Association between childhood Celiac Disease, Hyperglycemia and Thyroid Disorder in Red Sea State, Sudan (2014 – 2017)

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Department of Clinical Chemistry
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July/ 2018
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قال تعالى:

(وَقَل رَبِّ زَدْنِي عِلْماً)

صدق الله العظيم

سورة طه: الآية 114
Dedication

To my Family, to my husband, Friends and colleagues

To all whom I love and respect

To everyone who support me in completion this Research

I dedicate this work

Nazik
I am Nazik Sir Elkhatum Bakhit Seliman to declare that the work concern the requirements of PhD degree in Medical laboratory Sciences (Association between childhood Celiac Disease, Hyperglycemia and Thyroid Disorder in Red Sea State, Sudan, (2014 – 2017)) at the University of Gezira, Sudan under the supervision of Prof. Ali Khidir Ali Ageep, Prof. Bakri Yousif Mohamed Nour, had been prepared by my own potential and it has not been copied from any other sources, also it had not been presented by any other researcher for scientific degree elsewhere.

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Association between childhood Celiac Disease, Hyperglycemia and Thyroid Disorder in Red Sea State, Sudan (2014 – 2017)

Nazik Sir Elkhatum BakhitSeliman

Abstract

This study was designed to assess association between celiac disease diabetes mellitus type 1 and thyroid disorder in Red Sea State, Sudan. Cross sectional, analytical, hospital base study was conducted during Dec.2014 to Dec.2017; the sample was consent of patients suspected to have celiac disease refer from pediatrics in some hospital. Total of one thousand Sudanese children suspect to have CD were taken, only 200 of them were confirmed positive for CD. Level of AGA and ATTG were considered positive when found to be more than 10 times of cut-off value. Level of serum RBS, HbA1C and CRP where measured used biosystem 310 analyzer (Germany), semi-automated spectrophotometer. Level of TSH, T3 and T4 were measured used ELISA instrument (USA), ESR was measured using quick method. In addition observation like Age, Gender, Tribe and common complain which were chronic diarrhea and weight loss. The result showed that the majority of patients were in age 6-10 years (45%), most of patients were having CD for less than 5 years (91.5%). The distribution of CD patients among the Red Sea State tribes showed that (60%) of patients having the disease were Bane Ammer then Hadandawa (16.5%). The result of our research showed that the most common clinical presenting symptom was chronic diarrhea (30%) followed by weight loss (16.5%). The result indicated that (73 from 200) (36.5%) of CD patients were discovered to have hyperglycemia and (7%) of patients were discovered to have thyroid disorder, thyroid disorders among CD patients showed that (93%) of patients have Euthyroid and (7%) of patients have hypothyrodisin, the result of correlation between CD, HbA1C and RBS showed there was significant association between variables but weak correlation between CD and Thyroid disorder. Our study concluded that in Sudanese
patients having CD were also carried risk for hyperglycemia and hypothyroidism, the only treatment for CD is gluten free diet (GFD).
الارتباط بين مرض الداء الزلاقي لدى الأطفال وارتفاع سكر الدم واعتلال
وظائف الغدة الدرقية بولاية البحر الأحمر، السودان (2014 – 2017م)

ملخص البحث

صممت هذه الدراسة لتقديم الإرباط بين مرض الداء الزلاقي وارتفاع معدل سكر الدم واعتلال الغدة الدرقية بولاية البحر الأحمر–السودان. أجربت هذه الدراسة التحليلية المقطعية لعينات مرضى بالمستشفيات في فترة ما بين ديسمبر 2014 وحتى ديسمبر 2017. وكانت العينة عبارة عن مجموعة من المرضى الذين يشتبه في إصابتهم بالداء الزلاقي تم تحولهم من اختصاصي الأطفال في بعض المستشفيات، تم أخذ مجموعة من 1000 طفل سوداني مصته في إصابتهم بالمرض. تم تأكيده 200 منهم فقط وأعتبر مستوى الأجسام المضادة (CRP) لكل من AGA و ATTTG و HbA1C (السكر التراكمي) و HbA1C (السكر التراكمي) و البروتين المناعي من نوع C (RBS) و TSH و T4 و T3 في المصل باستخدام جهاز بابوست两点 310 الألماني الصنع. تم قياس مستوى غلوكوز الدم في جميع المرضى باستخدام جهاز جهاز جهازات وآلات تول الصناع. تمت استيفاء نظام التحليقات مثل العمر، الجنس، القبائل وأكثر الاعلامات السريرية إزائدة والتي كانت الأعراض المزمنة وقدان الوزن. أظهرت النتائج أن غالبية المرضى كانوا من سن 6-10 سنوات (45.5)، وكان معظم المرضى لديهم مرض فترة أقل من 5 سنوات (91.5%). أظهر توزيع مرض الداء الزلاقي بين فتيات ولاية البحر الأحمر أن (60%) من المرضى المصابين من قبلة بني عام ثم الهدنة بنسبته (16.5%). أظهرت نتائج البحث أن أكثر الأعراض السريرية شيوعاً هي الإسهال المزمن (30%) بليه تعداد الوزن (16.5%). أشارت النتائج إلى أن (73 من 200) (36.5%) من مرضى الداء الزلاقي أكتشف لديهم ارتفاع في سكر الدم وسكر التراكمي . (7%) من المرضى لديهم اعتلال في الغدة الدرقية. أظهرت النتائج أن إضطرابات الغدة الدرقية بين مرض الداء الزلاقي (93%) من المرضى سويغ الغدة الدرقية و (7) لديهم نقص الغدة الدرقية ابهرت الدراسة أن هناك علاقة إرباط بين إرتفاع السكر الدم ونقص الغدة الدرقية P value 0.00 (P value 0.496) مع وجود ارتباط ضعيف مع اعتلال الغدة الدرقية. نتائج هذه الدراسة أن المرضى السودانيين الذين يعانون من إرتفاع سكر الدم كانا أيضًا عرضة للإصابة بارتفاع سكر الدم ونقص الغدة الدرقية والعلاج الوحيد للداء الزلاقي هو نظام غذائي خالي من القليلين.
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**Abbreviation**

AGA = anti glaidin antibodies

ARA = anti reticlin antibodies

ATTG = anti tissue tranlgutamate antibodies

CD = Celiac disease

CRP = C- reactive protein

CSF = Cerebrospinal Fluid

DM1 = diabetes mellitus type 1

ELISA = enzyme – linked immunosorbent assay

EMA = anti endomysium antibodies

ESR = erythrocyte sedimentation rate

GFD = glutine free diet

GIT = gastro-intestinal tract

HLA = human leukocyte antigen

HRP = horse reddish peroxidase

IBS = irritable bowel syndrome

IFCC = International Federation of Clinical Chemistry Standardied Method

MHC = major histocompatibility

NGSP = National Glycohemoglobin Standardization Program Certified Method

NICE = national institute for health clinical excellence

PTH = ParaThyroid Hormone

SPSS = statistical package for social science
TBG = thyroxine-binding globulin

TMB = tetra methyl benzidin

TRH = thyrotropin-releasing hormone

USA = united state of American
Chapter one

Introduction
Chapter one

1-Introduction

Celiac disease is an autoimmune disorder of the small intestine that occur in genetically predisposed people of all ages. Coeliac disease appears to be polyfactorial, both in that more than one genetic factor can cause the disease and in that more than one factor is necessary for the disease to manifest in a person.

It is caused by a reaction to gliadin, a prolamin - gluten protein - found in wheat, and similar proteins found in the crops of the tribe Triticeae. Frequently, the symptoms are ascribed to irritable bowel syndrome - IBS, only later to be recognized as coeliac disease; a small proportion of people with symptoms of IBS have underlying coeliac disease, and screening for coeliac disease is recommended for those with IBS symptoms.

Historically, celiac disease was thought to be rare, with prevalence of about 0.02%. Recent increases in the number of reported cases may be due to changes in diagnostic practical.

Celiac disease is more prevalent in woman than men. The disease is thought to affect 1 in 1750 -defined as clinical disease- to 1 in 105 (defined by present of IgA TG in blood) people in united states.

Coeliac disease is associated with a number of other medical conditions, many of which are autoimmune disorders: diabetes mellitus type 1, autoimmune thyroiditis, some factors can trigger symptoms include: surgery, pregnancy, infection and emotional stress.

Diabetes mellitus D, is a group of metabolic diseases in which a person has high blood sugar. Thyroid autoimmunity is familial disease in which the body interprets the thyroid glands and its hormone products T3, T4 and TSH as threats.
1-2 Rationale:

From our observation there was increasing of number of patients suffering from celiac disease with non-etiology which become a serious problem recently, which need specific investigation to diagnosis. Also large proportion of celiac disease remain undiagnosed; this is due to, in part, to many clinicians being unfamiliar with condition. Historically, coeliac disease was thought to be rare in Sudan, with a prevalence of about 0.02% (3). But recent increases in the number of reported cases may be due to changes in diagnostic practice. The prevalence of clinically diagnosed disease is 0.05–0.27% in various studies (10).

However, population studies from parts of Europe, India, South America, Australia and the USA using serology and biopsy indicate that the prevalence may be between 0.33 and 1.06% in children, and 0.18–1.2% in adults. Other populations at increased risk for coeliac disease, with prevalence rates ranging from 5% to 10%, include individuals with type 1 diabetes, and autoimmune thyroid disease, including both hyperthyroidism and hypothyroidism.

Diabetes mellitus is common disease in Sudanese people, lead to serious complication. In 2011 it resulted in 1.4 million deaths worldwide making it the 8th leading cause of death. the incidence of type 1 diabetes has been increasing by about 3 % per year. Untreated, diabetes mellitus is ultimately fatal (11). Thyroiditis is familial, lead to situation interval with celiac disease.

The aim of this research was to confirm the presence of CD in the Red Sea State, Sudan and to assess any association between celiac disease, hyperglycemia and thyroid disorder in Red Sea State, Sudan 2014, no pervious study was done. This data will help sensitize our local health staff towards celiac disease, thus encouraging delivery of appropriate clinical management and health care facilities to these patients.
Chapter two

Literature
2- chapter two

Literature review

2-1 Celiac disease

Is an autoimmune disorder of the small intestine that occurs in genetically predisposed people of all ages from middle infancy onward.

This condition has several other names, including celiac disease, coeliac sprue, nontropical sprue, endemic sprue and gluten enteropathy. The term coeliac derived from the Greek - koiliakos, "abdominal" - and was introduced in 19th century in a translation of what is generally regarded as an ancient Greek description of the disease by Aretaeus of Cappadicia (12).

2-1-1 Epidemiology;

The disease is thought to affect between 1 in 1750 defined as clinical disease with limited digestive tract symptoms, to 1 in 105 defined by presence of IgA TG in blood donors people in the United States (6). The prevalence of clinically diagnosed disease symptoms prompting diagnostic testing is 0.05 – 0.27 % in various studies. However, population studies from parts of Europe, India, South America, Australia and USA using serology and biopsy indicate that the prevalence may be between 0.33 and 1.06 % in children, and 0.18 – 1.2 % in adult. Among those in primary care population who report gastrointestinal symptoms, the prevalence of celiac disease is about 3 %.

People of Africa, Japanese and Chinese descent are rarely diagnosed (13). This reflects a much lower prevalence of the genetic risk factor, such as HLA-B8. Population studied also indicate that a large proportion of celiac remain undiagnosed; this is due to, in part, to many clinicians being unfamiliar with the condition (14). Celiac disease is more prevalence in women than in men (5).

A large multicentre study in the U.S. found a prevalence of 0.75% in not-at-risk groups, rising to 1.8% in symptomatic people, 2.6% in second-degree relatives of a person with coeliac disease and 4.5% in first-degree relatives. This profile is similar to
the prevalence in Europe\(^{(15)}\). Other populations at increased risk for coeliac disease, with prevalence rates ranging from 5% to 10%, include individuals with Down and Turner syndromes, type 1 diabetes, and autoimmune thyroid disease, including both hyperthyroidism and hypothyroidism\(^{(3)}\). Historically, coeliac disease was thought to be rare, with a prevalence of about 0.02\%. Recent increases in the number of reported cases may be due to changes in diagnostic practice\(^{(4)}\).

2-1-2 Causes

It was caused by a reaction to gliadin, a prolamin-gluten protein found in wheat and similar proteins found in the crops of the tribeTriticeae - which includes other common grains such as barley and rye -\(^{(1)}\). Other grains also induce symptoms like Wheat subspecies - spelt and durum- and related species such as barley, rye, triticale and Kamut. A small minority of people with celiac disease also react with oats maybe due to cross-contamination with other grains in the field or in the distribution channel. Therefore, oats are generally not recommended. Other cereals such as maize, millet, sorghum, teff, rice, and wild rice are safe for people with coeliac to consume, as well as noncereals such as amaranth, quinoa, and buckwheat\(^{(16,17)}\). Noncereal carbohydrate-rich foods such as potatoes and bananas do not contain gluten and do not trigger symptoms.

2-1-3 Prolamins

The majority of the proteins in food responsible for the immune reaction in coeliac disease are the prolamins. These are storage proteins rich in proline (prol-) and glutamine –amin; that dissolve in alcohols and are resistant to proteases and peptidases of the gut\(^{(18)}\). Prolamins are found in cereal grains with different grains having different, but related prolamins: wheat (gliadin), barley (hordein), rye (secalin), corn (zein) and as a minor protein, avenin in oats. One region of \(\alpha\)-gliadin stimulates membrane cells, enterocytes, of the intestine to allow larger molecules around the sealant between cells. Disruption of tight junctions allow peptides larger than three amino acids to enter circulation.

2-1-4 Genetics
The vast majority of people with celiac have one of two types of the HLA-DQ protein\(^{19}\). HLA-DQ is part of the MHC class II antigen-presenting receptor also called the human leukocyte antigen system and distinguishes cells between self and non-self for the purposes of the immune system. The two subunits of the HLA-DQ protein are encoded by the HLA-DQA1 and HLA-DQB1 genes located on the short arm of the sixth chromosome. There are seven HLA-DQ variants. Over 95% of people with celiac have the isoform of DQ2 or DQ8 which is inherited in families. The reason these genes produce an increase in risk of celiac disease is that the receptors formed by these genes bind to gliadin peptides more tightly than other forms of antigen-presenting receptor, thereby, these forms of receptor are more likely to activate T lymphocytes and initiate the autoimmune process\(^{10}\).

Other genetic factors have been repeatedly reported in CD; however, involvement in disease has variable geographic recognition. Only the HLA-DQ loci show a consistent involvement over the global population\(^{20}\).

The prevalence of CD genotypes in the modern population is not completely understood. Given the characteristics of the disease and its apparent strong heritability, it would normally be expected that the genotypes would undergo negative selection and to be absent in societies where agriculture has been practiced the longest. This expectation was first proposed by Simoons (1981)\(^{21}\).

By now, however, it is apparent that this is not the case; on the contrary, there is evidence of positive selection in CD genotypes. It is suspected that some of them may have beneficial by providing protection against bacterial infection\(^{22,23}\).

People of African, Japanese and Chinese descent are rarely diagnosed\(^{17}\); this reflects a much lower prevalence of the genetic risk factors\(^{24}\). Coeliac disease is more prevalent in women than in men\(^{25}\).

There are various theories as to what determines whether a genetically susceptible individual will go on to develop coeliac disease. Major theories include infection by rotavirus\(^{24}\) or human intestinal adenovirus\(^{25}\). Some researches has suggested that smoking is protective against adult-onset coeliac disease\(^{26}\). Early introduction of grains is protective against grain allergies\(^{27}\). Breastfeeding may also reduce risk with prolonging breastfeeding until the introduction of gluten-containing grains into the
diet associated with a 50% reduced risk of developing coeliac disease in infancy; whether this persists into adulthood is not clear\(^{(28)}\). Factors that can trigger symptoms include: surgery, pregnancy, infection and emotional stress\(^{(29)}\).

2-1-5 Signs and symptoms

Severe coeliac disease leads to the characteristic symptoms of pale, lose and greasy stool (steatorrhoea) and weight loss or failure to gain weight in young children. People with milder coeliac disease may have symptoms that are much more subtle and occur in other organs than the bowel itself. It is also possible to have coeliac disease without any symptoms whatsoever\(^{(1)}\). Many adults with subtle disease only have fatigue or anaemia\(^{(10)}\).

There was diarrhoea, Abdominal pain and cramping, bloatedness with abdominal distension thought to be due to fermentative production of bowel gas, and mouth ulcers\(^{(30)}\) may be present. As the bowel becomes more damaged, a degree of lactose intolerance may develop. Frequently, the symptoms are ascribed to irritable bowel syndrome (IBS), only later to be recognized as coeliac disease; a small proportion of people with symptoms of IBS have underlying coeliac disease, and screening for coeliac disease is recommended for those with IBS symptoms\(^{(31)}\). Coeliac disease leads to an increased risk of both adenocarcinoma and lymphoma of the small bowel.

This risk return to baseline with diet. long -standing and untreated disease may lead to other complication .

Related to malabsorption

The change in bowel become make it less able to absorb nutrients, minerals and the fat-soluble vitamins A, D, E, and K\(^{(32)}\).

- The inability to absorb carbohydrates and fats may cause weight loss (or failure to thrive/stunted growth in children) and fatigue or lack of energy.
- : iron malabsorption may cause iron deficiency anemia, and folic acid and vitamin B12 malabsorption may give rise to megaloblastic anemia.
- Calcium and vitamin D malabsorption .
• A small proportion have abnormal coagulation due to vitamin K deficiency, make them at risk for abnormal bleeding.
• Coeliac disease is also associated with bacterial overgrowth of the small intestine (33).

Coeliac disease has been linked with a number of conditions. In many cases, it is unclear whether the gluten-induced bowel disease is a causative factor or whether these conditions share a common predisposition, like IgA deficiency is present in 2.3% of people with coeliac disease, this was increased risk of infections and autoimmune disease (34).

Dermatitis herpetiformis; this itchy cutaneous condition has been linked to a transglutaminase enzyme in the skin (35,36).

Growth failure and/or pubertal delay in later childhood can occur even without obvious bowel symptoms or severe malnutrition. Evaluation of growth failure often includes coeliac screening. also recurrent miscarriage and unexplained infertility (1). Hyposplenism (37) this occurs in about a third of cases, Abnormal liver function tests randomly can be detect.

Coeliac disease is associated with a number of other medical conditions, many of them are autoimmune disorders: diabetes mellitus type 1, autoimmune thyroiditis (38), primary biliary cirrhosis, and microscopic colitis. A more controversial area is group of disease in which antigliadin antibodies are sometimes detected, but no small bowel disease can be demonstrated. Sometimes these conditions improve by removing gluten from the diet. This includes cerebellar ataxia, peripheral neuropathy, schizophrenia and autism (39).

2-1-6 Pathophysiology:

Coeliac disease appears to be polyfactorial, both in that more than one genetic factor can cause the disease and in that more than one factor is necessary for the disease to manifest in a person. Almost all people with celiac disease have either the variant HLA-DQ2 allele or the HLA-DQ8 -less commonly. However, about 20 – 30 % of people without celiac disease have also inherited either of these alleles (23).
suggests additional factors are need for celiac disease to develop; that is, the predisposing HLA risk allele is necessary but not sufficient to develop celiac disease. Furthermore, around 5% of those people who do develop celiac disease do not have typical HLA-DQ2 or HLA-DQ8 alleles.

2-1-7 Diagnosis

There are several tests that can be used to assist in diagnosis. The level of symptoms may determine the order of the tests, but all tests lose their usefulness if the person is already eating a gluten-free diet. Intestinal damage begins to heal within weeks of gluten being removed from the diet, and antibody levels decline over months. The prediction rule recommends that people with high-risk symptoms or positive serology should undergo endoscopic biopsy of the second part of the duodenum. The study defined high-risk symptoms as weight loss, anemia, or diarrhoea (26).

Serological blood tests are the first-line investigation required to make a diagnosis of coeliac disease.

1. Antiendomysial antibodies of the immunoglobulin A (IgA) type can detect celiac disease with a sensitivity and specificity of 90% and 99%, respectively (40).
2. Serology for anti-tTG antibodies was initially reported to have a higher sensitivity of 99% and specificity of >90% for identifying coeliac disease. However, it is now thought to have similar characteristics to anti-endomysial antibody. It is an easier test to perform.
3. Historically three other antibodies were measured: anti-reticulin (ARA), anti-gliadin (AGA) and anti-endomysium (EMA) antibodies, anti-gliadin performing somewhat better than other tests in children under five (41). Serology tests are based on indirect immunofluorescence (reticulin, gliadin and endomysium) or ELISA (gliadin or tissue transglutaminase tTG) (42).

Professional guideline recommend that a positive blood test is still followed by an endoscopy/gastroscopy and biopsy which still consider as gold standard (37,43,44). Also Guidelines recommend that a total serum IgA level is checked in parallel, as people with celiac with IgA deficiency may be unable to produce the antibodies on which
these tests depend ("false negative"). In those people, IgG antibodies against transglutaminase (IgG-tTG) may be diagnostic\(^{(45)}\). Antibody testing and HLA testing have similar accuracies. However, widespread use of HLA typing to rule out celiac disease is not currently recommended\(^{(46)}\).

At the time of diagnosis, further investigations may be performed to identify complications, such as:

- iron deficiency by full blood count and iron studies.
- folic acid and vitamin B12 deficiency.
- Blood glucose level, because CD may associate with diabetes mellitus.
- hypocalcaemia.
- Thyroid function tests may be requested during blood tests to identify hypothyroidism, which is more common in people with coeliac disease.

2-1- 7-1 Tissue transglutaminas

Anti-transglutaminase antibodies to the enzyme tissue transglutaminase (tTG) are found in an overwhelming majority of cases. Tissue transglutaminase modifies gluten peptides into a form that may stimulate the immune system more effectively. These peptides are modified by tTG in two ways, deamidation or transamidation\(^{(47)}\).

Deamidation is the reaction by which a glutamate residue is formed by cleavage of the epsilon-amino group of a glutamine side chain. Transamidation, which occurs three times more often than deamidation, is the cross-linking of a glutamine residue from the gliadin peptide to a lysine residue of tTg in a reaction which is catalysed by the transglutaminase. Crosslinking may occur either within or outside the active site of the enzyme. The latter case yields a permanently covalently linked complex between the gliadin and the tTg\(^{(48)}\). This results in the formation of new epitopes which are believed to trigger the primary immune response by which the autoantibodies against tTg develop\(^{(49)}\).

Upon exposure to gliadin, and specially to three peptides found in prolamin, the enzyme tissue transglutaminase modifies the protein and immune system cross reacts
with small bowel tissue, causing an inflammatory reaction that lead to a truncating of the villi ling the small intestine called villous atrophy.

2-1-8 Screening:

Due to its high sensitivity, serology has been proposed as a screening measure, because the presence of antibodies would detect previously undiagnosed cases of coeliac disease and prevent its complications in those people. Some studies suggest that early detection would decrease the risk of osteoporosis and anemia. In contrast, a cohort study in Cambridge suggested that people with undetected celiac disease had a beneficial risk profile for cardiovascular disease less overweight, lower cholesterol levels. There is limited evidence that screen-detected cases benefit from a diagnosis in term of morbidity and mortality; hence, population-level screening is not presently thought to be beneficial.

In the United Kingdom, the National Institute for Health and Clinical Excellence (NICE) recommends screening for coeliac disease in people with newly diagnosed chronic fatigue syndrome and irritable bowel syndrome, as well as in type 1 diabetics, especially those with insufficient weight gain or unexplained weight loss. It is also recommended in autoimmune thyroid disease, dermatitis herpetiformis, and in the first-degree relatives of those with confirmed coeliac disease.

2-2 Diabetes mellitus

Diabetes mellitus (DM) or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar which is due to either the pancreas not producing enough insulin, or because cells of the body do not respond properly to the insulin that is produced. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. There are three main types of diabetes mellitus:

- Type 1 DM results from the body's failure to produce insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes".
• Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes also with an absolute insulin deficiency. This form was previously referred to as non-insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes".

• Gestational diabetes, is the third main form and occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level.

• **2-2-1 Epidemiology**

Globally, as of 2010, an estimated 382 million people have diabetes worldwide, with type 2 making up about 90% of the cases\(^{53,54}\). This is equal to 3.3% of the population, with equal rates in both women and men\(^{55}\). In 2011 diabetes resulted in 1.4 million deaths worldwide, making it the 8th leading cause of death. Its rate has increased, and by 2030, this number is estimated to almost double.

Diabetes mellitus occurs throughout the world, but is more common especially type 2 in more developed countries. The greatest increase in rates is, however, expected to occur in Asia and Africa, where most people with diabetes will probably be found by 2030. The increase in rates in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental such as dietary effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented\(^{56}\).

• **2-2-2 Signs and symptoms**

The classic symptoms of untreated diabetes are weight loss, polyuria-frequent urination, polydipsia -increased thirst and polyphagia -increased hunger. Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes.

Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. Blurred vision is a
common complaint leading to a diabetes diagnosis. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes.

2-2-3 Causes

Diabetes mellitus is classified into four broad categories: type 1, type 2, gestational diabetes, and "other specific types". The "other specific types" are a collection of a few dozen individual causes. The term "diabetes", without qualification, usually refers to diabetes mellitus.

2-2-3-1 Diabetes mellitus type 1:

The term "type 1 diabetes" has replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes mellitus (IDDM).

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin\(^{(57)}\). Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

Still, type 1 diabetes can be accompanied by irregular and unpredictable hyperglycemia, frequently with ketosis, and sometimes with serious hypoglycemia. Other complications include an impaired counterregulatory response to hypoglycemia, infection, gastroparesis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease)\(^{(58)}\). These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1 diabetes\(^{(59)}\).
Type 1 diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection or diet. Unlike type 2 diabetes, the onset of type 1 diabetes is unrelated to lifestyle.

2-2-3-2 Diabetes mellitus type 2

Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.

Type 2 diabetes is due primarily to lifestyle factors and genetics. A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity, lack of physical activity, poor diet, stress, and urbanization.

2-2-3-3 Gestational diabetes;

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2-10% of all pregnancies and may improve or disappear after delivery. However, after pregnancy approximately 5-10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases insulin may be required.

Untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central
nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment.

2-2-3-4 Other types

Prediabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM. Many people destined to develop type 2 DM spend many years in a state of prediabetes.

Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than etiology. Some cases of diabetes are caused by the body's tissue receptors not responding to insulin -even when insulin levels are normal, which is what separates it from type 2 diabetes; this form is very uncommon.

Genetic mutations autosomal or mitochondrial can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes such as, chronic pancreatitis and cystic fibrosis.

Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes which is typically resolved once the hormone excess is removed. Many drugs impair insulin secretion and some toxins damage pancreatic beta cells.

2-2-4 Diabetic emergencies:

People usually with type 1 diabetes may also experience episodes of diabetic ketoacidosis, a type of metabolic problems characterized by nausea, vomiting and abdominal pain, the smell of acetone on the breath, deep breathing known as Kussmaul breathing, and in severe cases a decreased level of consciousness. A rare
but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration\(^{62}\)

### 2-2-5 Diagnosis:

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following\(^{63}\):

- Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol/l (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test
- Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)
- Glycated hemoglobin (Hb A1C) ≥ 6.5%\(^{64}\).

According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus.

Per the World Health Organization people with fasting glucose levels from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose\(^{65}\), people with plasma glucose at or above 7.8 mmol/L (140 mg/dL), but not over 11.1 mmol/L (200 mg/dL), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease\(^{66}\).

The American Diabetes Association since 2003 uses a slightly different range for impaired fasting glucose of 5.6 to 6.9 mmol/l (100 to 125 mg/dl)\(^{67}\). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause\(^{68}\). The rare disease diabetes insipidus has similar symptoms to diabetes mellitus, but without disturbances in the sugar metabolism and does not involve the same disease mechanisms.

### 2-2-6 Treatment and Prevention
Metformin is generally recommended as a first line treatment for type 2 diabetes, as there is good evidence that it decreases mortality\(^{(69)}\). Routine use of aspirin, however, has not been found to improve outcomes in uncomplicated diabetes\(^{(70)}\). Type 1 diabetes is typically treated with a combination of regular and NPH insulin, or synthetic insulin analogs. When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications. Doses of insulin are then increased to effect.

A pancreas transplant is occasionally considered for people with type 1 diabetes who have severe complications of their disease, including end stage renal disease requiring kidney transplantation\(^{(71)}\). Prevention often involve a healthy diet, physical exercise, not using tobacco, and being a normal body weight. Blood pressure control and proper foot care are also important for people with the disease. Type 1 diabetes must be managed with insulin injections. Type 2 diabetes may be treated with medications with or without insulin\(^{(52)}\). Insulin and some oral medications can cause low blood sugar, which can be dangerous. Gastric bypass surgery has been successful in many with severe obesity and type 2 DM. Gestational diabetes usually resolves after the birth of the baby. Active smoking is also associated with an increased risk of diabetes, so smoking cessation can be an important preventive measure as well. There is no known preventive measure for type 1 diabetes.

**2-2-7 Management**

Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing hypoglycemia. This can usually be accomplished with diet, exercise, and use of appropriate medications insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes.

Learning about the disease and actively participating in the treatment is vital for people with diabetes, since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar levels\(^{(72,73)}\).
The goal of treatment is an HbA1C level of 6.5%, but should not be lower than that, and may be set higher. Attention is also paid to other health problems that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. Specialised footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet.

2-2-6 Complications

All forms of diabetes increase the risk of long-term complications. These complications typically develop after many years (10–20), but may be the first signs or symptoms in those who have otherwise not received a diagnosis before that time. The major long-term complications relate to damage to blood vessels. These complications can be grouped into microvascular disease and macrovascular disease.

The primary micro vascular complications of diabetes include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and potentially blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems such as diabetic foot ulcers may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness.

The primary macrovascular complications of diabetes include coronary artery disease, angina and myocardial infarction, stroke, and peripheral vascular disease. About 75% of deaths in diabetics are due to coronary artery disease.

2-3 Thyroid
The thyroid gland, one of the largest endocrine glands in the body, consists of two connected lobes. It is found in the anterior neck, below the laryngeal prominence (Adam's apple). There is occasionally 28%-55% of population, mean 44.3% (77) a third lobe present called the pyramidal lobe of the thyroid gland. The pyramidal lobe is also known as Lalouette's pyramid (78).

In a healthy person the gland is not visible yet can be palpated as a soft mass. Examination of the thyroid gland includes the search for abnormal masses and the assessment of overall thyroid size. The thyroid gland controls rate of use of energy sources, protein synthesis, and controls the body's sensitivity to other hormones. It participates in these processes by producing thyroid hormones, the principal ones being thyroxine (T₄) and triiodothyronine (T₃), which is more active.

These hormones regulate the growth and rate of function of many other systems in the body. T₃ and T₄ are synthesized from iodine and tyrosine. The thyroid also produces calcitonin, which plays a role in calcium homeostasis. Hormonal output from the thyroid is regulated by thyroid-stimulating hormone (TSH) produced by the anterior pituitary, which itself is regulated by thyrotropin-releasing hormone (TRH) produced by the hypothalamus (79).

The thyroid may be affected by some frequent thyroid diseases. Hyperthyroidism occurs when the gland produces excessive amounts of thyroid hormones, the most common cause being Graves' disease—an autoimmune disorder. In contrast, hypothyroidism is a state of insufficient thyroid hormone production. Worldwide, the most common cause is iodine deficiency. Thyroid hormones are important for development, and hypothyroidism secondary to iodine deficiency remains the leading cause of preventable intellectual disability (80). In iodine-sufficient regions, the most common cause of hypothyroidism is Hashimoto's thyroiditis—also an autoimmune disease.

Autoimmune thyroiditis, or Chronic Autoimmune thyroiditis, is a disease in which the body interprets the thyroid glands and its hormone products T3, T4 and TSH as threats, therefore producing special antibodies that target the thyroid’s cells, thereby destroying it. It presents with hypothyroidism or hyperthyroidism and the presence or absence of goiters. Specialists clinically separate autoimmune thyroiditis into two
categories. If goiters are present, it is understood as Hashimoto’s Thyroiditis. On the other hand, if the thyroid is atrophic, but does not present goiters, it is denominated Atrophic Thyroiditis. If the symptoms of thyroiditis appear in women after giving birth, it is attributed to such and therefore called Postpartum Thyroiditis. The effects of this disease are not permanent but transient. Symptoms may come and go depending on whether the patient receives treatment, and whether the treatment is effective.

2-3-1 Genetics

“Thyroid autoimmunity is familial.” The disease is said to be inherited as a dominant trait since it has been reported that as many as fifty percent of the first degree relatives of patients with some type of autoimmune thyroiditis present thyroid antibodies in serum.

2-3-2 Age

It has been shown that “the prevalence of positive tests for thyroid antibodies increases with age, with a frequency as high as 33 percent in women 70 years old or older.” The mean age of prevalence in women is higher than in men by one year, 58 and 59 years old respectively. Autoimmune thyroiditis can also affect children. Although it is very rare for children under the age of five, it can occur, and it accounts for around 40 percent of the cases in adolescents with goiters. In the case of hypothyroidism, patients over the age of 45 have more chances of developing autoimmune thyroiditis (^81).

2-3-3 Physiology

The primary function of the thyroid is production of the hormones T3, T4 and calcitonin. Up to 80% of the T4 is converted to T3 by organs such as the liver, kidney and spleen. T3 is several times more powerful than T4, which is largely a prohormone, perhaps four or even ten times more active(^82).

2-3-3-1 Iodide sequestration:
Iodide—the ionized form of iodine—is essential for proper thyroid function. Iodide is taken up by follicular cells through the sodium-iodide symporter (NIS) present on the basolateral membrane, which transports two sodium cations and one iodide ion into the cell. It works against the iodide concentration gradient and uses energy of sodium gradient maintained by the sodium-potassium pump and therefore acts by secondary active transport. Thus, NIS helps to maintain a 20 to 40-fold difference in iodide concentration across the membrane. This iodide is transported to the follicular space through the apical membrane of the follicular cell with the help of the iodide-chloride antiporter pendrin. This iodide is then oxidized to iodine and attached to thyroglobulin by the enzyme thyroid peroxidase to form the precursors of thyroid hormones.

2-3-3-1-1 Significance of iodine

In areas of the world where iodine is lacking in the diet, the thyroid gland can become considerably enlarged, a condition called endemic goiter. Pregnant women on a diet that is severely deficient of iodine can give birth to infants with thyroid hormone deficiency congenital hypothyroidism, manifesting in problems of physical growth and development as well as brain development—a condition referred to as endemic cretinism. In many developed countries, newborns are routinely tested for congenital hypothyroidism as part of newborn screening. Children with congenital hypothyroidism are treated supplementally with levothyroxine, which facilitates normal growth and development.

The use of iodised salt is an efficient way to add iodine to the diet. It has eliminated endemic cretinism in most developed countries, and some governments have made the iodination of flour, cooking oil, and salt mandatory. Potassium iodide and sodium iodide are typically used forms of supplemental iodine. As with most substances, either too much or too little can cause problems. Recent studies on some populations are showing that excess iodine intake could cause an increased prevalence of autoimmune thyroid disease, resulting in permanent hypothyroidism.

2-3-3-2 \( T_3 \) and \( T_4 \) production and action:
Thyroxine (T4) is synthesised by the follicular cells from the tyrosine residues of the protein called thyroglobulin (Tg). Iodine is captured with the "iodine trap" by the hydrogen peroxide generated by the enzyme thyroid peroxidase (TPO) and linked to the 3' and 5' sites of the benzene ring of the tyrosine residues on Tg sequentially on tyrosine residue forming monoiodotyrosine (MIT) and then diiodotyrosine (DIT) (iodination). Two DIT can couple (coupling) to form T4 hormone attached to thyroglobulin releasing one alanine. Upon stimulation by the thyroid-stimulating hormone (TSH), the follicular cells reabsorb Tg and cleave the iodinated tyrosines from Tg in lysosomes, forming free T4, DIT, MIT, T3 and traces of RT3 (in T3 and RT3 has three iodine atom while T4 has four), and releasing T3 and T4 into the blood. Deiodinase releases the sequestred iodine from MIT and DIT. Deiodinase enzymes convert T4 to T3 and RT3, which is a major source of both RT3 (95%) and T3 (87%) in peripheral tissues. Thyroid hormone secreted from the gland is about 80-90% T4 and about 10-20% T3.

Cells of the developing brain are a major target for the thyroid hormones T3 and T4. Thyroid hormones play a particularly crucial role in brain maturation during fetal development. A transport protein that seems to be important for T4 transport across the blood–brain barrier (OATP1C1) has been identified. A second transport protein (MCT8) is important for T3 transport across brain cell membranes. In the blood, T4 and T3 are partially bound to thyroxine-binding globulin (TBG), transthyretin, and albumin. Only a very small fraction of the circulating hormone is free (unbound) - T4 0.03% and T3 0.3%. Only the free fraction has hormonal activity.

2-3-3-3 T3 and T4 regulation:

The production of thyroxine and triiodothyronine is primarily regulated by thyroid-stimulating hormone (TSH), released by the anterior pituitary. The thyroid, and thyrotropes in the anterior pituitary, form a negative feedback loop: TSH production is suppressed when the free T4 levels are high. The negative feedback occurs on both the hypothalamus and the pituitary, but it is of particular importance at the level of the pituitary. The TSH production itself is modulated by thyrotropin-releasing hormone (TRH), which is produced by the hypothalamus. This is secreted at an increased rate in situations such as cold exposure to stimulate thermogenesis which is prominent in
case of infants. TSH production is blunted by dopamine and somatostatin (SRIH) which act as local regulators at the level of the pituitary, in response to rising levels of glucocorticoids and sex hormones estrogen and testosterone, and excessively high blood iodide concentration. An additional hormone produced by the thyroid contributes to the regulation of blood calcium levels. Parafollicular cells produce calcitonin in response to hypercalcemia. Calcitonin stimulates movement of calcium into bone, in opposition to the effects of parathyroid hormone (PTH). However, calcitonin seems far less essential than PTH, as calcium metabolism remains clinically normal after removal of the thyroid (thyroidectomy), but not the parathyroids.

2-3-3-4 Physiological action of T3 and T4:

T3 of particular physiological importance produced mainly in tissue after deiodination. It has calorigenic, cardiovascular, neural and other metabolic actions. It increases oxygen consumption of all tissues except brain, uterus, testis (though it is important for normal fertility) lymph node, spleen, and its source, anterior pituitary, mainly by its action on sodium potassium pump and fat metabolism. It is helps in conversion of carotene into vitamin A in hepatic cells, therefore, hypothyroidism may lead to high levels of carotene in the blood, resulting in yellowish tint of only skin (and not the mucous membrane, like sclera). It increases growth and results in positive nitrogen usage. Protein anabolism however high levels result in protein catabolism. Produced potassium due to catabolism appears in urine.

Decreased level of thyroid hormone result in retention of hyaluronic acid and chondroitin sulfuric acid in the skin, which results in water retention (due to polyolic nature) and myxedema. It also has significance in proper mentation. Increased levels results in irritability. Decreased levels results in poor mentation and increased protein level in CSF. It has significance in proper development of cochlea, and hypothyroidism may lead to low IQ and deaf mutism. Many cardiovascular effects are reported. Increased peripheral resistance, increased rate and force of heart beat occurs by effects of circulatory T3. It increases carbohydrate absorption. It is also reported to decrease cholesterol levels.

2-3-4 Thyroid Function Tests;

<table>
<thead>
<tr>
<th>Test</th>
<th>Abbreviation</th>
<th>Normal ranges[^96]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum thyrotropin/thyroid-stimulating</td>
<td>TSH</td>
<td>0.5–6.0 μU/ml</td>
</tr>
<tr>
<td>hormone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Free thyroxine | FT<sub>4</sub> | 7–18 ng/l = 0.7–1.8 ng/dl
--- | --- | ---
Serum triiodothyronine | T<sub>3</sub> | 0.8–1.8 μg/l = 80–180 ng/dl
Radioactive iodine-123 uptake | RAIU | 10–30%
Radioiodine scan (gamma camera) | N/A | N/A - thyroid contrasted images
Free thyroxine fraction | FT4F | 0.03–0.005%
Serum thyroxine | T<sub>4</sub> | 46–120 μg/l = 4.6–12.0 μg/dl
Free thyroxine index | FT4I | 4–11
Free triiodothyronine l | FT<sub>3</sub> | 230–619 pg/d
Free T3 Index | FT3I | 80–180
Thyroxine-binding globulin | TBG | 12–20 μg/dl T4 +1.8 μg
TRH stimulation test | Peak TSH | 9–30 μIU/ml at 20–30 min.

As of early 2015, in the United States, new guidelines for TSH levels have been implemented as endorsed by The American Association of Clinical Endocrinologists. The new range is a TSH of 0.45 to 4.12.<sup>(92)</sup>

### 2-3-5 Clinical significance

Thyroid disorders include:

1. hyperthyroidism (abnormally increased activity),
2. hypothyroidism (abnormally decreased activity)
3. thyroiditis, inflammation of the thyroid

All these disorders may give rise to a goiter, that is, an enlarged thyroid.

#### 2-3-5-1 Hyperthyroidism :

Hyperthyroidism, or overactive thyroid, is defined as an overproduction of the thyroid hormones T<sub>3</sub> and T<sub>4</sub>. This condition is most commonly caused by the development of Graves' disease, an autoimmune disease in which anomalous antibodies stimulate the thyroid to secrete excessive quantities of thyroid hormones.<sup>(93)</sup> The disease can progress to the formation of a toxic goiter as a result of thyroid growth in response to a lack of negative feedback mechanisms. It presents with symptoms such as a thyroid goiter, protruding eyes (exophthalmos), palpitations, excess sweating, diarrhea, weight loss, muscle weakness and unusual sensitivity to heat. The appetite is often increased. Beta blockers are used to decrease symptoms of hyperthyroidism such as increased
heart rate, tremors, anxiety and heart palpitations, and anti-thyroid drugs are used to decrease the production of thyroid hormones, in particular, in the case of Graves' disease. These medications take several months to take full effect and have side-effects such as skin rash or a drop in white blood cell count, which decreases the ability of the body to fight off infections. These drugs involve frequent dosing (often one pill every 8 hours) and often require frequent doctor visits and blood tests to monitor the treatment, and may sometimes lose effectiveness over time. Due to the side-effects clarification needed and inconvenience of such drug regimens, some patients choose to undergo radioactive iodine-131 treatment. Radioactive iodine is administered in order to destroy a portion of or the entire thyroid gland, since the radioactive iodine is selectively taken up by the gland and gradually destroys the cells of the gland. Alternatively, the gland may be partially or entirely removed surgically, though iodine treatment is usually preferred since the surgery is invasive and carries a risk of damage to the parathyroid glands or the nerves controlling the vocal cords. If the entire thyroid gland is removed, hypothyroidism results.

2-3-5-2 Hypothyroidism :

Hypothyroidism is the underproduction of the thyroid hormones T3 and T4.

Hypothyroid disorders may occur as a result of

- congenital thyroid abnormalities (Thyroid deficiency at birth)
- autoimmune disorders such as Hashimoto's thyroiditis,
- iodine deficiency (more likely in poorer countries) or
- the removal of the thyroid following surgery to treat severe hyperthyroidism and/or thyroid cancer.

Typical symptoms are abnormal weight gain, tiredness, baldness, cold intolerance, and bradycardia. Hypothyroidism is treated with hormone replacement therapy, such as levothyroxine, which is typically required for the rest of the patient's life. Thyroid hormone treatment is given under the care of a physician and may take a few weeks to become effective\(^{(95)}\). Negative feedback mechanisms result in growth of the thyroid gland when thyroid hormones are being produced in sufficiently low quantities, as a means of increasing the thyroid output; however, where hypothyroidism is caused by
iodine insufficiency, the thyroid is unable to produce T3 and T4 and as a result, the thyroid may continue to grow to form a non-toxic goiter. It is termed non-toxic as it does not produce toxic quantities of thyroid hormones, despite its size.

2-3-5-3 Thyroiditis

There are two types of thyroiditis where initially hyperthyroidism presents which is followed by a period of hypothyroidism; the overproduction of T3 and T4 followed by the underproduction of T3 and T4. These are Hashimoto's thyroiditis and postpartum thyroiditis. Hashimoto's thyroiditis or Hashimoto's Disease is an autoimmune disorder whereby the body's own immune system reacts with the thyroid tissues in an attempt to destroy it. At the beginning, the gland may be overactive, and then becomes underactive as the gland is damaged resulting in too little thyroid hormone production or hypothyroidism. Some patients may experience "swings" in hormone levels that can progress rapidly from hyper-to-hypothyroid sometimes mistaken as severe mood swings, or even being bipolar, before the proper clinical diagnosis is made. Some patients may experience these "swings" over a longer period of time, over days or weeks or even months. Hashimoto's is more common in females than males, usually appearing after the age of 30, and tends to run in families, meaning it can be seen as a genetic disease. Also more common in individuals with Hashimoto's thyroiditis are type 1 diabetes and celiac disease.

Postpartum thyroiditis occurs in some females following the birth of a child. After delivery, the gland becomes inflamed and the condition initially presents with overactivity of the gland followed by underactivity. In some cases, the gland may recover with time and resume its functions. In others it may not. The etiology is not always known, but can sometimes be attributed to autoimmunity, such as Hashimoto's thyroiditis or Graves' disease. There are other disorders that cause inflammation of the thyroid, and these include subacute thyroiditis, acute thyroiditis, silent thyroiditis and Riedel's thyroiditis.
2-4 Objective

2-4-1 General Objective:

To assess the association between celiac disease, hyperglycemia and thyroid disorder in Red Sea State, Sudan.

2-4-2 Specific Objective:

- To measure present of anti TTG IgA in patients in Red Sea State, Sudan.
- To measure present of anti Gliadin IgG
- To measure level of Random blood Glucose and HbA1C.
- To measure level of TSH.
- To measure level of T3.
- To measure level of T4.
- To measure level of CRP.
Chapter three

Material and Method
Chapter Three
3-Material and Method

3-1 Study design:

This is an observational, analytical, cross-sectional, hospital-based study.

3-2 Study area and period:

Red Sea is one of the 18 wilayat or states of Sudan. It has an area of 212,800 km² and an estimated population of 1,396,000 (2010). Port Sudan is a port city in Eastern Sudan, and the capital of the state of Red Sea. As of 2007, it has 489,725 residents. Located on the Red Sea, it is the Republic of Sudan's main port city. The original inhabitants of the State are the Beja Tribes, who constitute above 65% of the current population with lower wealth and power in the region. Geographically, in the East, bordering the state, is the Red Sea. Inland, mountains run from north to south, which are interrupted by arid plains. To the northwest is the Nubian Desert. The Siyal Islands are located in the disputed area between Egypt and Sudan in the northeast.

The Red Sea state was divided into the eight administrative areas: Bur Sudan (Port Sudan), Gebiet Elma’din, Halayib, Haya, Sawakin, Sinkat, Gunob Awlieb, Derodieb, Tokar and Ageeg. This study was done in patients suspected to have celiac disease in Almwani hospital, Educational Red Sea hospital, in Red Sea State during the period from Dec. 2014 to Dec. 2016.

3-3 Study population:

patients suspected to have CD in Red Sea State, Sudan, referral by pediatrics having severe Diarrhea or weight loss.

3-4 Ethical consideration:

Permission of this study was obtained from the medical director of the some hospital. An informed consent was obtained from all parents of child participants in this study, and the objectives of the study were explained to all individual participating in this study.
3-5 Selection criteria:
Selection criteria: Patients diagnosed with CD.
Exclusion criteria: any non-diagnosed CD patients or infectious and malignant patients.

3-6 Sample Size:
One thousand patients suspected to have celiac disease referred by pediatrics from some hospital were examined at Alyousif – Medical Laboratory, Port Sudan, Sudan, and 50 healthy people as control.

3-7 Clinical assessment and sample collection:

Interview and questionnaire:
Interview with the patient were done to explain the objective of the study and to obtain data to fill questionnaire (appendix 1 page 73) which specifically designed to obtain information which help in either including or excluding certain individuals in or from the study which contain many question like age, gender, tribe and history of disease, ……el.

Blood sample:
Two samples of blood (3 cc for each) were collected from each patients, one in heparinized containers, other in plain containers, then refrigerated in 2-8 °C till analyzed.

3-8 Biochemical measurement:
Serum Random Blood glucose, HbA1C and C-reactive protein were measured using a Biosystem 310 spectrophotometer Germany, this analyzer is semi-automated spectrophotometer and reagent kits from BioSystem companies.

Serum anti tTG, serum anti Gliadin, TSH, T3 and T4 has been estimated using ELISA instrument and reagent kits.
3-9 methodology :

3-9-1 Instrument :

Biosystem BTS 310 spectrophotometer (Germany) was used in this study. This analyzer is semi-automated spectrophotometer for blood glucose samples.

ELISA automated hormone detector, with microplate 1×96 coated antibodies, for detected of, assay was perform as manufacturer's instruction.

The ELISA test kit provides a semi quantitative or quantitative in vitro assay for human auto antibodies of the IgA class against tissue transglutamine in serum or plasma. the test kit contain micro titer strips each with 8 break-off reagent wells coated with human tissue transglutaminase. in the first reaction step, diluted patient samples are incubated in the wells. in the case of positive sample, specific IgA antibodies is carried out using an enzyme-labbelled anti-human IgA ( enzyme conjugate ) catalyzing a colour reaction.

2-9-2 Antigliadin antibody assay :

a. Principle of the assay: Microwells are pre-coated with the gliadin antigen. The calibrators, controls and diluted patient samples are added to the wells and autoantibodies recognizing the gliadin antigen bind during the first incubation. After washing the wells to remove all unbound proteins, purified peroxidase labeled eabit anti-human IgG or IgA ,conjugate is added. The conjugate binds to the captured human autoantibody and the excess unbound conjugate is removed by a further wash step. The bound conjugate is visualized with tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point colour, which is read at 450 mm.

b. Assay method: Each serum sample (100 μL) to be tested was added to the wells in quadruplicate at 1:100 dilution in diluents, then the plate was
incubated at room temperature for 30 minutes, then washed three times with wash buffer. Subsequently, 100 μL of either peroxidase – conjugated goat antihuman IgA or IgG was added. This was followed by incubation for 30 minute at room temperature then the plate was washed three times. 100 μL of TMB substrate solution were added to each well and incubated in the dark at room temperature for 30 minutes. To stop the reaction 100 μL sulphuric acid was added to each well, this causes a change in colour from blue to yellow, optical density was read at 450 nm using an automated ELISA reader within 30 minutes of stopping the reaction.

Results and quality control: in order for the assay to be valid 50 calibrators (with known concentration of AGA autoantibodies), and positive and negative controls were included in each run. The obtained values for the calibrator and control should be in the range specified on the quality control, certificate provided with the kit. A calibration curve was plotted, then the level of antigliadin autoantibody in the diluted sample was read directly from the curve.

The cut-off value of the test was 10 RU/ml.

**Reagent composition preparation and stability:**

See Appendix no 2 page 74

**2-9-3 Anti-tissue Transglutaminase :**

The assays were carried out as per the manufacturer’s instructions. Briefly, patient sera were first diluted 1:20 in sample buffer. A 100 μl each of the calibrators, negative controls, positive controls and diluted samples were next transferred into the individual microplate wells and then incubated for 30 minutes. Subsequently the wells were emptied and washed three times using 300 μl of working strength wash buffer for each well. Thereafter 100 μl of enzyme conjugate (peroxidase-labelled anti-human IgG) washed to each
well and incubated for another 30 minutes. Post incubation the same washing procedure was repeated, followed by addition of 100 µl of chromogen/substrate solution to each well and 15 minutes of incubation in the dark. Finally 100 µl of stop solution was added to each well in the same order and at the same speed as the substrate solution was introduced.

Photometric measurement of color intensity was recorded by an ELISA reader at a filter 450 nm within 30 minutes of adding the stop solution. The cut-off value of the test was 20 RU/ml.

**Reagent composition preparation and stability :**

See Appendix no 3 page 75

**3-9-4 Measurement of serum glucose (RBS):**

**Principle of the method**

Glucose in sample originates by means of the coupled reaction below, a colored complex that can be measured by spectrophotometer.

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{glucose oxidase} \rightarrow \text{Gluconate} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{-aminophenazine} + \text{phenol peroxidase quinoneimine} + 4\text{H}_2\text{O}
\]

**Reagent composition preparation and stability :**

See Appendix no 4 page 76

**Procedure :**

Reagent must be warmed as company instructed, three test tube had been labeled as Blank, STD and Test, 1.0 ml of glucose reagent was transferred to each tube, then 10 µl of sample were added to test tube, 10 µl of standard reagent were added to STD tube. The content of each tube were mixed, incubated for 10 minute at room temperature. Absorbance (A) had been recorded for sample and standard at 500 nm against the blank, the colored was stable for at least 2 hours.
Calculation:

Plasma blood glucose mg/dl:

A sample × concentration of standard
A standard

Linearity:

This method is linear up to 500 mg/dl.

3-9-5 measurement of HbA1c:

Hemoglobin A1C

After preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin. Hemoglobin A1c (HbA1c) is specifically eluted after washing away the hemoglobin A1a+b fraction (HbA1a+b), and is quantified by direct photometric reading at 415 nm. The estimation of the relative concentration of HbA1c is made by the measure of total hemoglobin concentration by direct photometric reading at 415 nm.

Sample:

whole blood collected by standard procedure

hemoglobin A1c is stable for 7 days at 2-8°C. Heparin or EDTA may be used as anticoagulants

procedure:

hemolysate preparation and labile fraction elimination

1- bring the columns and reagents to room temperature (21-26°C)

2- pipette into a test tube:

<table>
<thead>
<tr>
<th>Blood</th>
<th>50 μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>200 μL</td>
</tr>
</tbody>
</table>

3- shake thoroughly and let it stand at room temperature for 10-15 minute. This hemolysate will be used in step 6 and 11.
Colum preparation (notes 2 and 3)

4- Remove the upper cap of the column and then snap the tip off the bottom.

5- using the flat end of s pipette , push the upper disc down to the resin surface taking care not to compress it . Let the column drain completely to waste .

Separation and Reading of HbA1c fraction

6- carefully pipette on the upper filter

| Hemolysate | 50 µL | Let the column drain to waste |

7- in order to drain any sample residue left above the upper disc , pipette

| Reagent 2 | 200 µL | Let the column drain to waste |

8- pipette :

| Reagent 2 | 2.0 ml | Let the column drain to waste |

9- place the column over a test tube and add :

| Reagent 3 | 4.0 ml | Collect the eluate ( HbA1c fraction ) |

10- shake thoroughly and read the absorbance (A) of the HbA1c fraction at 415 nm against distilled water (A_{HbA1c}). The absorbance is stable for at least one hour .

Reading of Hb total

11- pipette into a test tube :

| Reagent 3 | 12.0 ml |
| Hemolysate | 50 µL |

12- shake thoroughly and read the absorbance ( A) at 415 nm against distilled water (A_{HbTOTAL}). The absorbance is stable for at least one hour .
Calculation

The HbA1c relative concentration in the sample is calculated using the following general formula:

\[
\frac{A_{HbA1c}}{A_{HbTOTAL}} \times V_{HbA1c} \times 100 = \% \text{HbA1c}
\]

The volume of HbA1c (\(V_{HbA1c}\)) is 4 mL, the volume of Hb total (\(V_{HbTOTAL}\)) is 12 mL. The following formula is deduced for the calculation of the concentration:

\[
\frac{A_{HbA1c}}{A_{HbTOTAL}} \times 100 \times \frac{100}{3} = \% \text{HbA1c}
\]

Reference value:

<table>
<thead>
<tr>
<th>%</th>
<th>-NGSP</th>
<th>mmol/mol - IFCC</th>
<th>Degree of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0 – 6.5</td>
<td></td>
<td>20- 48</td>
<td>Non Diabetic</td>
</tr>
<tr>
<td>6.0 – 7.0</td>
<td></td>
<td>42 - 53</td>
<td>Goal</td>
</tr>
<tr>
<td>7.0 – 8.0</td>
<td></td>
<td>53 – 64</td>
<td>Good Control</td>
</tr>
<tr>
<td>≥ 8.0</td>
<td></td>
<td>≥ 64</td>
<td>Action suggested</td>
</tr>
</tbody>
</table>

Reagent composition preparation and stability:

See Appendix no 5 page 77

3-9-6 measurement of serum TSH:

Principle:

Immunoenzymometric assay:
The essential reagents required for an immunoenzymometric assay high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, In excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of amicroplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.
Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies. Without competition or steric hindrance, to form a soluble sandwich complex.

**Reagent Materials Provided : Store at 2-8°C**

<table>
<thead>
<tr>
<th>(TSH) Kit Contents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyrotropin Calibrators, in 7 levels, &amp; TSH Antigen level as mentioned on the label.</td>
<td>7 × 1.0ml</td>
</tr>
<tr>
<td>TSH Enzyme Reagent</td>
<td>1 × 13ml</td>
</tr>
<tr>
<td>Streptavidin coated plate</td>
<td>96 Wells</td>
</tr>
<tr>
<td>Wash Solution Concentrate</td>
<td>1 × 20ml</td>
</tr>
<tr>
<td>Single Substrate solution</td>
<td>1 × 12ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 × 8ml</td>
</tr>
<tr>
<td>Product insert</td>
<td>1</td>
</tr>
</tbody>
</table>

**Specimen Collection and Preparation :**

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop veripuncture tube without tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the samples(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. when assayed in duplicate, 0.100ml of the specimen is required.

**Test Procedure :**

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100ml (100µl) of the TSH Enzyme Reagent to each well.
It is very important to dispense all reagents close to the bottom of the coated well.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 60 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.

   An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.

8. Add 0.100 ml (100µl) of substrate solution to all wells.

   Always add reagents in the same order to minimize reaction time differences between wells.

   **DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds.

   Always add reagents in the same order to minimize reaction time differences between wells.

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

   **Expected value: 0.2-5.1**

   **Reagent composition preparation and stability:**

   See Appendix no 6 page 78

**3-9-7 measurement of serum T4**

**Principle:**

**Competitive Enzyme Immunoassay**

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate native antigen.
Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme antigen conjugate for a limited number of insolubutzed binding sites.

**Reagent Materials Provided : Store at 2-8°C**

<table>
<thead>
<tr>
<th>(T4) Kit Contents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum References, for Thyroxine 6 levels, concentrations as indicated on vials ug/dl</td>
<td>6 × 1.0ml</td>
</tr>
<tr>
<td>T4 Enzyme Reagent</td>
<td>1 × 1.5ml</td>
</tr>
<tr>
<td>T3/T4 Conjugate Buffer</td>
<td>1 × 13ml</td>
</tr>
<tr>
<td>T4 Antibody coated plate</td>
<td>96 Wells</td>
</tr>
<tr>
<td>Wash Solution Concentrate</td>
<td>1 × 20ml</td>
</tr>
<tr>
<td>Single Substrate solution</td>
<td>1 × 12ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 × 8ml</td>
</tr>
<tr>
<td>Product insert</td>
<td>1</td>
</tr>
</tbody>
</table>

**Specimen Collection and Preparation :**

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed for accurate comparison to established normal values, a fasting morning serum sample should be obtained.

The blood should be collected in a plain redtop veripuncture tube without tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Specimen(s) may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) cannot be assayed within 48 hours, the samples(s) may be stored at temperatures of -20°C for up to 30 days. Before assay, allow the specimens to equilibrate to ambient temperature (20°C-27°C). when assayed in duplicate, 0.05ml of the specimen is required.

**Test Procedure :**

Before proceeding with assay, bring all reagents, serum referenves and controls to room temperature (20-27°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate.

Replace any unused micro well strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of Working Reagent A, T4-enzyme conjugate solution to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add 0.100 ml (100µl) of single Substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells.

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

Expected value:
- Male 4.4 - 10.8 ug/dl
- Female 4.8 - 11.6 ug/dl

Reagent composition preparation and stability:

See Appendix no 7 page 79

3-9-8 measurement of serum T3

Principle:
Competitive Enzyme Immunoassay
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.
Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme antigen conjugate for a limited number of insolubutzed binding sites.

**Reagent Materials Provided : Store at 2-8°C**

<table>
<thead>
<tr>
<th>(T3) Kit Contents</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum of 6 levels as indicated on vials in ng/dl</td>
<td>6 x 1.0ml</td>
</tr>
<tr>
<td>Total T3 Enzyme Conjugate</td>
<td>1 x 1.5ml</td>
</tr>
<tr>
<td>T3/T4 Conjugate Buffer</td>
<td>1 x 13ml</td>
</tr>
<tr>
<td>T3 Antibody coated Microplate</td>
<td>96 Wells</td>
</tr>
<tr>
<td>Wash Solution Concentrate</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>Single Substrate solution</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 8ml</td>
</tr>
<tr>
<td>Product insert</td>
<td>1</td>
</tr>
</tbody>
</table>

**Specimen Collection And Preparation :**
The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed for accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop veripuncture tube. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Specimen(s) may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) cannot be assayed within 48 hours, the samples(s) may be stored at temperatures of -20°C for up to 30 days. Before assay, allow the specimens to equilibrate to ambient temperature (20°C-27°C). when assayed in duplicate, 0.10ml of the specimen is required.

**Test Procedure :**
Before proceeding with assay, bring all reagents, serum references and controls to room temperature (20-27°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate.Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100ml (100µl) of Working Reagent A, T3-enzyme conjugate solution to all wells ( see Reagent Preparation Section ).
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add 0.100 ml (100µl) of single signal reagent solution to all wells (see Reagent Preparation Section).
   Always add reagents in the same order to minimize reaction time differences between wells.
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
    Always add reagents in the same order to minimize reaction time differences between wells.
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within thirty (30) minutes of adding the stop solution.

   Expected value: 0.52 - 1.85 ng/dl

Reagent composition preparation and stability:

See Appendix no 8 page 80

3-9-9 measurement of serum C-reactive protein

Principle of the method

Serum C-reactive protein causes agglutination with human Creactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry.
Procedure:

Followed the company instructed worm reagent then take 1.0 ml of working reagent in cuvette, add 7 µl of sample or stander, mix and insert cuvette into the spectrophotometer, the stop watch was started, and the absorbance of the standard or sample were measured at 540 nm after 10 second (A1) and after 2 minutes (A2).

Calculation:

CRP concentration mg/L:

$$\frac{(\text{Absorbance A2 – Absorbance A1}) \text{ of test}}{(\text{Absorbance A2 – Absorbance A1}) \text{ of stander}} \times \text{Concentration of stander}$$

Linearity:

This method is linear up to 150 mg/L.

Reagent composition preparation and stability:

See Appendix no 9 page 81
Chapter four

Result
Chapter four

4- Result

A total of a thousand Sudanese children suspected to have CD were involved in this study, only 200 of them were confirmed positive for Celiac Disease. The remaining eight hundred negative patients were excluded from further analysis, The patients were considered positive for Celiac Disease when the titer of Anti-TTG and AGA was found to be more than 10 times of the cut-off value\(^{(97)}\), the testes were duplicated by doing ATTG and AGA IgA for confirmation of the result which represent in table (1).

In this study male to female ratio was (1.06:1), that represent male and female semi equally of percent table (2) and figure (1).

The age of the patients were illustrated in figure (2). The commonest age of presentation was between 6-10 years (45.5 %), while (24.5 %) of patients between 11-15 years, the next common group were (23%) of patients between 1-5 years and (7%) were more than 15 years.

Our study show the percent of male and female in patients with celiac disease regarding to age, which represent that the age 6-10 years the female were affected more than male( 1.2: 1 ) respectively, but in age more than 15 years the male show high percent than female ( 3.2: 1 ) Figure (3).

Our patients were further classified according to the duration of the disease from the first day of complain. Most of them (91.5%) were having CD from less than 5 years, and (8 % ) of the patients were having the disease for six to ten years, and only (0.5 %) of patients were having the disease for more than ten years, figure (4).

The distribution of celiac disease patients among the Red Sea state tribes in the area were as follow, (60%) of patients having the disease were Banne Amer tribe, Hadandwa tribe had taken fraction (16.5 %), then the Northen Sudan tribes were (12.5%). The Rashyda were (7.5 %) of the studied CD patients. The less affected group were belong to Western Sudan tribe (4 %) figure (5).

The result of our research shows the clinical presentation among the patients, the commonest presenting symptom was Chronic Diarrhea (30 %) followed by Weight loss (16.5 %) . The third common presentation was noted to be Stunted growth (13%)
following by Anemia (11%), Bone and Join disease (10.5 %), Other GIT symptoms (10%), Skin manifestation (7%) and less common presenting symptom was Infertility (2%) table (3).

In this research, (36.5 % ) of CD patients were discovered to have DM1 and (7 %) of CD patients were discovered to have thyroid disorder, (56.5%) of patient have no other associated disease table (4).

The state of hyperglycemia was graded according to the level of RBS and HBAIC in Celiac disease patients to mild hyperglycemic patients, the level of RBS from 130-200 mg/dl and HBA1C 6.5-7% were (50.5% ) . Moderate glycemic patients, level of RBS from 200-250 mg/dl and HBA1C 7-8 % were (37 %) and sever glycemic patients, level of RBS was above 250 mg/dl and HBA1C above 8% was reported (12.3 %), table (5,6,7).

According to our result thyroid disorder among CD patients showed ( 93 %) of patients have Euthyroid, ( 7 %) of patients had hypothyroidism and no thyrotoxic patients . table (8).

The reading of CRP level more than 5 g/dl and reading of was used as indicator for inflammatory disordered of thyroid disease (98) .In this work high level of CRP was found in (7%) of CD patients i.e they were hypothyroid patients . table (9).

To assess the correlation between CD, HbA1C and RBS the result showed there was significant association between variables , table (10).

To assess the correlation between CD and Thyroid disorder we found that there was no significant association between two variables , table (11).
Table (1)

Mean ± Standard deviation for value of Anti-TTG and AG Antibodies.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TTG</td>
<td>162.4</td>
<td>57.2</td>
</tr>
<tr>
<td>AGA</td>
<td>216.2</td>
<td>51.1</td>
</tr>
</tbody>
</table>
Table (2) Frequency of patients with Celiac Disease (CD) according to gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>CD</th>
<th>N</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td>103</td>
<td>282.3689</td>
</tr>
<tr>
<td></td>
<td>Valid N (listwise)</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>CD</td>
<td>97</td>
<td>288.8660</td>
</tr>
<tr>
<td></td>
<td>Valid N (listwise)</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>
Figure (1) Percent of patients with Celiac Disease (CD) according to gender.
Figure (2) Frequency of patients with Celiac Disease (CD) according to age.
Figure (3) Show the percent of male and female in patients with Celiac Disease (CD) comparing to age.
Figure (4) Frequency of patients with Celiac Disease (CD) according to duration (years) of illness.
Figure (5) Percent of Celiac Disease (CD) patients in Red Sea State related to Tribe
Table (3) Shows the Clinical presentation of Celiac disease in different sex groups

<table>
<thead>
<tr>
<th>Presentation complain</th>
<th>Males</th>
<th>Females</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic diarrhea</td>
<td>28</td>
<td>32</td>
<td>60 (30%)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>21</td>
<td>12</td>
<td>33 (16.5%)</td>
</tr>
<tr>
<td>Stunted growth</td>
<td>16</td>
<td>10</td>
<td>26 (13%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>11</td>
<td>11</td>
<td>22 (11%)</td>
</tr>
<tr>
<td>Bone and joint pain</td>
<td>7</td>
<td>14</td>
<td>21 (10.5%)</td>
</tr>
<tr>
<td>Other GIT symptoms</td>
<td>11</td>
<td>9</td>
<td>20 (10%)</td>
</tr>
<tr>
<td>Skin manifestation</td>
<td>9</td>
<td>5</td>
<td>14 (07%)</td>
</tr>
<tr>
<td>Infertility</td>
<td>0</td>
<td>4</td>
<td>4 (02%)</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>97</td>
<td>200 (100%)</td>
</tr>
</tbody>
</table>
Table (4) Frequency of other disease among patients with Celiac Disease (CD).

<table>
<thead>
<tr>
<th>Other</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>73</td>
<td>36.5</td>
<td>36.5</td>
<td>36.5</td>
</tr>
<tr>
<td>THYROIDITIS</td>
<td>14</td>
<td>7.0</td>
<td>7.0</td>
<td>43.5</td>
</tr>
<tr>
<td>None</td>
<td>113</td>
<td>56.5</td>
<td>56.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
Figure (6) Frequency of other disease among patients with Celiac Disease (CD)
Table (5) Shows patients newly discovered as DM1.

<table>
<thead>
<tr>
<th>Patient with DM</th>
<th>Number of patients</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients newly diagnosed</td>
<td>73</td>
<td>36.5 %</td>
</tr>
</tbody>
</table>

Table (6) Shows number of patients has D.M type 1 according to level of RBS in patients.

<table>
<thead>
<tr>
<th>Level of RBS (mg/dl)</th>
<th>Number of patients</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild hyperglycemic patients Between 130-200</td>
<td>37</td>
<td>50.7 %</td>
</tr>
<tr>
<td>Moderate hyperglycemic patients Between 200-250</td>
<td>27</td>
<td>37.0 %</td>
</tr>
<tr>
<td>Sever hyperglycemic patients Above 250</td>
<td>9</td>
<td>12.3 %</td>
</tr>
</tbody>
</table>

Table (7) Shows number of patients has D.M type 1 according to level of HBA1C in patients.

<table>
<thead>
<tr>
<th>Level of HBA1C (%)</th>
<th>Number of patients</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild hyperglycemic patients Above 6.5 %</td>
<td>37</td>
<td>50.7 %</td>
</tr>
<tr>
<td>Moderate hyperglycemic patients Between 7.0 - 8.0 %</td>
<td>27</td>
<td>37.0 %</td>
</tr>
<tr>
<td>Sever hyperglycemic patients Above 8.0 %</td>
<td>9</td>
<td>12.3 %</td>
</tr>
</tbody>
</table>
Table (8) Level of thyroid hormones.

<table>
<thead>
<tr>
<th>Thyroid hormones</th>
<th>T3</th>
<th>T4</th>
<th>TSH</th>
<th>Frequency ( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>186</td>
<td>186</td>
<td>186</td>
<td>93 %</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>7 %</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (9) Level of CRP.

<table>
<thead>
<tr>
<th>CRP level more than 5 g/dl</th>
<th>Number of patients</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14</td>
<td>7 %</td>
</tr>
</tbody>
</table>
Table (10) correlation between CD, HbA1C and RBS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD and RBS</td>
<td>0.000</td>
</tr>
<tr>
<td>CD and HbA1C</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**: Correlation is significant at the 0.05 level (2-tailed).

Table (11) correlation between CD and Thyroid disorder.

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD and Thyroid</td>
<td>0.496</td>
</tr>
</tbody>
</table>

**: Correlation is significant at the 0.05 level (2-tailed).
Chapter Five

5.1 Discussion

Until 1990, the presence of celiac disease in Sudan was considered low. However, with the introduction of assays for anti-tissue transglutamate antibodies (ATTGA) and antigliadin antibodies (AGA) CD has been more readily detected and reported from Sudan. Celiac disease was first reported from Sudan in 1978 when 7 children were diagnosed by intestinal biopsy (99). The second report was done by Mohammed et al in Khartoum (100).

We believe that the prevalence of CD in the Red Sea state may be underestimated due to lack of awareness and low suspicion of the disease by the medical practitioner's. Unfortunately, some doctors, especially in the rural areas, still do not know when to suspect celiac disease. Many physicians still do not realize there is no “typical” celiac disease patient. Celiac disease can begin at any age, persists for life and can affect multiple organs.

Thus high levels of the serological markers remain the test of choice for detection of CD in our studied region (101). In our study, two hundred patients were diagnosed positive for CD. The diagnosis of CD was confirmed by serological detection of the AGA and ATTGA when the titer of Anti-TTG and AGA was found to be more than 10 times of the cut-off value (97). Endoscopic intestinal biopsy was not available in the region.

In the present study, the prevalence of CD in female was higher than male. People with celiac disease can be thin, obese or have normal weight. In both children and adults the symptoms of celiac disease can be extremely variable or there may be no symptoms at all. Unfortunately owing to the heterogeneity in the clinical presentation and lack of a standard clinical profile in CD, some patients go undiagnosed for years together despite sever consultations with different doctors.

The clinical presentation of CD varies from silent to severe intestinal manifestation (102,103), in this study, chronic diarrhea was the most common presenting symptom (30%). In Iran, Lebanon, Iraq, Saudia Arabia and Kuwait, CD has been reported to be one of the most common causes of chronic diarrhea (104). This is similar
to our findings. The second common symptom was weight loss (16.5%). In Egypt, 4.7% of children presenting with diarrhea and weight loss had CD\(^{(105)}\). The variation in the prevalence of clinical manifestation of CD across different studies, may be due to low number of patients evaluated or a delay in their presentation. Stunted growth represented the third most common presentation (13%). About one third of children with CD in Western countries develop short stature\(^{(106)}\). Anemia was noted in (11%) of the patients.

This reported that, the majority of celiac disease cases were noted to occur in the Benne Ammer tribe (63%) this tribe represents 28% of the whole population in our study, the CD patients in the Benne Ammer tribe were similar to this findings (60%). Genetic and environmental factor may play a role in the higher prevalence of CD in this tribe. CD is strongly associated with the human leukocyte antigen (HLA) DQ2 and DQ8 alleles\(^{(107)}\).

The term “associated conditions” refers to conditions that are found at an increased frequency in celiac disease but that are not thought to be due to gluten ingestion.

One of the most intensely disease studied associated with celiac disease is type 1 diabetes. Approximately 5–10% of patients with type 1 diabetes have positive TG antibodies, with up to 75% having abnormalities on small intestinal biopsy\(^{(108,109)}\). Celiac disease and type 1 diabetes share HLA risk genotypes. A strong association between DM1 and CD is well documented. In the present study, the level of HBA1C and RBS were used to diagnosis of DM1 in paediatrics patients. In our study they was 36% of patients were discovered to have DM1. The known association between the two disorders is likely largely related to shared genetic risk.

Patients with type 1 diabetes and celiac disease are closely related. In some patients, the diagnosis of celiac disease may precede the diagnosis of type 1 diabetes. In a report analyzing the risk for type 1 diabetes in patients with celiac disease are at a 3.9 increased hazard for the development of diabetes by age 20 years\(^{(110)}\). Observations such as these have lead to hypotheses regarding a common environmental etiology for celiac disease and type 1 diabetes. Given that the environmental cause of celiac disease is known (gluten) it has been hypothesized that gluten may play an important role in the development of type 1 diabetes. Through prospective studies of high-risk
infants for type 1 diabetes and celiac disease, it has been shown that early introduction of gluten (prior to 4 months of age) is associated with an increased risk for autoimmunity associated with both conditions \(^{(11,12,13)}\). In contrast, diabetes prevention trials using a gluten free diet have not shown any efficacy in delay of diabetes onset\(^{(14)}\). Therefore, the relationship between the two disorders is likely quite complex.

Therefore, significant debate in the literature exists regarding the significance of positive celiac disease-related antibodies in the population with type 1 diabetes and the importance of ongoing screening and treatment with a gluten free diet. Currently, the American Diabetes Association recommends screening for celiac disease related autoantibodies at diagnosis of diabetes and with signs or symptoms of celiac disease or vesaversa \(^{(15,16)}\).

Celiac disease also shares genetic risk factors with autoimmune thyroid disease. Patients with autoimmune thyroid disease are at an increased risk for celiac disease related antibodies. There are no current recommendations for screening for celiac disease in the setting of autoimmune thyroid disease, at the very least a complete review of systems should be elicited, poor growth and pubertal status, weight loss or other signs or symptoms of hypothyroidism. should be monitored and any evidence for growth failure or symptoms of celiac disease should be addressed with screening for TG autoantibodies.

In our study 7 % of patients were diagnosed as hypothyroidism and they were confirm by positive for CRP and ESR \(^{(98)}\) they were used as markers for presence of thyroiditis. Two parameter show elevation in result, CRP was more than 15 g/dl and ESR was more than 20 mm/hr .

Currently the only treatment for celiac disease is a gluten-free diet \(^{(117)}\). Dietary avoidance of gluten leads to symptoms improvement in 70 % of patients within 2 weeks \(^{(118)}\).
5-2 Conclusion

1. The clinical spectrum of celiac disease continues to evolve what was once thought to be a rare disorder effecting young children is now recognized to be very common.

2. The combination of AGA IgG and AT TG IgA tests represents a good diagnostic method for diagnosis of suspected cases of celiac disease.

3. Celiac disease patients also carries risk for both hyperglycemia and autoimmune thyroid disorder.

4. The only treatment for celiac disease is a gluten free diet.
Chapter five

Conclusion and recommendation
5-3 Recommendation:

1. Screening for celiac disease has become relatively easily with reliable antibodies against self-antigens have been done recently, which was AGA and ATTG Antibodies.

2. HbA1C and thyroid function test should be measured routinely for patients diagnosed with CD.

3. A combined effort on the part of health care workers and researchers to increase health education and help in clarification of the specific risk factors for CD in this region.

4. Introduce of AGA and ATTG as tool of differential diagnosis for diarrhea disease in children and enhance to improve treatment and management.

5. Biopsy should be available in paediatric.
Reference
Reference:


77. Kim DW, Jung SL, Baek JH et al. The prevalence and features of thyroid pyramidal lobe, accessory thyroid, and ectopic thyroid as assessed by computed tomography: a multicenter study. Thyroid 2013 Jan;23(1):84-91.


95. Treatment for Thyroid disease Retrieved on 2010-02-07


Appendices
Questionnaire

Date: .................................. Number ..........................

Name of the patient: ........................................

Age: .............................................................

Sex: male ............. female ..............................

History of the disease:

- Diabetes Mellitus ......................
- Hyperthyroidism ......................
- Hypothyroidism ......................

Duration: ..........................................................

Tribe: Hadandawa □ Banne Amer □ Rashayda □
Northern Sudan □ Western Sudan □

Laboratory investigation:

- Serum anti tTG IgA ; .........................
- Serum anti Gliadin IgG ; .....................
- Random blood sugar level ; ..............
- HbA1c level : ........................................
- Blood TSH level ; ............................
- Blood T3 level ; ..............................
- Blood T4 level ; ..............................
- CRP ..............................
- ESR ..............................
## Anti-Gliadin (GAF-3X) ELISA (IgA)

**Test instruction**

<table>
<thead>
<tr>
<th>ORDER NO.</th>
<th>ANTIBODIES AGAINST</th>
<th>IG-CLASS</th>
<th>SUBSTRATE</th>
<th>FORMAT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV 3011-9601 A</td>
<td>Gliadin (GAF-3X)</td>
<td>IgA</td>
<td>Ag-coated microplate wells</td>
<td>96 x 01 (96)</td>
</tr>
</tbody>
</table>

### Indications:
Gluten-sensitive enteropathy (coeliac disease), Dermatitis herpetiformis Duhring.

### Principles of the test:
The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgA class against gliadin (GAF-3X) in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with gliadin (GAF-3X). In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgA antibodies (also IgG and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgA anti-human IgA (enzyme conjugate) causing a colour reaction.

### Contents of the test kit:

<table>
<thead>
<tr>
<th>Component</th>
<th>Colour</th>
<th>Format</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microplate wells, coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use</td>
<td>—</td>
<td>12 x 8</td>
<td>STRIPS</td>
</tr>
<tr>
<td>2. Calibrator 1</td>
<td>dark red</td>
<td>1 x 2.0 ml</td>
<td>CAL 1</td>
</tr>
<tr>
<td>200 RU/ml (IgA, human), ready for use</td>
<td>3. Calibrator 2</td>
<td>red</td>
<td>1 x 2.0 ml</td>
</tr>
<tr>
<td>26 RU/ml (IgA, human), ready for use</td>
<td>4. Calibrator 3</td>
<td>light red</td>
<td>1 x 2.0 ml</td>
</tr>
<tr>
<td>2 RU/ml (IgA, human), ready for use</td>
<td>5. Positive control</td>
<td>blue</td>
<td>1 x 2.0 ml</td>
</tr>
<tr>
<td>(IgA, human), ready for use</td>
<td>6. Negative control</td>
<td>green</td>
<td>1 x 2.0 ml</td>
</tr>
<tr>
<td>(IgA, human), ready for use</td>
<td>7. Enzyme conjugate</td>
<td>orange</td>
<td>1 x 12 ml</td>
</tr>
<tr>
<td>peroxidase-labelled anti-human IgA (rabbit), ready for use</td>
<td>8. Sample buffer</td>
<td>light blue</td>
<td>1 x 100 ml</td>
</tr>
<tr>
<td>ready for use</td>
<td>9. Wash buffer</td>
<td>colourless</td>
<td>1 x 100 ml</td>
</tr>
<tr>
<td>10x concentrate</td>
<td>10. Chromogen/substrate solution</td>
<td>colourless</td>
<td>1 x 12 ml</td>
</tr>
<tr>
<td>TMB/H$_2$O$_2$, ready for use</td>
<td>11. Stop solution</td>
<td>colourless</td>
<td>1 x 12 ml</td>
</tr>
<tr>
<td>0.5 M sulphuric acid, ready for use</td>
<td>12. Test instruction</td>
<td>—</td>
<td>1 booklet</td>
</tr>
<tr>
<td>—</td>
<td>13. Protocol with reference values</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>LOT</td>
<td>Lot</td>
<td>Storage temperature</td>
</tr>
<tr>
<td></td>
<td>IVD</td>
<td>in vitro determination</td>
<td></td>
</tr>
</tbody>
</table>

### Storage and stability:
The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

### Waste disposal:
Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.
# Anti-tissue Transglutaminase ELISA (IgA) Test instruction

## ORDER NO. 9510-9601 A 

<table>
<thead>
<tr>
<th>ANTIBODIES AGAINST</th>
<th>IGG-CLASS</th>
<th>SUBSTRATE</th>
<th>FORMAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>human tissue transglutaminase (endomysium)</td>
<td>IgA</td>
<td>Ag-coated microplate wells</td>
<td>96 x 01 (96)</td>
</tr>
</tbody>
</table>

**Indications:** Gluten-sensitive enteropathy (coeliac disease), Dermatitis herpetiformis Duhring.

**Principle of the test:** The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human autoantibodies of the IgA class against tissue transglutaminase in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with human tissue transglutaminase. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgA antibodies (also IgG and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgA (enzyme conjugate) catalysing a colour reaction.

## Contents of the test kit:

**Component** | Colour | Format | Symbol |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microplate wells, coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use</td>
<td>—</td>
<td>12 x 8</td>
<td>STRIPES</td>
</tr>
<tr>
<td>2. Calibrator 1</td>
<td>dark red</td>
<td>1 x 2.0 ml</td>
<td>CAL 1</td>
</tr>
<tr>
<td>200 RU/ml (IgA, human), ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Calibrator 2</td>
<td>red</td>
<td>1 x 2.0 ml</td>
<td>CAL 2</td>
</tr>
<tr>
<td>20 RU/ml (IgA, human), ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Calibrator 3</td>
<td>light red</td>
<td>1 x 2.0 ml</td>
<td>CAL 3</td>
</tr>
<tr>
<td>2 RU/ml (IgA, human), ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Positive control</td>
<td>blue</td>
<td>1 x 2.0 ml</td>
<td>POS CONTROL</td>
</tr>
<tr>
<td>(IgA, human), ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Negative control</td>
<td>green</td>
<td>1 x 2.0 ml</td>
<td>NEG CONTROL</td>
</tr>
<tr>
<td>(IgA, human), ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Enzyme conjugate</td>
<td>orange</td>
<td>1 x 12 ml</td>
<td>CONJUGATE</td>
</tr>
<tr>
<td>peroxidase-labelled anti-human IgA (rabbit), ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Sample buffer</td>
<td>light blue</td>
<td>1 x 100 ml</td>
<td>SAMPLE BUFFER</td>
</tr>
<tr>
<td>ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Wash buffer</td>
<td>colourless</td>
<td>1 x 100 ml</td>
<td>WASH BUFFER 10x</td>
</tr>
<tr>
<td>10x concentrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Chromogen/substrate solution</td>
<td>colourless</td>
<td>1 x 12 ml</td>
<td>SUBSTRATE</td>
</tr>
<tr>
<td>TMB/H2O2, ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stop solution</td>
<td>colourless</td>
<td>1 x 12 ml</td>
<td>STOP SOLUTION</td>
</tr>
<tr>
<td>0.5 M sulphuric acid, ready for use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Test instruction</td>
<td>—</td>
<td>1 booklet</td>
<td></td>
</tr>
<tr>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Protocol with target values</td>
<td>—</td>
<td>1 protocol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Storage and stability:
The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

**Waste disposal:** Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents are to be disposed of according to official regulations.
HEMOGLOBIN A1C

**CONTENTS**

1. Reagent 1: 1 x 30 mL
2. Reagent 2: 1 x 50 mL
3. Reagent 3: 1 x 400 mL
4. Microcolumns: 1 x 20

**COMPOSITION**

1. Reagent: Potassium phosphate 10 mmol/L, detergent 5 g/L, pH 7.6, sodium azide 0.95 g/L.
2. Reagent: Phosphate buffer 1.5 mmol/L, pH 6.5, sodium azide 0.95 g/L.
3. Reagent: Phosphate buffer 72 mmol/L, pH 6.5, sodium azide 0.95 g/L.
4. Microcolumns: Contain a preweighed amount of resin equivalent to phosphate buffer 72 mmol/L, pH 6.5, sodium azide 0.95 g/L.

Use only microcolumns (4) and reagents 2 and 3 of the same kit.

**STORAGE**

Store at 15-30°C.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contamination is prevented during their use.

Indications of deterioration:
- Reagents: Presence of particulate material, turbidity.
- Microcolumns: Absence of buffer over the resin bead.

**ADDITIONAL EQUIPMENT**

- Spectrophotometer or photometer with a 415 nm filter (405-425 nm)

**SAMPLES**

Whole blood collected by standard procedures.

Hemoglobin A1C is stable for 7 days at 2-8°C. Heparin or EDTA may be used as anticoagulants.

**PROCEDURE**

Hemolyzate Preparation and Labile Fraction Elimination
1. Bring the columns and reagents to room temperature (21-26°C) (Note 1).
2. Pipette into a test tube:

   Blood: 50 µL
   Reagent 1: 200 µL
   Reagent 2: 400 µL
   Reagent 3: 400 µL

3. Shake thoroughly and let it stand at room temperature for 10 minutes. This hemolyzate will be used in steps 6 and 11.

**COLUMN PREPARATION** (Notes 2 and 3)

4. Remove the upper cap of the column and then snap the tip off the bottom.

5. Using the flat end of a pipette, push the upper disc down to the resin surface, taking care not to compress it. Let the column drain completely to waste.

**SEPARATION AND READING** of HbA1c fraction

6. Carefully pipette on the upper filter:

   Hemolyzate: 50 µL
   Let the column drain to waste
   Reagent 2: 200 µL
   Let the column drain to waste

7. Place the column over a test tube and add:

   Reagent 3: 40 µL
   Collect the eluate (HbA1c Fraction)

8. Shake thoroughly and read the absorbance (A) of the HbA1c fraction at 415 nm against distilled water (Awater). The absorbance is stable for at least one hour.

9. **READING OF HbT**

11. Pipette into a test tube:

   Reagent 3: 12.0 µL
   Hemolyzate: 50 µL

12. Shake thoroughly and read the absorbance (A) at 415 nm against distilled water (Awater). The absorbance is stable for at least one hour.

**CALCULATIONS**

The HbA1c relative concentration in the sample is calculated using the following general formula:

\[
\text{A}_{\text{sample}} \times \frac{\text{A}_{\text{water}}}{\text{A}_{\text{sample}}} = 100 \times \% \text{HbA1c}
\]

The volume of HbA1c (Vsample) is 4 mL, the volume of Hb total (Vinitial) is 12 mL. The following formula is deduced for the calculation of the concentration:

\[
\% \text{HbA1c} = \frac{\text{A}_{\text{sample}} \times 100}{\text{A}_{\text{water}}} \times 3
\]

The results obtained with the present method are equivalent to a US National Glycohemoglobin Standardization Program certified method (NSGSP) and can be converted into equivalent to the International Federation of Clinical Chemistry standardized method (IFCC), using the internationally recommended master equation:

\[
\text{HbA1c}_{\text{IFCC}} = 10.83 \times \text{HbA1c}_{\text{NSGSP-DCCT}} - 23.5
\]

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THYROTROPIN (TSH)
ELISA

Use

The Quantitative Determination of Thyrotropin Concentration in Human Serum by the Mammalian Immunoassay Method

Summary

The assay uses an enzyme immunoassay (EIA) method, which is a general technique for the quantification of thyroid-stimulating hormone (TSH) in human serum. The assay is designed to provide a rapid and reliable method for the quantification of TSH in clinical samples. The assay utilizes a monoclonal antibody that binds specifically to TSH in human serum. The monoclonal antibody is conjugated to an enzyme, and the resulting antibody-enzyme complex is used to detect the presence of TSH in the sample. The assay is performed in a 96-well microtiter plate format, which allows for the simultaneous measurement of TSH in multiple samples. The assay is sensitive and specific for TSH, and it can be used to measure TSH in a wide range of clinical samples, including serum, plasma, and urine.

Materials Provided (Store at 2-8°C):

- Thyrotropin Antibody
- Thyrotropin Standard
- Thyrotropin Reagent
- Wash Solution Concentrate
- Singlet Substrate Solution
- Stop Solution

Product Insert:

- Protocol:
  1. In 96-well plates, add 100 μL of sample or standard to each well.
  2. Add 100 μL of the antibody conjugate to each well.
  3. Incubate at room temperature for 1 hour.
  4. Wash the plate three times with wash buffer.
  5. Add 100 μL of substrate solution to each well.
  6. Incubate at room temperature for 30 minutes.
  7. Stop the reaction by adding 100 μL of stop solution to each well.
  8. Measure the absorbance at 450 nm.

- Reagents:
  - Thyrotropin Antibody
  - Thyrotropin Standard
  - Thyrotropin Reagent
  - Wash Solution Concentrate
  - Singlet Substrate Solution
  - Stop Solution

- Procedure:
  1. Dilute the standards and samples in assay buffer to the desired concentrations.
  2. Add 100 μL of each dilution to a 96-well plate.
  3. Add 100 μL of the antibody conjugate to each well.
  4. Incubate at room temperature for 1 hour.
  5. Wash the plate three times with wash buffer.
  6. Add 100 μL of substrate solution to each well.
  7. Incubate at room temperature for 30 minutes.
  8. Stop the reaction by adding 100 μL of stop solution to each well.
  9. Measure the absorbance at 450 nm.

- Interpreting the Results:
  - The absorbance values are used to calculate the concentration of TSH in the sample.
  - The concentration of TSH is calculated using a standard curve.
  - The standard curve is generated by plotting the absorbance values against the corresponding concentrations of TSH.
  - The concentration of TSH in the sample is determined by interpolation from the standard curve.

- Quality Control:
  - Positive controls should be run with each assay.
  - Negative controls should be run with each assay.

- Reference Ranges:
  - Adults: 0.5-5.0 mIU/L
  - Children: 0.5-10.0 mIU/L

- Notes:
  - The assay is sensitive and specific for TSH.
  - The assay is not affected by thyroid hormones or other substances.

- Limitations:
  - The assay is not suitable for the detection of TSH in pathological conditions.
  - The assay is not suitable for the detection of TSH in patient populations with altered TSH levels.

- References:

- For more information, please contact our technical support team at 1-800-123-4567.
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