Correlation between diabetic retinopathy and sensory-motor neuropathy : a cohort study

Gunderlach, Daniel Hyun

UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE

Daniel H. Gunderlach

CORRELATION BETWEEN DIABETIC RETINOPATHY AND SENSORY-MOTOR NEUROPATHY - A COHORT STUDY

Diploma thesis

Academic year:
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TABLE OF CONTENTS

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1. INTRODUCTION

1.1. Diabetes

Dr. Margaret Chan, former Director-General of the World Health Organization (WHO), addresses the global issue of the chronic disease diabetes, in the current Global Report on Diabetes, released in 2016 by the WHO, with the following words: “Diabetes is on the rise. No longer a disease of predominantly rich nations, the prevalence of diabetes is steadily increasing everywhere, most markedly in the world’s middle-income countries. (…) Please join me in ensuring (…) that we may indeed halt the rise in diabetes.”(1). Diabetes and its associated diseases affect the everyday lives of millions of people worldwide.

Diabetes mellitus (DM) is a chronic disease that occurs either due to the failure of the pancreas to produce sufficient quantities of the blood sugar regulating hormone insulin, or when the body is incapable of effectively using the insulin it produces. This disease is such an important public health issue, that it belongs to one of four priority noncommunicable diseases targeted for action by world leaders (2).

The International Diabetes Federation estimated that diabetes will cause 4 million deaths worldwide in 2017 of people aged between 20 and 79. This would be the equivalent to one death every 8 seconds and would make up a total of 10.7% of the global all-cause mortality among people in the previously defined age group. The latest release of the Diabetes Atlas, by the International Diabetes Federation, approximated that 451 million people worldwide, over the age of 18 years, were affected by diabetes in 2017 (3). Numbers have been increasing steadily over the past 3 decades, as well as associated risk factors such as obesity and overweight, which are also on the rise (4). These millions of people are not only suffering from the consequences of diabetes itself but are at a high risk for associated diseases. Today we know that diabetes is an important cause of blindness, kidney failure, as well as lower limb amputation, and other long-them consequences that have a significant impact on the quality of life (5).

The term “triopathy” has been established since the 1950s and appeared in the paper *Triopathy of Diabetes*. The term "triopathy" has been applied to patients with diabetes, who have shown first clinical evidence of neuropathy, then diabetic retinopathy, and finally the nephropathy of diabetes (6). Since then the effort in understanding, diagnosing, and treating the disease itself, as well as its “triopathy” has been of great interest to the medical community and the general population.

The purpose of this thesis therefore was to examine and statistically analyze the correlation of diabetic retinopathy and diabetic neuropathy. Therefore, we selected a well-

defined group of 29 patients, with type 2 diabetes mellitus, in the population of Dalmatia and tried to establish a correlation between those two well defined complications of diabetes.

1.2. Anatomy and Physiologic Anatomy of the Human Pancreas

The pancreas lies mostly posterior to the stomach and extends across the posterior abdominal wall from the duodenum, on the right, to the spleen, on the left. The pancreas consists of the uncinate process, head, neck, body, and tail, and lies secondarily retroperitoneally, except for a small part of its tail. The pancreatic duct begins in the tail of the pancreas and joins the bile duct in the head of the pancreas and form the hepatopancreatic ampulla (ampulla of Vater). The arterial supply of the pancreas includes the gastroduodenal artery, superior and inferior pancreaticoduodenal arteries, and dorsal pancreatic artery (7).

The pancreas is a large compound gland and is composed of two major types of tissues: the acini, which secrete digestive juices, and islets of Langerhans, which secrete insulin and glucagon directly into the blood (8). The acini secrete digestive enzymes, for digesting all three major types of food (proteins, carbohydrates, fats) and large volumes of sodium bicarbonate, which aid in neutralizing the acidity of chyme, that empty directly into the duodenum through the papilla of Vater (8). Furthermore, the pancreas secretes hormones such as somatostatin, amylin, and pancreatic polypeptide (8). These hormones will not be described any further in this thesis, since the main purpose of this section is to focus on the physiological roles of two other pancreatic hormones, insulin and glucagon, to later on elaborate on the pathophysiology of the disease diabetes mellitus (8).

In addition to its digestive functions, the human pancreas contains 1 to 2 million islets of Langerhans, organized around small capillaries into which its cells directly secrete their hormones into the blood circulation (8). The physiologic anatomy of the islets of Langerhans are being presented in Figure 1. These islets are made up of three major cell types: alpha (approximately make up 25% of all cells of the islets), beta (60%), and delta cells (10%) (8). Each type of cells is differentiated by its morphological and staining characteristics (8). Further alpha cells are responsible for the secretion of the hormone glucagon, beta cells secrete insulin and amylin, and delta cells secrete somatostatin. The two afore mentioned hormones, insulin and glucagon, that are necessary for the appropriate regulation of glucose, lipid, and protein metabolism, will be the main focus of the following section (8).

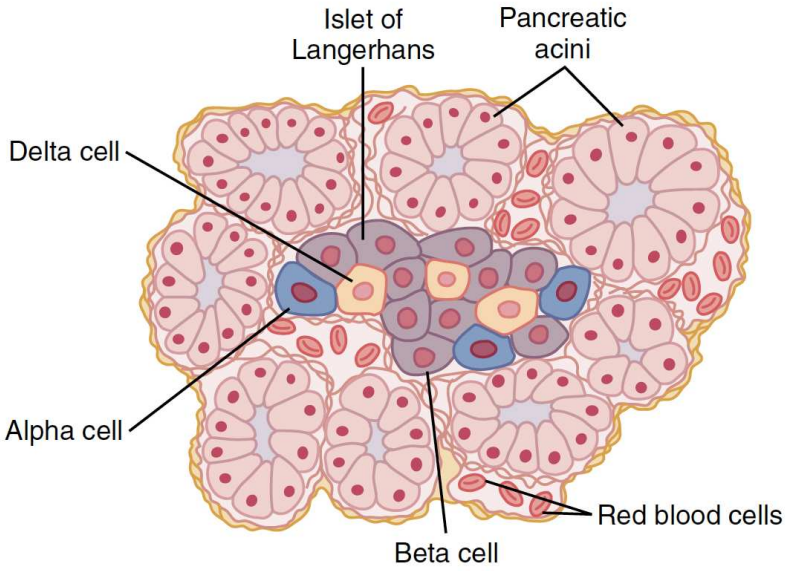


Figure 1. Physiologic anatomy of an islet of Langerhans in the pancreas (8).

1.3. Insulin

Insulin is a hormone associated with energy abundance. When there is an increased availability of energy-giving foods in the diet, especially high amounts of carbohydrates, insulin secretion increases in response. This consequently plays an important role in storing this excess energy in specific tissues. Excess carbohydrates are stored mainly in the liver and muscles as glycogen. In a state, in which carbohydrates are available in concentrations too high that they cannot be stored as glycogen, they are converted to fats and stored in adipose tissue under the direct stimulus of insulin. Insulin further plays a key role in protein metabolism, by having a direct effect in promoting amino acid uptake by cells and their consequent conversion into proteins. Furthermore, it inhibits the breakdown of proteins that are already available within the cells (8).

Human insulin is a small protein that has a molecular weight of 5808, is composed of two amino acid chains that are connected by disulfide linkages. It is synthesized in the pancreatic beta cells, beginning with translation of the insulin ribonucleic acid (RNA) by ribosomes attached to the endoplasmic reticulum to form preproinsulin, which is then cleaved in the endoplasmic reticulum to form proinsulin. The majority of the proinsulin is further cleaved in the Golgi apparatus to form insulin (8).

After insulin has been secreted into the blood via secretory granules, it has a plasma halflife of approximately 6 minutes and is mainly cleared from the circulation within 10 to 15 minutes, mostly by the enzyme insulinase in the liver and to a lesser extent in the kidneys and

muscles and slyghtly by most other tissues. This rapid removal from the plasma is important to rapidly turn off or turn on the control functions of insulin (8).

For the hormone to initiate its effects on target cells, it has to first bind with and activate a specific membrane receptor protein. This activated membrane receptor is then responsible for the subsequent effects. The insulin receptor, representing an enzyme-linked receptor, is made up of four subunits, two alpha and two beta subunits, that penetrate the cell membrane and protruding into the cytoplasm. Insulin then binds to the extracellular alpha subunits causing the beta subunits protruding into the cell to be autophosphorylated. Autophosphorylation activates a local tyrosine kinase, which further phosphorylates multiple other inreacellular enzymes including a group of insulin-receptor enzymes (IRS). These IRS are expressed in different types of tissues throughout the body and the net effect is to activate and inactivate some of these enzymes. By this mechanism, insulin directs the intracellular metabolic machinery to cause the desired effects on carbohydrate, fat, and protein metabolism (8).

The outcome and end-effects of insulin stimulation are summarized as following:

1. Within seconds after the activation of insulin receptors, approximately 80% of the body's cell's membranes increase their uptake of glucose, especially in muscle and adipose tissue, which is immediately transported into the cells and phosphorylated, and becomes a substrate for all the usual carbohydrate metabolic functions (8).
2. The cell's membrane permeability increases towards amino acids, potassium ions, and phosphate ions, causing an increased influx of these substances into the cell (8).
3. Within the next 10 to 15 minutes, activity levels of many intracellular metabolic enzymes change, as a result of phosphorylation of the enzymes (8).
4. After hours to several days slower effects continue to be seen. These result from changes in rates of translation of messenger RNA (mRNA) at the ribosomes to form new proteins and changed rates of transcription of deoxyribonucleic acid (DNA) in the cell nucleus. Thus, insulin remolds the cellular enzymatic machinery to achieve its metabolic goals (8).

1.4. Diabetes Mellitus

The syndrome of diabetes mellitus is comprised of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or a decreased sensitivity of the tissues to the hormone. It is defined as a fasting plasma glucose level of greater than or equal to 7.0 mmol/L or on medication for diabetes a raised blood glucose value (1). Diabetes mellitus

is categorized into two general types. Firstly, type 1 diabetes mellitus, which is caused by a lack of insulin secretion. Secondly, type 2 diabetes mellitus, that is caused by a decreased sensitivity of target tissues to the metabolic effect of insulin. Often this reduction in sensitivity to insulin is called insulin resistance. In both type 1 diabetes mellitus and type 2 diabetes mellitus we can find a change in metabolism of all the main foodstuff. The basic outcome of this insufficiency, either due to lack or resistance, on glucose metabolism is the prevention of efficient uptake and utilization of glucose by most cells of the body. Thus, glucose concentration increases, cell utilization of glucose decreases progressively, and utilization of fats and proteins increase (8).

1.5. Type 2 Diabetes Mellitus

Approximately 90 to 95 percent of all cases of diabetes mellitus is due to type 2 diabetes mellitus (8). The usual age of onset, for type 2 DM, occurs after the age of 30 and often commences between the ages of 50 to 60 years of age. Therefore the syndrome has been also referred to as adult-onset diabetes (8). In the past, mainly middle-aged and elderly people were affected, but within recent years, type 2 DM occurs increasingly frequently in children and young people (1). This trend appears to be caused by a marked increase in the prevalence of obesity in this vulnerable population and is the main risk factor for type 2 DM (8).

Type 2 DM is associated with hyperinsulinemia, hence. This results out of a compensatory response, by the beta cells of the pancreas, for diminished sensitivity of the target tissue to insulin. This diminished responsiveness to the hormones' metabolic effects is also referred to as insulin resistance. This insulin resistance consequently impairs carbohydrate utilization and storage, thus raising blood glucose levels and stimulating a compensatory increase in insulin secretion (8).

Currently the diagnosis of type 2 DM is based on glucose tolerance, which is classified into three broad categories: normal glucose homeostasis, diabetes mellitus and impaired glucose homeostasis. The glucose tolerance can be assessed using the fasting plasma glucose (FPG), oral glucose challenge, or the hemoglobin A1C (A1C). An FPG of less than 5.6 mmol/L, a plasma glucose of less than 11.1 mmol/L after an oral glucose challenge, and an A1C of less than 5.6% are considered to define normal glucose tolerance. Further, the International Expert Committee has issued diagnostic criteria for diabetes mellitus based on the following items: the FPG, the response to an oral glucose challenge, and A1C differ among individuals, and DM is defined as the level of glycemia at which diabetes-specific complications occur, rather than on deviations from a population based mean (9).

These diabetes-specific chronic complications affect a great range of organ systems and are responsible for the majority of morbidity and mortality associated with the disease. These chronic complications can be divided into vascular and non-vascular complications. Vascular complications can further be subdivided into microvascular complications, such as retinopathy, neuropathy, as well as nephropathy, and macrovascular complications, for example coronary heart disease, cerebrovascular disease, or peripheral arterial disease (9).

1.6. Anatomy of the Human Eye

The human eye is a globe-shaped organ, occupying the anterior part of the orbit. The eyeball in its rounded shape is disrupted anteriorly by the transparent cornea, which bulges outward, and represents about one-sixth of the total eye surface. Going in order from front to back, posterior to the cornea, we can find the anterior chamber, iris, pupil, the posterior chamber, lens, the postremal (vitreous) chamber, the retina, and the choroid (7) as seen in Figure 2.

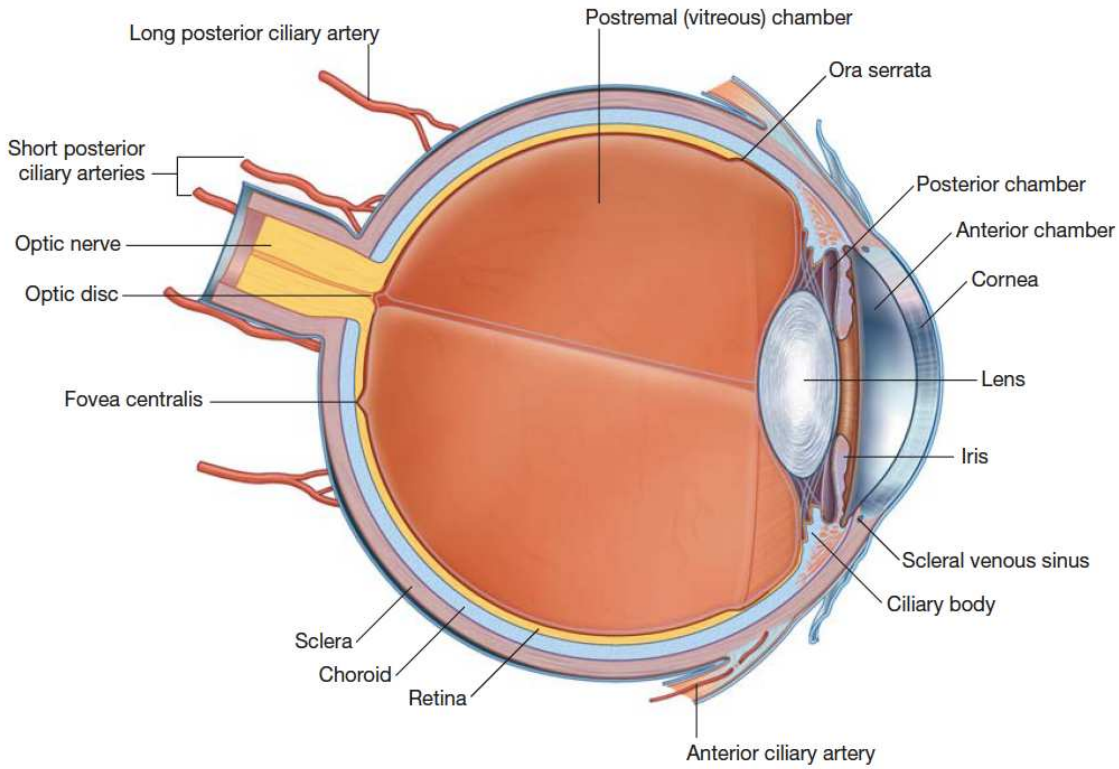


Figure 2. Cross sectional view of the human eyeball (7).

The part of interest, to this thesis, is the inner layer of the eyeball, the retina. The retina consists of two parts, the optic part of the retina and nonvisual part. The optic part takes up the posterior and lateral aspects of the retina and is sensitive to light. Further, the optic part of the retina consists of two layers, an inner neural and an outer pigmented layer. In contrast the nonvisual part is limited to the anterior surface and covers the internal surface of the ciliary body and the iris. These two parts are being divided at their junction by an irregular line called the ora serrata (7).

Several other features can be noted on the posterior surface of the optic part of the retina. The optic disc is an area which is lighter than the surrounding retina and is the location where the optic nerve leaves the retina. It is also the location at which branches of the central retinal artery spread from outward, to supply the retina. Since there is no light sensitive tissue in this area, it is also being referred to as the so-called blind spot in the retina. Beyond that, we can find another structure lateral to the optic disc. This small yellowish colored area is the macula lutea with its central depression, called the fovea centralis. Here we find the thinnest area of the retina which stands out in its high visual sensitivity relative to the surrounding tissue. This is due to decreased number in rods, the light-sensitive receptor cells that function in dim light and are not sensitive to color, and higher concentration of cones, the counterpart, which are light-sensitive receptor cells that respond to bright light and are sensitive to color (7).

1.7. Diabetic Retinopathy

The short definition of diabetic retinopathy is, that diabetic retinopathy is a neurovascular disease. This neurovascular disease is one of the main causes of acquired blindness at the age of 30 to 60 years of age in industrialized countries and approximately 90% of patients with DM will have retinopathy after 20 years. Further, 90% of all visual impairments in diabetic patients are caused by diabetic retinopathy and the overall prevalence accounts for 7% in industrialized countries (10).

As previously mentioned, diabetic retinopathy is a microvascular complication or in more specific terms, a microangiopathy (10). Hyperglycemia is considered to play an essential part in the pathogenesis of retinal microvascular damage. Numerous metabolic pathways have been suspected in hyperglycemia-induced vascular damage. These include the polyol pathway, advanced glycation end products accumulation, protein kinase C pathway, and the hexosamine pathway (11).

This microangiopathy ultimately leads to the thickening of the basement membrane of the vessels and a loss in pericytes and vascular endothelial cells. As afore mentioned, hyperglycemia, as described in patients with DM, plays a key role at its early stage. Later and due to the prolonged injury to the vessels, capillary closure occurs, which eventually leads to a hypoxic environment and consequently to retinal ischemia. In the ischemic retina angiogenic factors are produced, such as vascular endothelial growth factor and insulin-like growth factor-1. These contribute to new vessel formation in the pre-retinal area and iris. If at any stage, the blood-retina barrier breaks down and an increased vascular permeability occurs, macular edema may develop (10).

During the progression of diabetic retinopathy, retinal neurodegeneration can be observed as an early event. Animal studies involving diabetic rats have shown, that apoptosis of retinal neurons can be observed as early as one month after induction of diabetes. Upregulation of pro-apoptotic molecules such as Fas, Bax, and cleaved caspase-3 was detected in retinal neurons of both diabetic animals and human subjects. Further mitochondrial dysfunction has been implicated in diabetic retinopathy's retinal degeneration. Currently there is growing evidence that retinal neurodegeneration could be a separate pathophysiology of diabetic retinopathy (11).

Presently the most common international nomenclature used to describe the various changes seen in the patient with diabetic retinopathy, is based on the classification of the Diabetic retinopathy Study. The classification relies on the distinction between non-proliferative and proliferative stages with the characteristics of mild, moderate, and high risk, as well as clinically significant macular edema and ischemic maculopathy (10). The following Table 1 is a gives a comprehensive overview of the Diabetic Retinopathy Disease Severity Scale and International Clinical Diabetic Retinopathy Disease Severity Scale (12).

Table 1. Diabetic Retinopathy Disease Severity Scale and International Clinical Diabetic Retinopathy Disease Severity Scale (12).

Disease Severity Level	Findings upon Dilated Ophthalmoscopy
No apparent retinopathy	No abnormalities
Mild NPDR (see Glossary)	Microaneurysms only
Moderate NPDR	More than just microaneurysms but less than severe NPDR
Severe NPDR (International Definition)	Anything of the following and no signs of proliferative retinopathy: • More than 20 intraretinal hemorrhages in each of four retinal quadrants • Definite venous beading in two or more quadrants • Prominent IRMA (see Glossary) in one or more quadrants
PDR (see Glossary)	One or both of the following • Neovascularization • Vitreous/preretinal hemorrhage

IRMA = intraretinal microvascular abnormalities; NPDR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy

Note:

- Any patient with two or more of the characteristics of severe NPDR is considered to have very severe NPDR.
- PDR may be classified as high-risk and non-high-risk.

Symptoms of diabetic retinopathy appear late throughout the course of disease. Therefore, only in the late stages and with macular involvement or vitreous hemorrhage will the affected individual notice visual impairment or even sudden blindness (10).

1.8. Physiologic Anatomy of the Human Nervous System

The human nervous system is formed by a network of many billion nerve cells, also called neurons, and forms by far the most complex system in the body. Further, all neurons are being supported by many cells called glial cells. Through a complex network of neurons, in which every neuron interconnects a hundredfold to other neurons, they form a very complex system for processing information and generating responses. The nervous system (NS) and its distribution throughout the body forms an integrated communications network. Viewed from an anatomical angle, the general organization of the NS has two main divisions. First, the central nervous system (CNS), consisting of the brain and spinal cord, and the peripheral nervous system (PNS), composed of the cranial, spinal, and peripheral nerves. Therefore, the PNS is responsible for conducting impulses to and from the CNS (respectively sensory and motor nerves) and ganglia (small aggregates of nerve cells outside the CNS) (13).

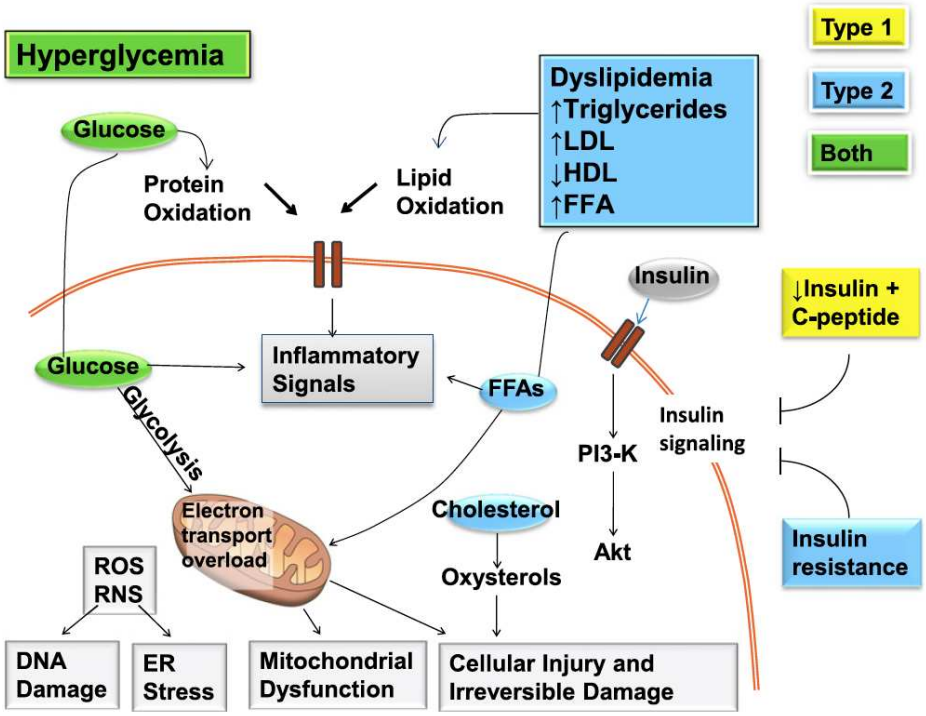
Neurons possess the capability to respond to environmental changes, also referred to as stimuli, by changing the ionic gradient that exists across their cell membranes. This gradient, also called an electrical potential, is universal to all cells. Only cells that can rapidly change this potential in response to a stimulus are said to be excitable. Neurons can instantly react to such stimuli through the reversal of the ionic gradient or in other terms via membrane depolarization. This depolarization usually spreads from the place of reception across the neuron's entire plasma membrane. This propagation called the action potential (AP) is capable of propagating long distances along neuronal processes. By this mechanism such signals are stimulating other neurons, muscles or even glands. Through collecting, analyzing, and integrating information as AP, the NS continuously stabilizes the intrinsic homeostasis of the organism within normal ranges and maintains behavioral patterns (13).

1.9. Diabetic Neuropathy

Diabetic neuropathies are the most common chronic complications of diabetes and are defined as "signs and symptoms of peripheral nerve dysfunction in a patient with diabetes mellitus in whom other causes of peripheral nerve dysfunction have been excluded" ((14)(15). Further, diabetic neuropathy is one of the most common causes of peripheral neuropathy and accounts for more frequent hospitalizations than any other complication of diabetes mellitus and above that it poses the most frequent indication for non-traumatic amputation (14).

The etiology of diabetic neuropathy remains unknown up to date, but experimental studies suggest a multifactorial pathogenesis, with ischemic and metabolic components implicated. A prevailing view of the pathogenesis is that inflammatory and oxidative stress may, in the context of metabolic dysfunction, damage nervous tissue (15).

Hyperglycemia is thought to play an essential part in the pathogenesis of the disease by inducing rheological changes. These changes increase the endothelial vascular resistance and thereby reduce blood flow to the nerve. Further hyperglycemia also causes depletion of the nerve's myoinositol, a glucose derivative, via a competitive uptake mechanism. The activation of the polyol pathway in the nerve leads to an accumulation of sorbitol and fructose in the tissue, thus inducing a non-enzymatic glycosylation of structural nerve proteins. Hyperglycemia also causes oxidative stress and activation of protein kinase C, which was linked to vascular damage in diabetic neuropathy. Through direct measurement of glucose, sorbitol, and fructose, in nervous tissue of patients with DM, a correlation with the severity of neuropathy could be established. All these changes lead to an abnormal neuronal, axonal, and Schwann cell metabolism, which ultimately cause an impaired axonal transport (14). A comprehensive overview of the involved mechanisms of diabetic neuropathy can be seen in the following Figure 3.



ER = endoplasmic reticulum; FFA = free fatty acids; PI3-K = phosphatidylinositol-3 kinase; RNS = reactive nitrogen species; ROS = reactive oxygen species

Note:

- Factors linked to type 1 diabetes (yellow), type 2 diabetes mellitus (blue), and both (green) cause DNA damage, endoplasmic reticulum stress, mitochondrial dysfunction, cellular injury, and irreversible damage.
- The relative importance of the pathways in this network will vary with cell type, disease profile, and time.

Figure 3. Mechanisms of diabetic neuropathy (15).

Until today, diabetic neuropathy is a diagnosis of exclusion and the most common forms encountered in practice are distal symmetric polyneuropathy and autonomic neuropathy. This heterogeneous group of conditions can affect different parts of the nervous system and may presents as a diverse clinical picture. The following Table 2 provides a comprehensive classification scheme, based on the position paper by the American Diabetes Association from 2017, for the diabetic neuropathies.

Table 2. Classification for diabetic neuropathies from Diabetic Neuropathy: A Position Statement by the American Diabetes Association

Diabetic neuropathies
A. Diffuse neuropathy
Distal Symmetric Polyneuropathy
Primarily small-fiber neuropathy
Primarily large-fiber neuropathy
Mixed small- and large-fiber neuropathy (most common)
Autonomic
Cardiovascular
Reduced HRV
Resting tachycardia
Orthostatic hypotension
Sudden death (malignant arrhythmia)
Gastrointestinal
Diabetic gastroparesis (gastropathy)
Diabetic enteropathy (diarrhea)
Colonic hypomotility (constipation)
Urogenital
Diabetic cystopathy (neurogenic bladder)
Erectile dysfunction
Female sexual dysfunction
Sudomotor dysfunction
Distal hypohydrosis/anhidrosis,
Gustatory sweating
Hypoglycemia unawareness
Abnormal pupillary function
B. Mononeuropathy (mononeuritis multiplex) (atypical forms)
Isolated cranial or peripheral nerve (e.g., cranial nerve III, ulnar, median, femoral, peroneal)
Mononeuritis multiplex (if confluent may resemble polyneuropathy)
C. Radiculopathy or polyradiculopathy (atypical forms)
Radiculoplexus neuropathy (a.k.a. lumbosacral polyradiculopathy, proximal motor amyotrophy)
Thoracic radiculopathy
Nondiabetic neuropathies common in diabetes
Pressure palsies
Chronic inflammatory demyelinating polyneuropathy
Radiculoplexus neuropathy
Acute painful small-fiber neuropathies (treatment-induced)

2. OBJECTIVES

The objective of this study was to provide a comprehensive insight into common pathological mechanisms of diabetic retinopathy and neuropathy. Thus, we aimed at studying patients with long-standing type 2 diabetes mellitus who were prone to have detectable and gradable diabetic retinopathy and evaluate a possible association with sensitive measures of diabetic neuropathy.

Hypothesis

1. There is no correlation between diabetic retinopathy and diabetic neuropathy.
2. There is no correlation between diabetic retinopathy and age.
3. There is a no correlation between diabetic retinopathy and duration of insulin therapy.
4. There is no correlation between diabetic retinopathy and duration of type 2 diabetes mellitus.
5. There is no correlation between diabetic sensory-motor neuropathy and age.
6. There is a no correlation between diabetic sensory-motor neuropathy and duration of insulin therapy.
7. There is no correlation between diabetic sensory-motor neuropathy and duration of type 2 diabetes mellitus.

3. MATERIALS AND METHODS

3.1. Study Design

This study was conducted at the Department of Ophthalmology and at the Department of Neurology of the University Hospital of Split. It was carried out in adherence to the tenets of the Declaration of Helsinki and Good Clinical Practice guidelines. According to the population-based design, this is a cohort study. The ethical approval for this study was obtained by the University of Split School of Medicine.

3.2. Subjects

The population-based cohort study included patients from the Split-Dalmatia county, creating a total sample size of twenty-nine examinees. Twenty-nine adult patients with type 2 diabetes mellitus, 11 female and 18 male patients, with a diagnosis of more or equal to 1 year were included into the study. Of those patients that were screened, 1 patient presented with no diabetic retinopathy or features of other ocular pathologies, such as non-proliferative diabetic retinopathy or macular edema.

3.3. Materials and Methods

After written informed consent was obtained of all examinees, all participants underwent a detailed screening for medical history, duration of type 2 diabetes mellitus diagnosis, duration of insulin therapy, grade of diabetic retinopathy and clinically significant macular edema, using the Diabetic Retinopathy Disease Severity Scale and International Clinical Diabetic Retinopathy Disease Severity Scale and a detailed ophthalmologic examination according to the protocol, as well as sensory-motor neuropathy and radiculopathy, using electromyographic studies with the Medelec Synergy 5 Channel EMG/EP Plinth System (Oxford Instruments Medical Ltd, U.K., 2004).

3.3.1. Grading of Diabetic Retinopathy and Clinically Significant Macular Edema

For the grading of diabetic retinopathy and clinically significant macular edema the Diabetic Retinopathy Disease Severity Scale and International Clinical Diabetic Retinopathy Disease Severity Scale were used as introduced by the Early Treatment of Diabetic Retinopathy Study (ETDRS). Diabetic retinopathy was classified as absent, mild, moderate, severe, or

proliferative in accordance to the criteria established in the Early Treatment Diabetic Retinopathy Study. This was achieved by comparing stereophotographs in seven standard color fundus photographic fields, with the examinees findings in those same seven defined fields (16).

Further, as introduced by the EDTRS, clinically significant macular edema (CSME) was defined upon slit lamp biomicroscopy as "(1) thickening of the retina at or within 500 μm of the center of the macula; or (2) hard exudate at or within 500 μm of the center of the macula associated with thickening of adjacent retina; or (3) a zone of retinal thickening 1 disc area or larger, any part of which is within 1 disc diameter of the center of the macula" (16).

3.3.2. Grading of Sensory-Motor Neuropathy and Radiculopathy

The diagnosis of diabetic neuropathy is principally a clinical one (15). In the evaluation of patients with neuropathy or nerve injury, it is important to assess the physiologic status of peripheral nerves with nerve conduction studies, such as electromyography, in order to assess and correlate their physiological status with their clinical symptoms (17). Therefore, with the support of the Department of Neurology of the Medical University of Split, we developed a grading system based on the electromyographic examination of the common peroneal nerve. For that purpose, the conduction velocity of the common peroneal nerve was recorded and on the basis of those findings the grading system was formed. The grading comprised of four grades ranging from absent neuropathy to very sever neuropathy. The following Table 3 will provide a comprehensive overview of the grading system for diabetic neuropathy, developed by the Department of Neurology of the Medical University of Split for the purpose of this study.

Table 3. Grading System for Diabetic Neuropathy as Developed by the Department of Neurology of the Medical University of Split

Grade	Description	Conduction Velocity
Grade 0	Without neuropathy	>45 m/s
Grade 1	Mild neuropathy	41-44 m/s
Grade 2	Moderate neuropathy	35-40 m/s
Grade 3	Severe neuropathy	30-34 m/s
Grade 4	Very severe neuropathy	<30 m/s

3.4. Statistical Analysis

Descriptive analysis included the presentation of data as absolute numbers and percentages for categorical and ordinal variables, further medians and interquartile range (IQR) were used in the case of a non-normal distribution of a numeric variable or a mean and standard deviation (SD) was used in the case of normally distributed numeric variable. The distribution of numeric variables was tested using a Kolmogorov-Smirnov test where non-parametric tests were used if we obtained a $P < 0.05$ while parametric tests were used if $P \geq 0.05$. Bivariate statistical analysis was performed using Spearman Rank correlation test (referred to as r) or Pearson's correlation coefficient.

The statistical analysis was performed, using the software STATISTICA 12 (TIBCO Software Inc. v12.0), and the *P* value significance threshold was set to <0.05.

4. RESULTS

4.1 Patient Characteristics

A comprehensive and detailed description of the examined participants' characteristics can be found in Table 2.

Table 2. Sociodemographic Characteristics of Participants

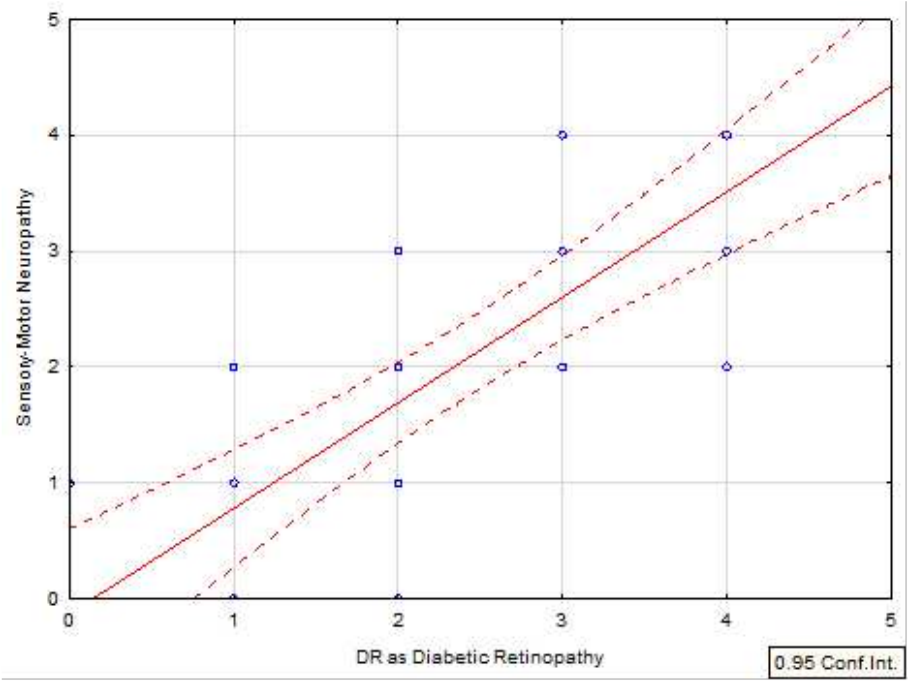
Gender; n (%)	
Male	18 (62.07)
Female	11 (37.93)
Age (years); (mean ± SD)	62.34 ± 11.73
Duration (years) of Type 2 DM Status; (mean ± SD)	15 ± 7.71
Duration (years) of Insulin Therapy; n (median [IQR]))	16 (4 [3-10])
Grade of Diabetic Retinopathy; n (median [IQR])	29 (2 [1-4])
Grade of Sensory-Motor Neuropathy; n (median [IQR])	29 (2 [1-3])
Grade of Diabetic Retinopathy; n (%)	
Grade 0	1 (3.45)
Grade 1	7 (24.14)
Grade 2	8 (27.59)
Grade 3	5 (17.24)
Grade 4	8 (27.59)
Clinically Significant Macular Edema; n (%)	
Yes	16 (55.17)
No	13 (44.83)
More affected Eye by Diabetic Retinopathy; n (%)	
Right	11 (37.93)
Left	11 (37.93)
Equal	7 (24.14)
Grade of Sensory-Motor Neuropathy; n (%)	
Grade 0	6 (20.69)
Grade 1	3 (10.34)
Grade 2	9 (31.03)
Grade 3	5 (17.24)
Grade 4	6 (20.69)
Radiculopathy; n (%)	
Yes	11 (37.93)
No	18 (62.07)

SD = Standard Deviation; IQR = Interquartile

4.2 Diabetic Retinopathy and Sensory-Motor Neuropathy

There was a significant strong positive correlation (r = 0.81) between sensory-motor neuropathy and diabetic retinopathy ($P < 0.001$). The following Figure 1 visualizes the correlation.

Figure 1. Correlation between sensory-motor neuropathy and diabetic retinopathy



Testing for the correlation between the grade of diabetic retinopathy and the age (years) of patients, we found a weak positive correlation (r = 0.21), which did not reach statistical significance ($P = 0.26$). Further, we found a negative, moderate (r = -0.57) correlation between the grade of diabetic retinopathy and duration of insulin therapy (years), that reached statistical significance ($P = 0.02$). The grade of diabetic retinopathy and duration of type 2 diabetes mellitus (years) patient status showed a very weak correlation (r = 0.0037) and no statistical significance ($P = 0.98$). A weak positive (r = 0.06) correlation between the grade of diabetic sensory-motor neuropathy and age (years), that did not reach statistical significance ($P = 0.76$), could be established. A significant negative moderate (r = -0.53) correlation was found between the grade of diabetic sensory-motor neuropathy and duration of insulin therapy ($P = 0.035$). Finally, there was no significant correlation between the grade of diabetic sensory-motor neuropathy and duration of disease (years) with type 2 diabetes mellitus (r = 0.08, $P = 0.666$).

5. DISCUSSION

Diabetes is a global burden and awareness is on the rise. Nevertheless, there is a lack of knowledge about the disease itself and its severe complications (18). In 2010 diabetic retinopathy caused 1.9% of visual impairment and 2.6% of blindness globally (19). Meanwhile, studies suggest that the prevalence of any retinopathy in patients with diabetes is 35%, at the same time as proliferative retinopathy is 7% (20). Diabetic neuropathy still is a diagnosis of exclusion and there are currently no approved disease modifying therapies (15). The pathogenesis of both complications, diabetic retinopathy and diabetic sensory-motor neuropathy, is the same and therefore the occurrence of any one complication may indicate the presence or even reflect on the severity of disease in an affected person (18). If detected early on and treated appropriately, both these complications may be reversible up to a certain extent (18).

To our knowledge, this is the first study to investigate correlations between the grade of diabetic retinopathy and the degree of sensory-motor neuropathy using objective diagnostic methods. Further we aimed at establishing further correlations between diabetic retinopathy and diabetic sensory-motor neuropathy to the patients' age, duration of insulin therapy, as well as duration of type 2 diabetes mellitus disease status, in the area of the Split-Dalmatia county. Evaluation of the data yielded three main results. First, we were able to establish a significant and strong positive correlation between the grade of diabetic retinopathy and the grade of diabetic neuropathy. Further, we could show that there is a significant, moderate negative correlation between the grade of diabetic retinopathy and the duration of insulin therapy. Finally, we were able to prove that there is a significant, moderate negative correlation that applies to the grade of diabetic sensory-motor neuropathy and duration of insulin therapy. By establishing a significant and strong positive correlation between the grade of diabetic retinopathy and the grade of diabetic neuropathy, we may be able to develop future screening programs and therapy approaches that take both pathologies into account. We are aware that these findings are limited by the sample size as well as the lack in long term observation of the patients. Nonetheless, we should see this as an early approach and incentive to continue research in this field.

The pathology and physiology of diabetic retinopathy and diabetic sensory-motor neuropathy have many triggering mechanisms in common. Hyperglycemia induces distinct metabolic pathways, such as the hexosamine pathway, activation of diacylglycerol and protein kinase C, non-enzymatic glycosylation polyol pathway, formation of advanced glycosylation end-products, and production of reactive oxygen species (21,22). Further, recent research has focused on the implications of chronic low-grade inflammation in the pathogenetic mechanisms

of diabetic retinopathy(23,24). Nevertheless, the complete mechanisms underlying neuronal degeneration in the retina is not yet fully comprehended, but elevated levels of glutamate, Müller cell activation, and the overexpression of the renin-angiotensin system by glial cells are currently reported to play an essential role (25–27). Although there is an extensive availability of diverse human and animal studies, mechanisms of possible associations between retinal neurodegeneration and vasculopathy remain unclear and require further research (28). Thus, understanding the underlying mechanisms of neurodegeneration is important for identifying new therapeutic targets and in the future prevention of advanced diabetic retinopathy (28). We hope that through the findings of this study future and further understanding of the pathophysiology of diabetic retinopathy and diabetic sensory-motor neuropathy and its relationship with diabetic systemic neuropathy may be facilitated.

In conclusion, this study should indicate timely and thorough ophthalmologic fundoscopic examination in diabetic patients presenting with clinical or subclinical sensory-motor neuropathy. Further, retinal changes, that indicate diabetic retinopathy, should entail a more detailed interrogation of peripheral nerve symptoms and a more stringent diabetic control is recommended in hopes of delaying future retinopathy (28).

6. CONCLUSION

This study investigated the correlation between the grade of diabetic retinopathy and the grade of diabetic neuropathy, which yielded the following main results.

1. A significant and strong positive correlation between the grade of diabetic retinopathy and the grade of diabetic neuropathy could be established.
2. A significant, moderate negative correlation between the grade of diabetic retinopathy and the duration of insulin therapy was observed.
3. A significant, moderate negative correlation that applies to the grade of diabetic sensory-motor neuropathy and duration of insulin therapy was proven.

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8. SUMMARY

Objectives: The objective of this study was to provide a comprehensive insight into common pathological mechanisms of diabetic retinopathy and neuropathy. Thus, we aimed at studying patients with long-standing type 2 diabetes mellitus who were prone to have detectable and gradable diabetic retinopathy and evaluate a possible association with sensitive measures of diabetic neuropathy.

Subjects and methods: The population-based cohort study included patients from the Split-Dalmatia county, creating a total sample size of twenty-nine examinees. Twenty-nine adult patients with type 2 diabetes mellitus, 11 female and 18 male patients, with a diagnosis of more or equal to 1 year, were included into the study. All participants underwent a detailed screening for medical history, duration of type 2 diabetes mellitus diagnosis, duration of insulin therapy, grade of diabetic retinopathy and clinically significant macular edema, using the Diabetic Retinopathy Disease Severity Scale and International Clinical Diabetic Retinopathy Disease Severity Scale and a detailed ophthalmologic examination according to the protocol, as well as assessment of sensory-motor neuropathy and radiculopathy, using electromyographic studies with the Medelec Synergy 5 Channel EMG/EP Plinth System (Oxford Instruments Medical Ltd, U.K., 2004).

Results: There was a significant strong positive correlation ($r = 0.81$) between sensory-motor neuropathy and diabetic retinopathy ($p < 0.001$). A negative, moderate ($r = -0.57$) correlation between the grade of diabetic retinopathy and duration of insulin therapy (years), that reached statistical significance ($p = 0.02$) could be established. A significant negative moderate ($r = -0.53$) correlation was found between the grade of diabetic sensory-motor neuropathy and duration of insulin therapy ($p = 0.035$).

Conclusions: We found a strong correlation between diabetic retinopathy and diabetic sensory-motor neuropathy. Thus, patients presenting with diabetic retinopathy should be further questioned and investigated for diabetic sensory-motor neuropathy.

9. CROATIAN SUMMARY

Naslov: Korelacija između dijabetičke retinopatije i motorno-senzorne neuropatije, kohortno istraživanje

Ciljevi: Cilj ovog istraživanja je bio pružiti sveobuhvatan uvid u zajedničke patološke mehanizme dijabetičke retinopatije i neuropatije. Stoga smo proučavali pacijente s dugogodišnjim dijabetesom tipa 2 koji imaju dijabetičku retinopatiju i procijenili moguću povezanost sa senzibilnim pokazateljima dijabetičke neuropatije.

Materijali i metode: Kohortno istraživanje uključivalo je bolesnike iz splitsko-dalmatinske županije, stvarajući ukupnu veličinu uzorka od 29 ispitanika. U istraživanju je uključeno 29 odraslih bolesnika s dijabetesom melitusom tipa 2 od kojih su 11 ženskog spola i 18 muškog spola, s dijagnozom više ili jednako 1 godine. Svi sudionici podvrgnuti su detaljnom pregledu povijesti bolesti, trajanja dijagnoze dijabetesa melitusa tipa 2, trajanja terapije inzulinom, stupnja dijabetičke retinopatije i klinički značajnog makularnog edema, uporabom Diabetic Retinopathy Disease Severity Scale i International Clinical Diabetic Retinopathy Disease Severity Scale, detaljnom oftalmološkom pregledu prema protokolu, kao i procjenu motorno-senzorne neuropatije i radikulopatije, korištenjem elektromiografskih metoda s Medelec Synergy 5 Channel EMG/EP Plinth System (Oxford Instruments Medical Ltd, U.K., 2004).

Rezultati: Postojala je značajna pozitivna korelacija (r = 0,81) između motorno-senzorne neuropatije i dijabetičke retinopatije (P <0,001). Može se utvrditi negativna, umjerena (r = -0.57) korelacija između stupnja dijabetičke retinopatije i trajanja terapije inzulinom, koja je postigla statističku značajnost (P = 0.02). Značajna negativna umjerena (r = -0,53) korelacija pronađena je između stupnja dijabetičke motorno-senzorne neuropatije i trajanja terapije inzulinom (P = 0,035).

Zaključci: Pronašli smo izraženu korelaciju između dijabetičke retinopatije i dijabetičke motorno-senzorne neuropatije. Stoga se pacijenti s dijabetičkom retinopatijom trebaju dodatno ispitivati i pregledati za dijabetičku motorno-senzornu neuropatiju.

10. CURRICULUM VITAE

Daniel Hyun Gunderlach

Address:	Matice Hrvatske 100
	21000 Split, Croatia
Phone:	+385 955 194 880
E-mail:	gunderlach@hotmail.de

Personal Information:

Date of Birth:	23. December 1991
Place of Birth:	Berlin, Germany
Nationality:	German

Education:

2018, September	Degree, Medical Doctor (MD), Medical Studies in English at the University of Split, School of Medicine, Split, Croatia
2011, June	General Higher Education Entrance Qualification in Germany, John F. Kennedy School, Berlin, Germany
2010, June	American High School Diploma, John F. Kennedy School, Berlin, Germany