,

p- aminobenzoate and l - glutamic acid.

The highest concentrations are found in liver and yeast (> 200 µ g per 100 g), spinach, other greens and nuts (> 100 µ g per 100 g). The total folate content of an average Western diet is about 250 µ g daily, but the amount varies widely according to the type of food eaten and the method of cooking. Folate is easily destroyed by heating, particularly in large volumes of water; over 90% may be lost.(A Victor, 2011).

**1.5.1 Body stores and requirement**

Total body folate in the adult is about 10 mg, the liver containing the largest store. Stores are only sufficient for about 4 months in normal adults, so severe folate deficiency may develop rapidly. Requirements are about 100 µ g/ day.(A Victor, 2011).

**1.5.2 Absorption**

The principal site of folate absorption is the upper small intestine, and there is a steep fall off in absorptive capacity in the lower jejunum and ileum. The absorption of all forms tested is rapid, a rise in blood level occurring within 15-20 min of ingestion.

The small intestine has a tremendous capacity to absorb folate

monoglutamates: about 90% of a single dose is absorbed regardless of whether this is small (100 μ g) or large (15 mg). A proton coupled high affinity folate transporter with a low pH optimum, termed PCFT/HCP1, is located at the apical brush border of the duodenal, and to a lesser extent jejunal mucosa and in other cells, including the blood – brain barrier. It accounts for the bulk of folate absorption including of folic acid itself, and loss of function in hereditary folate malabsorption is not compensated by other folate transporters expressed on intestinal cells (A Victor, 2011).

The absorption of folate polyglutamates with higher numbers of glutamate residues is less. This may be due to the limited capacity of the small intestine to hydrolyse these compounds or to their limited transfer in the mucosal cell. On average, about 50% of food folate is absorbed.Polyglutamate forms are hydrolysed by pteroyl-polyglutamate hydrolase to the monoglutamate derivatives, either in the lumen of the intestine or within the mucosa; they do not enter portal blood intact. Monoglutamate or polyglutamate forms of dietary folate, which are already partly or completely reduced, are converted to 5 -methyl-THF within the small intestinal mucosa before entering the portal plasma. The monoglutamates are actively transported across the enterocyte by a carrier mediated mechanism. Pteroylglutamic acid at doses greater than 400 μ g is absorbed largely unchanged and converted to natural folates in the liver (Antony, 2016).

**1.5.3 Folate deficiency**

Folic acid is an element essential for amino acid and nucleic acid metabolism. Adequate folicacid is required for normal hematopoiesis and development of the nervous system. Its deficiency may cause

fatigue, irritability, diarrhea, poor growth, and smooth and tender tongue(Antony, 2016).

Contributors to folate deficiency include:

- Diseases in which folic acid is not well absorbed in the digestive system (such as Celiac diseaseor Crohn disease).
- Drinking too much alcohol.
- Eating overcooked fruits and vegetables.
- Hemolytic anemia.
-Certain medicines (such as phenytoin, sulfasalazine, or trimethoprim-sulfamethoxazole).
- Eating an unhealthy diet that does not include enough fruits and vegetables**.**
- Kidney dialysis(Antony, 2016).

**1.6 Previous studies**

Many studies were done concerning serum folate in celiac disease.

Prospective study of 39 consecutive biopsy-proven celiac disease patients (32 women, seven men; median age 48 yr, range 22-77 yr),A total of 16 (41%) patients were vitamin B12 deficient (<220 ng/L) and 16 (41%) patients (11 women and five men) were anemic. Concomitant folate deficiency was present in only 5/16 (31%) of the vitamin B12 patients (Dahele andGhosh, 2001).

The haematological status, as well as the fractional absorptions of folic acid was studied longitudinally in 20 celiac children aged 1.2-16.6 yr (mean 7.5 yr) during periods of gluten-free and gluten containing diets. The folate status (erythrocyte-folate) showed significant variations related to dietary changes. However, few patients were folate depleted (Hjelt and Krsilnioff,

1990).

A study included 22 children with CD in whom the disease remained undiagnosed until they had presented with hematological abnormalities. Anemia was present alone in 19 (86.3%) patients, and leukopenia coexisted with anemia in 2 (9%) patients. Thrombocytopenia was found alone in 1 (4.5%) patient. Twelve patients had an iron deficiency anemia. Iron deficiency coexisted with zinc and vitamin B12 deficiency in 3 patients, folate and vitamin B12 deficiency in two, vitamin B12 deficiency in two, zinc deficiency in two and one patient had combined iron, zinc, and copper deficiency (Çatal *et al.*, 2015).

A cross-sectional study enrolled a total of 20 celiac patients ($36.3 \pm 13.7$ years old; 65% women), following strict gluten-free diet (GFD) and 39 healthy controls matched by sex and age. The dietary intake was assessed by 3-day food records, and serum concentrations of folate were determined after overnight fasting. Celiac patients had lower serum folate concentrations ($7.7 \pm 3.5$ ng/mL, $P < 0.05$) than controls. All celiac patients had folate intake below the Estimated Average Requirement (EAR) ($130.8 \pm 53.6$ μg/dl) (Valente *et al.*, 2015).

A case- control study in 80 newly diagnosed adult CD-patients were included (n = 80, 42.8 ± 15.1 years) and a comparable sample of 24 healthy Dutch subjects was added to compare vitamin concentrations. Nutritional status and serum concentrations of folic acid, haemoglobin (Hb) (before prescribing gluten free diet). Almost all CD-patients (87%) had at least one value below the lower limit of reference. Specifically, folic acid 20%and 32% had anaemia. Vitamin deficiencies were barely seen in healthy controls, with the exception of vitamin B12 (Wierdsma *et al.*, 2013).

In a cohort study of patients seen at a tertiary care center for CD to assess

the characteristics of anemia in this population. Hematological parameters measured <or=3 months of diagnosis and degree of villous atrophy from 405 patients diagnosed >1995 was analyzed. Folate deficiency was seen in approximately 12% of the total sample. Anemia was present in approximately 20% of the cohort. Macrocytic anemia with concurrent B12 or folate deficiency was rare (3%) (Harper *et al.*, 2007).

A total of 93 patients with a valid CD diagnosis were identified. In total, 30 % had anemia; 40% iron deficiency; 20% folate deficiency. After introduction of a gluten-free diet, 28% had normalized TGS antibody levels after 6 months, and 56% did after 12 months (Scholser *et al.*, 2015).

Thirty adults with coeliac disease (mean age, 55 years; range, 45-64 years; 60% women), in biopsy-proven remission following 8-12 years of dietary treatment, were studied. We measured the total plasma homocysteine level, a metabolic marker of folate. Coeliac patients showed a higher total plasma homocysteine level than the general population, indicative of a poor vitamin status. In accordance, the plasma levels of folate and pyridoxal 5'-phosphate (active form of vitamin B-6) were low in 20% and 37%, respectively, and accounted for 33% of the variation of the total plasma homocysteine level ($P < 0.008$). Half of the adult coeliac patients carefully treated with a gluten-free diet for several years showed signs of a poor vitamin status. The results may suggest that, when following up adults with coeliac disease, the vitamin status should be reviewed, (Hallert *et al.*,2002).

## Chapter two

# Rationale and Objectives

## 2.1 Rationale

CD is a digestive and autoimmune disorder that results in damage to the lining of the small intestine when foods with gluten are eaten, resulting in malabsorption of micronutrients; such as folic acid that is absorbed in lower of small intestines (the jejunim and ilieum), so defect in this sites due toCD lead to malbsorption resulting in anaemia

According to Ibn Sinaa Hospital records, recently there is increase incidence of celiac disease among Sudanese.To our knowledge there is no Sudanese study adressing folate deficiency as a cause of anaemia in CD patients and serum folate level is not routinely investigated for celiac patients; so estimation of serum folate levelmay reflect the abnormalities and improve patient's management protocols.

## 2.2 Objectives

### 2.2.1 General objective

To study serum folate level in patients with CD and to evaluate its effect on RBCs parameters.

### 2.2.2 Specific objectives

- To estimate serum folate level in patients with CD using immunoassay.
- To correlate serum folate level with patient's age, gender and RBCs parameters.
- To evaluate the effect of dietary program of celiac disease patients on serum folate level.
- To correlate between disease duration and serum folate level.

# Chapter Three

# Materials and Methods

## 3.1 Materials

### 3.1.1 Study design

This is study is descriptive cross sectional study

### 3.1.2 Study area

The study was conducted at Ibn Sinaa hospital; Khartoum, Sudan

### 3.1.3 Study duration

The study was conducted in the period from April- August 2016

### 3.1.4 Study population

Sudanese patients with CD referred to Ibn Sinaa hospital

### 3.1.5 Inclusion criteria

Sudanese patients with CD

### 3.1.6 Exclusion criteria

Patients with conditionsknown to have effect on haematological parameters-such as pregnancy, chronic blood loss, malaria, and renal failure, were excluded from the study.

## 3.2 Method

### 3.2.1 Sample collection

Three ml of blood were collected in plain container from each patients.

### 3.2.2 Sample preparation

Serum was prepared from clotted samples and used for measurement of serum folate(25 µ l) by immunoassay (Cobas e411, Germany).

### 3.2.3 Principle

Electro chemiluminscence or chemilumenscence is a kind ofluminescence produced during electrochemical reactions in solutions. In electrogenerated chemiluminescence, electrochemically generated intermediates undergo a highly exergonic reaction to produce an electronically excited state that then emits light upon relaxation to a lower-level state. This wavelength of the emitted photon of light corresponds to the energy gap between these two states.

### 3.3 Data collection and analysis

Patients' data were collected using structured questionnaire, hematological parameters were taken from patients' record; then analyzed using statistical package for social science software (SPSS).

### 3.4Ethical considerations

This study was approved by Ministry of health; Khartoum, Sudan and by Ibn Sinaa hospital management. Informed consent was taken from all participants before sample collection, and for children consent was taken from their parents. No risk is known as a result of sample collection. Patient with abnormal haematological parameters was advised to visit the suitable physician. Patient's data were kept confidentially.

# Chapter Four

## Results

This study was conducted on 40 patients with celiac disease referred to Ibn Seina Hospital; Khartoum- Sudan. In the period from April to August 2016, of which 10 (25%) were males and 30 (75%) were females, (Figure1).

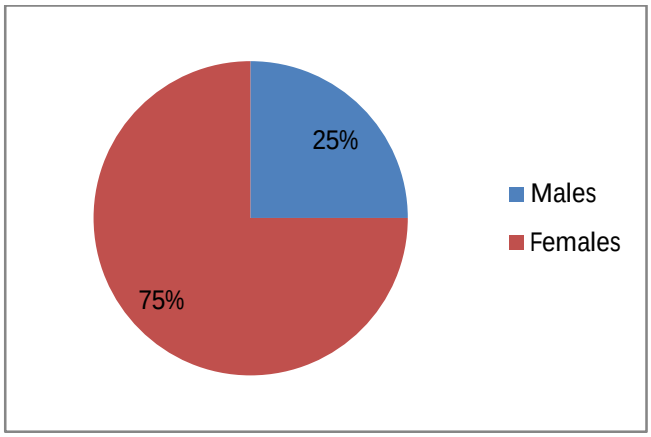



**Figure 4.1:** Gender distribution in study population

Age of study population ranged from 2 – 43 years (Mean ± SD: 16.5 ±10.2); 17 (42.5%) of the 40 patients were children and 23 (57.5%) were adults, (Figure 2).

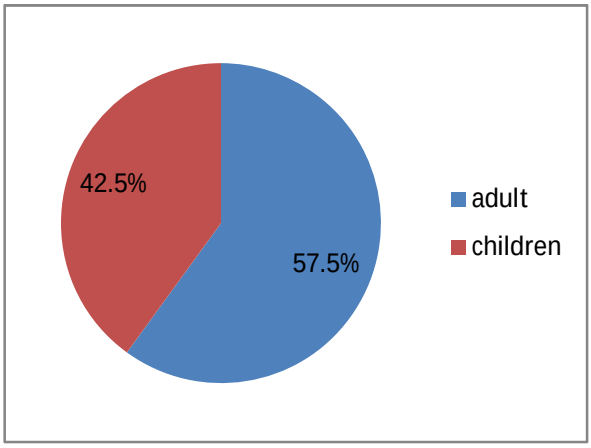



**Figure 4.2:** Distribution of study population according to age group

Four (10%) of the patients were not committed to gluten free diet while 36 (90%) were committed, (Figure3).

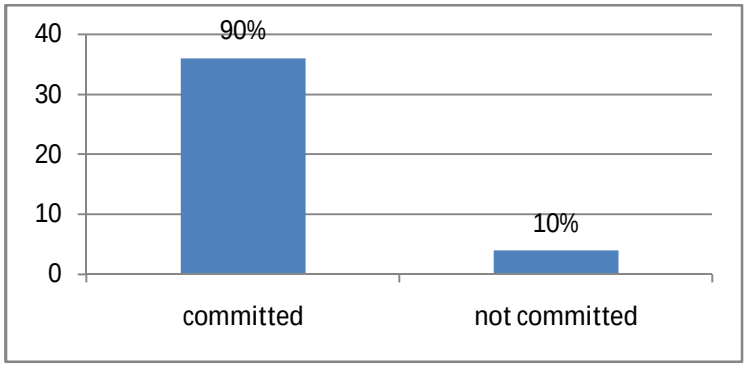



**Figure 4.3:** Commitment to gluten free diet in study population

Duration of disease among patients was ranged from one month to 240 month (Mean ± SD: 38.9 ± 47.0). Results of red blood cells parameters, duration and serum folate level are shown in Table 4.1

**Table 4.1:** Red cells parameters, serum folate and duration of disease in study population

| **Parameters** | **Mean** | **SD** |
|---|---|---|
| RBCs (u/l) | 4.77 | 0.51 |
| Hb (g/dl) | 10.6 | 2.2 |
| PCV (%) | 35.9 | 4.6 |
| MCV (fl) | 75.7 | 9.2 |
| MCH (pg) | 23.3 | 4.0 |
| MCHC (g/dl) | 30.7 | 2.4 |
| Folate(ng/ml) | 5.0 | 2.0 |
| Duration(months) | 38.9 | 47.0 |

Concerning serum folate level (referance values for adults 2-20 ng/ml; for children 5-20ng/ml) there were 11 (27.5%) patients with deficiency, 6(15%) were males and 5 (12.5%) were females; 29 (72.2%) with normal serum folate, 4 (10%) were males and 25 (62.5%) were females.

There was statistically significant correlation between gender and folate deficiency Table 4.2.

**Table 4.2:** Correlation between gender and folate deficiency

| **Folate level** | **Gender** | | | | P.value |
|---|---|---|---|---|---|
| | **Male** | | **Female** | | |
| | N | % | N | % | |
| Normal | 4 | 10% | 25 | 62.5 % | 0.008 |
| Deficient | 6 | 15% | 5 | 12.5% | |

The mean of age in patients with normal and low level of serum folate was significantly difference; as all of the deficient patients were children (< 12 years), Table 4.3.

**Table 4.3:** Comparison of Age in patients with and without folate deficincy

| **Age** | **Patients with normal folate** | | **Patients with folate deficiency** | | P.value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| | 20.0 | 9.9 | 7.5 | 3.1 | 0.00 |

There was no significant correlation between serum folate level and red cell parameters, as well as duration of disease, Table 4.4.

**Table 4.4:** Correlation of serum folate with red cells parameters & disease duration

| **Parameters** | **Pearson correlation** | P. value |
|---|---|---|
| RBCs (u/l) | -0.08 | 0.62 |
| Hb (gldl) | -0.27 | 0.09 |
| PCV (%) | -0.26 | 0.87 |
| MCV (fl) | -0.27 | 0.87 |
| MCH (pg) | 0.04 | 0.80 |
| MCHC (g/dl) | 0.025 | 0.87 |
| Duration(months) | -0.16 | 0.31 |

Regarding serum folate level and commitment with dietary program no significant difference was found, Table 4.5.

**Table 4.5:** Comparison of folate level according to diet program commitment

| | **Committed** | | **Not committed** | | P. value |
|---|---|---|---|---|---|
| **Serum folate level** | Mean | SD | Mean | SD | 0.89 |
| | 5.0 | 2 | 4.9 | 1.7 | |

Comparison of red cells parameters in patients with normal serum folate and those with low serum folate; showed no significant difference in RBCs count, Hb, PCV, MCHC, and duration of the disease. But there was a significant difference in MCV and MCH, Table 4.6.

**Table 4.6:** Comparison of red cells parameters and duration of disease in patients with and without folate deficiency

| **Red cells parametes** | **Patients with normal folate level** | | **Patients with folate deficiency** | | P. value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| RBCs(u/l) | 4.7 | 0.5 | 4.9 | 0.42 | 0.16 |
| Hb (g/dl) | 10.9 | 2.4 | 10.1 | 1.3 | 0.34 |
| PCV (%) | 36.6 | 4.7 | 33.9 | 4.2 | 0.10 |
| MCV (fl) | 78.0 | 8.9 | 69.7 | 7.2 | 0.009 |
| MCH (pg) | 24.1 | 4.0 | 20.7 | 3.1 | 0.016 |
| MCHC(g/dl) | 31.0 | 2.6 | 29.8 | 1.7 | 0.20 |
| Duration (months) | 40.3 | 53.8 | 35.1 | 21.9 | 0.76 |

# Chapter Five

# Discussion, Conclusion and Recommendations

## 5.1 Discussion

CD is an autoimmune disorder characterized by a permanent intolerance to gluten, a protein found in wheat, rye, barley, and other grains. Celiac disease causes inflammation and damage to the small intestine, leading to the malabsorption of iron, folate, calcium, and vitamin D, and the longer it remains untreated, the fat-soluble vitamins (A, D, E, and K), carbohydrates, and fats. This malabsorption can lead to multi-systemic conditions such as anemia, osteoporosis, fertility problems, and delayed growth (Ciclitira, 1999).

This is a hospital based descriptive cross sectional study, included 40 patients with CD referred to Ibn Seina Hospital; Khartoum, Sudan. In the period from April to August 2016. It aimed to assess serum folate level among CD patients; and correlate the result with red cell parameters, patients' gender and age, duration of disease, and commitment to dietary program.

In this study folate deficiency was present in 27.5 % of the patients; this finding agrees with previous studies reported a frequency of 20% for folate deficiency in newly diagnosed CD patients (Wierdsma *et al.,* 2007 and Schoslr *et al*., 2015), also agrees with study done by Dahele *et al.*, (2001), who reported 30% of CD patients with low serum folate. Our finding disagrees with previous studies done by Harper *et al,* and catal *et al,* who reported folate deficiency in 12% and 9% of CD patients respectively, (Harper *et al.*2007, and catal *et al.* 2015).

When correlate serum folate level and age a statistically significant difference was found (*P.value*< 0.05), as all the deficient patients were children; this may due to increase of demand of folic acid in children more than adult (Hoffbrand and Moss,2011).

We found that there was statistically significant correlation between gender and folate deficiency (*P.value*<0.05). This may due to that number of males in this study was less than femaleand most of deficient patients were males.

In the present study there was no statistically significant difference in RBCs parameters in patients with and without folate deficiency, except for MCV and MCH; and no patients with macrocytosis noted, this agrees with study done by Harper *et al,* (2007). Who found that macrocytosis was rare. This may due to concomitant iron deficiency is commonly seen in CD patients; so patients with deficiency of folate may not present with the characteristic macrocytosis,(Thorvardur*et al*.,2007)

Concerning dietary commitment and serum folate level we found that no statistically significant correlation was found. A study by Valente *et al.*(2015), found that patients who strict to gluten free diet had low serum folate and this in agree with our study, another study by Hjelt *et al.* (1990), reported that folate status showed significant variations related to dietary changes and this disagree with our findings,this may because of gluten-free products aren't subject to the same fortification standards as conventional foods, even a healthful gluten-free diet can contain suboptimal levels of iron, folate, thiamin, riboflavin, and fiber, which are nutrients commonly supplemented by conventional food, (Fasano *et al.,* 2003).

Regarding folate level and duration of disease no significant correlation was found in this study, which disagree with a study by Hallert *et al*, who found that half of the CD patients carefully treated with a gluten-free diet

for several years showed signs of a poor vitamin status,(Hallert *et al.,* 2002). This may due that patients are take supplementation that compensate the deficiency (Fasano *et al.*, 2003).

## 6.2 Conclusion

- 27.5% of the patients had folate deficiency.
- All patients with folate deficiency were children.
- A significant correlation was found between folate deficiency and gender, because most of deficient patients were males.
- MCV and MCH were significantly lower in patients with folate deficiency.
- No significant correlation was found between serum folate level and duration of disease and dietary program commitment.

## 6.3Recommendations

- A study with increased sample size should be carried out
- Patients with CD, particularly children should be assessed frequently for folate level**.**

# References


**Alaa R, Joseph A,and Martin F, (2006).** Medical Position Statement on Celiac Disease, *Gastroenerology*. **131**(6): 1977–1980.

**Alberts and Bruce, (2012).** Blood Cells. *Molecular Biology of the Cell*. NCBI Bookshelf.

**Antony AC,(2016).** Megaloblastic anemias. In: Goldman L, Schafer AI, eds. *Goldman's Cecil Medicine*. 25th ed. Philadelphia, PA: Elsevier Saunders; chap 164.

**A victor H, (2011).** Post Graduate Haematology. 6th Edition. Wiley Blackwell UK. P: 76.

**Bailey A, Lynn B, (2009).**Folate in Health and Disease, Second Edition. CRC Press. P: 198.

**Çatal F, Topal E, Ermiştekin H, Yildirim Acar N, Sinanoğlu MS, Karabiber H, Selimoğlu MA, *et al.*,(2015).** The hematologic manifestations of pediatric celiac disease at time of diagnosis and efficiency of gluten-free diet. *Turk J Med*. **45**(3); P: 663-7.

**Ciclitira P, (1999).** Coeliac Disease. In: **Yamada T**, **Alpers D, Laine L, Owayang C, Powell D,** editors. Text book of Gastroenterology. 3rd ed. Philadelphia: Lippircott Williams and Wilking. P: 1660 – 76

**Cotran, Kumar, Fausto, Robbis, Stanley, abbas *et al.*, (2005).**Robbins and Cotran pathologic basis of disease. St, Louis, Mo: Elsevier. P: 637.

**DACIE S.M., BAIN B.J., and BATES I, (2006).**Dacie and Lewis, Practical Hematology.10th ed. Churchill Livingstone.

**Dahele A.1., & Ghosh S, (2001).** Vitamin B12 deficiency in untreated celiac disease. *Am J Gastroenterol*. **96**(3); P: 745-50.

**Farrie RJ, & Kelly C.P, (2002).** Celiac sprue and refractory sprue. In: **Feldman M**, (edi.). *Sleisenger & Fordtran's Gastrointestinal and Liver Disease Pathophysiology/ Diagnosis/ Management*. Philadelphia, PA, USA: Elsevier Science. P: 1818–1841.

**Fasano A, Berti I, Gerarduzzi T, Colletti RB, Drago S, Elitsur Y, *et al.*, (2003).** Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study, *Arch Intern Med*. **163**:286–292

**Feighery C, (2001).** Coeliac disease. *Br Med J*. **319**(7204): 236 – 9.

**Fine J.D, (1996).** Immunobullous diseases. In: SAMS, W.M. Jr, LYNCH, P.J. (eds.). *Principles and practice of dermatology*. 2nd ed. New York: Churchill Livingstone. P: 461.

**Green PH, Cellier C, (2007).** Celiac disease. *N Engl J Med*. **357**:1731–43

**Green P.H.R, & Jabri B, (2006).** Celiac disease. *Annu Rev Med* . **57** (2); P:207–221.

**Guanlini S, (2008**). Celiac disease: risk assessment diagnosis and monitoring. *Mol Diagn Ther*. **12** (3); P:289–298.

**Hadithi M, von Blomberg BM, Crusius JB, Bloemena E, Kostense PJ, Meijer JW,*et al,* (2007).** Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease. *Ann Intern Med*. **147** (4); P:294–302.

**Halfdanrson T, LitzowM, and MurrayJ, (2007).** Hematologic manifestations of celiac disease. *Blood*. **10**(9); P:412–421.

**Hallert C, Grant C, Grehn S, Gränno C, Hultén S, Midhagen G, *et al.,* (2002).**Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years.*Aliment Pharmacol Ther.***16**(7):1333-9.

**Harper JW, Holleran SF, Ramakrishnan R, Bhagat G, Green PH, *et al.*, (2007).** Anemia in celiac disease is multifactorial in etiology.*J Hematol*. **82**(11); P: 996-1000.

**Hjelit K, & Krsilnioff P.A, (1990).** The impact of gluten on haematological status, dietary intakes of haemopoietic nutrients vitamin B12 and folic acid absorption in children with coeliac disease. *Acta Paediatr Scand.***79**(10); P: 911-9.

**HOFFBRAND A.V., & MOSS P.A.H.,(2011).**Essential Haematology. 6th ed. Wiley Blackwell UK. P: 26-28.

**Hopper A.D, Hadjivassiliou M, Hurlstone DP, Lobo AJ, McAlindon ME, Egner W, *et al*, (2008).** What is the role of serologic testing in celiac disease? A prospective, biopsy confirmed study with economic analysis. *Clin Gastroenterol Hepatol* . **6**(3); P:314–320.

**Jones R.B., Robin G.G, and Howdle P.D, (2006).** Advances in celiac disease. *Curr Opin Gastroenterol* . **22**(5); P:117–123

**Logan R.F.A, (1992).**Epidemiology of celiac disease. Oxford: Blackwell Scientific. P: 192–4.

**Mulder CJ, van Bodegraven AA, *et al.*, (2013).** Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients. *Nutrients.* **5**(10); P: 3975-92.

**Parveno A.L., (2011).** Hematological disorders in celiac disease.*Ter Ark* . **83**(7); P: 68-73.

**Purves, willim k, sadava,david,orians,Gordon,*et al.*, (2004)**.The science biology.7th ed. Sunderland, mass: Sinauer b associates . P.54

**Reeves G, Squance ML, Duggan AE, Murugasu RR, Wilson RJ, Wong RC, *et al*, (2006).** Diagnostic accuracy of coeliac serological tests: a prospective study. *Eur J Gastroenterol Hepatol.***18** (5); P:493–501.

**Robert , Tallistsch, Martini, Frederic, Timmons, Micheal,(2006).** Human anatomy. 5th ed.San Francisco; pearson-Benjamin cummings. P:529

**Robinson S.H, & Reich P.R, (2013).**Hematology: Pathophysiologic Basis For Clinical Practice. 6th ed. Little Brown and Company.

**Schoslar, L, Christensen LA, and Hvas CL1, (2016).**Symptoms and findings in adult onset celiac disease in a historical Danish patient cohort. *Scand J Gastroenterol* .**51**(3);P: 288-94.

**Setty M, Hormaza L, and Robinson S.H, & Reich P.R, (2013).**Hematology: Pathophysiologic Basis For Clinical Practice. 6th ed. Little Brown and Company.

**Sulieman G, (1978).** Celiac disease in Sudanese Childern. *Gut*. **19**(2):121-5.

**Thorvardur R, Mark R,and Joseph A, (2007).** Haematologic manifestiation of celiac disase. *Blood*. 15; **109**(2): 412–421.

**Valentex F.X, Tatiana C , Luís M, Helen H , Leandro C, Helena M, *et al.*, (2015).** B vitamins related to homocysteine metabolism in adult's celiac disease patients: a cross-sectional study. *Nutr J*. 20(10).

**Wierdsma NJ, van Bokhorst D, van der Schueren MA, Berkenpas M, Mulder CJ, van Bodegraven AA, *et al.*, (2013).** Vitamin and mineral deficiencies are highly prevalent in newly diagnosed celiac disease patients. *Nutrients.* **5**(10): 3975-92.

# Appendix one

Sudan University of Science and Technology

College of Graduate Sciences

**Evaluation of Serum Folate Level and its Effects on Red Cells Parameters in Patients with Celiac disease**

# Questionnaire

ID …….

Gender ……..

Age …….…….

Duration of disease: ……

Strict to gluten free diet:

Yes ……. No ……

Any chronic disease: ……

Serum folate: ……

RBCs……..……

Hb……...……

MCV……...……

MCH……

MCHC……..

# Appendix Two

## Equipments, Disposables and Reagents

-70% alcohol

-Syringe

-Cotton

-Plaster

-EDTA contatiers

-Plain ontainers

-Folate reagents:

Folate kits contain two reagents:

- Reagent one: pretreatment reagent which contain potassium hydroxide, micro particle, conjugate and specific diluents containing borate buffer.
- Reagent two: contain dithioretiol in acetic acid buffer, and TRIS buffer for sample dilution.