Biochemical Changes in Disease

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PREFACE

This book aims to understand and identify the main biochemical changes that occur in several diseases, namely pancreatic, cardiovascular, musculoskeletal, psychiatric, nutritional and infectious diseases. It is also focused on the recognition of possible biochemical markers of each disease, highlighting how they can be used in diagnosis.

Pancreatitis, cystic fibrosis and diabetes mellitus are the diseases of the pancreatic system, and many biochemical changes occur during the development and progress of these diseases. Cystic fibrosis is a common autosomal recessive disease and pancreas is mostly affected by cystic fibrosis. Diabetes mellitus occur due to a decrease in the amount of pancreatic beta cells as well as their dysfunction.

Cardiovascular diseases, mainly ischemic heart disease and stroke, with atherosclerosis as the key underlying factor, are one of the most common causes of death in both developing and developed countries worldwide, with an ever-increasing prevalence. Atherosclerosis results from low-grade chronic inflammation that arises from an interaction between immunological mechanisms and metabolic abnormalities within the vessel wall.

When considering musculoskeletal diseases, more than 150 possible diagnoses of conditions that affect the locomotor system should be considered. These conditions are characterised by pain, decreased physical abilities, big impacts in mental health and are a strong cause for the development of other chronic diseases such as obesity, since sedentarism and often incorrect food intake are associated. Musculoskeletal disorders are divided into three subgroups according to the affected organ or tissue, namely bone, joint and muscle diseases.

The American Psychiatric Association defines a mental disorder as a syndrome characterized by clinically significant disruption in an individual's cognition, behaviour, or emotion regulation, due to dysfunctions in psychological, biological or developmental processes concerning mental functioning. Nowadays common mental disorders are depression, anxiety and schizophrenia. Solid evidence on the aetiology and pathophysiology of these disorders are relevant for clinical psychiatry.

Nutritional diseases are any of the nutrient-related disorders and conditions that cause illness. These disorders occur when dietary intake does not contain the right amount of nutrients for healthy functioning of the body, or when nutrients from food cannot be properly absorbed. Nutritional diseases include a wide range of conditions, such as widespread undernutrition (malnutrition), overnutrition that leads to obesity, and the eating disorders such as anorexia nervosa, bulimia nervosa, binge eating disorder, and orthorexia nervosa and bigorexia.

Infectious diseases, caused by viruses, bacteria, and parasites, are a public health problem that has emerged in recent decades with new specificities. Infectious diseases can be acquired through direct contact with the infectious agent or through exposure to contaminated water or food, as well as through the respiratory or sexual route. Often, these diseases can also be transmitted from person to person. Different infectious diseases, namely COVID-19, AIDS, tuberculosis, *Helicobacter pylori*, *Tinea* and *Candida* infections, toxoplasmosis and malaria, can alter different biochemical parameters.

CHAPTER 1

BIOCHEMICAL CHANGES IN PANCREATIC DISEASES

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List of abbreviations

ADA: American Diabetes Association
ADIPOQ: Adiponectin
Akt: Protein kinase B
AP: Acute pancreatitis
ASL: Airway surface liquid
ATF6: Activating transcription factor 6
ATP: Adenosine triphosphate
BMI: Body mass index
CF: Cystic fibrosis
CFRD: Cystic fibrosis related diabetes
CFTR: Cystic fibrosis transmembrane regulator
CRP: C-reactive protein
DM: Diabetes mellitus
DNA: Deoxyribonucleic acid
EPO: Erythropoietin
ETS: Electron transport system
FoxO: Forkhead transcription factor

GAD: Glutamic acid decarboxylase
GADA: Glutamic acid decarboxylase autoantibodies
GCK: Glucokinase
GCKR: Glucokinase regulator
G-CSF: Granulocyte-colony stimulating factor
GDM: Gestational diabetes mellitus
GLUT-1: Glucose transporter 1
GSH: Glutathione
GSSG: Glutathione reductase
HbA1c: Glycated haemoglobin A1c
HGFs: Hematopoietic growth factors
IA-2: tyrosine phosphatase-related islet antigen *2*
IAA: İnsulin autoantibodies
ICAM-1: Inter-cellular adhesion molecule 1
ICM: Intestinal current measurement
IL: Interleukin
IRS1: İnsulin receptor substrate 1
IκB: Inhibitor of NF-κB
KCNQ1: Potassium voltage-gated channel subfamily Q member 1
M-CSF: Macrophage-colony stimulating factor
METSIM: The metabolic syndrome in men study
miRNA: microRNA
MTNR1B: Melatonin receptor 1B
NAD^+: Nicotinamide adenine dinucleotide
NEAPC: Neutrophil elastase antiprotease complex
NF-κB: Nuclear factor-kappa B
NMN: Nicotinamide mononucleotide
NPD: Nasi potential differential
OGTT: Oral glucose tolerance test
PCT: Procalcitonin
PI: Exocrine pancreatic insufficiency
PI3K: Phosphoinositide 3-kinase
PMN: Polymorphonuclear granulocyte
PPAR: Peroxisome proliferator-activated receptor
RNA: Ribonucleic acid
ROS: Reactive oxygen species
RXR: Retinoid X receptor
SAA: Serum amyloid A
SHIP1: Src homology 2-containing inositol 5-phosphatase 1
SLPI: Secretory leukocyte peptidase inhibitor
SNP: Single nucleotide polymorphisms

TAP: Trypsinogen activation peptide
TCA: Tricarboxylic acid cycle
TCF7L2: Transcription factor 7 like 2
TNF- α: Tumour necrosis factor alpha
ZnT8: Zinc transporter-8

Introduction

Pancreatitis, cystic fibrosis (CF) and diabetes mellitus are the diseases of the pancreatic system, and many biochemical changes occur during the development and progress of these diseases. Over the past decades, especially in Western countries, an increase in both the incidence rate and hospital admissions have been reported for pancreatitis patients. Cystic fibrosis is a common autosomal recessive disease and pancreas is mostly affected by cystic fibrosis. According to the patient registry records of the Cystic Fibrosis Foundation, each year, almost 1000 new cases are being diagnosed with CF in the United States. Diabetes mellitus occur due to a decrease in the amount of pancreatic beta cells as well as their dysfunction. According to the American Diabetes Association (ADA), in 2018, approximately 10.5% of the population had diabetes mellitus and every year, more than 1.5 million new cases are being diagnosed in the United States.

Pancreas is an organ of the digestive system responsible for the production of insulin and other hormones as well as some important enzymes to breakdown foods. The pancreas plays a dual role: both an endocrine function and an exocrine function. It has an endocrine function as it secretes insulin and glucagon to regulate blood sugar. It has also an exocrine function as it secretes enzymes to help digestion. Among these enzymes, lipase digests fats, amylase digests carbohydrates and chymotrypsin and trypsin digest proteins.

In this section, the pancreatic system diseases are divided into three subgroups as pancreatitis, CF and diabetes mellitus. Biochemical changes that could be defined as the markers of the diseases are evaluated.

1.1. Pancreatic disorders

1.1.1. Pancreatitis

This is an inflammation of the pancreatic tissue. It may be caused by the pancreatic enzymes before they reach the duodenum. Acute pancreatitis

(AP) can be identified by a sudden and severe abdominal pain commonly caused by the blockage of the main pancreatic duct by gallstones. Fever and vomiting mostly accompany abdominal pain. It has also been reported that AP could be caused by taking too much alcohol. The morbidity and mortality rate is high in AP and it can turn into a chronic condition.

Biochemical markers in pancreatitis

C-reactive protein (CRP)

CRP is a biomarker for inflammatory diseases. After the onset of AP symptoms, CRP reaches its peak level within 72-96 hour. Mayer *et al.* (1984) reported that the severity of AP could be predicted by evaluating CRP levels. CRP has the advantages of having high prognostic value, being cheap and available in the routine clinical setting. However, liver disease can influence CRP levels and if the AP patient is obese and/or alcoholic, liver disease is mostly inevitable. Mikó *et al.* (2019) investigated the severity and mortality of AP by evaluating biochemical markers together with CRP and they found out that it has 71% sensibility and 87% specificity.

Procalcitonin (PCT)

PCT is the biologically inactive form of calcitonin and has been in use to detect the severity of AP during the last decade. It is superior to CRP as it can differ mild and severe AP within 24 hours. Since PCT is also the biomarker of both bacterial and fungal infections, sepsis and organ failure, it is nonspecific to AP. Khanna *et al.* (2013) reported that PCT could predict organ failure with 100% sensitivity and can predict severe AP with 86.4% sensitivity. Despite its high sensitivity, PCT is an expensive biomarker.

Interleukins (IL)

IL-1 is an important proinflammatory cytokine that can predict pancreatic necrosis within 48 to 72 hours with 88% sensitivity. Among the interleukins, IL-6 is the most promising biomarker for use in clinical routine. Soyalp *et al.* (2017) reported an elevation of IL-6 level in accordance with the severity of pancreatitis. Jiang *et al.* (2004) found 100% sensitivity and 89.7% specificity for the assessment of AP. Its major drawback is its high cost and the rapid drop of its serum concentration. IL-8 was found to be elevated significantly with the severity of AP. Rau *et al.* (1997) reported an association between IL-8 and pancreas necrosis. IL-8 increases rapidly (24 hours) after the onset of disease symptoms, but it is not recommended for AP diagnosis.

Other biochemical markers

Serum amyloid A (SAA) levels were found to elevate faster than CRP, but its sensitivity and specificity to AP were found to be slightly lower than CRP (69% and 67% versus 71% and 74%). SAA was also reported as being superior to CRP in terms of distinguishing mild and severe AP.

Trypsinogen activation peptide (TAP) is the amino terminal of trypsinogen which is known to be released from trypsinogen to peritoneum, serum and urine during AP. It induces the activation of proteases. A positive correlation was found between the level of pancreatic injury and activated proteases. TAP is superior to CRP as it has 100% sensitivity and 85% specificity to AP, but it cannot predict the progression of the disease as its concentration in the urine decreases rapidly.

Polymorphonuclear granulocyte (PMN) elastase is a serine protease found in the neutrophil granules. PMN elastase is an important AP biomarker as it is being released similarly to other factors such as proteolytic enzymes, reactive oxygen species, eicosanoids, cationic peptides and microbicidal products and reaches its peak concentration within 24 hours.

Tumour necrosis factor (TNF)-α is crucial for the detection of AP pathogenesis. High levels of TNF-α receptors were found in AP, being related to the severity of the disease.

Tissue factor is a transmembrane glycoprotein and *Hepcidin* is a circulating peptide hormone, both of which can assess AP severity better than CRP.

Soluble E-selectin and *soluble thrombomodulin* are endothelial markers which are found to be the predictive markers of mortality in AP.

Moreover, Milnerowicz *et al.* (2013) reported a strong correlation with AP severity and elevated *endothelin I* levels. Guo *et al.* (2012) verified a strong association between pancreatic necrosis and high *matrix metalloproteinase-9* levels. Zhou *et al.* (2019) reported *red blood distribution width* as a reliable marker for AP severity.

The elevation of *blood urea nitrogen* was also found to be correlated with mortality in AP patients. Elevated level of *haematocrit* was shown to be associated with pancreatic necrosis and AP severity. On the other hand, *hypoalbuminemia* and low serum *calcium* levels were reported as a predictor of severe AP. A link between pancreatic necrosis and elevated *creatinine* concentration was also reported by Muddana *et al.* (2009).

Low levels of *proteinuria* can be used as a marker to evaluate AP severity. Urine dipsticks are mostly being used for an easy and inexpensive detection of proteinuria. In severe AP patients, organ failure has been reported, being verified by measuring the level of *angiopoietin-2* which is known as an angiogenic growth factor that plays a role as a modulator of vascular permeability.

Intercellular adhesion molecule 1 (ICAM-1) can also be used as a marker for AP severity with the advantages of being a simple, rapid and reliable method.

1.1.2. Cystic fibrosis

Among the autosomal recessive diseases, CF is quite common and affects many organs, primarily the lungs and pancreas. This is due to a mutation in the cystic fibrosis transmembrane regulator (*CFTR*) gene. The *CFTR* gene is responsible for the formation of the CFTR protein. This protein has a channel structure in the membranes of cells that produce digestive, mucus, sweat, and salivary enzymes and plays a role in the passage of chloride ions into and out of these cells. Mutations in this gene can alter the production, structure or stability of the CFTR protein. Chloride cannot be transported to the cell surface as the CFTR protein becomes non-functional. With the deterioration of the structure of the mucus in the lungs, narrowing and obstructions are experienced in the airways. Similarly, it inhibits the secretion of digestive enzymes in the pancreas. As a result, eating disorders and growth retardation occur.

In CF, all organs in which the CFTR protein functions, are damaged. Lungs are among the most damaged organs with a frequency of 99%. After the lungs, the most affected organs are the reproductive organs and the pancreas. Obstructive azoospermia in the reproductive organs (97%), exocrine pancreatic insufficiency (87%), diabetes (32%) and pancreatitis (2%) may occur in CF patients. In addition, fatty liver (25-60%), cirrhosis (10%), cholecystolithiasis (15%) as well as meconium ileus (20%) and distal intestinal obstruction syndrome (6%) may occur.

In individuals with positive new-born screening or with CF symptoms or a family history of CF, the *sweat chloride test* is usually chosen for the diagnosis of this disease. This test measures the amount of chloride in sweat. A sweat chloride level of 30-59 mmol/L indicates the possibility of CF and if so, an additional test is needed. A chloride level of 60 mmol/L and above

is one of the diagnostic criteria for CF. CFTR genetic testing and CFTR function tests are also being used as additional tests for the diagnosis of CF.

Biochemical changes in lung, liver and pancreas

There is a loss of chloride and bicarbonate transport due to CFTR in the lung. As a result, airway surface fluid (ASL) dehydration and increased mucus concentration occur. The mucin content of goblet cells is elevated. Disruption of the mucus structure in the airways leads to airway obstruction and increased inflammation. Children with CF born with normal lung structure, but the occlusion of the bronchioles occurs when they become 4 months old. TNF-α, IL-1β, IL-6, and IL-8 were found to be elevated in the lung secretions of CF patients. Biliary obstruction occurs when CFTR function is impaired. This blockage causes various liver diseases. The most common of these diseases are hepatic steatosis, focal biliary cirrhosis and micro gallbladder. Studies show that the mechanical effects of flow in the apical membrane of cholangiocytes stimulate ATP release and affect chloride secretion, which further regulates bile secretion. Therefore, decreased bile flow resulting from CFTR dysfunction may exacerbate abnormalities in bile formation.

Pancreatic ductal cells secrete isotonic fluid in response to food intake. CFTR plays a crucial role in the production of this alkaline solution which helps to send the digestive proenzymes secreted from the pancreatic duct tree into the duodenum. It also neutralizes the acidic cumin entering the proximal part of the small intestine and the protons that are co-released during digestive enzyme secretion by the pancreatic acinar cells.

Damage to the pancreas is found in almost all patients with CF at the earliest stage. Pancreatic injury causes not only severe inflammation but also occlusion of ducts by mucoprotein plugs. Pancreatic cyst formation and fibrosis occur by the secretion of viscous and protein-rich fluid. The changes begin in utero and eventually lead to pancreatic insufficiency (PI), which manifests itself in 83% of all CF patients. The clinical symptoms of PI are abdominal pain, digestive upset and low BMI. Exocrine PI is a common gastrointestinal complication that affects people with cystic fibrosis. Pancreatic insufficiency results from a progressive fibrotic process that begins in the uterus. Pancreatic cells are damaged by the deposits of dehydrated pancreatic secretions and are replaced by fibrous scar tissue. The pancreas no longer functions effectively and produces reducing amounts of enzymes necessary for digestion. PI is the main cause of improper digestion of dietary macronutrients, including fat, protein and carbohydrate. If left

untreated, it will result in malabsorption symptoms such as malnutrition status, impaired growth and development, deficiency of fat-soluble vitamins and steatorrhea.

Cystic fibrosis related diabetes (CFRD)

CFRD has similar and different characteristics to type 1 and type 2 diabetes mellitus (DM). CFRD is characterized by insulin deficiency and insulin resistance as a result of the destruction of pancreatic islets. The prevalence of CFRD increases markedly with age and affects approximately 2% of children, 19% of adolescents, and 40% to 50% of adults. About 80% of individuals with severe mutations, have CFRD after the age of 40 and women have a higher prevalence. Features unique to CFRD include partial loss or dysfunction of pancreatic islets leading to the deficiency of insulin secretion. There is a chronic underlying inflammation that flares periodically during the infection which causes fluctuating levels of insulin resistance. Diabetes develops asymptomatically in CF patients and an annual screening with OGTT is recommended.

Biochemical markers in cystic fibrosis

The function of CFTR protein has to be monitored to identify CF. Biomarkers used for CF measure the function of CFTR protein in different tissues and organs. Today, *sweat chloride test*, *nasal potential difference (NPD)* measurements and *intestinal flow measurements (ICM)* are being performed to identify CF.

In the *sweat chloride test*, the sweat glands are stimulated with pilocarpine and the sweat is collected in a gauze or a collector. The amount of chloride in sweat is determined. A chloride level above 60 mmol/L indicates insufficient chlorine reabsorption and diagnosis of cystic fibrosis. *NPD* and *ICM* measure the voltage potential or electric current, respectively, arising from epithelial ion fluxes at the mucosal surface. The *NPD* measurement is thought to provide information on both sodium absorption and chloride excretion. When these two methods detect voltage potential or electric current difference, they help to estimate the ion transitions in cells, especially chloride.

Sweat chloride is a biomarker only suitable for systemic treatments in clinical trials. Significant changes in sweat chloride occurred after administration of the CFTR enhancer ivacaftor to CF patients with the G551D mutation. Subjects homozygous for the F508del mutation had minor changes after intervention with the CFTR corrector VX-809 and moderate

changes occurred during combination therapy with ivacaftor and VX-809. In patients with the nonsense mutation, ataluren improved NPD, but not sweat chloride.

Pulmonary biomarkers

As a result of finding the inflammation in the airway in CF and discovering the changes resulting from inflammation, various parameters have become candidate biomarkers for CF such as cytokines, neutrophil chemo attractants, proteases, antiproteases, adhesion molecules, antioxidants, nitric oxide metabolites, antimicrobial proteins, eicosanoids, mucins and components of signalling cascades.

In the lung tissues of CF patients, peroxisome *proliferator activating receptor* (PPAR) was found to be deficient when compared to the healthy controls. When activated, PPAR forms a heterodimer with the activated retinoid X receptor (RXR), which can modulate inflammation. PPAR typically exerts its attenuating effects by inhibiting NF-κB activity through upregulation of IκB or by competing with NF-κB for helicases. CF airway epithelial cell lines appear to have less PPARγ activity than non-CF airway epithelial cell lines. Therefore, decreased PPAR expression also contributes to the imbalance between IκB and NF-κB, possibly promoting increased inflammation in CF.

Metabolomic biomarkers

To understand epithelial dysfunction associated with CF mutations and to discover the biomarkers for therapeutic development, non-targeted metabolomic analysis was performed on primary human airway epithelial cell cultures of three individual CF patients and non-CF individuals. Statistical analysis revealed a number of reproducible and significant metabolic differences between CF and non-CF cells. Alongside changes consistent with known CF effects, such as decreased cellular regulation to oxidative stress and osmotic stress, new observations on cellular metabolism in disease have been established. In CF cells, the levels of various purine nucleotides were significantly reduced, which may function to regulate cellular responses through purinergic signalling. Moreover, CF cells exhibited reduced glucose metabolism in the glycolysis, pentose phosphate pathway and sorbitol pathway, which can further exacerbate oxidative stress and limit the epithelial cell response to environmental pressure. Taken together, these findings reveal novel metabolic abnormalities associated with the pathological process of CF and identify a

panel of potential biomarkers for therapeutic development using this model system.

It was observed that in CF cells, the levels of various purine nucleotides are significantly reduced. Moreover, CF cells exhibit reduced glucose metabolism in the glycolysis, pentose phosphate pathway and sorbitol pathway, which can further exacerbate oxidative stress. In a study, nucleotide, tryptophan, glutathione, glucose metabolisms and osmolytes were examined. One of the most important differences between CF and non-CF cells was found in nucleotide metabolism, during purine biosynthesis. In CF cells, the purine metabolites such as adenosine, inosine, hypoxanthine and guanosine were reported as significantly reduced. The cytidine metabolite of the pyrimidine metabolism also showed a decrease in CF cells. During tryptophan metabolism, 1-methylnicotinamide showed a dramatic 24-fold increase, while the level of its precursor, nicotinamide, was significantly reduced. A ~2-fold increase was observed in kynurenine and anthranilate levels in CF cells. Glutathione and its associated metabolites also demonstrated significant differences between CF and non-CF cells. Both oxidized glutathione (GSSG) and reduced glutathione (GSH) levels in CF cells were reduced to 30% of the amount present in non-CF cells. In addition, ophthalmate (Glu-2-aminobutyrate-gli), a metabolite involved in GSH synthesis, also showed similar decreases. S-lactoylglutathione, a metabolite derived from glutathione detoxification, was significantly lower in CF cells. Glucose has a central function in cellular metabolism to produce energy and biosynthetic precursors of nucleotides and fatty acids. The levels of glucose and various glycolytic intermediates, including glucose-6-phosphate, fructose-6-phosphate and lactate, were significantly reduced in CF cells. Ribulose-5-phosphate levels in the pentose phosphate pathway, malate levels in the tricarboxylic acid cycle, and sorbitol and fructose levels in the sorbitol pathway were found to be reduced. In general, these findings may show that glucose metabolism is suppressed in CF cells. The levels of the two main cellular osmolytes, sorbitol and glycerophosphorylcholine, were also found to be significantly reduced in CF cells compared to non-CF cells.

MicroRNA (miRNA) biomarkers

miRNAs are small, non-coding RNAs that participate in post-transcriptional gene expression. They are involved in many biological processes such as growth, development, differentiation, proliferation and cell death. In recent years, it has been shown that the expression of miRNAs in cystic fibrosis cell lines and tissues has changed, and studies have demonstrated that they

are one of the promising treatment approaches for the future. As a result of studies with different disease models, miRNAs that directly bind to CFTR mRNA or indirectly affect inflammation, fibrosis formation, CFTR protein folding, and many different mechanisms were shown to affect disease severity.

In CF, due to the formation of dehydrated mucus in the lung, chronic lung infection occurs following the reduction of CFTR channel activity. miRNAs indirectly affect disease severity in patients with CF by targeting genes involved in the immune response. In a study by Oglesby *et al.* (2010), it was found that the expression of miR-126 was decreased in primary bronchial cell cultures of patients with CF. In another study, it was shown that miR17 directly targets IL-8 and inflammation is triggered by reduced expression of miR17 in patients. miR-145, whose expression is increased in the nasal epithelial cell culture of CF patients, has been shown to trigger IL-8 release and inflammation by targeting SMAD3. In a study performed with primary bronchial cell cultures created from healthy and cystic fibrosis patients, it was determined that the expression level of miR-122, which directly targets the Activating Transcription Factor 6 (*ATF6*) gene, was increased in CF. ATF6 directs misfolded proteins to the degradation pathway. The CFTR protein, which cannot be destroyed, accumulates and creates endoplasmic reticulum stress.

Numerous miRNAs involved in inflammation pathways in cystic fibrosis have been identified. miR-155 is one of them. As a result of several studies in different cell models, it was found that miR-155 targets genes involved in the IL-8-mediated inflammation response. One of these genes, *SHIP1*, is a phosphatase enzyme involved in the PI3K/Akt signalling pathway. It was found that the *SHIP1* mRNA level decreased and the expression of miR-155 targeting *SHIP1* increased in the primary cell culture formed from nasal swabs taken from patients.

1.1.3. Diabetes mellitus

DM is a metabolic disease manifested as hyperglycaemia in the clinic under the influence of various genetic and environmental factors, resulting a decrease in pancreatic beta cells and their dysfunctions. All diabetics with hyperglycaemia are at risk for the same chronic complications, but the severity and progression of complications can vary. According to ADA criteria, DM can be classified under 3 headings:

Type I DM

Type 1 DM is defined as the persistent presence of two or more autoantibodies, together with clinical hyperglycaemia, having HbA1c levels between 5.7-6.4%, or more than 10%.

Type II DM

The first occurrence in the emergence of type 2 DM is insulin resistance. When insulin resistance occurs in the individual, the suppression mechanism of hepatic glucose production is impaired, and the use of insulin-mediated glucose in the muscles and liver decreases. Over time, this leads to loss of function due to excessive insulin secretion from the pancreatic β-cells and type 2 DM occurs.

Gestational DM (GDM)

GDM is a type of diabetes that can occur in women during pregnancy. It can be diagnosed in the second or third trimester of pregnancy. This situation is risky for the foetus and new-born. According to ADA, there are one-step and two-step strategies to diagnose GDM. One step strategy involves an oral glucose tolerance test (OGTT) with the ingestion of 75 grams of glucose. The OGTT needs to be performed in the morning after at least 8 hours of fasting. A value of fasting plasma glucose (FBG) above 92 mg/dL, together with a value of 1-h plasma glucose (1-h PG) more than 180 mg/dL and a two-hour plasma glucose (2-h PG) above 153mg/dL after OGTT, represents the presence of GDM.

According to the two-step strategy, a first non-fasting glucose load test (GLT), with ingestion of 50 grams of glucose, has to be applied. If the 1h-PG is between 130-140 mg/dL, then OGTT should be performed after fasting for at least 8 hours, with ingestion of 100 grams of glucose. In order to diagnose GDM, at least two of the four following PG levels must be present: FBG above 95 mg/dL; 1-h PG above 180 mg/dL; 2-h PG above 155 mg/dL; and 3-h PG above 140 mg/dL.

Biochemical markers in diabetes mellitus

Glycated haemoglobin A1c (HbA1c)

HbA1c is the most important marker of blood glucose levels in patients with diabetes mellitus. This marker occurs as a result of changes in the haemoglobin molecule. It is widely used for routine glycaemic monitoring

in patients with type 1 and type 2 diabetes. HbA1c (a sub-fraction of glycosylated haemoglobin), produced at a rate dependent on substrate (glucose) concentration, is continuously formed *in vivo* by glucose forming a keto amine at the N-terminal of the haemoglobin beta chain. Glucose enters erythrocytes at a rate proportional to extracellular concentration via constitutively active GLUT1 channels, so the intracellular and extracellular glucose environments are nearly equivalent. The unique microvascular complications of diabetes (nephropathy, neuropathy, and retinopathy) occur in tissues that also express the GLUT1 channel and are likely caused by intracellular glucose toxicity. Since the half-life of erythrocytes is 120 days, HbA1c measurement estimates the average glycaemic index of the last 3 months. HbA1c measurement has several advantages over fasting glucose measurement and OGTT. These advantages can be counted as not having to fast, not requiring preanalytical stability and not being affected by environmental and personal factors. During pregnancy, HbA1c shows biphasic changes that decrease between the first and second trimesters and increase in the third trimester.

Fructosamine

Fructosamine is an alternative diagnostic criterion used as a glycaemic marker. Fructosamine is a glycoprotein that is formed by a covalent linkage between a sugar (such as glucose or fructose) and total serum proteins, particularly albumin, thus forming keto amines. It reflects the average blood glucose level over the past 14 weeks. This method is cost-effective and easy. It is also a good indicator for microvascular complications. It is often used to evaluate glycaemic control to monitor the effectiveness of treatment, often when a patient changes medication or insulin. Recently, fructosamine has been associated with all-cause and cardiovascular disease mortality and morbidity in haemodialysis patients. Gounden *et al.* (2021) reported a reference range for fructosamine in non-diabetic individuals as 200 to 285 µmol/L. Fructosamine levels increase in conditions of high glucose concentrations, such as diabetes. In a study conducted by Kalia *et al*. (2004), it was found that the fructose values of patients with a long-term history of diabetes and chronic hyperglycaemia were higher than the fructose values of healthy people without diabetes.

Glycated albumin (GA)

GA represents glucose levels for a 2-weeks period, similar to fructosamine, and increases in conditions of high glucose concentration such as diabetes. In some special cases like renal failure and haemolytic anaemia, it may be

a better indicator than HbA1c. Under conditions of hyperglycaemia, albumin glycation can occur as a result of spontaneous, non-enzymatic Maillard reaction.

Albumin is known to be more sensitive to glycation than haemoglobin. Pathological conditions such as nephrotic syndrome, hepatic cirrhosis and thyroid diseases can affect blood levels of glycated albumin. During diabetes treatment, the level of glycated albumin decreases more rapidly than HbA1c. Therefore, it is thought that glycated albumin may be important in providing therapeutic observation. Since it is not affected by anaemia and related treatments, it has become a prominent biomarker in glycaemic control in dialysis patients.

Iron deficiency is common in the first trimester of pregnancy. In this period of gestational diabetes patients, measurement of glycated albumin instead of HbA1c may provide an advantage in blood glucose monitoring since it is not affected by iron deficiency treatment.

Insulin and C-peptide

Insulin and *C-peptide* serum concentrations elevate in case of insulin resistance that occurs before the development of Type 2 DM. The deterioration of beta cell function over time causes a reduction in insulin and C-peptide serum concentrations. After proinsulin formation in the endoplasmic reticulum, proinsulin is packaged in the form of granules and, by limited proteolysis, cleaved into insulin and C-peptide. Upon stimulation (e.g., by glucose), both peptides rapidly secrete into the circulation at equimolar rates. Because insulin is rapidly degraded by liver, peripheral blood insulin concentrations are significantly reduced when compared to C-peptide levels. Under *in vivo* conditions, C-peptide has a longer plasma half-life and is less affected by haemolysis than insulin, and standardization studies are already well advanced. Determination of C-peptide concentration is also important for the distinction between type 1 and type 2 diabetes and for classification of diabetes subtypes.

Wnt proteins

Wnt proteins determine embryonic cell proliferation, cell differentiation and multiple cellular functions for the survival of cells including neurons, cardiomyocytes, endothelial cells, red blood cells, tumours, and adipose tissue. Recent studies have found that an abnormality in the Wnt pathway increases the risk of type 2 DM and a strong association with obesity was reported. In patients with DM, an elevated expression of Wnt-5b in adipose

tissue, liver and pancreas was found. It was also noticed that the expression of Wnt-3a and Wnt-7a was increased in hyperglycaemic mice fed with high amounts of fat.

Intact Wnts increase glucose tolerance and insulin sensitivity. It has also been observed that intact Wnts can protect glomerular mesangial cells from high glucose-induced apoptosis. Improved glucose homeostasis and reduced hyperinsulinemia were observed in mice overexpressing Wnt-10 on a high-fat diet. Wnt-1, controlled by erythropoietin (EPO), protects cells from high glucose exposure.

Wnt improves cellular protection in patients with DM through regulation of protein kinase B (Akt). Akt activation supports cell survival in cases of cell proliferation, progenitor cell development, permeability of the blood-brain barrier, and inflammation. In addition, Akt can modulate microglial cell activation, regulate transcription factors, inhibit cytochrome c release, and block caspase activity. Thus, Wnt can prevent the occurrence of oxidative stress through Akt in diabetes. Silencing *Akt* gene expression and inhibition of the phosphatidylinositol 3-kinase (PI3-K) pathway can prevent Wnt from blocking apoptotic damage.

Nicotinamide/nicotinic acid

Nicotinamide/nicotinic acid is the water-soluble form of vitamin B3 and is rapidly absorbed by the gastrointestinal epithelium. Nicotinic acid is converted into nicotinamide in the liver or by NAD^+ hydrolysis. It is the precursor of adenine dinucleotide (NAD^+). Nicotinamide can be converted into nicotinamide mononucleotide (NMN) by nicotinamide adenylyl transferase. NAD^+ synthase or nicotinamide riboside kinase catalyses the conversion of NMN to NAD^+. NAD^+ takes part in energy metabolism in the tricarboxylic acid (TCA) cycle. It plays an active role in the production of ATP, DNA synthesis and repair in the electron transport system (ETS).

In case of exposure to reactive oxygen species (ROS) products, nicotinamide/nicotinic acid can increase endothelial cell viability. Just before DNA damage occurs in the cell, nicotinamide intervenes. Thus, it can prevent apoptotic damage. This suggests that apoptotic damage may be reversible. Nicotinamide/nicotinic acid provides protection against free radical formation in neuronal cells.

Since nicotinamide plays an important role in cellular energy management, it is thought to be important during diabetic complications. It maintains fasting blood sugar in streptozotocin induced diabetic mice. In a clinical

study, it has been shown that oral nicotinamide administration (1200 mg/m^2/day) inhibits type 1 DM islet cell antibody formation and protects pancreatic beta cell function.

Some studies have shown that long-term nicotinamide exposure reduces pancreatic beta cell function. They stated that it slows down cell growth and may promote DM. It can also cause the progression of diseases such as Parkinson's by inhibiting cytochrome P450 and hepatic metabolism.

A new transcription factor that can control some of the beneficial effects of nicotinamide in DM is forkhead (FoxOs). They bind to DNA and activate or inhibit target genes. FoxO proteins play a cytoprotective role in DM and cell metabolism. FoxO-1 improves glucose tolerance and insulin sensitivity by its expression in adipose tissue during high-fat diet in mice. FoxO3a controls apoptotic damage via caspase. Thus, it contributes to cell viability. Some studies have shown that FoxO1, FoxO3a and FoxO4 reduce post-translational phosphorylation and can initiate cellular apoptosis. Like the Sirt-1 activator resveratrol, increased transcriptional activity of the *FoxO1* gene may reduce insulin-mediated glucose uptake and insulin resistance may occur. Overexpression of *FoxO1* in skeletal muscles reduces muscle mass and attenuates glycaemic control in mice. Nicotinamide inhibits the activity of FoxO proteins. It blocks apoptotic cell damage by FoxO3a phosphorylation and inhibits caspase 3 activity.

Nicotinamide prevents oxidant-induced apoptotic damage in a certain serum concentration range. Nicotinamide protects cells against oxidative stress at a concentration of 5-25 mmol/L.

Erythropoietin (EPO)

EPO, a growth factor and cytokine, is approved by the Food and Drug Administration for the treatment of anaemia. But clinical studies have shown that apart from anaemia, it can also be a treatment agent in conditions such as Alzheimer, depression, cardiovascular diseases, spinal cord injury, ocular and gastrointestinal disorders.

The main organs where EPO is produced are the liver, kidney, brain, and uterus. The amount of EPO is relatively lower in patients with DM, regardless of anaemia. EPO secretion increases in gestational diabetes. EPO is thought to be elevated when the body defends itself against DM. In animal experiments, EPO can reduce apoptotic pathways in the developing brain under the conditions of hyperoxia. In addition, EPO can prevent the toxic effects of agents used in cognitive control such as haloperidol.

In diabetic and non-diabetic patients with severe congestive heart failure, EPO reduces fatigue, significantly reduces hospital stay, and increases left ventricular ejection fraction. Also, EPO can reverse complications of anaemia that can occur during DM.

EPO can drive the modulation of FoxO proteins and Wnt signalling. In this way, it can have protective effects both in the haematological and vascular system. Cell culture studies have shown that Wnt1 protein is sufficient for cellular protection at high glucose exposure. EPO is involved in the maintenance of mitochondrial membrane potential. In case of disruption of membrane mitochondrial potential, apoptotic damage may occur in the cell. It has been shown that EPO can provide protection in cellular apoptosis and prevent mitochondrial depolarization.

When metabolite biomarkers were examined, a relationship between type 2 DM and amino acid metabolism was determined. Some amino acids, such as valine, leucine, isoleucine and tryptophan, were found to have higher serum concentrations in T2DM patients. In a meta-analysis study by Long *et al.* (2020), some serum metabolites of T2DM patients were examined. The levels of valine, leucine, isoleucine, proline, tyrosine, lysine and glutamate were found to be lower and glycine higher than normal levels. In the Male Metabolic Syndrome (METSIM) cohort study, which included only men and had 4.5 years of follow-up, various metabolites were measured and their relationship with type 2 DM was examined. Among the metabolites, mannose was shown to have the strongest association with type 2 DM. Mannose was found to be inversely proportional to insulin sensitivity and insulin secretion, but its mechanism was not elucidated. Mannose is required for glycoprotein synthesis. In addition, proinsulin, lipids, glycerol, non-cholesterol sterols, isoleucine and alanine, acetoacetate and some inflammatory markers were shown to be associated with type 2 DM. The effect of lifestyle and environmental factors on pancreatic beta cell functions is lower than in insulin sensitivity. Therefore, the measurement of pancreatic beta cell functions is the main focus in biomarker studies for Type 2 DM.

Autoantibodies

Autoantibodies are being used to distinguish between autoimmune (type 1) and non-autoimmune (mainly type 2) diabetes, and to predict the requirement for insulin therapy. Clinically, adult-onset diabetes, that is mostly seen over the age of 30, does not show any signs of ketoacidosis and weight loss. It is a slow-growing form of autoimmune type 1 DM, also called GADA

(Glutamic Acid Decarboxylase Autoantibodies). It is often misdiagnosed as type 2 DM and treatment is performed accordingly. Therefore, it is important to conduct autoantibody tests in diabetic adults to determine the correct diagnosis and treatment.

Classical biomarkers that come to the fore in type 1 DM are serum autoantibodies against beta cell antigens, including *insulin (IAA), glutamic acid decarboxylase (GAD), tyrosine phosphatase-like protein (IA-2)* and *zinc transporter 8 (ZnT8).* The formation of beta cell autoantibodies is generally observed 6 months after birth. The first autoantibodies formed are usually IAA at 9-24 months of age and GADA at 36 months. While 70% of individuals with diabetes have three or four autoantibodies, only 10% have a single autoantibody. Diabetes diagnosed in childhood mostly contains IAA autoantibodies. Since IA-2 autoantibodies are seen in almost all type 1 DM patients, it is considered as an important biomarker. Similarly, ZnT8 autoantibodies are commonly seen in type 1 DM patients, thus facilitating the predictability of the disease. High levels of IAA and IA-2 increase the development of type 1 DM according to the results of the TEDDY study.

Single nucleotide polymorphism (SNP)

Many studies reported a relationship between *single nucleotide polymorphism (SNP)* and GDM. The transcription factor 7-like 2 (*TCF7L2*) gene encodes a transcription factor involved in Wnt signalling, an important signalling pathway in regulating glucose homeostasis. Most studies investigating four different SNPs in this gene (rs7903146, rs4506565, rs7901695 and rs12255372) found an association between the T allele (rs7903146) and GDM.

Adiponectin (ADIPOQ), an adipokine that regulates glucose and lipid metabolism, has been associated with GDM. G allele of rs266729 and rs2241766 SNPs formed in this *ADIPOQ* gene was associated with GDM. The melatonin receptor 1B (*MTNR1B*) gene encodes one of the melatonin receptors involved in regulating insulin signalling and glucose metabolism. Three studies investigating Rs1387153, one of the SNPs in this gene, reported an association between the T allele and GDM. Studies with glucokinase (*GCK*) and glucokinase regulator (*GCKR*), insulin receptor substrate 1 (*IRS1*), potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) genes SNPs demonstrated that some variants are associated with GDM.

Conclusion

In this chapter, most commonly seen pancreatic disorders (pancreatitis, CF and diabetes mellitus) were briefly described. The biochemical changes reported in the outcome of several clinical studies were evaluated. These biochemical changes reflect the differences in cellular pathways and help to understand the metabolism dependent mechanisms of the diseases. In pancreatitis, although nonspecific to AP, PCT is more sensitive to AP than CRP. IL-6 is reported as the most promising biomarker for clinical usage as it has high specificity and sensitivity to AP. Biomarkers (pulmonary, metabolomic and miRNA) of CF measure CFTR gene in diverse tissues and organs. In diabetes mellitus, together with glucose, HbA1c is the most crucial diagnostic marker. For the classification of diabetes mellitus, biomarkers such as insulin, C-peptide and autoantibodies are mostly being used. Standardization of each biomarker for the related condition is important to be able to list in the clinical practice guidelines to ease the diagnosis, classification and monitoring of the diseases. We believe that the information being provided in this chapter will enlighten future studies.

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