

NEURODIAB 2017

27TH ANNUAL MEETING

OF THE DIABETIC NEUROPATHY STUDY GROUP OF THE
EUROPEAN ASSOCIATION FOR THE STUDY OF DIABETES (EASD)



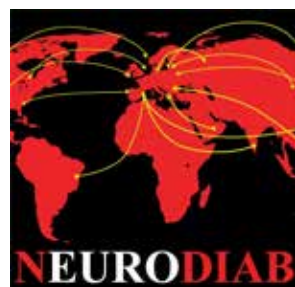
PROGRAM AND
ABSTRACTS

9 to 11
SEPTEMBER

2017
Hotel Vila Galé

COIMBRA
PORTUGAL

www.neurodiab2017.com



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General Information

UNIVERSITY TOWER

The clock has played a central role in the University daily life for the activities of the institution have long been organised according to it.

GENERAL INFORMATION

ABOUT COIMBRA

Coimbra is a very beautiful and peaceful town situated on the Mondego River, approximately 185 km Northeast of Lisbon and 98 km Southeast of Oporto. The climate is mild and the rainfall averages 750 mm annually. The town is very well served of public transports and is easily accessible both by bus or fast trains either from Lisbon or Oporto.

The life of the city depends primarily on the University of Coimbra. As in medieval universities, the students wear long black capes and ribbons of varying color to distinguish the various faculties. The institution was founded in 1290 and it is one of the oldest universities in Europe. Like an acropolis, the white buildings of the University now dominate the hilltop overlooking the north bank of the river.

Coimbra has museums of natural history and archaeology and a botanical garden. The first cathedral, Sé Velha, in the middle of the old city, is one of the best examples of Romanesque churches in Portugal. The new Cathedral, Sé Nova, begun by the Jesuits in the late 16th century and consecrated a cathedral in 1772, has a single nave in the Roman style. Santa Cruz, a church dated mostly from the Renaissance, is famed for its cloisters and for the tombs of Afonso Henriques and his son Sancho I, the first two kings of Portugal. In the convent of Santa Clara, built on the height overlooking the south bank of the Mondego, are the remains of the sainted Queen Elisabeth, patroness of the city and of the University.



A multi-tiered stone fountain is the central focus, set in a lush garden. The fountain has a conical top with water cascading down through several tiers. The surrounding foliage is vibrant, with trees showing autumnal red and orange leaves on the left and dense greenery on the right. The scene is captured in bright, natural light, creating a serene and romantic atmosphere.

About Neurodiab

Botanical Garden of the University of Coimbra

Central Square / Fountain

This terrace can be considered the “cradle” of the garden. The typical characteristics of the neoclassical style are well represented here. Diversity reigns in this place, where varied Magnolias, Garden Cherry, Azaleas, among others, flank a great Central Fountain, transmitting to this area all the atmosphere of Romanticism.



Simona Frontoni MD, PhD

Chairman, Neurodiab

*Professor of Endocrinology,
University of Rome Tor Vergata
Rome, Italy*

*Chief, Endocrinology and
Diabetes – San Giovanni
Calibita Fatebenefratelli
Hospital, Rome, Italy*



Isaura Tavares

*Associate Professor with Tenure
Faculdade de Medicina do Porto*

*PhD in Human Biology
Faculdade de Medicina do Porto*

*Research at I3S – Institute
for Research and Innovation
in Health, University of Porto,
Portugal*

Dear Friends and Colleagues,

It is our great pleasure to welcome you to the **27th Annual Scientific Meeting of NEURODIAB** at Hotel Vila Galè, Coimbra, Portugal.

The 2017 meeting will be, as in past editions, the place to meet old friends and make new ones from all over the world, and to share clinical and experimental experiences in the field of diabetic neuropathy.

Young researchers in the field will have the opportunity to exchange their research ideas and to spend time with senior scientists in order to get advice and improve their projects.

The meeting will include oral and poster sessions, keynote lectures and symposia, in the intimate atmosphere always present among the small number of attendees at the Neurodiab meetings.

We really hope you'll enjoy the meeting!

Simona Frontoni
Chairman of Neurodiab

Isaura Tavares
On behalf of the Local
Organising Committee



Program Overview

Botanical Garden of the University of Coimbra

The Botanical Garden of the University of Coimbra, located in the heart of the city of Coimbra since 1772.

The collections of plants that fill each space transport us to different latitudes and regions of the world, transforming the Garden into a real living museum.

SATURDAY – 9 SEPTEMBER 2017

13h30 – 14h00 Registration & Coffee

14h00 – 14h30 **Welcome**
Simona Frontoni

14h30 – 15h30 **Göran Sundkvist Clinical Award**
Chairs: Simona Frontoni and Sanjeev Sharma
Presenter: Fabiana Picconi
Presenter: Anna Korei

15h30 – 17h30 Oral Session: **Young Investigators Oral Presentations**
Chairs: Henning Andersen and Abd Tahrani

17h45 – 18h45 **Sponsored Symposium IMPETO MEDICAL: SUDOSCAN a new point-of-care device for DPN**
Chair: Peter Kempler
Introduction
Sudomotor dysfunction early in diabetes and in painful and painless DPN
Speaker: Dan Ziegler
The One Stop Microvascular Screening Service
Speaker: Solomon Tesfaye
Discussion

SUNDAY – 10 SEPTEMBER 2017

- 08h00 – 08h30** **Pain modulation from the brain during Diabetic Neuropathy**
Chairs: Rayaz Malik and Christian Quattrini
Speaker: Isaura Tavares
- 08h30 – 10h00** Oral Session: **Small Fibers**
Chairs: Rayaz Malik and Christian Quattrini
- 10h00 – 10h15** **Coffee and Exhibition**
- 10h15 – 10h45** **Visualization of pancreatic islet cell signal – transduction, applying the anterior chamber of the eye as a transplantation site and the cornea as a natural body window**
Chairs: Peter Kempler and Vincenza Spallone
Speaker: Per Olof Berggren
- 10h45 – 12h15** Oral Session: **Autonomic Neuropathy**
Chairs: Peter Kempler and Vincenza Spallone
- 12h15 – 13h15** **Sponsored Symposium WÖRWAG PHARMA: Neuropathy in Diabetic Children – Never Forget Pathogenetic Treatment**
Chairs: Solomon Tesfaye and Peter Kempler
Opening and Introduction
Summary on neuropathy in diabetic children and adolescents
Speaker: Laszlo Barkai
Update on pathogenetic vs symptomatic treatment of diabetic polyneuropathy
Speaker: Dan Ziegler
Discussion
- 13h15 – 14h15** **Lunch**
- 14h15 – 16h15** Poster Session: **Young Investigators Poster Presentations**
Chairs: Isaura Tavares and Gerry Rayman
- 16h15 – 16h30** **Coffee and Exhibition**
- 16h30 – 18h30** Poster Session: **Pathogenesis and treatment**
Chairs: Mark Yorek and Luciano Bernardi
- 16h30 – 18h30** Poster Session: **Autonomic neuropathy**
Chairs: Paul Valensi and Rodica Pop-Busui
- 16h30 – 18h30** Poster Session: **Diagnosis and Small Fibers**
Chairs: Tamás Varkonyi and Soroku Yagihashi
- 18h30 – 19h00** **Lifetime achievement award ceremony**
- 19h00 – 20h00** **General Assembly**

MONDAY – 11 SEPTEMBER 2017

08h00 – 08h30 Painful Neuropathy in Diabetes: a clinical approach

Chairs: Dan Ziegler and Eirik Softeland

Speaker: Troels Jensen

08h30 – 10h00 Oral Session: **Treatment**

Chairs: Dan Ziegler and Eirik Softeland

10h00 – 10h30 **Coffee and Exhibition**

10h30 – 11h00 **Sponsored Lecture SIGMA-TAU: Pharmacological management of painful sensorimotor polyneuropathy**

Speaker: Paolo Marchettini

11h00 – 12h30 Oral Session: **Pathogenesis and Diagnosis**

Chairs: Solomon Tesfaye and Prashanth Vas

12h30 – 12h45 **Closing Remarks**

Simona Frontoni



Daily Program

Chapel of St. Michael of the University of Coimbra

The Chapel of the University of Coimbra - Chapel of St. Michael- dates back to the sixteenth century, probably built on an old chapel of the XII century, Manueline style.

The background is a solid red color. Overlaid on this are several thick white lines that intersect to form a network of irregular polygons. Three of these intersection points are highlighted with solid white circles. The lines and circles are arranged in a way that suggests a network or a map. The text is positioned in the lower right quadrant of the image.

SATURDAY
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Presenter: Fabiana Picconi
Presenter: Anna Korei

15h30 – 17h30 Oral Session: **Young Investigators Oral Presentations**
Chairs: Henning Andersen and Abd Tahrani

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|----|--|--|
| 01 | A magnetic resonance imaging study of brain volume changes in diabetic peripheral neuropathy | <i>Francesca Heiberg-Gibbons, United Kingdom</i> |
| 02 | Simultaneous pancreas kidney transplantation in Type 1 diabetes <i>mellitus</i> is associated with an early small and later large nerve fibre improvement | <i>Shazli Azmi, United Kingdom</i> |
| 03 | Abnormal somatomotor network functional connectivity in subjects with painful diabetic neuropathy: a resting state functional magnetic resonance imaging study | <i>Mohammed Awadh, United Kingdom</i> |
| 04 | Differential association of cardiac autonomic function with insulin sensitivity and secretion in recently diagnosed Type 1 and Type 2 diabetes | <i>Gidon Bönhof, Germany</i> |
| 05 | Increased dermal microvasculature in painful diabetic neuropathy | <i>Pallai Shillo, United Kingdom</i> |
| 06 | Study of the impact of the degree of peripheral neuropathy associated to diabetes on pressure ulcer incidence and healing process | <i>Noëlle Remoué, France</i> |
| 07 | Cardiovascular autonomic neuropathy is associated with bone metabolism and possibly mediated through kidney function in patients with Type 1 diabetes | <i>Christian Stevns Hansen, Denmark</i> |
| 08 | Elevated Proprotein convertase subtilisin/kexin-type 9 (PCSK9) is associated with small fibre neuropathy. | <i>Shazli Azmi, United Kingdom</i> |

17h45 – 18h45 **Sponsored Symposium IMPETO MEDICAL: SUDOSCAN a new point-of-care device for DPN**
Chair: Peter Kempler
Introduction
Sudomotor dysfunction early in diabetes and in painful and painless DPN
Speaker: Dan Ziegler
The One Stop Microvascular Screening Service
Speaker: Solomon Tesfaye
Discussion

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**SUNDAY
10 SEPTEMBER 2017**

SUNDAY – 10 SEPTEMBER 2017

08h00 – 08h30 Pain modulation from the brain during Diabetic Neuropathy

Chairs: R ayaz Malik and Christian Quattrini

Speaker: Isaura Tavares

08h30 – 10h00 Oral Session: Small Fibers

Chairs: Rayaz Malik and Christian Quattrini

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| 09 | Langerhans cell density in recent-onset Type 2 diabetes recovers after five years in association with preserved intraepidermal nerve fiber density | <i>Alexander Strom, Germany</i> |
| 010 | Corneal confocal microscopy detects small fibre neuropathy and nerve regeneration after an improvement in glycaemic control in subjects with LADA | <i>Uazman Alam, United Kingdom</i> |
| 011 | Small fibre dysfunction in chemotherapy induced peripheral neuropathy (CIPN) – A prospective study using methods of small fibre function and structure | <i>Sanjeev Sharma, United Kingdom</i> |
| 012 | Corneal endothelial damage is not related to corneal nerve fibre loss in patients with diabetes | <i>Maryam Ferdousi, United Kingdom</i> |
| 013 | Unusual cases of isolated diabetes small fibre neuropathy complicated by charcot neuroarthropathy of the foot | <i>Sanjeev Sharma, United Kingdom</i> |
| 014 | Corneal confocal microscopy detects corneal nerve damage in patients admitted with acute Ischemic stroke | <i>Adnan Khan, Qatar</i> |

10h00 – 10h15 Coffee and Exhibition

10h15 – 10h45 Visualization of pancreatic islet cell signal – transduction, applying the anterior chamber of the eye as a transplantation site and the cornea as a natural body window

Chairs: Peter Kempler and Vincenza Spallone

Speaker: Per Olof Berggren

10h45 – 12h15 Oral Session: Autonomic Neuropathy

Chairs: Peter Kempler and Vincenza Spallone

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|-----|---|---|
| 015 | Consequences of obstructive sleep apnoea syndrome on autonomic nervous system and resting oxygen saturation | <i>Lucio Bianchi, France</i> |
| 016 | Changes in autonomic function, adipokines, gut hormones and inflammatory markers after sleeve gastrectomy in obese subjects with and without diabetes | <i>Henri Parson, United States of America</i> |
| 017 | Short-term progression of coronary artery calcification in type 1 diabetes is not related to presence of autonomic neuropathy | <i>Henrik Hjortkjær Ø., Denmark</i> |

SUNDAY – 10 SEPTEMBER 2017

O18	Acute cardiovascular changes during hypoxia-induced sympathetic activation in patients with an obstructive sleep apnoea syndrome	<i>Paul Valensi, France</i>
O19	Internal carotid artery flow resistivity and Type-1 diabetes duration	<i>Iain D. Wilkinson, United Kingdom</i>
O20	Acute glycemic changes and measures of cardiovascular autonomic function in Type 1 diabetes	<i>Lynn Ang, United States of America</i>

12h15 – 13h15

Sponsored Symposium WÖRWAG PHARMA: Neuropathy in Diabetic Children – Never Forget Pathogenetic Treatment

Chairs: Solomon Tesfaye and Peter Kempler

Opening and Introduction

Summary on neuropathy in diabetic children and adolescents

Speaker: Laszlo Barkai

Update on pathogenetic vs symptomatic treatment of diabetic polyneuropathy

Speaker: Dan Ziegler

Discussion

13h15 – 14h15

Lunch

14h15 – 16h15

Poster Session: Young Investigators Poster Presentations

Chairs: Isaura Tavares and Gerry Rayman

P1	Symptoms of diabetic polyneuropathy are related to falls in patients with Type 2 diabetes	<i>Karolina Snopek, Denmark</i>
P2	Motor unit number estimation in diabetic patients in relation to the presence of diabetic polyneuropathy	<i>Anders Stouge, Denmark</i>
P3	Pharmacological modulation of mitochondrial chaperones and ATP dependent proteases in experimental diabetic neuropathy	<i>Anil Kumar Kalvala, India</i>
P4	Corneal confocal microscopy: A Imaging surrogate end point for mild cognitive impairment and dementia	<i>Georgios Ponirakis, Qatar</i>
P5	Keratocyte density is related to corneal nerve damage in patients with and without neuropathy	<i>Alise Kalteniece, United Kingdom</i>
P6	The impact of obstructive sleep apnoea on foot insensitivity and foot ulceration in patients with Type 2 diabetes: A longitudinal pilot study	<i>Quratul-ain Altaf, United Kingdom</i>
P7	Pyridoxamine prevents memory deficits in experimental diabetes	<i>Sarah Al-Adham, United Kingdom</i>
P8	Metabolic and clinical features associated with severity of erectile dysfunction in male patients with Type 2 diabetes	<i>Nino Cristiano Chilelli, Italy</i>
P9	Optimization of threshold for diagnosis of CAN using kidney function	<i>Christian Stevns Hansen, Denmark</i>
P10	Therapeutic effects of novel mesenchymal stem cells in a diabetic neuropathy rat model: do soluble adhesion molecules play a role?	<i>Sandra Marisa Oliveira, Portugal</i>
P11	Attenuation of diabetic neuropathy by isoquercitrin is mediated via Wnt/beta-catenin pathway	<i>Kahkashan Resham, India</i>
P12	Retinal neurodegeneration in Type 1 diabetes mellitus as an early marker of diabetic neuropathy	<i>Fabiana Picconi, Italy</i>

SUNDAY – 10 SEPTEMBER 2017

16h15 – 16h30

Coffee and Exhibition

16h30 – 18h30

Poster Session: **Pathogenesis and treatment****Chairs:** Mark Yorek and Luciano Bernardi

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| P13 | Non-invasive measurements of AGEs products in the crystalline lens can distinguish subjects with prediabetes and Type 2 diabetes from healthy control subjects and strongly correlated with level of small fibre neuropathy | <i>Mitra Tavakoli, United Kingdom</i> |
| P14 | Hyperglycaemia induced spinal cord vasculopathy and hypoxia; contributing factors in the development of diabetic neuropathic pain | <i>Marlene Da Vitoria Lobo, United Kingdom</i> |
| P15 | Assessing ethologically relevant behavioural changes in the streptozotocin-induced rat model of diabetic neuropathy | <i>Natalie Gardiner, United Kingdom</i> |
| P16 | Immortalized Schwann cells IKARS1 from aldose reductase-deficient mice as a useful tool to study polyol pathway and aldehyde metabolism | <i>Kazunori Sango, Japan</i> |
| P17 | Adjuvant effects of diabetes on hypertensive neuropathy in spontaneously hypertensive rats | <i>Hitoshi Nukada, Japan</i> |
| P18 | Role of descending pain pathway in diabetic peripheral neuropathy | <i>Louis S. Premkumar, United States of America</i> |
| P19 | Peripheral neuropathy in patients with sarcopenia and Type 2 diabetes <i>mellitus</i> | <i>Julia Onuchina, Russia</i> |
| P20 | Metal levels are not dysregulated in the sciatic nerve and dorsal root ganglia of diabetic rats | <i>Sarah Al-Adham, United Kingdom</i> |
| P21 | Diabetic peripheral neuropathy in SDT fatty rat, a new animal model of obese Type 2 diabetes | <i>Tomohiko Sasase, Japan</i> |
| P22 | Risk factors for cognitive impairments in patients with Type 2 diabetes <i>mellitus</i> | <i>Nadiia Zherdova, Ukraine</i> |
| P23 | Efficacy of administration for two years of ACE-inhibition on diabetic peripheral and autonomic neuropathy in patients with diabetes <i>mellitus</i> | <i>Triantafyllos Didangelos, Greece</i> |
| P24 | Treatment of neuropathy in Type 2 diabetic mice: menhaden oil vs resolvin D1, E1 or D1 or D2 methyl esters | <i>Mark Yorek, United States of America</i> |
| P25 | Effect of dietary oils on peripheral neuropathy related endpoints in dietary obese Sprague-Dawley rats | <i>Mark Yorek, United States of America</i> |
| P26 | Comparison of efficacy and toxicity of Diode Laser and Capsaicin on pigskin innervation as a potential treatment for painful diabetic neuropathy | <i>Mikhail I. Nemenov, United States of America</i> |

SUNDAY – 10 SEPTEMBER 2017

P27 N-3 polyunsaturated fatty acids promote neurite outgrowth via PI3K and JNK-mediated signaling pathways in neuro2a cells *Koichi Kato, Japan*

P28 Effects of gamma linoleic acid in people with diabetic peripheral neuropathy *Chong Hwa Kim, South Korea*

P29 The comparison of neuroprotective effect between glucose control and alpha lipoic acid in the STZ-induced diabetic rats *Tae Sun Park, Korea*

16h30 – 18h30

Poster Session: Autonomic neuropathy

Chairs: Paul Valensi and Rodica Pop-Busui

P30 Role of autonomic activity in hypertension and left ventricle function in obese patients *Amel Rezki, France*

P31 The impact of obstructive sleep apnoea on cardiac autonomic neuropathy in patients with Type 2 diabetes: A longitudinal study *Quratul-ain Altaf, United Kingdom*

P32 The impact of the interaction between obstructive sleep apnoea and cardiac autonomic neuropathy on eGFR decline in patients with Type 2 diabetes: A longitudinal study *Quratul-ain Altaf, United Kingdom*

P33 Cardiovascular autonomic neuropathy and renal function in participants in the preventing early renal loss in Type 1 diabetes trial *Mamta Jaiswal, United States of America*

P34 Impaired cardiovascular autonomic function and peripheral sensory nerve function are present among subjects with high risk for the development of Type 2 diabetes *mellitus* screened by the FINDRISC questionnaire *Orsolya Erzsébet Vági, Hungary*

P35 Diabetic retinal sensory neuropathy progression predicts cardiovascular autonomic neuropathy in Type 1 diabetes *Rodica Pop-Busui, United States of America*

P36 Relationship between abnormal handgrip test results and Ambulatory Blood Pressure Monitoring (ABPM) parameters *Anna Körei, Hungary*

P37 Association between indices of heart rate variability and metabolic syndrome in an adult Chinese population *Kara Mizokami-Stout, United States of America*

P38 The role of autonomic neuropathy in the pathogenesis of glycemic variability in Type 1 diabetes *Tamás Várkonyi, Hungary*

P39 Correlation between symptoms of gastroparesis and ¹³C-Octanoic acid breath test in patients with diabetes *Iryna Kostitska, Ukraine*

P40 Diagnosis of diabetic cardiac autonomic neuropathy by high-frequency ultrasonic dopplerography *Tatiana Zelenina, Russia*

SUNDAY – 10 SEPTEMBER 2017

P41	Association of cardiovascular autonomic neuropathy and structural characteristics of myocardium in patients with type 2 diabetes <i>mellitus</i>	<i>Oleksandra Briukhova, Ukraine</i>
P42	In patients with Type 1 diabetes and cardiac autonomic neuropathy the microvessel density in dermal endothelial cells is markedly increased	<i>Aleksandra Araszkiwicz, Poland</i>
P43	Relationship of heart rate variability with peripheral arterial stiffness in patients with Type 2 diabetes	<i>Seong-Su Moon, Korea</i>
P44	The association between serum 25 (OH) D levels and cardiac autonomic neuropathy	<i>Juyoung Shin, Korea</i>
P45	Relationship between hypoglycemia unawareness, driving and cognitive function in T1DM patients with diabetic autonomic neuropathy.	<i>Olga Svetlova, Russia</i>
P46	Diagnostic value of different autonomic symptoms assessed by COMPASS 31 for cardiovascular autonomic neuropathy and diabetic polyneuropathy	<i>Carla Greco, Italy</i>
P47	Changes in thyrotropic axis are not associated with cardiac autonomic dysfunction in the obese population	<i>Camille Pillegand, France</i>
16h30 – 18h30	Poster Session: Diagnosis and Small Fibers Chairs: Tamás Varkonyi and Soroku Yagihashi	
P48	Small fibre neuropathy in obese non-diabetic patients: Relationship to abnormalities in lipoproteins and inflammation	<i>Shazli Azmi, United Kingdom</i>
P49	New insights into diabetic neuropathy provided by nociceptor fiber-selective stimulation	<i>Mikhail I. Nemenov, United States of America</i>
P50	Assessment of diabetic polyneuropathy in Zanzibar: comparison between traditional methods and a point-of-care nerve conduction device (NC-Stat DPNCheck)	<i>Eirik Søfteland, Norway</i>
P51	Utility of <i>in vivo</i> corneal confocal microscopy in comparison with other measures of small fiber function in subjects with type-2 diabetes	<i>Carolina Casellini, United States of America</i>
P52	Corneal confocal microscopy detects severe small fibre neuropathy in diabetic patients with Charcot foot	<i>Ioannis Petropoulos, Qatar</i>
P53	Decline in CMAP amplitude of the tibialis anterior muscle indicates advanced diabetic polyneuropathy	<i>Masayuki Baba, Japan</i>
P54	Adaptation and validation of the neuropathy-foot ulcer specific quality of life instrument (NEURQoL) for the arabic language	<i>Amal Madanat, Saudi Arabia</i>
P55	Correlations between skin autofluorescence, diabetic neuropathy diagnostic scores and quality of life in patients with diabetes	<i>Norina Alinta Gavan, Romania</i>

SUNDAY – 10 SEPTEMBER 2017

P56	Diabetic Neuropathy: an assessment of screening quantitative sensory testing	<i>Ariel Odriozola, Spain</i>
P57	Patterns of nerve conduction studies in patients with hyperesthesia and diabetic peripheral neuropathy	<i>Jong Chul Won, Korea</i>
P58	Five year prospective study on the progression of diabetic polyneuropathy in Japanese patients with early type 2 diabetes–multicenter study	<i>Soroku Yagihashi, Japan</i>
P59	More branching of corneal nerve fibres in patients with painful compared to painless diabetic polyneuropathy	<i>Gidon Bönhof, Germany</i>
P60	Patterns of small and large fiber dysfunction in painful and painless diabetic polyneuropathy	<i>Gidon Bönhof, Germany</i>
P61	The role of sudomotor function testing in the diagnostic pathway of diabetic neuropathy	<i>Carla Greco, Italy</i>
P62	Relation of oxidative stress and glycemic variability with <i>in vivo</i> corneal confocal microscopy parameters in Type 1 diabetes <i>mellitus</i>	<i>Fabiana Picconi, Italy</i>
P63	Smoking influences skin autofluorescence in patients with sensorimotor diabetic neuropathy	<i>Cosmina Ioana Bondor, Romania</i>
P64	Selective stimulation of silent, mechano-insensitive C nociceptive fiber in humans	<i>Mikhail I. Nemenov, United States of America</i>
P65	Association between the risk of cardiac neuropathy and blood pressure in patients with Type 2 diabetes	<i>Bogdan Florea, Romania</i>
18h30 – 19h00	Lifetime achievement award ceremony	
19h00 – 20h00	General Assembly	

The background is a solid red color. Overlaid on this are several thick white lines that intersect to form a network of irregular polygons. Three of these intersection points are marked with solid white circles. The circles are located in the top-left, top-right, and bottom-left areas of the frame. The lines extend from these circles and other points across the page, creating a sense of connectivity and structure.

MONDAY
11 SEPTEMBER 2017

MONDAY – 11 SEPTEMBER 2017

08h00 – 08h30

Painful Neuropathy in Diabetes: a clinical approach

Chairs: Dan Ziegler and Eirik Softeland

Speaker: Troels Jensen

08h30 – 10h00

Oral Session: **Treatment**

Chairs: Dan Ziegler and Eirik Softeland

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| 021 | Effect of ENTRESTO vs valsartan on vascular and neural complications in Type 2 diabetic rats | <i>Mark Yorek, United States of America</i> |
| 022 | Polyneuropathy before and after bariatric surgery: association with serum triglyceride levels? | <i>Helena Schmid, Brazil</i> |
| 023 | Cell therapy using human dental pulp stem cells bypasses impacts of aging and diabetes and ameliorates diabetic polyneuropathy | <i>Keiko Naruse, Japan</i> |
| 024 | Effects of saxagliptin on cardiovascular autonomic nervous system activity and hemodynamic parameters in obese patients with impaired glucose tolerance | <i>Amel Rezki, France</i> |
| 025 | Topiloxostat, a novel xanthine oxidase inhibitor, ameliorates murine diabetic polyneuropathy | <i>Hiroki Mizukami, Japan</i> |
| 026 | Study of parameters of macro- and microcirculation in patients with diabetes <i>mellitus</i> Type 2 at administration of serum deproteinized derivates bovine (Actovegin®). | <i>Anastasiia Fokina, Russia</i> |

10h00 – 10h30

Coffee and Exhibition

10h30 – 11h00

Sponsored Lecture SIGMA-TAU: Pharmacological management of painful sensorimotor polyneuropathy

Speaker: Paolo Marchettini

11h00 – 12h30

Oral Session: **Pathogenesis and Diagnosis**

Chairs: Solomon Tesfaye and Prashanth Vas

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| 027 | Functional reorganisation of the somatosensory cortex determines clinical presentation in diabetic neuropathy | <i>Dinesh Selvarajah, United Kingdom</i> |
| 028 | Impaired hemodynamic response to thermal pain in patients with painful diabetic neuropathy: a dynamic susceptibility contrast MRI study | <i>Marni Greig, United Kingdom</i> |
| 029 | Enhanced subclinical inflammation in painful compared to painless diabetic polyneuropathy. A multimarker approach | <i>Dan Ziegler, Germany</i> |

MONDAY – 11 SEPTEMBER 2017

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| 030 | Diabetes-induced microvascular complications at the level of the spinal cord and neuropathic pain development | <i>Richard Hulse, United Kingdom</i> |
| 031 | Evaluation of diabetic polyneuropathy by photoacoustic and ultrasound imaging | <i>Soroku Yagihashi, Japan</i> |
| 032 | RAGE signaling is associated with macrophage activation and insulin resistance during the development of experimental diabetic polyneuropathy | <i>Hiroki Mizukami, Japan</i> |

12h30 – 12h45

Closing Remarks
Simona Frontoni



Oral Abstracts

São Sebastião Aqueduct

The São Sebastião Aqueduct, popularly known as the “Arcos do Jardim”, is located on the Martim de Freitas sidewalk, in front of the Botanical Garden of the University of Coimbra

The background features a complex network of white lines and circles on a solid red field. The lines intersect at various points, creating a web-like structure. Three prominent white circles are located at different nodes of this network: one in the top-left corner, one in the upper-middle section, and one in the lower-left section. The text is positioned in the lower-right area, overlapping the network lines.

**YOUNG INVESTIGATORS
ORAL PRESENTATIONS**

[O1] A MAGNETIC RESONANCE IMAGING STUDY OF BRAIN VOLUME CHANGES IN DIABETIC PERIPHERAL NEUROPATHY

Heiberg-Gibbons F.¹, Pallai S.², Wilkinson I. D.³, Ghandi R.², Tesfaye S.², Selvarajah D.⁴

1 – University of Sheffield, Medical Student, United Kingdom

2 – Sheffield Teaching Hospitals, Diabetes, United Kingdom

3 – University of Sheffield, Head of Adult Neuroimaging Research, United Kingdom

4 – University of Sheffield, Diabetes, United Kingdom

Rationale & hypothesis

Diabetic neuropathy (DN) is a serious complication affecting up to 50% of patients with diabetes. DN is associated with chronic pain which leads to reduced quality of life. Hitherto considered a disease of the peripheral nervous system, we have conducted a series of magnetic resonance imaging (MRI) experiments to examine central nervous system involvement in DN. The study hypothesis was alterations occur to structural brain volumes in DN.

Methods

136 subjects [102 with diabetes (34 No DN, 34 Painless DN & 34 Painful DN) and 34 healthy volunteers] underwent detailed clinical and neurophysiological assessments to quantify the severity of neuropathy. All subjects underwent 3-dimensional T1-weighted brain MRI (3.0T, Philips). Brain volume analysis was performed using published software (SIENAX www.fmrib.ox.ac.uk/fsi). Further volumetric analysis was performed using the freesurfer image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>). Results presented use family-wise error correction ($p < 0.05$) and ANOVA where appropriate.

Results

No statistical significant difference in age or gender distribution between the groups ($p > 0.05$). Total brain volume was significantly lower in both neuropathy groups; painful DN [1401.7 (10.7) ml], painless DN [1393.5 (69.6) ml] compared to the HV group [1457.2 (79.2) ml] and No DN [1437.2 (60.9) ml]; ANOVA $p < 0.01$. Both neuropathy groups had significantly lower total grey matter volume; painful DN [713.9 (67.1) ml], painless DN [717.2 (42.4) ml] compared to controls (HV [758.4 (46.5)]; $p < 0.01$; No DN [747.3 (41.1)]; $p = 0.015$). There were no significant differences in white matter (ANOVA $p = 0.18$) and CSF (ANOVA $p = 0.23$) volumes across study groups. More detail analysis using FreeSurfer showed subjects with painful DN had significantly lower cortical thickness in the right postcentral gyrus [1.83 (0.14) mm vs HV [1.91 (0.13) mm]; ($p = 0.02$); the right precentral gyrus [2.27 (0.14) mm vs HV [2.37 (0.13) mm]; ($p < 0.01$) and no DN [2.36 (0.13) mm]; ($p < 0.01$); the right insula [2.81 (0.17) mm vs HV [2.93 (0.16) mm]; ($p < 0.01$); the left precentral gyrus [2.31 (0.16) mm vs HV [2.39 (0.12) mm]; ($p = 0.02$) and no DN [2.38 (0.14) mm]; ($p = 0.04$); and left insula [2.81 (0.15) mm] vs HV [2.97 (0.14) mm]; ($p < 0.01$). Significant correlations between the DN4 pain score and the left postcentral gyrus ($r = 0.44$, $p < 0.01$) right postcentral gyrus ($r = 0.35$, $p = 0.05$) and right precentral gyrus ($r = 0.35$, $p = 0.05$) within the painful DN group.

Discussion

This is the largest cohort study of brain volume changes in subjects with DN examined to date. We have demonstrated significant reduction in grey matter volume in painful and painless DN subjects. In painful DN this is localised within the somatomotor cortex and insula. These findings highlight significant CNS involvement in DN that provides clues to the pathogenesis of this condition.

[O2] SIMULTANEOUS PANCREAS KIDNEY TRANSPLANTATION IN TYPE 1 DIABETES MELLITUS IS ASSOCIATED WITH AN EARLY SMALL AND LATER LARGE NERVE FIBRE IMPROVEMENT

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Objectives

Simultaneous pancreas kidney transplantation (SPK) can normalise glucose levels in patients with type 1 diabetes (T1DM). However, it has shown limited early benefit on neuropathy.

Methods

A detailed assessment of small and large fibre neuropathy was undertaken at baseline, 6 months and annually over 3 years following SPK in 36 patients with T1DM.

Results

HbA1c improved significantly (69.1 ± 16.9 v 39.0 ± 3.0 , $p < 0.0001$). Corneal nerve fibre density (CNFD) (9.4 ± 1.0 v 12.2 ± 1.7 , $p = 0.005$), corneal nerve branch density (CNBD) (9.8 ± 0.05 v 13.2 ± 2.7 , $p = 0.05$) and corneal nerve fibre length (CNFL) (7.2 ± 0.5 v 8.2 ± 0.8 , $p = 0.05$) and mean dendrite length (MDL) (10.9 ± 0.8 v 15.6 ± 1.4 , $p = 0.02$) in skin biopsies, improved significantly at 12 months with no change in neuropathic symptoms, quantitative sensory testing, neurophysiology or intra-epidermal nerve fibre density. However, at 36 months there was a significant improvement in the neuropathy symptom profile (5.3 ± 0.9 v 3.1 ± 1.2 , $p = 0.04$), peroneal nerve conduction velocity (32.1 ± 1.8 v 38.7 ± 2.7 , $p = 0.05$) and sural nerve amplitude (3.4 ± 0.5 v 6.9 ± 0.4 , $p = 0.02$), with a continued improvement in CNFD (9.4 ± 1.0 v 14.4 ± 1.6 , $p = 0.01$), CNFL (7.2 ± 0.5 v 10.3 ± 0.6 , $p = 0.001$) and MDL (10.9 ± 0.8 v 18.0 ± 1.3 , $p = 0.02$).

Conclusion

Corneal confocal microscopy and skin biopsy demonstrate an improvement in small fibres within 12 months of SPK, followed by an improvement in neuropathic symptoms and neurophysiology at 36 months. These data provide further support for the use of CCM as an early surrogate end-point in clinical trials of diabetic neuropathy.

[O3] ABNORMAL SOMATOMOTOR NETWORK FUNCTIONAL CONNECTIVITY IN SUBJECTS WITH PAINFUL DIABETIC NEUROPATHY: A RESTING STATE FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY

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Rationale and hypothesis

Painful diabetic peripheral neuropathy (Painful-DPN) is a common disabling condition, which has a negative impact on quality of life. As there are no objective biomarkers of painful-DPN, current treatments are less than optimal. Recent imaging studies using resting-state functional magnetic resonance imaging (rs-fMRI) have identified a network of brain structures that are active and connected during task-free paradigms, when the brain is at rest. We therefore investigated the status of the resting state networks in painful DPN.

Methodology and Findings

46 patients with diabetes (No DPN, n=16; Painful DPN, n=15; Painless DPN, n=15) and 16 healthy volunteers underwent detailed clinical and neurophysiological assessments. Rs-fMRI data were acquired at 3T (Achieva, Philips Healthcare). Data analysis was performed using FSL. Independent component analysis showed that patients with painful DPN had reduced functional connectivity in the post central gyrus - sensorimotor network (x, y, z MNI coordinates, -42, -22, 56) (TFCE, corrected $p < 0.05$), Precuneous - default mode network DMN (x, y, z MNI coordinates, -6, -46, 40) (TFCE, corrected $p < 0.05$), Superior frontal gyrus (x, y, z MNI coordinates, 34, 62, 60) (TFCE, corrected $p < 0.05$), Heschl's gyrus (x, y, z MNI coordinates, -42, -22, 12) (TFCE, corrected $p < 0.05$), Insular cortex (x, y, z MNI coordinates, 34, 62, 60) (TFCE, corrected $p < 0.05$) and Superior parietal lobule (x, y, z MNI coordinates, -22, -42, 68) (TFCE, corrected $p < 0.05$). Independent sample T-test form sensorimotor region of interest analysis ROI (5mm spherical mask) confirmed this finding ($p = 0.007$). Further correlation analyses using the ROI parameter estimates and quantitative pain score scales revealed a significant correlation between SF36 (Pearson correlation, $r = -0.55$; $p = 0.03$) and CPAQ-ACTIVITY ($r = -0.52$; $p = 0.045$).

Conclusion

This preliminary study suggests abnormal functional connectivity in patients with painful DPN, which correlates with measures of pain and behaviour. These findings demonstrate that chronic pain has a widespread impact on overall brain function in diabetes, and suggests that disruptions of the resting state networks may underlie the cognitive and behavioural impairments accompanying chronic pain.

[O4] DIFFERENTIAL ASSOCIATION OF CARDIAC AUTONOMIC FUNCTION WITH INSULIN SENSITIVITY AND SECRETION IN RECENTLY DIAGNOSED TYPE 1 AND TYPE 2 DIABETES

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Objectives

It is unclear to which extent reduced insulin sensitivity and/or secretion contribute to the development of diabetic cardiovascular autonomic neuropathy (CAN) characterized by diminished heart rate variability (HRV). We sought to determine whether HRV indices are associated with measures of insulin sensitivity and secretion in type 1 and type 2 diabetes.

Methods

HRV indices were assessed in 275 individuals with type 1 diabetes (T1D), 450 persons with type 2 diabetes (T2D) (diabetes duration ≤ 1 year), and 81 glucose-tolerant controls from the baseline cohort of the German Diabetes Study (GDS): (T1D/T2D/controls [mean \pm SD]: age: 36.0 \pm 11.8/52.7 \pm 10.5/47.4 \pm 13.8 years, male: 62/64/68%, HbA1c: 6.6 \pm 1.2/6.4 \pm 0.9/5.3 \pm 0.3%). Four time domain and frequency domain HRV measures each, reflecting vagal and/or sympathetic modulation over 3 h were determined during a hyperinsulinemic-euglycemic clamp. Insulin sensitivity was calculated by the M value, while fasting and glucagon-stimulated C-peptide and their ratio served as measures of insulin secretion.

Results

After adjustment for sex, age, BMI, and smoking, M value remained significantly lower in the groups with T1D and T2D compared to controls (8.6 \pm 3.2 and 6.1 \pm 2.6 vs 10.8 \pm 3.9 mg/kg,min; both $p < 0.05$). Multiple linear regression analyses showed that in the T1D group 5 out of 8 and in T2D participants 7 out of 8 HRV measures were positively associated with M value (e.g. SDNN T1D: $\beta = 0.193$; T2D: $\beta = 0.218$, both $p < 0.005$; very low frequency (VLF) power: T1D: $\beta = 0.219$; T2D: $\beta = 0.219$, both $p < 0.001$). The low-frequency/high-frequency (LF/HF) power spectrum as an indicator of sympathovagal balance was positively associated with the C-peptide ratio in T1D participants ($\beta = 0.221$, $p = 0.001$) and with stimulated C-peptide in those with T2D ($\beta = 0.119$, $p = 0.016$).

Conclusions

In both well controlled recent-onset T1D and T2D, lower cardiac vagal activity is linked to insulin resistance, while predominant sympathetic tone is associated with higher insulin secretion.

[O5] INCREASED DERMAL MICROVASCULATURE IN PAINFUL DIABETIC NEUROPATHY

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Aim

Painful diabetic peripheral neuropathy (painful-DPN) can be intractable with major impact, yet the underlying pain mechanisms remain uncertain. While distal leg intra-epidermal nerve fibre density is recommended for the diagnosis of small fibre neuropathy, we found that intra-epidermal nerve markers do not differentiate between painful-DPN and painless-DPN. However, as impaired skin microvascular reactivity has been described in painful-DPN we investigated neuronal and vascular biomarkers in carefully phenotyped subjects with painful- and painless-DPN.

Methods

42 T2DM subjects and 13 healthy volunteers (HV) underwent detailed clinical and neurophysiological assessments and were subsequently divided into three groups based on the neuropathy composite score of the lower limbs [NIS (LL)] plus 7 tests (16 Painful-DPN, 14 Painless-DPN and 12 No-DPN). All subjects underwent calf skin punch biopsy, and immunohistochemistry was used to quantify intra-epidermal (IENF) and sub-epidermal (SENF) nerve fibres with structural marker PGP9.5, regenerating fibres with GAP43, sensory fibres with neuropeptide CGRP, and the dermal blood vessels with von Willebrand Factor (vWF).

Results

IENF density was severely decreased ($p < 0.001$) in both DPN groups, with no differences for PGP9.5, GAP43, CGRP, or GAP43/PGP9.5 ratio. There was significant increase of vWF in Painless-DPN and no-DPN groups compared to controls, but this was markedly greater for painful-DPN, and significantly higher than painless-DPN ($p < 0.0001$). The ratio of SENF CGRP:vWF showed a significant decrease in painful-DPN vs Painless-DPN ($p = 0.014$).

Conclusions

In established neuropathy increased dermal vasculature and its ratio to nociceptors may differentiate painful-DPN from painless-DPN. Increased small blood vessels following tissue ischaemia/hypoxia associated with disproportionate and abnormal nerve fibres (irritable nociceptors) may lead to a "painful vaso-neuropathy".

[O6] STUDY OF THE IMPACT OF THE DEGREE OF PERIPHERAL NEUROPATHY ASSOCIATED TO DIABETES ON PRESSURE ULCER INCIDENCE AND HEALING PROCESS

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Objectives

The present study aims to determine the influence of the degree of peripheral neuropathy (PN) associated to diabetes on 1) skin pressure ulcer incidence, 2) healing time and process, 3) the quality of the healed skin.

Methods

C57Bl6 male mice were randomly assigned to vehicle (control mice) or STZ intra-peritoneal injection (inclusion criteria: glycaemia ≥ 300 mg/dl) (n=10 per group). Tail Flick latency (TFL) and motor nerve conduction velocity (MNCV) on sciatic nerve were measured to establish a moderate PN with only small nerve fiber impairment (4wk diabetes) and a severe PN with both small and large nerve fiber impairment (8wk diabetes). We performed repetitive cycles of skin compression using magnets to induce skin pressure ulcer in STZ and aged-matched control mice. The wound healing process until wound closure was followed using skin imaging, histological and gene expression analysis.

Results

Both epidermal and dermal thicknesses were decreased in diabetic groups compared to control groups. After induction of skin pressure ulcer, maximal lesion area was reached at day 4. Histological analysis revealed cutaneous muscle degradation by inflammatory cells, and dermis and epidermis damages in all groups, indicating a grade 3-ulcer formation. Maximal lesion surface was not different between control groups whereas it was 1.8 and 2.4 fold increased in 4wk- and 8wk-diabetic mice, respectively compared to controls (Figure 1). Closure of the wound was reached at day 19 in both control mice and delayed at day 22 and day 27 in 4wk- and 8wk-diabetic mice, respectively. Gene expression kinetic during healing showed increased MMP 8, 9 and 10, and decreased matrix proteins in diabetic compared to control groups.

Conclusions

This study suggests that the degree of PN associated to diabetes impairs skin resistance to pressure, but not the healing process, showing that the large nerve fibers do not influence the healing process. Further physiological, nerve and vascular studies are in progress to decipher the mechanisms.

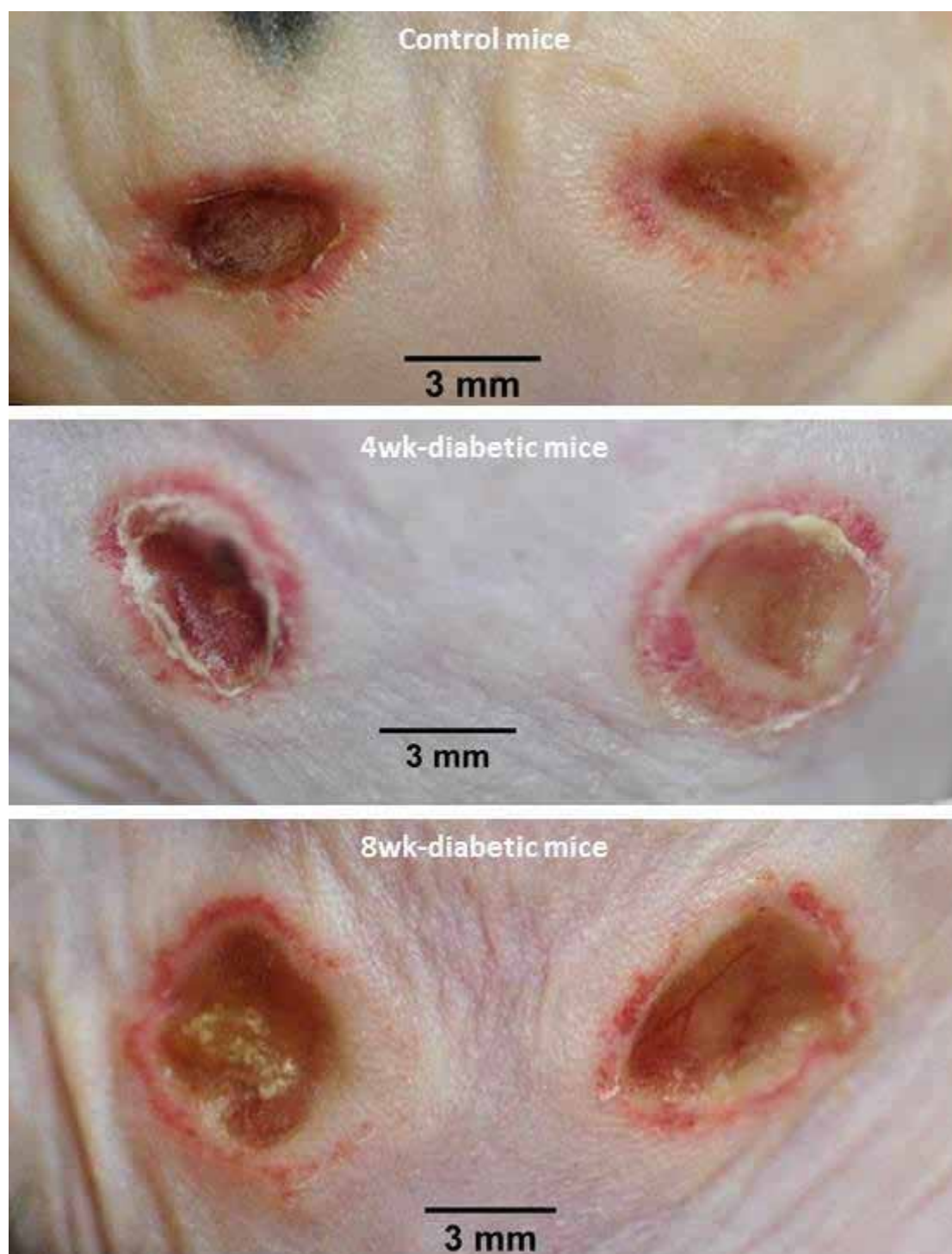


Figure 1: Skin pressure ulcer 4 days after induction by magnets application in control, 4 and 8 weeks diabetic mice

[O7] CARDIOVASCULAR AUTONOMIC NEUROPATHY IS ASSOCIATED WITH BONE METABOLISM AND POSSIBLY MEDIATED THROUGH KIDNEY FUNCTION IN PATIENTS WITH TYPE 1 DIABETES

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Objectives

Patients with type 1 diabetes have an increased risk of both osteoporosis and cardiovascular autonomic neuropathy (CAN). As both bone vascular supply and osteoclast and osteoblast activity are subjected to autonomic regulation – we investigated the possible association between CAN and bone metabolism in patients with type 1 diabetes.

Methods

In 329 type 1 patients CAN was assessed by cardiovascular reflex tests (CARTs): heart rate response to deep breathing (E/I ratio), to standing (30/15 ratio) and to the Valsalva manoeuvre (VM). Moreover, 2 minutes resting heart rate (rHR) and the standard deviation of normal-to-normal R-R-intervals (SDNN) were obtained. Two or three pathological CARTs defined CAN. Bone mineral density of the femoral neck (BMDfn) was assessed by dual energy x-ray absorptiometry. Bone markers were serum parathyroid hormone (PTH), ionized calcium and phosphorus. Stepwise linear regression analyses were applied to assess associations.

Results

Patients were (mean (SD)) aged 55.6 (9.4) years, 52% were male, diabetes duration was 40 (8.9) years, HbA1c was 61.7 (8.7) mmol/mol, estimated glomerular filtration ratio (eGFR) 77.5 (26.3) ml/min/1.73m², 36% was diagnosed with CAN and BMDfn was 0.7 (0.1) g/cm². In models adjusted for age, sex, diabetes duration, HbA1c, serum vitamin D3, body mass index, smoking, exercise level and beta blocker use, a doubling of E/I ratio, VM and rHR was associated with change in BMDfn of 14.2% (95% CI 0.8; 29.3 p=0.04), 7.5% (95% CI 0.08; 15.5 p=0.04) and -16.6% (95% CI -22.4; -10.4 p=0.02) respectively. A doubling of 30/15 ratio, VM and rHR was associated with changes in PTH of -43% (-64.2; -8.5 p=0.02), -29.8% (-45.2; -10.2 p=0.005) and 36.3% (4.6; 77.2 p=0.02), respectively. Patients with CAN had 4.2% (-8.0; -0.2 p=0.04) lower BMDfn and 33.6% (14.3; 53.8 p=0.0002) higher PTH compared to patients without CAN. Only higher rHR remained associated with higher PTH and lower BMDfn after additional and exploratory adjustment for eGFR, p<0.0001 and 0.04 respectively. Standardised estimates for models including BMDfn and PTH are shown in Figure 1.

Conclusions

The presence of CAN was associated with reduced BMDfn and increased levels of PTH, thus, type 1 diabetes patients with CAN could be at increased risk of osteoporosis and subsequent bone fractures. Whether CAN directly affects bone metabolism detrimentally or if this association is mediated via decreased kidney function should be investigated further.

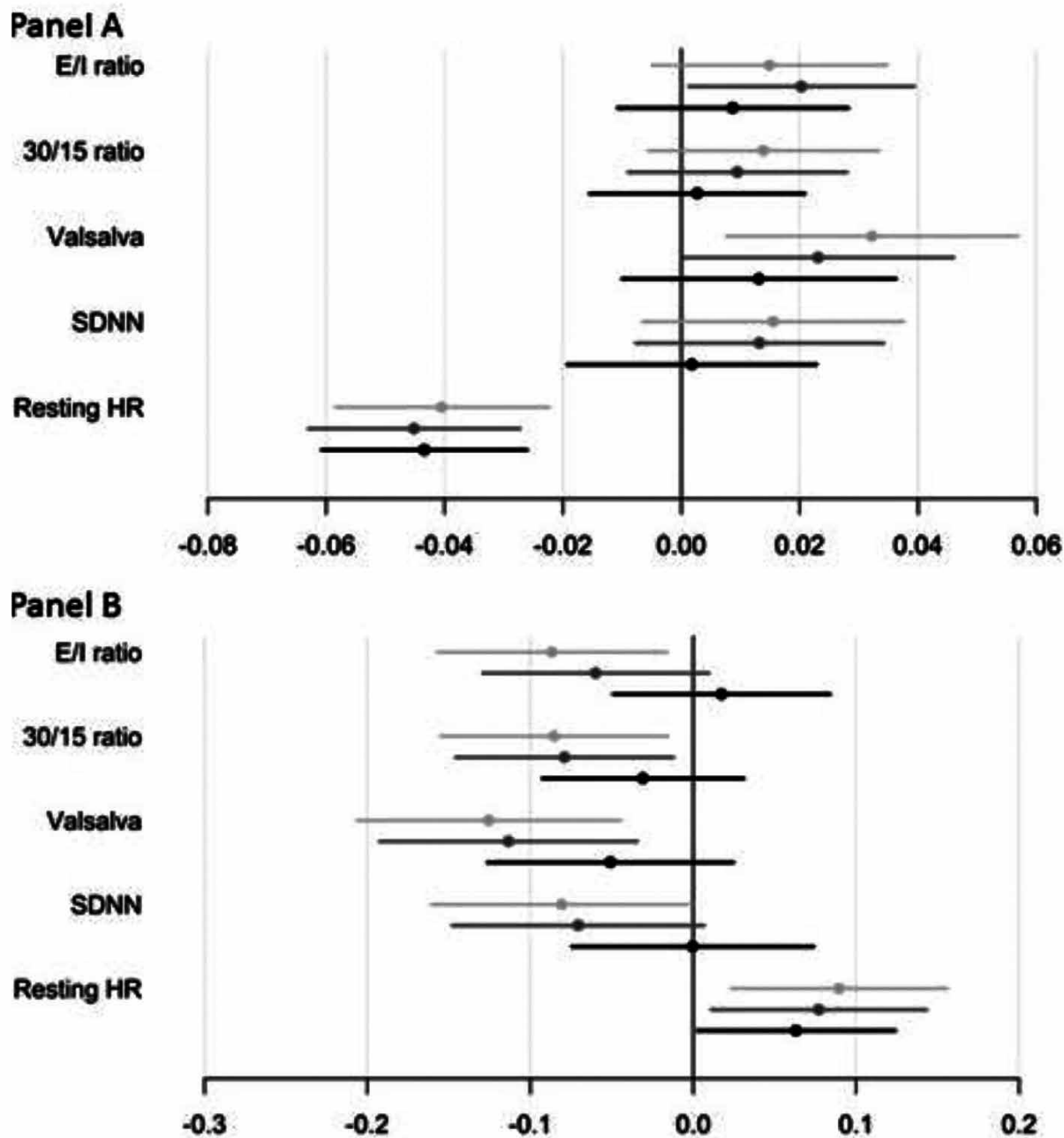


Figure 1 – Standardized regression coefficients and 95% confidence intervals of linear regression analyses of associations between CAN measures and bone mineral density of the femoral neck (Panel A) and serum levels of parathyroid hormone (Panel B). Estimates are in log scale and presented as a one SD increase of determinants on a log2 scale. Light grey: adjusted for age and sex. Dark grey: further adjusted for diabetes duration, HbA1c, serum vitamin D₃, body mass index, smoking, exercise level and beta blocker use. Black: further adjusted for estimated glomerular filtration ratio.

[O8] ELEVATED PROPROTEIN CONVERTASE SUBTILISIN/KEXIN-TYPE 9 (PCSK9) IS ASSOCIATED WITH SMALL FIBRE NEUROPATHY

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Objectives

A high expression of proprotein convertase subtilisin/kexin-type 9 (PCSK9) is associated with increased LDL and accelerated coronary artery disease and PCSK9 inhibitors represent a treatment for elevated LDL. There is increasing evidence for the role of LDL-C and triglycerides in the pathogenesis of diabetic neuropathy.

Methods

34 morbidly obese subjects underwent detailed neuropathy phenotyping using corneal confocal microscopy (CCM) and assessment of circulating PCSK9 and lipids levels.

Results

Subjects were divided into two groups: With and without small fibre neuropathy (SFN) based on a corneal nerve fibre length (CNFL) <2SD of an age matched control group. Age, measures of obesity (waist circumference, BMI, HbA1c, total cholesterol, HDL and triglycerides did not differ, however, levels of PCSK9 and LDL-C were significantly higher in the group with SFN.

	No SFN (n=20)	SFN (n=14)	p
Age (Years)	45.5±1.8	47.3±2.5	NS
Waist (cm)	136.2±4.1	131.7±3.0	NS
BMI (Kg/m²)	49.3±1.8	48.1±2.5	NS
IFCC (mmol/mol)	44.7±3.4	47.4±5.2	NS
Cholesterol (mmol/l)	4.4±0.2	4.3±0.3	NS
Triglyceride (mmol/l)	1.4±0.2	2.0±0.4	NS
HDL-C (mmol/l)	1.1±0.1	0.9±0.1	NS
LDL-C (mmol/l)	2.4±0.2	2.7±0.2	0.04
oxLDL (U/l)	40.9±2.5	38.8±2.4	NS
CRP (mg/l)	8.4±1.6	7.6±1.4	NS
PCSK9 (ng/ml)	833.2±77.9	1096.7±108.5	0.05

PCSK9 inversely correlated with CNFD (r=-0.54, p=0.003), CNBD (r=-0.49, p=0.007) and CNFL (r=-0.56, p=0.002). Multiple regression analysis showed that PCSK9 was independently associated with CNFL (-0.49, p=0.017).

Conclusion

There is an association between increased PCSK9 and small fibre neuropathy assessed using CCM. PCSK9 inhibitors are currently used for lowering LDL and have been shown to have an impact on coronary artery disease. However, these data encourage further work and potential clinical trials of PCSK9 inhibitors in the management of neuropathy.



SMALL FIBERS

[O9] LANGERHANS CELL DENSITY IN RECENT-ONSET TYPE 2 DIABETES RECOVERS AFTER FIVE YEARS IN ASSOCIATION WITH PRESERVED INTRAEPIDERMAL NERVE FIBER DENSITY

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Aim

To assess the predictive value of distinct cutaneous biomarkers for the changes in sensorimotor function and intraepidermal nerve fiber density (IENFD) in recently diagnosed type 2 diabetes (T2D) patients over 5 years.

Methods

We assessed nerve conduction velocity (NCV), quantitative sensory testing (QST), and skin biopsies at baseline and after five years in 45 glucose-tolerant individuals (Control) and 34 type 2 diabetes (T2D) patients. Baseline characteristics (Control/T2D): age: 55.8±13.8/56.2±11.1 [mean±SD] years; male: 58/77%; BMI: 25.1±3.7/33.0±7.0 kg/m²; Neuropathy Disability Score (NDS): 0.70±1.33/1.85±1.95 points; T2D: diabetes duration: 12.8±11.7 months; HbA1c: 6.7±1.2%. Five-year follow-up (Control/T2D): BMI: 25.5±4.4/32.2±6.0 kg/m²; T2D: HbA1c: 7.2±1.3% (p=0.022 vs baseline). Dermal endothelial cell and mitochondrial superoxide dismutase (SOD2) areas were assessed using anti-CD31 and anti-SOD2 antibodies, respectively. Epidermal Langerhans cell density (LCD) was assessed using an anti-langerin antibody.

Results

After five years, there were no differences in the changes from baseline between the groups for NDS, NCV, and QST. IENFD (Control: 10.2±3.9 vs 10.3±4.1 fibers/mm; T2D: 8.1±2.8 vs 7.6±3.9 fibers/mm), dermal endothelial cell area (Control: 2.27±1.82 vs 2.29±1.27%; T2D: 2.51±1.13 vs 2.44±0.83%), and SOD2 area (Control: 0.29±0.22 vs 0.19±0.20%; T2D: 0.31±0.23 vs 0.27±0.16%) also remained unchanged over five years. Epidermal LCD was higher at baseline in Control compared to T2D individuals (453±148 vs 371±145 cells/mm²; p<0.05), whereas after five years no difference between the groups was found (401±148 vs 409±130 cells/mm²). In the T2D group, an increase in LCD over five years was strongly associated with an increase in IENFD after adjustment for sex, age, BMI, and diabetes duration ($\beta=0.618$, p=0.003).

Conclusions

In well-controlled recent-onset type 2 diabetes patients, Langerhans cell density recovered and was associated with preserved IENFD after 5 years, while no significant deterioration in peripheral nerve function and morphology was observed. These findings suggest that an improved cutaneous immunogenic balance toward reduced inflammation could prevent IENF loss in type 2 diabetes.

[O10] CORNEAL CONFOCAL MICROSCOPY DETECTS SMALL FIBRE NEUROPATHY AND NERVE REGENERATION AFTER AN IMPROVEMENT IN GLYCAEMIC CONTROL IN SUBJECTS WITH LADA

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Objectives

Latent Autoimmune Diabetes in Adults (LADA) is often misdiagnosed, and a prolonged period of poor glycaemic control may increase the risk of diabetic neuropathy.

Methods

We have undertaken a detailed longitudinal assessment of neuropathy in control subjects (C) (n=12), subjects with type 2 DM (T2DM) (n=12) and LADA (n=12) by quantifying: neuropathy disability score (NDS), vibration perception threshold (VPT), peroneal and sural nerve conduction velocity and amplitude (PMNCV, PMNAmp, SSNCV, SSNAmp), cold threshold (CT), warm threshold (WT), Neuropad® (%), corneal nerve fibre density (CNFD), branch density (CNBD) and fibre length (CNFL) at baseline and after 1 year.

Results

There was no significant difference in age (47.8±5.9 vs 52.7±6.1 vs 49.8±13.9 years, p=NS), duration of diabetes (T2DM: 9.4±11.2 vs LADA: 13.0±10.6 years, p=NS), blood pressure, total cholesterol, HDL, triglycerides and eGFR between T2DM and LADA. HbA1c (37.3±2.5 vs 53.2±7.2 vs 84.0±26.3 mmol/mol, p=0.01) was higher and BMI (27.2±4.9 vs 30.9±4.9 vs 25.7±4.0 kg/m², p=0.03) was lower in LADA compared to T2DM. There were no significant differences in NDS (0.3±0.7 vs 2.2±2.5 vs 4.2±3.5, p=NS), VPT (4.4±3.0 vs 10.0±6.1 vs 11.3±9.5 volts, p=NS), PMNCV (49.3±2.1 vs 42.1±8.7 vs 40.4±8.2 m/s, p=NS), PMNAmp (5.5±2.5 vs 4.1±2.7 vs 3.2±2.4 mV, p=NS), SSNCV (52.4±5.5 vs 46.3±5.7 vs 43.4±4.7 m/s, p=NS), SSNAmp (21.0±7.5 vs 11.6±8.1 vs 11.2±6.4 μV, p=NS), WT (37.8±2.8 vs 41.0±3.3 vs 42.9±5.0 °C, p=NS), CT (28.1±3.2 vs 27.0±2.3 vs 23.6±4.7 °C, p=NS) and Neuropad® (89±20 vs 86±21 vs 67±38 %, p=NS), but CNFD (29.0±7.3 vs 29.1±9.0 vs 19.4±7.1 no/mm², p=0.04), CNBD (42.2±14.1 vs 43.1±23.5 vs 22.0±10.3 no/mm², p=0.007) and CNFL (18.2±3.1 vs 17.0±4.7 vs 12.0±3.2 mm/mm², p=0.04) were significantly lower in LADA compared to T2DM. Comparing baseline to follow up at 1 year, there were no change in BMI, lipids, NDS, VPT, electrophysiology, CT, WT and Neuropad® in C, T2DM and LADA. HbA1c, CNFD, CNBD and CNFL did not differ in C and T2DM. However, HbA1c was lower and approached significance (84.0±26.3 vs 68.4±17.4 mmol/mol, p=0.06) and CNBD (22.0±10.3 vs 34.8±16.3 no/mm², p=0.005) and CNFL (12.0±3.2 vs 14.3±4.0 mm/mm², p=0.02) were increased with no change in CNFD (19.4±7.1 vs 22.3±5.9 no/mm², p=NS) in LADA.

Conclusion

Subjects with LADA have a significant small fibre neuropathy due to poorer glycaemic control, but an improvement glycaemic control is associated with an improvement in corneal nerve branching and length. CCM not only detects early small fibre neuropathy, but also can monitor early nerve regeneration and therefore could be an ideal surrogate end-point marker in therapeutic trials of diabetic neuropathy.

(O11) SMALL FIBRE DYSFUNCTION IN CHEMOTHERAPY INDUCED PERIPHERAL NEUROPATHY (CIPN) – A PROSPECTIVE STUDY USING METHODS OF SMALL FIBRE FUNCTION AND STRUCTURE

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Objectives

Methods to assess neural small fibre function (SFF) and structure (SFS) are being increasingly employed in the study of diabetes neuropathy. We have used our experience with these methods to investigate their potential value in small fibre neuropathy in other conditions.

Chemotherapy-induced peripheral neuropathy (CIPN) is the commonest neurological complication of cancer treatment. However its accurate diagnosis remains a clinical challenge. Conventional methods like nerve conduction studies and quantitative sensory testing are limited by their inability to detect subclinical changes and have not been shown to correlate with severity of patients' symptoms. In this prospective study, two established methods – the LDI (laser doppler imager) _{FLARE} technique and Corneal confocal microscopy (CCM) have been used to study SFF and SFS respectively.

Methods

40 patients [20 each on Taxanes and Platinum analogues chemotherapy] were assessed at baseline and 3 months after successful completion. Assessments included detailed neurological examination including vibration perception thresholds. Small fibre assessments included the LDI _{FLARE} technique and CCM for corneal nerve fibre density (CNFD). Large fibre assessments included vibration perception threshold and sural nerve conduction velocity and amplitude. The QLQ-CIPN20 questionnaire was used to assess symptom severity. 20 age-matched healthy controls (H_c) were also used as comparators and assessed at baseline and after 1 year.

Results

Group	Method	Baseline	3 months after completion of chemotherapy (After 1 year in HC)		Significance of change compared to baseline
		Mean (±SD)	Mean (±SD)	Correlation with QLQ-CIPN20	
Taxanes (n=20)	LDI _{FLARE} (cm ²)	7.84±2.39	5.01±2.78	p=<0.001	p=<0.001
	CNFD using CCM	50.88±10.29	34.67±8.87	p=0.008	p=<0.001
Platinum Analogues (n=20)	LDI _{FLARE} (cm ²)	7.11±1.58	3.89±1.78	p=<0.001	p=<0.001
	CNFD using CCM	52.02±11.56	38.90±12.56	p=0.002	p=<0.001
Healthy controls (n=20)	LDI _{FLARE} (cm ²)	8.25±1.54	8.01±2.40	-	NS
	CNFD using CCM	61.13±8.98	58.78±5.59	-	NS

The significant correlations between both methods to assess SFN with QLQ-CIPN20 scores are shown in the table above. No significant change was seen in any of the large fibre methods ($p > 0.05$) and similarly, no correlation was observed between large fibre methods and the QLQ-CIPN20 scores ($p > 0.05$).

Conclusions

Our prospective study shows that both the LDI_{FLARE} and CCM method are sensitive indicators of progressive small fibre dysfunction in CIPN. Moreover, they strongly correlate with symptom scores and functional impairment due to CIPN. We propose that these methods should be considered not only in the diagnosis but also for prospective assessment of CIPN. Finally, our study also shows that these methods, already shown to be useful in the assessment of diabetes neuropathy, may also have value in the assessment of SFN in other conditions.

(O12) CORNEAL ENDOTHELIAL DAMAGE IS NOT RELATED TO CORNEAL NERVE FIBRE LOSS IN PATIENTS WITH DIABETES

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Aim

The purpose of this study was to assess corneal endothelial cell morphology in relation to corneal nerve fibre damage using corneal confocal microscopy (CCM).

Methods

We have studied 15 diabetic subjects (age; 48.2±2.3, IFCC; 68.3±5.6, duration of diabetes; 16±4 years) and 10 age-matched controls (age; 51.2±2.65) using CCM. Corneal endothelial cell density (cell/mm²), area (µm²), polymegathism (Coefficient of Variation) and pleomorphism (Hexagonality Coefficient) and corneal nerve fibre density (no./mm²), length (mm/mm²) and branch density (no./mm²) were assessed in images obtained from the central cornea using the Corneal Endothelium Analysis System (CEAS) and CCMetrics software.

Findings

Corneal endothelial cell density (3380.98±75.52 vs 3592±133.82, p=0.07) and polymegathism (52.95±1.01 vs 56.21±1.62, p=0.08) were reduced and endothelial cell area (240.47±6.24 vs 227.60±10.05, p=0.05) and pleomorphism (32.69±1.53 vs 30.92±2.30, p=0.07) were increased in diabetic patients compared to controls. Corneal nerve fibre density (27.37±1.83 vs 33.60±1.78, p=0.04), branch density (69.78±9.23 vs 98.87±7.32, p=0.05) and length (20.63±3.15 vs 25.7±2.02, p=0.03) were reduced in diabetic patients compared to controls. Duration of diabetes correlated significantly with polymegathism (r=0.6, p=0.03) and pleomorphism (r=-0.62, p=0.02). There were no association between corneal endothelial cell morphology and corneal nerve morphology.

Conclusion

This pilot study shows corneal endothelial cell abnormalities and corneal nerve damage in patients with diabetes. However, there was no association between corneal endothelial and nerve fibre abnormalities.

(O13) UNUSUAL CASES OF ISOLATED DIABETES SMALL FIBRE NEUROPATHY COMPLICATED BY CHARCOT NEUROARTHROPATHY OF THE FOOT

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Objectives

Charcot neuropathic osteoarthropathy (CN) is a chronic, progressive destructive arthropathy of the foot and ankle leading to fixed deformities, ulceration and bone loss. Pathophysiologically, it is associated with diabetes large fibre neuropathy (LFN) leading to repeated trauma, increased blood flow and increased expression of nuclear factor kappa-B ligand (RANKL). CN has also been associated with small fibre neuropathy (SFN). Previous studies from our unit have shown that patients with CN have significantly higher maximum microvascular hyperaemia (MMH) as compared to those with neuropathy but without CN. We describe two atypical cases of CN in whom the neuropathy was confined only to small fibre (SF) involvement.

Methods and Results

Patient A, a 49-year old man presented with a red swollen left foot 2 days after wearing a new pair of shoes and without any preceding trauma. Examination revealed absence of ulceration, but loss of the longitudinal arch and a 4° C temperature difference. Plain radiographs showed Lisfranc ligament separation and MRI further confirmed CN with bone marrow oedema of the navicular and cuneiform bones (Brodsky type 4 CN). SF function (SFF) assessed by the modified laser Doppler imager (LDI_{FLARE}) was significantly reduced (flare size 1.6 cm² compared with healthy control range of 8.1–10.7cm²) and SF structure (SFS) assessed by confocal microscopy (CCM) showed reduced indices: corneal nerve fibre density (CNFD) 34.56 no/mm³; nerve branch density (CNBD) 23.32 mm/mm³ and nerve fibre length (CNFL) 19.82 mm/mm³. LFN assessment by Tibio-peroneal and sural nerve assessments were entirely normal and vibration perception threshold (VPT) was also normal at 11 mV. In keeping with our previous findings MMH was high at 1182 perfusion units (PU).

Patient B, a 58-year old man presented with a 3-week history of painless swelling of his right foot and inability to bear weight on this right leg. He recollects tripping over a brick side-walk 4 weeks prior to presentation. Examination revealed a midfoot deformity. There was 5° C temperature difference but absence of any ulceration. Plain radiographs showed bone destruction and periosteal reaction confined to the bases of the first to fifth metacarpals consistent with Brodsky Type 1 CN. There was significant SFN as evidenced by a LDI_{FLARE} of only 1.1cm² and reduced CNFD 31.32 no/mm³; CNBD 25.56 mm/mm³ and CNFL 21.76 mm/mm³. There was no evidence of LFN with normal Tibio-Peroneal velocities, latency and amplitude. VPT was normal at 9 mV. MMH was high at 1288 PU.

Conclusions

These two cases highlight that CN can develop in diabetes patients without large fibre neuropathy. The absence of LFN suggests that SFN may be integral to the pathogenesis of CN. Furthermore, the intriguing finding of normal to high MMH in these and the cases described in our previous study requires further study to understand their role in the development of CN.

[O14] CORNEAL CONFOCAL MICROSCOPY DETECTS CORNEAL NERVE DAMAGE IN PATIENTS ADMITTED WITH ACUTE ISCHEMIC STROKE

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Objectives

Corneal confocal microscopy (CCM) can identify corneal nerve damage in patients with peripheral and central neurodegeneration. To determine the utility of CCM in patients presenting with acute ischemic stroke.

Methods

130 patients (57 without diabetes (NGT), 32 with impaired glucose tolerance (IGT) and 41 with type 2 diabetes *mellitus* (T2DM)) admitted with acute ischemic stroke and 28 age-matched healthy control participants underwent CCM to quantify corneal nerve fibre density (CNFD), corneal nerve branch density (CNBD) and corneal nerve fibre length (CNFL).

Results

There was a significant reduction in CNFD, CNBD and CNFL in stroke patients with NGT ($p < 0.001$, $p < 0.001$, $p < 0.001$), IGT ($p = 0.004$, $p < 0.001$, $p = 0.002$) and T2DM ($p < 0.001$, $p < 0.001$, $p < 0.001$) compared to controls. HbA1c and triglycerides correlated with CNFD ($r = -0.187$, $p = 0.03$, $r = -0.229$, $p = 0.01$), CNFL ($r = -0.228$, $p = 0.009$, $r = -0.285$, $p = 0.001$) and CNBD ($r = -0.187$, $p = 0.033$, $r = -0.229$, $p = 0.01$). BMI correlated with CNFL ($r = -0.395$, $p = 0.007$).

Conclusions

Corneal confocal microscopy is a rapid non-invasive ophthalmic imaging technique that identifies corneal nerve fibre loss, which relates to HbA1c, triglycerides and BMI in patients with acute ischemic stroke.

An abstract graphic on a solid red background. It features three white circles of varying sizes connected by white lines. One circle is at the top left, another is in the upper right, and the largest is at the bottom left. Lines radiate from these circles, creating a network-like structure. The text 'AUTONOMIC NEUROPATHY' is written in white, bold, uppercase letters, positioned to the right of the bottom-left circle.

AUTONOMIC NEUROPATHY

[O15] CONSEQUENCES OF OBSTRUCTIVE SLEEP APNOEA SYNDROME ON AUTONOMIC NERVOUS SYSTEM AND RESTING OXYGEN SATURATION

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Objectives

Obstructive sleep apnoea syndrome (OSAS) is a frequent sleep-disordered breathing in type 2 diabetes (T2D) and obesity. It could affect the autonomic nervous system and cardio-respiratory control and thus worsen cardiovascular prognosis.

Methods

We studied 70 patients: 41 type 2 diabetic patients (age 55 ± 13 yrs, 30 males, BMI 33.4 ± 7.3 kg/m², HbA1c $8.03 \pm 1.56\%$) and 29 non diabetic obese patients (50 ± 13 yrs, 8 males, BMI 39.2 ± 5.3 kg/m²); 33 patients with OSAS (OSAS+, 21 T2Ds, age 56 yrs) and 37 without OSAS (OSAS-, 20 T2Ds, age 51 yrs); and 19 healthy subjects (55 ± 15 yrs, 7 males, BMI 22.0 ± 2.3 kg/m²). We continuously monitored oxygen saturation (SaO₂), heart rate and blood pressure (to measure baroreflex sensitivity (BRS)) for 5 minutes in supine position. Cardiac autonomic dysfunction (CAD) was assessed using standard tests (deep-breathing, lying-to-standing, Valsalva), and the autonomic score (AS) was calculated.

Results

OSAS+ patients had lower BRS compared to patients without OSAS (4.06 ± 2.4 vs 5.30 ± 2.1 ms/mmHg, $p < 0.05$; pvalue for age: NS). AS was mildly increased in both groups as compared to controls (AS=0, $p < 0.001$), and was slightly higher in OSAS+ (1.37 ± 0.9 vs 1.14 ± 0.8 in OSAS-, $p = \text{NS}$). OSAS- patients had lower BRS compared to controls (5.30 ± 2.1 vs 9.22 ± 4.9 ms/mmHg, $p < 0.01$). OSAS+ patients had lower SaO₂ at rest compared to OSAS- patients (95.9 ± 2.0 vs $97.5 \pm 1.4\%$, $p < 0.01$) and to healthy subjects ($97.8 \pm 1.7\%$, $p < 0.01$). A similar decrease in SaO₂ was seen among OSAS+ patients when taking separately T2Ds and obese patients, even if T2Ds tended to be more hypoxic (95.7 ± 1.8 vs $96.3 \pm 1.2\%$, NS).

Conclusions

OSAS patients showed autonomic dysfunction. BRS was more capable than AS to detect autonomic abnormalities in OSAS. Considering the antagonistic interaction between baroreflex and chemoreflex the latter should be increased in condition of depressed BRS, but despite an increased stimulus to ventilation, SaO₂ was still low. All these findings highlight the serious consequences of OSAS on cardio-respiratory control resulting in subclinical resting hypoxia.

[O16] CHANGES IN AUTONOMIC FUNCTION, ADIPOKINES, GUT HORMONES AND INFLAMMATORY MARKERS AFTER SLEEVE GASTRECTOMY IN OBESE SUBJECTS WITH AND WITHOUT DIABETES

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Objective

Gastric bypass induces significant long-lasting weight loss and ameliorates obesity-associated co-morbidities, including type 2 diabetes (T2DM). This form of surgery bypasses the duodenum as well as sacrificing vagal integrity. In contrast sleeve gastrectomy (SG) does not bypass the duodenum and the vagus remains intact, yet the metabolic response remains salutary. However, the exact mechanisms by which these interventions improve metabolic alterations are still unclear. The aim of this study was to evaluate the impact of SG on adipokines, inflammatory markers, circulating gut hormones and autonomic function in obese subjects with different levels of glycemic control.

Methods

Patients were evaluated at baseline and 24 weeks after SG. Adiponectin, leptin, Plasminogen Activator Inhibitor Type 1 (PAI-1) (multiplex analysis; Millipore, Billerica, MA), Interleukin (IL) -6 (HS ELISA), glucagon like peptide (GLP) -1 and glucose-dependent insulinotropic polypeptide (GIP) (RIA) were measured. Cardiac and peripheral autonomic function was assessed with Sudoscan™ that measures electrochemical skin conductance (ESC) of hands and feet, and cardiac autonomic reflex tests using time and frequency domain analysis of heart rate variability (HRV).

Results

Eighty-one subjects completed 24-weeks of follow-up (26 non-T2DM, 28 pre-DM and 27 T2DM). Feet ESC (62.18±2.45 to 71.80±2.65; p=0.018) and HRV measures of sdNN (34.48±3.35 to 45.83±3.55; p=0.025) and rmSSD, a measure of parasympathetic function (23.70±3.53 to 37.50±3.75; p=0.012) improved significantly in T2DM. GLP-1 improved significantly in non-T2DM (33.33±2.36 to 47.52±2.81; p=0.0004) and pre-T2DM (29.47±2.82 to 41.65±3.61; p=0.014) and GIP improved significantly in non-T2DM (12.00±4.06 to 33.58±4.82; p=0.002) and pre-DM (10.22±2.30 to 18.78±2.89; p=0.04) but not in T2DM. Adiponectin/leptin ratio improved significantly in pre-DM (2.61±0.32 to 0.91±0.35; p=0.001) and T2DM (6.05±1.0 to 2.56±1.00; p=0.02). IL-6 did not improve significantly in any group when compared to baseline.

Conclusion

This report shows that autonomic function, inflammatory markers and circulating gut hormones behave differently after SG depending on baseline glycemic state. Gastric sleeve surgery was associated with an improved Adiponectin/Leptin ratio only in T2DM patients. In contrast, GLP-1 and GIP improved significantly in non-T2DM and pre-DM, but failed to reach significance in T2DM patients. Moreover parasympathetic function improved in T2DM. The lack of improvement on GLP-1 and GIP levels in the T2DM patients is surprising and questions the role for GLP-1 and GIP in the rapid recovery of glycemic control in T2DM whereas the improved parasympathetic function may play an important role in the metabolic recovery after sleeve gastrectomy.

[O17] SHORT-TERM PROGRESSION OF CORONARY ARTERY CALCIFICATION IN TYPE 1 DIABETES IS NOT RELATED TO PRESENCE OF AUTONOMIC NEUROPATHY

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Background and aims

Type 1 diabetes is associated with increased cardiovascular (CV) morbidity and mortality by mechanisms not fully understood. Coronary artery calcium (CAC) is associated with CV disease and progression of CAC is an independent predictor of mortality. The aim of this study was to examine whether short-term progression of CAC is increased in patients with type 1 diabetes with and without autonomic neuropathy compared with matched controls.

Materials and methods

Fifty-three normoalbuminuric patients with long-term (>10 years) type 1 diabetes were matched in a 1:2 ratio with 106 controls from the general population according to age, gender, and baseline CAC score and was examined through cardiac computed tomography scans at a mean (\pm SD) of 25 (3) months and 29 (5) months, respectively, for re-measurement of CAC score. Progression of CAC was determined according to the square root method, where progression was defined as a change ≥ 2.5 between the square root transformed values of follow-up and baseline CAC volume score.

Results

Forty of the 159 individuals had progression of CAC. Among patients with type 1 diabetes, 18 (34%) had progression of CAC compared to 22 (21%) of the controls ($p=0.08$). Neither CAN (42% of non-progressors vs 38% of progressors, $p=0.77$) nor decreased HRV (14 in non-progressors vs 15 in progressors) was associated with progression of CAC. Due to adherence to treatment guidelines, patients with diabetes were more often on treatment with blood pressure-lowering medication and statins. In type 1 diabetes compared to controls blood pressure was mean (\pm SD) of 123/74 (13/7) mmHg vs 142/88 (21/10) mmHg ($p<0.001$), LDL cholesterol was 2.4 (0.6) mmol/l vs 3.3 (0.9) mmol/l ($p<0.001$), and BMI was 24.5 (3.0) kg/m² vs 25.8 (3.7) kg/m² ($p=0.03$). There was no difference in smoking status. In multivariable logistic regression, adjusted for these risk factors of CV disease and risk factors of progression, including scan interval, type 1 diabetes was associated with an odds ratio of 4.0 (95% CI 1.5–10.9, $p<0.01$) for progression of CAC. In patients with type 1 diabetes, HbA1c was 63 (11) mmol/mol and diabetes duration was 33 (12) years.

Conclusions

To our knowledge, this is the first study to examine short-term progression of coronary artery calcium in patients with type 1 diabetes compared with matched controls from the general population. We found that even in well-treated, normoalbuminuric patients with type 1 diabetes, there was an odds ratio of 4.0 for short-term progression of CAC, but this was not related to the presence of autonomic neuropathy. This might explain some of the increased CV morbidity and mortality in patients with type 1 diabetes.

[O18] ACUTE CARDIOVASCULAR CHANGES DURING HYPOXIA-INDUCED SYMPATHETIC ACTIVATION IN PATIENTS WITH AN OBSTRUCTIVE SLEEP APNOEA SYNDROME

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Objectives

Obstructive sleep apnoea syndrome (OSAS) is characterised by cardio-respiratory reflex imbalance. Several studies showed that slow breathing (SLB) can improve baro-chemoreflex interaction and oxygen saturation (SaO₂). However we recently showed that SLB can shift an ill-balanced cardio/respiratory reflex interaction and acutely trigger OSAS-related respiratory abnormalities. Sympathetic activation is a physiological consequence of apnoea. The present study aimed to investigate the acute cardiovascular changes occurring during induced apnoea in patients with OSAS.

Methods

In 25 patients with OSAS without CPAP (16 diabetics: age 57±11yrs, 10 males, BMI 36.6±7.7 kg/m², HbA1c 7.6±1.7% and 9 non diabetic obese patients: age 48±9yrs, 2 males, BMI 39.4±3.2 kg/m²) and 20 healthy subjects (age 55±15yrs, 7 males, BMI 22.0±2.3 kg/m²) we continuously monitored ECG, blood pressure (BP, Finapres), SaO₂, peripheral finger blood flow (PPG, photoplethysmography) and ventilation (inductance plethysmography) during spontaneous respiration (5min), 5-min of SLB at 6 cycles/min and 10-min follow-up under spontaneous breathing (POST-SLB). We calculated also baroreflex sensitivity (BRS) and autonomic score (AS) through standard reflex tests (orthostatic test, deep breathing, Valsalva).

Results

At baseline patients with depressed BRS had lower DD% (diastolic duration/ heart period %) (p<0.05). After SLB all patients developed apnoeas or hypopnoeas, conversely none of the controls developed respiratory abnormalities. During each apnoea there was a decrease in SaO₂ (-6.0±3.4%), an increase in heart rate (+6.2±2.9 bpm), systolic BP (+25.0±7.5 mmHg), diastolic BP (+11.2±3.8mmHg), a decrease in systolic duration (-16.5±8.0msec), diastolic duration (-75.7±52.8msec, decrease 4.6 times greater than systolic duration) and in PPG (-46.4±12.4%); as consequences of a sympathetic activation. Heart rate increase correlated negatively with AS (r=-0.609, p<0.01) and positively with BRS (r=+0.55, p<0.01).

Conclusions

In patients with OSAS the SLB test detects respiratory abnormalities and modelizes spontaneous nocturnal apnoeas. During inducible apnoea, sympathetic activation produces dramatic cardiovascular changes. Among OSAS patients, those with severe autonomic dysfunction and depressed BRS respond less in term of heart rate adaptation. All these data highlight the major cardiovascular effects of apnoeas and strongly advocate an accurate diagnosis and treatment of OSAS.

[O19] INTERNAL CAROTID ARTERY FLOW RESISTIVITY AND TYPE-1 DIABETES DURATION

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Objectives

Few studies have focused on brain small vessel disease (SVD) as a primary complication in type 1 diabetes *mellitus* (T1DM). Qualitative and quantitative Magnetic Resonance Imaging (MRI) offers various techniques that can monitor the presence of and consequences of SVD. This study investigates white matter and ventricular / sulcal CSF status plus internal carotid artery (ICA) flow resistivity index (RI) using MRI in patients at varying durations of T1DM.

Methods

66 subjects (49 T1DM and 17 HV) with no history of cardiovascular disease / stroke were assessed and grouped by diabetes duration [Short≤15yrs, Medium=15–30yrs; Long≥30yrs]. Lacunes, white matter WMH and atrophy were rated on T2 MRI. Quantitative ICA MR angiography assessed carotid RI.

Results

There were significant differences in group mean ICA RI (HV=0.54±0.05, Short=0.56±0.08, Medium=0.63±0.06, Long term=0.65±0.06; p<0.001). Post-hoc analysis indicated higher RI in Long and Medium-term compared to both Short-term (p<0.005; p<0.05) and HV (p<0.001; p<0.001) groups. There were no significant group differences in the prevalence of imaging abnormalities ($\chi^2=7.064$, p=0.07). In cases of no imaging abnormalities, ICA RI varied with retinopathy stage (none=0.53±0.05, pre-proliferative=0.56±0.08, proliferative=0.63±0.07; p<0.05). ICA RI correlated with duration of diabetes (r=0.6, p<0.001), prevalence of retinopathy (r=0.6, p<0.001), peripheral neuropathy (r=0.3, p<0.05) and higher SVD score (r=0.3, p<0.04), adjusting for age.

Conclusions

Changes in ICA RI paralleled diabetes duration. Prevalence of diabetic retinopathy, polyneuropathy and higher severity of cerebral SVD (all microangiopathic complications) were associated with ICA RI. Even in patients without evidence of cerebral SVD on standard MRI, ICA RI was higher in those with retinopathy than those without. These data suggest RI is an early direct marker for cerebrovascular involvement. As such, RI may help to elucidate the mechanistic basis of diabetic-vascular-neurodegeneration as well as aid initiation of and monitor prophylactic therapies.

[O20] ACUTE GLYCEMIC CHANGES AND MEASURES OF CARDIOVASCULAR AUTONOMIC FUNCTION IN TYPE 1 DIABETES

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Objective

To assess the effects of acute glyceimic changes on measures of cardiovascular autonomic function and subsequent arrhythmogenesis risk in type 1 diabetes (T1D) subjects with various stages of disease.

Methods

T1D subjects with no complications (N=7), mild (N=5) and advanced complications (N=7), mean age 36±15 years, diabetes duration 21±14 years, HbA1c 8.1±1.3%), and age-matched healthy controls (HC) (N=6) underwent sequentially euglycemic (100±20mg/dl), hyperglycemic (300±20mg/dl) and hypoglycemic (45±10mg/dl) clamp studies to mimic real life scenarios. All clamps were done in the same day after an overnight fast in the clinical research center while holding any other medications. CAN was assessed by time [standard deviation of normal RR interval (SDNN), root mean square of successive differences (RMSSD)] –and–frequency–domain [low frequency (LF), high frequency (HF) and their ratio (LF/HF)] indices of heart rate variability (HRV) derived from continuous ECG recordings during this sequence using the ANX 3.1 (ANSAR Inc. Philadelphia, PA). Blood and urine samples were obtained at 30-min intervals for stress counter–regulatory hormones and metabolomics markers.

Results

Indices of HRV were similar between T1D with no complications and HC during euglycemic and hyperglycemic clamp conditions. However low frequency power was reduced in T1D vs HC during hypoglycemic clamp study. In addition, several time and frequency indices of HRV were significantly lower during euglycemic (LF 1.9±1.9 vs 1.8±2.2 vs 5.0±2.1 ms²; HF 0.3±0.1 vs 1.2±2.1 vs 2.5±1.5 ms²; SDNN 35±15 vs 44±26 vs 71±25 ms; RMSSD 12±5 vs 20±21 vs 38±18 ms. p<0.05 for all), hyperglycemic (LF 1.0±1.0 vs 2.2±2.6 vs 4.9±2.5 ms²; HF 0.3±0.5 vs 0.9±1.3 vs 2.6±1.9 ms²; SDNN 32±15 vs 97±1 vs 84±35 ms; RMSSD 10±5 vs 27±1 vs 42±26 ms, p<0.05 for all), and hypoglycemic (LF 1.7±1.0 vs 2.4±2.6 vs 5.0±1.6 ms²; HF 0.3±0.1 vs 0.4±0.4 vs 2.9±1.1 ms²; p<0.05 for all) clamp studies in T1D with advanced complications compared with those with mild complications and without complications respectively.

Conclusion

These preliminary data suggest that acute glyceimic changes affect cardiovagal balance in T1D patients with complications, which may potentially explain the link between CAN and cardiac arrhythmias.



TREATMENT

[O21] EFFECT OF ENTRESTO VS VALSARTAN ON VASCULAR AND NEURAL COMPLICATIONS IN TYPE 2 DIABETIC RATS

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Objective

Determine the efficacy of ENTRESTO, a drug consisting of an angiotensin II receptor blocker and neutral endopeptidase inhibitor vs valsartan, an angiotensin receptor blocker, on vascular and neural complications in a type 2 diabetic rat model.

Methods

Type 2 diabetic rats after 4 weeks of hyperglycemia and control rats were treated for 16 weeks with ENTRESTO (68 mg/kg) or valsartan (31 mg/kg) daily by gavage. The endpoints evaluated included vascular relaxation to acetylcholine by epineurial arterioles, motor and sensory nerve conduction velocity, thermal nociception, corneal sensitivity and innervation of sensory nerves in the cornea and skin.

Results

Our findings show that ENTRESTO or valsartan did not improve hyperglycemia or glucose utilization (data not shown). Untreated diabetic rats were thermal hypoalgesic, had impaired corneal sensitivity, reduced motor and sensory nerve conduction velocities and decrease innervation of the cornea and skin (see Table below). Vascular relaxation to acetylcholine by epineurial arterioles, blood vessels that provide circulation to the sciatic nerve, was also significantly decreased in untreated diabetic rats. These endpoints were all significantly improved in diabetic rats treated with ENTRESTO which was more efficacious than valsartan treatments.

Conclusions

The studies suggest that ENTRESTO could be an effective treatment for neural and vascular complications associated with diabetes. ENTRESTO has been approved by the Food and Drug Administration for the treatment of heart failure and could quickly advance to clinical trials.

Determination	Control (12)	Control + ENTRESTO (6)	Control + Valsartan (6)	Diabetes (12)	Diabetes + ENTRESTO (11)	Diabetes + Valsartan (10)
MNCV (m/sec)	55.4±1.4	62.1±3.9	53.3±1.2	41.8±1.6 ^a	49.6±1.6 ^b	47.1±2.1 ^a
SNCV (m/sec)	33.7±1.7	30.9±0.8	31.7±0.9	26.5±0.6 ^a	29.7±0.6	28.8±0.6 ^a
IENF (profiles/mm)	20.3±1.1	20.0±1.2	18.5±1.8	14.9±1.0 ^a	21.2±1.7 ^b	16.9±1.3
Corneal nerve fiber length (mm/mm ²)	9.5±0.4	9.1±0.6	9.2±0.5	4.5±0.4 ^a	7.5±0.3 ^{ab}	5.5±0.3 ^a
Thermal nociception (sec)	11.4±0.5	11.7±0.5	12.0±0.5	19.6±0.9 ^a	12.3±0.6 ^b	16.5±0.8 ^{ab}
Corneal sensitivity (cm)	5.75±0.08	5.96±0.04	5.92±0.06	4.73±0.17 ^a	5.91±0.05 ^b	5.75±0.08 ^b

Data are presented as the mean±SEM. a) p<0.05 compared to control; b) p<0.05 compared to diabetic. Parentheses indicate the number of experimental animals.

[O22] POLYNEUROPATHY BEFORE AND AFTER BARIATRIC SURGERY: ASSOCIATION WITH SERUM TRIGLYCERIDE LEVELS?

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Objective

To define prevalence of polyneuropathy (PNP), abnormal neurological tests and associated factors in patients from an obesity treatment center before and 6 to 18 months after being submitted to bariatric surgery (BS).

Methods

In a cross-sectional study, obese, no diabetic patients were evaluated before BS (n=294) and after gastrectomy (n=113; Y-in-Roux or Sleeve). PNP was defined by the Michigan Neuropathy Screening Instrument (MNSI) and by the Neuropathy Disability Score (NDS). By using univariate analysis, associations with values/abnormalities of age, waist circumference (WC), body weight (BW), height, body mass index (BMI) and serum 25-hydroxy-vitamin D (25-OH-D), B12 vitamin, HDL-cholesterol, LDL-cholesterol, triglycerides (TG), hemoglobin-A1c, glucose before and 2 hours after 75 grams of oral glucose, magnesium, iron, ferritin and presence of metabolic syndrome (MS) were tested. Variables with a p<0.2 on univariate analysis were evaluated with Pearson's multivariate models.

Results

Before BS, prevalence of PNP was 26.2% with the MNSI and 5.2% with the NDS. After BS, prevalence of PNP was 18.6% with MNSI and 4.4% with NDS. By using the MNSI, before and after BS, presence of PNP was associated with higher serum levels of 25-OH-D (p=0.023 and p=0.044, respectively), on univariate analysis. By using NDS, no significant associations were found, but evaluation of the results of two NDS tests that are not used on MNSI (temperature sensation and pin-prick) showed a positive association with serum levels of TG (p=0.035 before BS and p=0.030 after BS) and with BW (p=0.016 after BS) on univariate analysis. In three models of Poisson multivariate analysis, after BS, positivity of temperature sensation and/or pin-prick test was associated with serum TG levels (table).

Conclusion

Abnormal small fiber tests are probably related to serum triglyceride levels after BS.

Post-BS	Model 1 p=0.04		Model 2 p=0.03		Model 3 p=0.03	
	PR (95%IC)	p	PR (95%IC)	p	PR (95%IC)	p
Age	0.94 (0.88-1.01)	0.05	0.95 (0.89-1.01)	0.13	0.95 (0.89-1.01)	0.09
WC	1.02 (0.98-1.07)	0.31	NA	NA	NA	NA
BW	NA	NA	1.02 (0.99-1.05)	0.09	NA	NA
BMI	NA	NA	NA	NA	1.05 (0.98-1.13)	0.15
TG	1.02 (1.01-1.03)	0.01	1.02 (1.01-1.03)	0.01	1.02 (1.01-1.03)	0.01

NA: not applicable

[O23] CELL THERAPY USING HUMAN DENTAL PULP STEM CELLS BYPASSES IMPACTS OF AGING AND DIABETES AND AMELIORATES DIABETIC POLYNEUROPATHY

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We previously demonstrated that the transplantation of progenitor/stem cells improved diabetic polyneuropathy using diabetic animals. However, aging and diabetes were reported to disrupt the efficacy of cell therapy using progenitor/stem cells. Dental pulp stem cells, a kind of mesenchymal stem cells, are a favorable stem cell source, because they can be isolated from extracted teeth by orthodontic reasons in youth or before the onset of diabetes and cryopreserved until the time of need. We hypothesized that the transplantation of cryopreserved human dental pulp stem cells (hDPSCs) ameliorates diabetic polyneuropathy. We collected human impacted third molars from 4 adults (13–23 years of age) at Aichi Gakuin University hospital. Written informed consent was obtained from each donor. Dental pulp was extracted and hDPSCs were isolated by collagen digestion methods. hDPSCs were expanded to the fourth passage and cryopreserved. Before transplantation, hDPSCs were thawed and re-cultured. Diabetes was induced by an intraperitoneal injection of STZ in 6 week-old BALB/cAJcl-nu/nu mice. Eight weeks after STZ injection, hDPSCs (1×10^5 cells/limb) were transplanted into unilateral hindlimb skeletal muscles of normal and diabetic mice. Four weeks after the transplantation, neurophysiological, gene expression and pathological assessments were performed. Diabetic mice showed significant delay of sciatic motor/sensory nerve conduction velocity (MNCV/SNCV) and decrease in sciatic nerve blood flow (SNBF) compared with the normal mice. hDPSC transplantation significantly ameliorated MNCV/SNCV and SNBF in the transplanted side of the diabetic mice. We confirmed that some of the transplanted hDPSCs located around the bundles of hindlimb muscles and expressed angiogenic and neurotrophic factors. Furthermore, conditioned medium of hDPSC culture increased neurite outgrowth of primary cultures of mouse dorsal root ganglion neurons. In conclusion, transplantation of hDPSCs may be a desirable tool for treatment of diabetic polyneuropathy.

[O24] EFFECTS OF SAXAGLIPTIN ON CARDIOVASCULAR AUTONOMIC NERVOUS SYSTEM ACTIVITY AND HEMODYNAMIC PARAMETERS IN OBESE PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE

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Objective

Impaired glucose tolerance (IGT) is associated with a high cardiovascular risk. Diabetes prevention should be proposed in this situation. In obese patients, cardiac output (CO) is increased and autonomic cardiovascular changes are common. Cardiovascular safety is an issue for all new anti-diabetic treatments. DPP4 inhibitors induce a slight increase in plasma GLP1 which might alter cardiovascular autonomic activity. The aim of this study was to examine the acute and long-term effects of saxagliptin on plasma glucose and cardiovascular autonomic activity and hemodynamic parameters before and after a standard breakfast in patients with IGT.

Patients and methods

We included 24 IGT patients, normotensive or with well-controlled hypertension, without any history of cardiovascular disease. The patients were randomized in a double blind trial with saxagliptin 5mg (S) vs placebo (P). The tested treatment was taken from the first to the second visit, during 12 weeks. The biological and physiological investigations were performed before and every hour after a standardized breakfast (75g carbohydrates) during 4 hours, at visit 1 (day 0) and visit 2 (day 90). Cardiac vagal activity (HF-HR), sympathetic activity (LF-HR), vascular sympathetic activity (LF-sBP) and sympatho-vagal balance (LF/HF-HR) were assessed by spectral analysis of heart rate and blood pressure variations; CO and left ventricular ejection time (LVET) were measured by thoracic impedance (Task Force Monitor®) at controlled breathing rate (12/min). Radial and central blood pressure and carotid-to-femoral pulse wave velocity were measured by arterial tonometry (Sphygmocor®). Mean cutaneous blood flow (CBF) was measured on the forearm during 6 minutes by laser doppler (Periflux®) and endothelial function using iontophoretic cutaneous infusion of acetylcholine (area under curve AUC-CBF).

Results

Sex-ratio (Females S: 83% vs p: 75%), age (S: 49.8±14.6 vs p: 40±10.7 years) and BMI (S: 36.2±5.6 vs p: 37.5±4.1kg/m²) were similar in both groups. Compared to the placebo group, patients treated by saxagliptin had similar level of plasma glucose at fasting at day 0 and day 90 but lower plasma glucose at 1 and 2 hours after breakfast by day 0 and at day 90 as compared to placebo-treated patients (p<0.01 to p<0.004). There was no significant difference for sympatho-vagal activity (LF-HR, LF-sBP, HF-HR and LF/HF-HR), CO, LVET, blood pressure and pulse wave velocity, mean CBF and AUC-CBF between the 2 groups at day 0 and day 90, before and after breakfast.

Conclusion

Saxagliptin has a beneficial effect on post-prandial plasma glucose in obese patients with IGT without altering cardiovascular autonomic activity, the hemodynamic parameters including cardiac output, nor artery stiffness, cutaneous microcirculation and endothelial function.

[O25] TOPILOXOSTAT, A NOVEL XANTHINE OXIDASE INHIBITOR, AMELIORATES MURINE DIABETIC POLYNEUROPATHY

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Objectives

Type 2 diabetes is known to be frequently complicated with hyperuricemia. Therefore, safe compound, which can concurrently treat type 2 diabetes and hyperuricemia, is expected. The final step of purine metabolism is catalysis of xanthine to urate by xanthine oxidase (XO). By this reaction, reactive oxygen species (ROS) are simultaneously generated, leading to cell damage. Hence, an inhibitor of XO, can decrease the production of urate and generation of ROS. Previous report showed a partial correction of peripheral nerve function in streptozotocin-induced diabetic rats by XO-inhibitor allopurinol. The aim of this study is to evaluate the effects of a new potent XO-inhibitor, topiloxostat (To), on polyneuropathy in *db/db* mice.

Methods

5-week of age *db/db* mice (*db/db*) and C57BL/6 mice (C57) were treated daily with 1mg/kg (dbT1) and 2mg/kg (dbT2) freely ingested with food for 8 weeks. During experimental period, HbA1c, 2g/kg oral glucose tolerance test (OGTT), nerve conduction velocity (NCVs) and tail flick test were performed. At end, after measurement of sciatic nerve blood flow (SNBF) by laser doppler, mice were killed for the evaluation of intra-epidermal nerve fiber density (IENFD) and phosphorylation of ERK (pERK) in sciatic nerve.

Results

To-treatment had no effects on the values of HbA1c and OGTT in either *db/db* or C57. There was significant delay of NCVs and elevated perception threshold of tail flick in *db/db* compared to C57. To-treatment improved these measures in a dose dependent manner ($p < 0.05$ dbT2 vs C57 for NCVs, and $p < 0.05$ *db/db* vs dbT1 and $p < 0.01$ *db/db* vs dbT2 for tail flick). To-treatment at a high dose also corrected SNBF and IENFD distribution in *db/db* ($p < 0.01$ dbT2 vs *db/db* for both). Expression of p-ERK in sciatic nerve was significantly increased in *db/db* compared to C57 ($p < 0.01$). To-treatment reduced the expression of p-ERK dose-dependently ($p < 0.05$ *db/db* vs dbT1 and $p < 0.01$ *db/db* vs dbT2).

Conclusions

A novel and potent XO-inhibitor, topiloxostat, ameliorated neuropathic changes functionally and structurally in obese type 2 diabetic *db/db* mice without an influence on glucose metabolism. Topiloxostat can be a promising compound for the patient with diabetic neuropathy and hyperuricemia.

[O26] STUDY OF PARAMETERS OF MACRO- AND MICROCIRCULATION IN PATIENTS WITH DIABETES MELLITUS TYPE 2 AT ADMINISTRATION OF SERUM DEPROTEINIZED DERIVATES BOVINE (ACTOVEGIN®)

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The changes of vessels in microcirculatory bloodstream are one of the most important manifestations of diabetes *mellitus* that determines the features of the course, complications and outcomes of this disease. Changes in vessels at diabetes occur as a result of metabolic changes and the development of tissue hypoxia. The administration of drugs, which have antihypoxic and antioxidant effects (for example, Actovegin), at the earliest stages of diabetes will prevent the development of severe complications, in the pathogenesis of which hypoxia, ischemia and oxidative stress play a role.

Goal

The goal of the study is to evaluate the effect of treatment by Actovegin on the parameters of the microcirculation of the capillary bed, the rigidity of the arteries and endothelial function in patients with diabetes *mellitus* type 2.

Materials and methods

A comparative study of parameters of microcirculation, rigidity of the arteries and endothelial function was conducted in patients with diabetes *mellitus* type 2 before and after administration of Actovegin (group A, n=20) and in patients with diabetes *mellitus* type 2 without administration of Actovegin (group B, n=20).

Results

The parameters of microcirculation were improved significantly: the dilatation of arterial part of the capillaries at administration of Actovegin (p=0.005). In patients with an initially reduced endothelial function, a significant increase in its function (p=0.017) was observed at administration of Actovegin, as well as a reduction of perivascular edema (p=0.059) was near-significant. It was found that treatment with Actovegin does not effect on the parameters of central hemodynamics and arterial rigidity in patients with diabetes *mellitus* type 2 (p=0.778).

Conclusion

The study showed that Actovegin has a marked effect on the parameters of the capillary bed by the reduce of the degree of perivascular edema, and normalizes the arterial capillary sections, as well as increases in endothelial function, especially in patients with diabetes *mellitus* type 2, in whom this index was initially lowered.

Long-term use of Actovegin can be used in endocrinological practice to correct microcirculatory disorders in patients with diabetes *mellitus* type 2.

Table 1. General characteristics of study subjects.

Parameters	Group A before treatment (n=20)	Group B Initial (n=20)	p
Gender, m/f, %	15/85	25/75	0,489
Age, years	58 (54; 62)	54 (52; 60)	0,347
Duration of DM, years	1 (0; 5)	4 (2; 5)	0,092
Duration of AH, years	6 (3; 10)	10 (8; 12)	0,428
BMI, kg/m ²	34,2 (31; 36)	32,0 (30; 34)	0,182
Glycated hemoglobin, %	6,4 (6,2; 7,0)	7,1 (6,7; 7,3)	0,073
Smoking, %	20	20	0,987
Family history of DM, %	40	45	0,567

The background features a complex network of white lines and circles on a solid red field. The lines intersect to form various polygonal shapes, while the circles are placed at several of these intersection points, creating a stylized, interconnected structure.

**PATHOGENESIS
AND DIAGNOSIS**

[O27] FUNCTIONAL REORGANISATION OF THE SOMATOSENSORY CORTEX DETERMINES CLINICAL PRESENTATION IN DIABETIC NEUROPATHY

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Cortical plasticity is a fundamental property of the human CNS that enables adjustment to nerve injury but it can have maladaptive consequences resulting in chronic pain. We have previously demonstrated significant brain volume loss localised to the primary somatosensory cortex (S1) in diabetic neuropathy (DN). More recently, we reported functional reorganisation of S1 in patients with painful DN who were also insensate. This study aims to examine the relationship between the degree of functional reorganisation and intensity of neuropathic pain.

Methods

Clinical, neurophysiological and magnetic resonance imaging (MRI) data were compared for 35 Type 1 diabetes subjects [n=9 No-DN (male 6, mean age 45.9±10.1), 9 painless DN (5, 46.3±12.0), 9 painful DN sensate (4, 48.4±12.0) and 8 painful DN insensate (6, 44.5±12.1)] and 9 healthy volunteers (4, 51.5±7.9)]. 3T MRI system (Acheiva, Phillips) and cortical reconstruction and volumetric segmentation by FreeSurfer software were used. Blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) was used to examine functional organisation of the S1 cortex in response to noxious thermal stimulation of the right foot. Linear regression analysis was performed to examine the contribution of brain structural changes (S1 cortical thickness), pain (neuropathic and evoked pain) and clinical parameters (age, neuropathy composite score, diabetes and pain duration) on observed S1 plasticity.

Results

Painful DN insensate subjects had significantly lower mean S1 cortical thickness [F (4,39) =5.78, p=0.001] compared to other study cohorts. No significant difference emerged between subjects in cortical thickness in the control region [frontal cortex F (4,39) =1.23, p=0.31. On average somatosensory cortical thickness was 12.2% lower in painful DN insensate subjects compared to healthy volunteers. S1 plasticity was significantly correlated with overall neuropathic pain score (NTSS-6, r=-0.45, p=0.04), evoked foot pain score (r=-0.46, p=0.03) and S1 cortical thickness (r=-0.57, p=0.01). The most parsimonious regression model relied solely on neuropathy composite score as the independent variable (R²=0.54, F (1,15) =17.9, p=0.01; adjusted R²=0.51) that explained most of the variance in S1 plasticity.

Conclusions

Subjects with painful DN who were also insensate had the greatest reduction in S1 cortical thickness. FMRI demonstrated greater S1 cortical neuronal plasticity which was significantly associated with pain intensity and the severity of neuropathy. We have demonstrated in these experiments how structural brain changes are related to functional reorganisation of the S1 cortex which ultimately determines the clinical presentation of DN. This may provide clues to the pathogenesis of different sensory phenotypes of DN.

[O28] IMPAIRED HEMODYNAMIC RESPONSE TO THERMAL PAIN IN PATIENTS WITH PAINFUL DIABETIC NEUROPATHY: A DYNAMIC SUSCEPTIBILITY CONTRAST MRI STUDY

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Aim

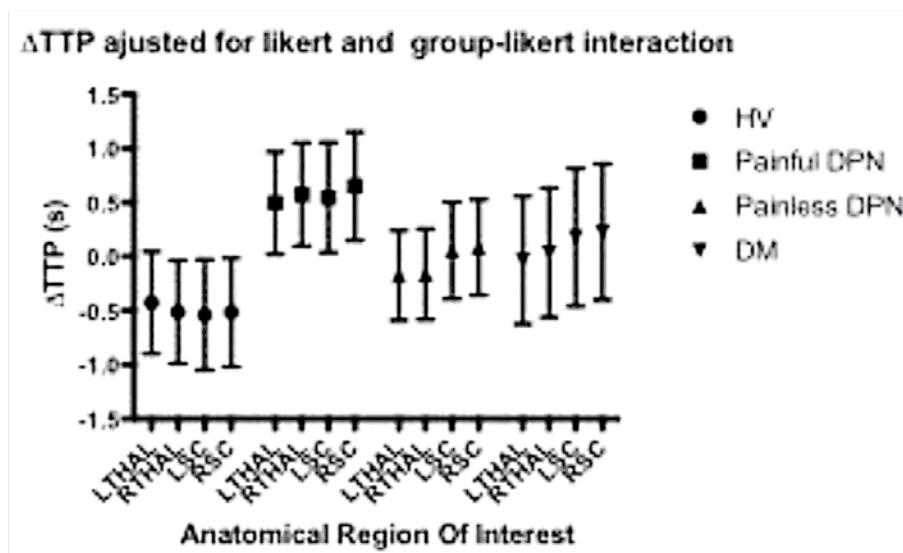
Painful diabetic peripheral neuropathy (P-DPN) causes distressing neuropathic pain that is only partially responsive to treatment. A better understanding of central nervous system CNS correlates of P-DPN is vital to develop more effective therapeutics. The aim of this study was to measure cerebral perfusion of the pain processing areas of the brain using Magnetic resonance-Dynamic susceptibility Contrast (MR-DSC) imaging during resting and under experimental pain condition.

Methods

54 T1DM subjects [19 P-DPN, 23 painless DPN, 13 DM (T1DM without DPN) and 19 Healthy Volunteers (HV)] underwent MR images at 3T using DSC, T2*-weighted technique (TR/TE=1250/35ms; 72 dynamics), to assess a bolus of intravenous gadolinium-chelate passing through the cerebral vascular bed. Subjects were scanned at baseline and during 90s of heat-pain applied to the right lateral thigh (non-neuropathic area). Contrast perfusion was determined to yield time-to-peak (TTP) concentration of gadolinium in regions of interest (ROI): right and left thalamus, right and left sensory cortices (RSC and LSC) and parieto-occipital white matter (POWM) as control. Δ TTP was calculated by subtracting TTP baseline from TTP pain.

Results

At baseline although the mean TTP (seconds) in the ROIs was numerically shorter in the P-DPN group [e.g. Rthal: M (SD): 9.22 (1.13) vs HV 9.83 (0.99), DM 9.59 (0.90), painless DPN 9.94 (0.97)] this was not statistically significant (p=0.058). However the Δ TTP in response to thermal pain was significantly different between the groups Lthal (p=0.021), Rthal (p=0.003), LSC (p=0.009), RSC (p=0.008) (Figure).



Whilst HV respond to the thermal pain by shortening the TTP the P-DPN group paradoxically lengthen the TTP indicating a breakdown in hemodynamic response to external pain.

Conclusion

This study demonstrated changes in perfusion of ROI associated with pain sensation. Subjects with P-DPN experience a paradoxical increase in TTP, indicating that chronic continuous neuropathic pain state may result in a failure to mount a hemodynamic response and descending inhibition. This novel finding may serve as an objective marker of P-DPN, and in the future may facilitate the development of novel treatments.

[O29] ENHANCED SUBCLINICAL INFLAMMATION IN PAINFUL COMPARED TO PAINLESS DIABETIC POLYNEUROPATHY. A MULTIMARKER APPROACH

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Objectives

The determinants and mechanisms contributing to the phenotype of diabetic sensorimotor polyneuropathy as a painful (DSPN+p) or painless (DSPN-p) entity remain unclear. Since inflammation has been implicated in the pathogenesis of neuropathic pain, we hypothesized that pro- and/or anti-inflammatory processes could be more relevant in DSPN+p than DSPN-p.

Methods

Using a new multimarker assay (Proseek Multiplex INF I assay, OLINK Proteomics), we measured 92 serum biomarkers including pro- and anti-inflammatory cytokines and chemokines as well as vascular and growth factors in 193 patients with DSPN+p and 171 with DSPN-p from the PROPANE study (DSPN+p/DSPN-p [mean±SD]: age: 65.7±9.9/68.0±10.3 years, male: 68/82%, BMI: 31.3±5.5/29.3±5.1 kg/m²; type 2: 86/83%, diabetes duration: 16.1±12.1/16.1±12.6 years, HbA1c: 7.6±1.4/7.3±1.2%). DSPN was diagnosed using modified Toronto Consensus (2011) criteria, while DSPN+p and DSPN-p were stratified using a cutpoint of 4 points on the Likert scale for chronic pain lasting >1 year in the distal lower limbs.

Results

After adjustment for sex, age, BMI, smoking, diabetes type, diabetes duration, and HbA1c, compared to patients with DSPN-p those with DSPN+p showed increased levels (normalized protein expression values) of the pro-inflammatory markers CUB domain-containing protein 1 (CDCP1: 3.29±0.64 vs 3.16±0.58), signaling lymphocytic activation molecule 1 (SLAMF1: 2.57±0.58 vs 2.71±0.57), and chemokine (C-C motif) ligand 20 (CCL20: 5.40±1.07 vs 5.74±1.31) as well as the anti-inflammatory markers interleukin 10 (IL-10: 2.37±0.43 vs 2.53±0.64) and osteoprotegerin (OPG: 10.23±0.39 vs 10.13±0.38) (all p<0.05). In patients with DSPN+p, several inflammatory markers correlated with diminished quantitative sensory tests (e.g. CDCP1 with cold thermal detection threshold on the hand: β =-0.241; p=0.002) and nerve conduction velocity (NCV) (e.g. SLAMF1 with peroneal motor NCV: β =-0.262; p=0.0005), whereas in those with DSPN-p these associations were either absent or markedly weaker.

Conclusions

Patients with painful DSPN show higher systemic levels of pro- and anti-inflammatory mediators than those with painless DSPN, pointing to a role of inflammatory processes in painful diabetic neuropathy.

[O30] DIABETES-INDUCED MICROVASCULAR COMPLICATIONS AT THE LEVEL OF THE SPINAL CORD AND NEUROPATHIC PAIN DEVELOPMENT

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Objectives

Dysfunction of the neurovascular interaction within the nervous system of diabetic patients is associated with the onset of many neurological disease states. However, to date the link between the neurovascular network within the spinal cord and regulation of nociception has not been investigated despite neuropathic pain being common in diabetic patients. It is hypothesised that hyperglycaemia induced endothelial degeneration in the spinal cord contributes to the development of diabetic neuropathic pain.

Methods

Female Sprague dawley rats (~200g) were used. A chemically induced model of type 1 diabetes (streptozotocin, insulin supplemented) was used and compared to age matched controls. The diabetic group was treated (intraperitoneal) biweekly with either vehicle or VEGF-A₁₆₅b, 20ng/g. Experiments were designed in accordance with UK Home Office legislation and ARRIVE guidelines. Mechanical (Von Frey hairs) and heat (Hargreaves test) nociceptive pain behaviour was carried out in all animals. Animals were split into experimental groups for histology and vascular permeability studies. Evans blue extravasation (intravenous 50mg/kg) was measured (solute flux µg/min/g) in the lumbar spinal cord. Lumbar spinal cord tissue was paraformaldehyde fixed and the dorsal horn imaged for endothelial cell (CD31/IB₄) and neuronal (c-fos/NeuN) markers. Additional spinal cord samples were collected for western blot analysis for endothelial markers (CD31/Occludin/VE-Cadherin).

Results

Diabetic animals developed mechanical allodynia and heat hyperalgesia as well as demonstrated spinal neuronal (c-fos) activation. This was associated with a reduction in the number of CD31/IB₄ positive blood vessels and reduction in Evans blue extravasation in the lumbar spinal cord of diabetic animals versus age-matched controls. Endothelial markers were downregulated in the spinal cord of the diabetic group versus controls, as well as a concurrent reduction of VEGF-A₁₆₅b expression. In diabetic animals, vascular endothelial growth factor (VEGF) -A₁₆₅b treatment restored normal Evans blue extravasation and prevented vascular degeneration, diabetes-induced central neuron sensitisation and neuropathic pain.

Conclusions

Hyperglycaemia leads to a dysfunction of endothelial function in the spinal cord, which was associated with diabetic neuropathic pain development. Treatment with the cytoprotective growth factor VEGF-A₁₆₅b prevents endothelial damage and consequent neuropathic.

[O31] EVALUATION OF DIABETIC POLYNEUROPATHY BY PHOTOACOUSTIC AND ULTRASOUND IMAGING

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Objective

Combined photoacoustic and ultrasound imaging is a novel approach to visualize tissue ischemia and pathologic changes in minute organs. This method enables to quantitate in situ tissue vascularity related to hemoglobin distribution. We applied this method to evaluate polyneuropathy in patients with type 2 diabetes.

Methods

We developed photoacoustic imaging attached with ultrasound detection system based on commercial medical ultrasound system using newly high-sensitivity 9 MHz probe and Alexandrite laser (Fujifilm Corp., Kanagawa, Japan). Imaging studies on the sural and median nerves were conducted on 59 Japanese type 2 diabetic subjects (age 66±10) and 12 healthy control subjects (age 46±10). Imaging was traced on the nerve bundle size and on blood hemoglobin images dually as scores of brightness (composite of hemoglobin concentration and blood oxygenation rate), vascularity (vessel area occupancy), and N-area (nerve bundle area). These parameters were analyzed for the correlation with clinical profile of neuropathy, nerve conduction data and other complications of diabetes. The imaging data were also examined for the correlation with other complications of diabetes, e.g. foot gangrene, retinopathy/nephropathy.

Results

The developed system well revealed cross and longitudinal features of multiple nerve fascicles of median and sural nerves showing honeycomb appearance and vessel distribution as yellow color in epi- and endoneurial vessels. N-area of sural and median nerve was enlarged in cases with diabetes, in particular with severe diabetic neuropathy complicated with foot gangrene. Brightness was significantly reduced in diabetic subjects with neuropathy compared to non-neuropathic subjects and there were significant correlations between brightness and F-wave latencies of both median and tibial nerves. Vascularity and N-area of sural nerve also correlated with reduced CMAP. There was an inverse correlation between pulse wave velocity and vascularity of sural nerve. Marked diminution of brightness was found in the sural nerve of gangrenous foot. Furthermore, there was a trend toward decrease in vascularity with progression of retinopathy.

Conclusion

The newly developed dual photoacoustic and ultrasound evaluation clearly disclosed reduced tissue vascularity and pathologic features of the nerve bundles. These results reflect the severity of neuropathy and other status of microvascular complications of diabetes. This novel and non-invasive method appeared to be valuable for the evaluation of neuropathy and may be useful to predict the risk for foot amputation.

[O32] RAGE SIGNALING IS ASSOCIATED WITH MACROPHAGE ACTIVATION AND INSULIN RESISTANCE DURING THE DEVELOPMENT OF EXPERIMENTAL DIABETIC POLYNEUROPATHY

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Objectives

Advanced glycation end products (AGEs) – RAGE signaling contributes to a major pathogenesis of diabetic complications including diabetic polyneuropathy (DPN). RAGE signaling exerts NF- κ B transcription, leading to macrophage activation. Although there is a fact of macrophage infiltration in the sciatic nerve (SN) of DPN, its implication in neuropathy remains controversial. The aim of this study is to elucidate the role of RAGE signaling in the development of neuropathy with special reference to the implication of macrophage infiltration and its relevance to insulin resistance (IR).

Methods

In vitro experiment: Schwann cells (IMS32) were co-cultured in transwell chamber with proinflammatory macrophages differentiated by 1mg/ml AGEs (R1) or anti-inflammatory macrophages (R2) differentiated by 100ng/ml IL-4 from RAW246 (R0). After 24 hours, they were stimulated with insulin for 20 minutes and the expression of phospho-AKT (pAKT) was evaluated.

In vivo experiment: Nerve conduction velocity (NCV) was evaluated in diabetic RAGE-deficient mice (RKOD) and C57BL6 mice (WD) 8 weeks after streptozotocin injection. Phenotype of infiltrated macrophages in SN was determined by immunohistochemistry and qPCR. IR of SN was evaluated by pAKT expression.

Results

In vitro: pAKT expression in Schwann cells co-cultured with R1 for 24 hours was markedly attenuated compared to those with R0 or R2. *In vivo*: WD exhibited significant delay of NCVs compared to non-diabetic counterpart, while NCVs delay was absent in RKOD. In sciatic nerve of WD, there emerged a significant increase in Iba-1-positive macrophages compared to that of W ($p < 0.05$). Surprisingly, the increase was more robust, composed mostly of R2 phenotype, in RKOD than WD ($p < 0.01$). Insulin-responsive pAKT expression in SN was attenuated in diabetic WT, whereas diabetic RAGE-KO preserved its expression to the extent in non-diabetic counterparts.

Conclusion

Our study suggests that activation of RAGE signaling exerts macrophage activation to promote proinflammatory reaction and insulin resistance, resulting in the development of DPN. Inhibition of RAGE signaling suppresses the inflammatory reaction towards predominance of anti-inflammatory macrophages and improvement of IR.



Poster Abstracts

Santa Cruz Monastery

The Santa Cruz Monastery was founded in 1131 by the Order of Regular Canons of St Augustine. The primitive Romanic church dates from the 12th century.

The quality of the artistic interventions in the monastery, particularly in the Manueline period, make this one of the main historical and artistic monuments of Portugal.

The background is a solid red color. Overlaid on this are several white circles of varying sizes and thick white lines. The circles are positioned at various points, and the lines connect them, creating a network-like or geometric pattern. One large circle is located in the lower-left quadrant, another in the upper-middle, and a third in the top-left corner. Lines radiate from these circles and intersect to form a complex web of shapes.

**YOUNG INVESTIGATORS
POSTER PRESENTATIONS**

[P1] SYMPTOMS OF DIABETIC POLYNEUROPATHY ARE RELATED TO FALLS IN PATIENTS WITH TYPE 2 DIABETES

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Objective

Distal sensorimotor polyneuropathy may cause impaired balance and unstable gait, which combined with decreased joint mobility and incoordination leads to an increased risk of falls. Falls may have serious consequences including decreased mobility, physical inactivity and higher morbidity and mortality.

Method

In the present study, we performed a cross-sectional analysis of survey data on patients with type 2 diabetes included in the cohort established by the Danish Center for Strategic Research in Type 2 Diabetes (DD2) in 2011. Questionnaires were sent to 7,011 patients with type 2 diabetes, and 5,755 questionnaires were completed and returned. Among study respondents, 5,440 had non-missing data on falls and neuropathic symptoms, which were included for further analysis. In addition, the survey consisted of data on age, gender, BMI, physical activity, alcohol consumption, frequency of falls and if the falls had led to contact to a physician or hospitalization. The Michigan Neuropathy Screening Instrument (MNSI) was used to record neuropathy symptoms. Univariate and multivariate logistic regression analyses were used to calculate the odds ratios of experiencing falls as a result of neuropathy. Possible moderating effects of alcohol consumption, smoking, age, BMI and gender were taken into consideration while performing the analysis of the association between falls and symptoms of neuropathy. The association was presented as an odds ratio (OR) with a 95% confidence interval by a positive neuropathy score of 4 or more points on the MNSI.

Results

Falls were reported in 16% of patients (944) during the past year, and 9% (505) had experienced 2 or more falls. Adjusted regression analysis showed a positive association between MNSI score and fall frequency. Patients with a MNSI score ≥ 4 had a higher risk for 1 fall over the past year (OR 3.3, 95% CI 2.76– 3.87) and for 2 or more falls (OR 4.2, 95% CI 3.40– 5.10) than those with an MNSI score <4 .

Conclusion

Cross-sectional data from this large national database show that patients with type 2 diabetes with 4 or more neuropathic symptoms have a 3–4 fold higher odds ratio of falls unrelated to alcohol consumption, smoking, physical activity, BMI, gender and age.

[P2] MOTOR UNIT NUMBER ESTIMATION IN DIABETIC PATIENTS IN RELATION TO THE PRESENCE OF DIABETIC POLYNEUROPATHY

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Objective

To evaluate the use of Multiple Point Stimulation (MPS) -MUNE, Motor Unit Number Index (MUNIX), and MscanFit MUNE (Mscan), in detecting early motor involvement in relation to the presence of diabetic polyneuropathy (DPN).

Methods

Twenty-five diabetic patients (7 non-DPN and 18 DPN patients, median age of both groups: 65 (60-70)), and twenty-five healthy control subjects, median age: 66 (29-76), were examined. MUNE methods were performed on abductor pollicis brevis (APB) by stimulating the median nerve. Presence of neuropathy was determined from clinical examinations using the Neuropathy Impairment Score (NIS), and nerve conduction studies (NCS) performed on the Median, Sural, Tibial and Peroneal nerve. Results from NCS were compared to the laboratory reference material.

Results

In MPS-MUNE the median (min-max) of non-DPN 98.6 (43.2-262.9) and DPN 94.5 (33.9-263.9) were lower than controls 188 (77-329) ($p < 0.01$). In MUNIX both the median value of non-DPN 128 (106-227) and DPN 124 (34-308) were lower than controls 248 (77-404) ($p < 0.01$). Furthermore, in Mscan the median of non-DPN 85 (61-120) and DPN 84.5 (28-116) were lower than controls 110 (61-193) ($p < 0.01$). No difference was observed when comparing DPN to non-DPN. Additionally, no correlation was observed in comparison of MUNE methods with the combined NCS sumscore¹ (MPS-MUNE: $R^2 = 0.15$, $p = 0.06$, MUNIX: $R^2 = 0.07$, $p = 0.20$, and Mscan: $R^2 = 0.12$, $p = 0.09$). No correlation was detected between NIS scores and MUNE methods.

Conclusions

Lower MUNE values suggest axonal loss not only in the DPN, but also in the non-DPN group compared to controls. This indicates a significant motor involvement in non-DPN patients preceding the presence of clinical signs. Even though, none of the methods were able to discriminate between non-DPN and DPN, knowledge regarding axonal loss and function could contribute with complementary information, as a supplement to NCS. The lack of an association between MUNE methods and NIS may be due to length dependent features of DPN, since NIS consists of a compound score evaluating both the sensory and motor nerve function of the entire body. This could also explain the fact, that only a tendency was observed between NCS sum-score and MUNE methods.

Significance

Motor unit number estimation methods (MUNE) may identify abnormalities in peripheral nerves earlier than conventional nerve conduction studies in diabetes.

1. Dyck *et. al.* Muscle Nerve. 2011

[P3] PHARMACOLOGICAL MODULATION OF MITOCHONDRIAL CHAPERONES AND ATP DEPENDENT PROTEASES IN EXPERIMENTAL DIABETIC NEUROPATHY

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Objective

This study assessed the pharmacological efficacy of modulating mitochondrial quality control i.e. ATP dependent proteases and chaperones activation in peripheral nerves by Resveratrol (RSV) in Streptozotocin (STZ) induced diabetic rats.

Materials & methods

RSV was administered at doses of 10 & 20 mg/kg (*i.p*) to the diabetic animals (STZ 55mg/kg, *i.p*). Animals were subjected to behavioral assessment and functional assessment to evaluate neuropathic severity. Sciatic nerves and dorsal root ganglion (DRG) were processed further to analyze protein expression.

Results

RSV administration for 2 weeks (7 & 8th week) ameliorated diabetes induced hyperalgesia and allodynia as evident from the enhanced tail withdrawal latencies to cold stimulus ($p < 0.001$), hot stimulus ($p < 0.001$), infrared radiation ($p < 0.001$) and increased paw withdrawal responses to mechanical stimuli ($p < 0.001$) in high dose of RSV treated diabetic rats. Chronic diabetes resulted in significantly compromised nerve conduction velocities ($p < 0.001$) and nerve blood flow ($p < 0.001$) in STZ induced diabetic rats. RSV administration reversed these conduction abnormalities by increasing conduction velocity (57.1 ± 1.4 vs 29.3 ± 2.2 m/s) and nerve blood flow (88.8 ± 5.2 vs 48.0 ± 3.5 PU) at 20 mg/kg dose. Molecular studies have revealed that chronic diabetes leads to significant reduction in the levels of mitochondrial ATP dependent proteases and chaperones in peripheral nerves of diabetic rats and thus compromising the mitochondrial integrity and vulnerability to oxidative damage. RSV treatment ameliorated the diabetes induced impairment in ATP dependent proteases and chaperones expression through restoring the protein expression of LON protease ($p < 0.001$), caseinolytic mitochondrial peptidase ($p < 0.001$), and HSP-60 ($p < 0.01$). Improved mitochondrial quality control by RSV further resulted in significantly increased mitochondrial function as evidenced by increased ATP levels ($p < 0.001$) and aconitase-2 ($p < 0.001$) protein expression.

Conclusion

RSV through ATP dependent proteases and chaperones activation improved mitochondrial quality control system and imparted mitochondrial protection in experimental DN. The study offers a new mechanistic indication to resveratrol in diabetic neuropathy.

[P4] CORNEAL CONFOCAL MICROSCOPY: A IMAGING SURROGATE END POINT FOR MILD COGNITIVE IMPAIRMENT AND DEMENTIA

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Background

Age-related vascular disease and neuronal dysfunction driven by diabetes are common in people with mild cognitive impairment and dementia. Corneal confocal microscopy (CCM) is an established imaging surrogate endpoint for neuronal loss in a range of peripheral neuropathies and in Parkinson's disease, stroke and multiple sclerosis.

Aims

Evaluate the diagnostic ability of CCM in patients with MCI and dementia and determine the association between corneal nerve fibre loss and cognitive impairment.

Methods

103 patients referred to a memory clinic underwent assessment of cognitive impairment using MoCA, disability using FIM and corneal nerve integrity with CCM by quantifying corneal nerve fibre density (CNFD), branch density (CNBD) and length (CNFL).

Results

34 subjects with MCI, 34 with dementia and 35 controls were studied. CNFD ($p < 0.0001$, $p < 0.0001$), CNBD ($p < 0.01$, $p < 0.0001$) and CNFL ($p < 0.001$, $p < 0.0001$) were significant lower in subjects with MCI and dementia compared to controls. The AUC/sensitivity and specificity for identifying patients with MCI were CNFD (0.726/62/74); CNBD (0.72/53/77); CNFL (0.75/74/71) and for dementia CNFD (0.84/77/74); CNBD (0.81/68/86); CNFL (0.82/79/80). Adjusted for age and duration of diabetes, CCM correlated with declining executive function, attention, orientation and overall cognitive ability and increased disability caused by dementia ($p = 0.03 - 0.0001$), but not memory loss ($p = 0.37 - 0.66$).

Conclusion

CCM detects axonal loss in patients with mild cognitive impairment and dementia. It has high diagnostic accuracy, sensitivity and specificity for diagnosing MCI and more so dementia. Corneal axonal loss is related to cognitive decline but not memory loss. CCM may act as a viable surrogate imaging end point for clinical trials in patients with MCI and dementia.

[P5] KERATOCYTE DENSITY IS RELATED TO CORNEAL NERVE DAMAGE IN PATIENTS WITH AND WITHOUT NEUROPATHY

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Introduction

Corneal confocal microscopy (CCM) is a rapid non-invasive ophthalmic end point, which has been utilized to quantify corneal nerve degeneration and regeneration and has become an established surrogate end point for the assessment of diabetic neuropathy (DN). The mechanisms underlying corneal nerve degeneration and regeneration are not clearly understood. It has been suggested that keratocytes (related to fibroblasts) contribute to corneal nerve degeneration and regeneration.

Methods

82 patients with type 1 and type 2 diabetes and 15 age-matched control subjects underwent assessment of the neuropathy disability score, quantitative sensory testing, electrophysiology and CCM. Based on Toronto criteria, patients were divided into those without (-DN) (n=48) and with (+DN) (n=34) neuropathy. Corneal nerve parameters and keratocyte density (KD) in the anterior, mid and posterior stroma were quantified.

Results

Anterior stromal KD was significantly reduced in the +DN group (396.37±174.82) compared to the -DN group (502.38±133.74, p=0.007) and control subjects (643.66±144.89, p<0.0001). Mid stromal KD was significantly reduced in the +DN (331.58±58.94, p<0.0001) and -DN (355.79±52.73, p=0.004) groups compared to controls (408.27±46.58). Posterior stromal KD was also significantly reduced in the +DN (329.74±51.45, p<0.0001) and -DN (341.92±56.57, p=0.002) groups compared to controls (397.87±53.29). Corneal nerve fiber density (CNFD) (19.83±5.97 vs 24.91±6.52, p=0.002), branch density (CNBD) (40.41±22.55 vs 60.86±31, p=0.004) and length (CNFL) (18.49±6.3 vs 23.03±6.42, p=0.005) were significantly reduced in the +DN compared to -DN group. CNFD (r=0.32, p=0.002 and r=0.206, p=0.04), CNBD (r=0.283, p=0.005 and r=0.275, p=0.007) and CNFL (r=0.292, p=0.004 and r=0.241, p=0.018) correlated significantly with mid and posterior KD.

Conclusions

This study shows a reduction in keratocyte density in patients with diabetes particularly those with diabetic neuropathy, and an association with corneal nerve damage.

[P6] THE IMPACT OF OBSTRUCTIVE SLEEP APNOEA ON FOOT INSENSITIVITY AND FOOT ULCERATION IN PATIENTS WITH TYPE 2 DIABETES: A LONGITUDINAL PILOT STUDY

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Background and aims

We have previously shown in a cross-sectional study that obstructive sleep apnoea (OSA) was associated with clinical evident peripheral neuropathy, foot insensitivity and foot ulceration in patients with Type 2 diabetes (T2DM). The aim of this longitudinal study was to assess the impact of OSA on the development of foot insensitivity and foot ulceration in patients with T2DM.

Methods

Patients were recruited from a single tertiary centre in the UK 2009–2011 and followed up in 2013–2014. Foot sensitivity was assessed using 10 gram monofilament. Foot ulceration was detected on foot exam. OSA was diagnosed using a portable multichannel cardiorespiratory device when the apnoea hypopnea index (AHI) was ≥ 5 events/hour. Patients with OSA at baseline were treated with continuous positive airway pressure (CPAP) if indicated clinically. By the end of follow-up we had 4 groups of patients: Group 1: no OSA (n=75); Group 2: mild OSA (n=77); Group 3: moderate to severe OSA not compliant with CPAP (n=31); and Group 4: moderate to severe OSA compliant with CPAP (n=17). Compliance with CPAP was defined as CPAP usage of ≥ 4 hours/night based on data downloaded from the CPAP equipment.

Results

200 patients included in the analysis. The OSA and foot insensitivity prevalence was 63% (n=126), and 36% (n=72) respectively. The average follow-up duration was 4.6 (0.5) years. During the follow-up, there was a trend of developing a combined outcome of foot insensitivity and/or foot ulceration in patients with OSA vs no OSA (20% (n=9) vs 6.1% (2); p=0.08). The development of the combined outcome was reduced in the CPAP compliant group (5.9% (n=2) vs 21.7% (n=5) vs 30.8% vs 0% (n=0) for groups 1–4 respectively; p=0.06). Examining the impact of OSA and CPAP on the individual components of the combined outcome showed that there was a non-significant trend of less progression to foot insensitivity (5.9% [n=2] vs 10% [n=2] vs 16.7% [n=2] vs 0% [n=0]; p=0.5 for groups 1–4 respectively) and development of foot ulcer (2.4% [n=1] vs 7.7% [n=3] vs 11.8% [n=2] vs 0% [n=0]; p=0.3 for groups 1–4 respectively) in patients compliant with CPAP.

Conclusion

Our findings suggest that OSA might contribute to the development of foot insensitivity and foot ulceration in patients with T2DM, and that CPAP treatment might have favourable impact on these outcomes. However, our small sample size limited our statistical power. Future larger cohort studies and randomized clinical trials are needed to assess whether CPAP can reduce the risk of developing foot ulceration in patients with T2DM.

[P7] PYRIDOXAMINE PREVENTS MEMORY DEFICITS IN EXPERIMENTAL DIABETES

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Objectives

Previous studies have demonstrated that pyridoxamine inhibits the formation of advanced glycation and lipoxidation endproducts, scavenges reactive oxygen species and toxic carbonyls and reduces hyperlipidemia. Here we assess the protective role of pyridoxamine in streptozotocin (STZ) -induced diabetic neuropathy, and perform untargeted metabolomic analyses of discrete regions of the central and peripheral nervous system.

Methods

Adult male Wistar rats were allocated into either age-matched control ($n=12$), diabetic (STZ 55mg/kg i.p.; $n=10$) or pyridoxamine-treated diabetic groups (400mg/L in drinking water from 1 week post-STZ ($n=9$)). Mechanical sensitivity was assessed at both 6 and 8 weeks. Cognitive function was assessed using a novel object recognition (NOR) test paradigm at 8 weeks. Motor and sensory nerve conduction velocity (NCV) was measured under terminal anaesthesia at 12 weeks and blood and tissues harvested for analysis. Total plasma cholesterol and triglyceride levels were measured by an enzymatic assay. Ultraperformance liquid and gas chromatography-mass spectrometry studies of the sciatic nerve, hippocampus and amygdala were performed followed by univariate analysis of data.

Results

Mechanical hypersensitivity in diabetic rats was not corrected with pyridoxamine ($p>0.05$) whereas NCV deficits were partially corrected. The NOR test revealed a significant memory deficit in the diabetic rats, which was reversed by pyridoxamine ($p<0.05$). Metabolomic analysis revealed a significant diabetes-associated increase in glucose and polyol pathway intermediates and changes in lipid metabolism in the sciatic nerve, hippocampus (Table 1) and amygdala. Evaluation of blood lipids showed a diabetes-induced increase in plasma triglyceride and cholesterol levels which were corrected by pyridoxamine ($p<0.05$), however there was no significant impact of pyridoxamine on the altered lipid profiles of the hippocampus, amygdala or sciatic nerve.

Conclusions

There was a significant perturbation of lipid metabolism in the sciatic nerve, hippocampus and the amygdala in experimental diabetes. Whilst pyridoxamine did not significantly alter the metabolomic dysfunction of these tissue, or indices of peripheral neuropathy, it did prevent diabetes-associated deficits in memory dysfunction, indicating a potential future therapeutic role.

A

Hippocampus Gas chromatography mass spectrometry metabolite features	Control Mean	Diabetic Mean	Difference	P value
Sorbitol	0.257199	-0.449876	0.707075	<0.000001
RH051 Fructose (Oxime)	-0.045551	-0.709977	0.664426	<0.000001
RH054 Fructose (Oxime)	-0.0932921	-0.773464	0.680172	<0.000001
Threitol	-0.083665	-0.595062	0.511397	0.000001
RH055 Glucose (oxime)	0.914664	0.000246342	0.91491	0.000012
RH058 Glucose (oxime)	0.0392986	-0.776576	0.815875	0.000031
RH092 Glucose-6-phosphate Peak2	-0.828001	-1.5131	0.685097	0.001143
RH091 Glucose-6-phosphate Peak2	-0.117021	-0.83097	0.713948	0.001173
Lactic acid, bis(trimethylsilyl)oxy-, ester	2.24105	2.07871	0.162337	0.002358
Fructose-6-phosphate	-1.04536	-1.45588	0.410523	0.002998
Mannitol	-1.28108	-1.39346	0.112376	0.012095
Urea	1.54926	1.34185	0.207408	0.012118
N-Acetyltyrosine, di-TMS	-2.36542	-2.13374	-0.231681	0.013279
Scyllo-inositol	-0.807267	-0.279328	-0.527939	0.000087

B

Hippocampus liquid chromatography mass spectrometry metabolite features	Control Mean	Diabetic Mean	Difference	P value
PE(22:6/0:0)	5.778	5.441	0.3376	0.0000990426 32704
PC(22:6/0:0)	6.349	6.079	0.2702	0.0013084244 25975
PC(16:0/22:6)	5.019	5.359	-0.3402	0.0002610885 5015
PC(18:0/18:2)	8.138	8.406	-0.268	0.0004644143 64234
TG(16:0/18:2/20:4)	5.483	6.299	-0.8166	0.0011188331 92129
PC(18:0/20:3)	7.038	7.323	-0.2843	0.0013140335 41645

A) Gas-chromatography-mass spectrometry (GC-MS) and B) Liquid-chromatography-mass spectrometry (LC-MS) reveal an effect of hyperglycaemia in the hippocampus of diabetic rats 12 weeks post-STZ. Significantly changed ($p < 0.05$) metabolite features upregulated shown in green and downregulated in red in control ($n=6$) vs. diabetic ($n=7$) tissue.

[P8] METABOLIC AND CLINICAL FEATURES ASSOCIATED WITH SEVERITY OF ERECTILE DYSFUNCTION IN MALE PATIENTS WITH TYPE 2 DIABETES

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Objective

Aim of this study was to assess endocrine, metabolic and clinical features significantly related to the severity of the erectile dysfunction (ED) in patients with type 2 diabetes (DM2).

Methods

244 male patients with DM2 and ED were studied. Data on age, BMI, waist circumference (WC), triglycerides (TG), total cholesterol, LDL-c, HDL-c, HbA1c, PSA, TSH, total testosterone, smoking and alcohol habits have been collected. We also evaluated the presence of hypertension, metabolic syndrome, nephropathy, cerebrovascular disease, peripheral vasculopathy, coronary artery disease (CAD), diabetic retinopathy, peripheral neuropathy (evaluated with the vibration perception threshold (VPT) of the right and left big toe and apex and base of the penis) and autonomic neuropathy (diagnosed by one or more of the following tests: lying to standing (LS), deep breathing (EI), Valsalva maneuver (VR) and orthostatic hypotension). Severity of ED was assessed through International Index of Erectile Function (IIEF-5) scoring system.

Results

Mean age at diagnosis was 59.9±7.3 yrs, mean duration of diabetes was 15±8 yrs and the average IIEF-5 score was 12.5±5.55. The bivariate analysis has shown that the factors directly associated with the severity of ED were: WC (p=0.020) and TG (p=0.040); total cholesterol (p=0.005), HDL-c (p=0.049), LDL-c (p=0.004) and total testosterone (p=0.035) were inversely correlated with severity of ED. Applying multivariate stepwise analysis, statistical significance remained only for TG (p=0.006) and total cholesterol (p=0.005). ANOVA detected that nephropathy (p=0.017), CAD (p=0.027), peripheral neuropathy (p=0.039), smoking (p=0.024) and metabolic syndrome (p=0.035) were also associated with ED. Among clinical tests for peripheral neuropathy, VPT right and left big toe (p=0.03 and 0.008) and VPT at apex and base of the penis (p=0.022 and p=0.030) correlated with ED, while those for autonomic neuropathy were LS (p=0.005), EI (p=0.0004) and orthostatic hypotension (p=0.018).

Conclusions

The severity of ED is clearly associated with several metabolic and endocrine features in males with DM2. Early screening of other micro and macrovascular complications, in particular with an accurate assessment of autonomic neuropathy, can help in planning a comprehensive approach to the DM2 patient with ED.

[P9] OPTIMIZATION OF THRESHOLD FOR DIAGNOSIS OF CAN USING KIDNEY FUNCTION

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Introduction

Measures of cardiovascular autonomic neuropathy (CAN) are independently associated with albuminuria and chronic kidney disease in patients with diabetes. Adding to this, CAN may predict renal function decline in patients with diabetes. Both CAN and albuminuria are independently associated with cardiovascular risk. Today the threshold for diagnosis of CAN is based on small healthy control groups which may lead to a reduced diagnostic sensitivity of the CAN test. The aim of this study was therefore to optimize the threshold for the diagnostic test for CAN using validated end organ biomarker in a group of both type 2 and type 1 diabetes patient.

Methods

In 669 type 2 and 526 type 1 diabetes patients from Steno Diabetes Center, Denmark, we examined urinary albumin and cardiovascular autonomic reflex tests (CARTs) including: Response-to-standing (RS), deep breathing (E:I) and the Valsalva maneuver (VM) using the Vagus device. We calculated the area under the ROC curve (AUC) for identifying CAN patients with microalbuminuria (albumin/creatinine ≥ 30 mg/g). Stepwise adjustments of CAN thresholds resulted in a new threshold for which CAN was closer related to microalbuminuria. We investigated the new CAN thresholds sensitivity and the ability to differentiate between patients based on baseline clinical data and diabetes complications.

Results

Optimal threshold for CAN were increased by 5% and 2% in type 1 and 2 diabetes patients respectively. AUC for microalbuminuria was increased from 0.63 to 0.72 and 0.58 to 0.62 in type 1 and type 2 diabetes patients respectively. Using the new CAN threshold we found 152 type 1 diabetes patients with CAN vs 84 using the old thresholds. For type 2 diabetes the new threshold found 174 patients vs 118 using the old threshold. The prevalence of other diabetic complications, peripheral neuropathy and retinopathy was unchanged in patients diagnosed by the modified thresholds.

Conclusion

Using microalbuminuria we were able to optimize the threshold for the diagnosis of CAN individually for type 1 and type 2 diabetes, resulting in a diagnosis closer associated to diabetic nephropathy.

Table 1 New threshold for type 2 diabetes patients

Age \ CARTs	20-29	30-39	40-49	50-59	60-69	70-79
RS	1.17	1.13	1.09	1.07	1.04	1.02
E:I	1.26	1.21	1.17	1.13	1.10	1.07
VM	1.45	1.38	1.32	1.26	1.21	1.17

Table 1 New threshold for type 1 diabetes patients

Age \ CARTs	20-29	30-39	40-49	50-59	60-69	70-79
RS	1.21	1.17	1.12	1.10	1.07	1.05
E:I	1.30	1.25	1.21	1.17	1.13	1.10
VM	1.49	1.42	1.35	1.30	1.25	1.21

[P10] THERAPEUTIC EFFECTS OF NOVEL MESENCHYMAL STEM CELLS IN A DIABETIC NEUROPATHY RAT MODEL: DO SOLUBLE ADHESION MOLECULES PLAY A ROLE?

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Objectives

Under the REDDSTAR project (<http://www.reddstar.eu/>), we used a streptozotocin (STZ) -induced rat model of diabetic neuropathy (DN) with associated altered pain responses and showed that one intravenous injection of human bone marrow CD362⁺ mesenchymal stem cells (MSCs) to STZ-diabetic rats was effective in preventing the development of behavioural signs of DN, without affecting metabolic parameters typical of this disease model (impaired weight gain, hyperglycemia, and elevated HbA1c levels). Further, as to the neurobiological mechanisms underlying the protective effects of this novel MSC population, we showed the existence of a potential peripheral effect through the modulation of sciatic nerve inflammation. However, given the observed protective effects of intravenously delivered CD362⁺ MSCs in not only our animal model of DN but also in animal models of other diabetes complications, namely diabetic kidney disease and diabetic retinopathy, a common mechanism of action relying on a systemic effect emerges as being also a plausible hypothesis. In this context, we here evaluated possible systemic paracrine actions of CD362⁺ MSCs intravenously delivered to our DN rats.

Methods

Briefly, control, STZ-diabetic, and STZ-diabetic rats administered CD362⁺ or CD362⁻ MSCs one week after the induction of diabetes were used in this study. Allowing a 5-hour fast, blood samples were collected from the tail vein before (0h), and 24h, 48h, 1 week, and 9 weeks after MSCs or vehicle solution (PBS) administration. Plasma samples were then used for the quantification of a panel of cytokines/chemokines/trophic factors/adhesion molecules by state-of-the-art Luminex Multiplex Array analyses.

Results

We show that, at baseline (0h), STZ-diabetic rats exhibited elevated levels of soluble ICAM-1 and L-Selectin adhesion molecules as compared to controls. After the administration of MSCs (or vehicle solution), only STZ-diabetic rats that received CD362⁺ MSCs maintained elevated levels of these soluble adhesion molecules at 9 weeks.

Conclusions

These results, in light of a body of literature that describes protective immunomodulatory actions of soluble forms of ICAM-1 in the context of diabetes, suggest that CD362⁺ MSCs may, indeed, exert systemic protective actions in our rat model of DN through the maintenance of elevated levels of immunoprotective circulating adhesion molecules.

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[P11] ATTENUATION OF DIABETIC NEUROPATHY BY ISOQUERCITRIN IS MEDIATED VIA WNT/ BETA-CATENIN PATHWAY

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Objectives

Peripheral diabetic neuropathy is a heterogeneous group of disorders with multifarious pathophysiological mechanisms, affecting both somatic and autonomic components of nervous system. A growing body of evidence have depicted that hyperglycemia can induce activation of the Wnt/beta-catenin pathway. The intent of the present study was to investigate whether inhibition of Wnt/beta-catenin pathway has protective effect in diabetic neuropathy.

Methods

Isoquercitrin (Wnt/beta-catenin inhibitor) administered intraperitoneally at a dose of 10 mg/kg (n=5) for two weeks, after 6 weeks of STZ-induced (50 mg/kg, i.p.) diabetes in male SD rats. After 8 weeks, functional (MNCV and NBF), behavioural (thermal and mechanical hyperalgesia), Rt-PCR and Western blotting studies of Wnt signaling proteins were performed.

Results

Diabetic rats showed significant reduction in nerve function parameters like motor nerve conduction velocity (MNCV) and nerve blood flow (NBF) as well as behavioral parameters like heat, cold and mechanical hyperalgesia as compared to the control animals. Isoquercitrin significantly ameliorated the alterations in MNCV (m/s) ($p < 0.01$), NBF (PU) ($p < 0.001$), thermal hyperalgesia thresholds (latency in seconds) ($p < 0.001$), cold hyperalgesia thresholds (latency in seconds) ($p < 0.001$) and mechanical hyperalgesia thresholds (in grams) ($p < 0.01$) in diabetic rats. Moreover, isoquercitrin also down-regulated the expression of Wnt/ β -catenin pathway genes and also protein expression of β -catenin as found in RtPCR and Western blotting respectively.

Conclusions

Results of this study suggest the potential of isoquercitrin in the treatment of diabetic neuropathy and its protective effect may be attributed to the inhibition of Wnt/ β -catenin signaling pathway. Thus, the present work substantiates the role of Wnt signaling activation in the etiology of diabetic neuropathy.

[P12] RETINAL NEURODEGENERATION IN TYPE 1 DIABETES MELLITUS AS AN EARLY MARKER OF DIABETIC NEUROPATHY

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Objectives

The predictive value of retinal neurodegeneration in the development of microvascular alterations in diabetic retinopathy (DR) has been already confirmed. However, no data are available on the association between neuroretinal dysfunction and diabetic peripheral neuropathy. Our study, therefore, was aimed to investigate the hypothesis that retinal neurodegeneration could be considered as an early marker of diabetic peripheral neuropathy (DPN).

Methods

15 type 1 diabetes *mellitus* (T1DM) patients with no symptoms/signs of peripheral polyneuropathy, without DR or with very mild non-proliferative DR, and 14 healthy controls (C) matched for age and gender were enrolled. To evaluate DPN, all patients underwent corneal confocal microscopy, including the number of fibers, the number of fiber beadings, the degree of fiber branching, and the degree of fiber tortuosity of corneal sub-basal nerve plexus. The following electrophysiological tests were also performed: standard nerve conduction studies (NCS), incremental motor unit number estimation (MUNE) from abductor hallucis (AH) and abductor digiti minimi (ADM) with assessment of AH and ADM average single motor unit potential (SMUP) size. Neuroretinal function was analyzed by multifocal electroretinogram measuring amplitude density (Amp) and implicit time (IT) of nasal (N) / temporal (T) / superior (S) / inferior (I) macular quadrants.

Results

Amp of all macular quadrants was significantly reduced in T1DM ($p < 0.001$) vs C. ADM MUNE and AH MUNE were significantly decreased in T1DM ($p = 0.039$; $p < 0.0001$, respectively), and AH-SMUP significantly increased ($p = 0.002$) vs C. A positive correlation between Amp in N and I quadrant and AH MUNE ($r = 0.368$, $p = 0.01$; $r = 0.288$, $p = 0.03$, respectively) was observed in T1DM patients. A negative correlation between degree of corneal sub-basal nerve plexus fiber tortuosity and Amp in N, I, T quadrants ($r = -0.780$, $p = 0.002$; $r = -0.583$, $p = 0.036$; $r = -0.571$, $p = 0.041$ respectively) and between degree of fiber tortuosity and AH MUNE ($r = -0.547$, $p = 0.043$) were observed in T1DM. No abnormalities of NCS were found in any subject.

Conclusions

The motor unit loss on the one hand and neuroretinal dysfunction on the other hand are already present in T1DM patients without DPN. The relationship between neuroretinal dysfunction and corneal confocal microscopy parameters and motor unit decline supports hypothesis that neuroretina represents a potential "window" to track the early neurogenic process in diabetes.

The background features a complex network of white lines and circles on a solid red field. The lines intersect to form various polygonal shapes, while the circles are placed at several of these intersection points, creating a stylized, interconnected structure.

**PATHOGENESIS AND
TREATMENT**

[P13] NON-INVASIVE MEASUREMENTS OF AGE_s PRODUCTS IN THE CRYSTALLINE LENS CAN DISTINGUISH SUBJECTS WITH PREDIABETES AND TYPE 2 DIABETES FROM HEALTHY CONTROL SUBJECTS AND STRONGLY CORRELATED WITH LEVEL OF SMALL FIBRE NEUROPATHY**Tavakoli M.**

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Background

The accumulation of advanced glycation end products (AGEs) in the body contributes to pathogenesis of many diseases including complications of diabetes including retinopathy and neuropathy. Proteins in the lens of the eye do not turnover and therefore give an indication of the average glucose levels over a very long period of time. These Advanced Glycated End Products (AGEs) have a unique measurable fluorescence, however non-invasive measurement of AGEs was not available until recently with introducing a new scanning confocal biomicroscope.

Aim

The main aims of the present study were to investigate if measurement of lens Auto Fluorescence (FL) can distinguish subjects with Impaired Glucose Tolerance (IGT) and Type 2 Diabetes (T2DM) from Healthy control subjects. Also to investigate the relationship between levels of FL. ratio, and corneal nerves morphology.

Methods

45 patients including 15 IGT, 20 T2DM and 10 Healthy aged matched control subjects underwent comprehensive medical and neurological assessments including corneal confocal microscopy with using HRT-III and measurement of Crystalline Lens Autofluorescence by using a new confocal bio microscope ClearPath DS-120.

Results

There was a significant difference at the level of fluorescence ratios in control subjects (FL ratio: 0.17 ± 0.008), IGT patient (FL ratio: 0.23 ± 0.01 ; $p=0.013$) and T2DM patients (FL ratio: 0.26 ± 0.011 ; $p<0.0001$). There was similar reduction in CNFL in IGT ($p=0.001$) and T2DM ($p<0.001$) subjects.

There was a significant correlation between FL. Ratios and level of Hb1Ac ($r=0.496$, $p=0.01$), and CNFL ($r=-0.721$, $p<0.0001$).

Conclusion

The results of this preliminary study showed the Lens fluorescence is significantly greater in patients with impaired glucose tolerance and type 2 diabetes compared to healthy subjects and supports the feasibility of lens autofluorescence to screen subjects for undiagnosed type 2 diabetes. The level of AGEs products were correlated with HbA1c and alterations in corneal nerves morphology. However the relationship with HbA1c was rather poor since Hb1Ac cannot completely reflect long-term glycation process.

Lens autofluorescence could be a robust marker of long-term diabetes control predicting future complication risks. However, confirmation of such hypothesis will need larger and long-term clinical studies.

[P14] HYPERGLYCAEMIA INDUCED SPINAL CORD VASCULOPATHY AND HYPOXIA; CONTRIBUTING FACTORS IN THE DEVELOPMENT OF DIABETIC NEUROPATHIC PAIN

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Objectives

Neuropathy is one of the most common neurological complications in diabetic patients. Chronic pain is typically described as hyperalgesia and allodynia accompanied by ongoing pains. The onset of this neurological disorder is strongly associated with a dysfunction in the neurovascular interaction within the central nervous system. This is a consequence of depletion in VEGF-A signalling. Our studies investigate a plausible link between the loss of vasculature and the development of neuropathy.

Methods

24 Sprague Dawley rats were used in this study. Procedures were carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986. Diabetes induction was carried out with intraperitoneal (i.p.) injection of streptozotocin (STZ) (50mg/kg) (n=12 untreated=Naïve). Rats positive for diabetes (blood glucose >15mmol) were supplemented with insulin. 24 transgenic mice (C57.bl6) were used (Tie2CreER^{T2} mice were crossed with *vegfr*^{2fl/fl}). All mice used were *vegfr*^{2fl/fl} and either Tie2CreER^{T2} positive (n=12) or Tie2CreER^{T2} negative (n=12) and dosed once daily by i.p. with 1mg tamoxifen for 5 consecutive days. Nociceptive behavioral testing was done using VonFreys and Hargreaves test. After 8 weeks, hypoxyprobe (60mg/kg) *i.p.* was injected 30 minutes before euthanasia in all animals. Spinal cord was collected and perfused fixed with 4% PFA. Spinal cords (40µm thickness) were stained using endothelial markers (IB₄, CD31), neuronal markers (NeuN) and Anti-hypoxyprobe. Confocal imaging of the dorsal horn of the lumbar spinal cord of all groups was performed. Imaris 8.1 software was used for 3D rendering.

Results

Diabetic rats and Vascular Endothelial Growth Factor Receptor 2 (VEGFR2KO) mice showed a reduction in mechanical withdrawal thresholds and an increase in sensitivity to thermal stimuli, when compared to their respective controls. A reduction in the number of IB₄/CD31 labelled blood vessels in the dorsal horn of the lumbar region of the spinal cord was observed in both Diabetic and KO groups. The intensity and cell number of hypoxyprobe staining was increased in the dorsal horn of VEGFR2KO and diabetic spinal cord. The percentage of hypoxic neurons in all laminae was also elevated.

Conclusions

The results from these experiments show a loss in endothelial function and development of hypoxia, in the diabetic and VEGFR2KO spinal cord. Therefore suggesting a correlation between vascular dysfunction and the development of neuropathy.

[P15] ASSESSING ETHOLOGICALLY RELEVANT BEHAVIOURAL CHANGES IN THE STREPTOZOTOCIN-INDUCED RAT MODEL OF DIABETIC NEUROPATHY

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Objectives

Experimental methods to test for sensory threshold changes in response to mechanical and thermal stimuli are well established for rodents with experimental diabetic neuropathy. Whilst methodologically more challenging, inclusion of non-stimulus evoked assays to assess ethologically-relevant behavioural changes may inform a more holistic view of the impact of diabetes and efficacy potential therapeutics. Here we measure burrowing performance over a time-course of streptozotocin (STZ) -induced diabetes and use a novel open-field arena to assess anxiolytic behaviours or locomotor deficits.

Methods

Baseline burrowing performance of adult male Wistar rats was assessed using a burrowing tube containing 2.5 kg of aquarium grade gravel presented for 1 hour in a test cage. Rats were pseudo-randomised into treatment groups following a ranking of baseline burrowing values to ensure equivalent group burrowing mean and variance. Diabetes was then induced using STZ (55 mg/kg i.p.). Study 1 consisted of non-diabetic age-matched control rats and untreated diabetic rats (for 10 weeks post-STZ). Study 2 consisted of control, untreated-diabetic rats and diabetic rats treated at 6 weeks post-STZ with either two subcutaneous slow-release insulin implants, or gabapentin (50mg/kg daily p.o.) for 2 weeks. Burrowing assessment was repeated over two consecutive days, at the same time of day, at fortnightly intervals. The mean amount of gravel displaced was calculated for each time-point. At 8 weeks post-STZ sensory thresholds were measured and behaviours were recorded in a novel open-field arena for later off-line video-analysis. Terminal sciatic motor and sensory nerve conduction velocity (NCV) were measured at the end of each study.

Results

Analysis of neuropathy indices revealed that diabetic rats displayed increased mechanical sensitivity and motor and sensory NCV deficits. Diabetic rats developed significant burrowing deficits from 2 to 8 weeks post-STZ relative to control rats which maintained their baseline burrowing performance over 8 weeks. Gabapentin treatment did not correct the burrowing deficit; however insulin supplementation restored burrowing performance. Open-field analysis revealed no locomotor deficits or anxiolytic behaviours in treated or untreated diabetic-rats compared to control rats.

Conclusions

Our results indicate no alteration in exploratory or anxiety behaviours, and locomotor activity suggesting that the reduced burrowing is not due to anxiety or immobility in diabetic rats, but could correspond to hyperglycaemia and/or mechanical hypersensitivity. Reversal of burrowing deficits may provide a new tool to fully explore clinically-relevant targets.

[P16] IMMORTALIZED SCHWANN CELLS IKARS1 FROM ALDOSE REDUCTASE-DEFICIENT MICE AS A USEFUL TOOL TO STUDY POLYOL PATHWAY AND ALDEHYDE METABOLISM

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Objectives

The increased glucose flux into the polyol pathway via aldose reductase (AR) is recognized as a major contributing factor for the pathogenesis of diabetic neuropathy, whereas little is known about the physiological roles of AR in the peripheral nervous system.

Methods

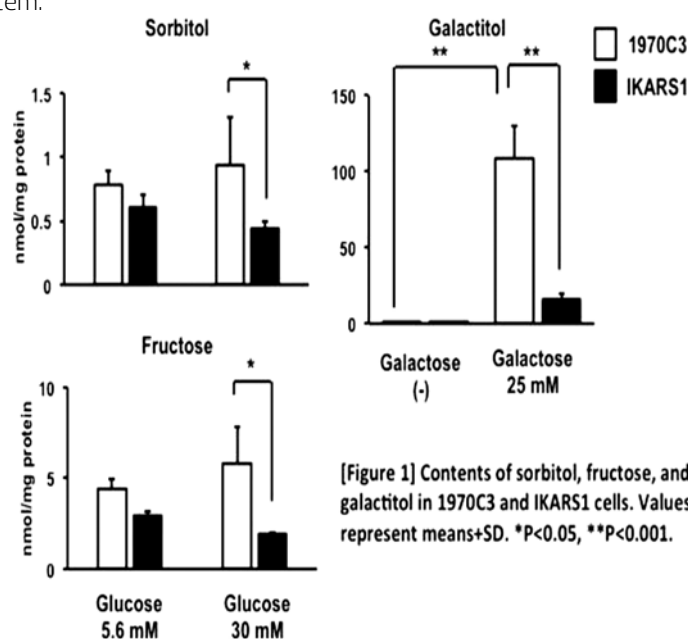
Spontaneously immortalized Schwann cell lines from long-term cultures of normal and AR-deficient C57BL/6 mouse peripheral nerves were designated as 1970C3 and IKARS1, respectively. The biological properties of these cell lines were characterized by using immunocytochemistry, western blotting, ELISA, liquid chromatography coupled to tandem mass spectrometry, and microarray/real time RT-PCR analyses.

Results

Both 1970C3 and IKARS1 cells exhibited distinct Schwann cell phenotypes, such as spindle-shaped morphology, immunoreactivity for S100 and p75 low-affinity neurotrophin receptor, and synthesis and secretion of neurotrophic factors (NGF and GDNF). The measurement of intracellular contents of sorbitol and fructose under high glucose (30 mM) load and content of galactitol under galactose (25 mM) load indicated the inactivation of the polyol pathway in IKARS1 cells (Fig.1). Microarray and subsequent real time RT-PCR analyses revealed significant down-regulation of mRNA expression for the polyol pathway-related enzymes, such as sorbitol dehydrogenase and ketohexokinase, in IKARS1 as compared with 1970C3. In contrast, significant up-regulation of mRNA expression for aldo-keto reductases (AKR1B8 and AKR1C14) and aldehyde dehydrogenases (ALDH1B1, ALDH1L2, ALDH3B1, ALDH5A1, and ALDH7A1) in IKARS1 cells as compared with 1970C3 cells was detected. Furthermore, exposure to the reactive aldehydes, such as 3-deoxyglucosone (0.5 mM), methylglyoxal (0.25 mM), or 4-hydroxynonenal (2.5 mM), significantly (>2.5 fold) up-regulated mRNA expression for the AR-related enzymes AKR1B7 and AKR1B8 in IKARS1, but not in 1970C3. Because we observed no significant differences in the viability between the two cell lines, the detoxification function might be taken over by AKR1B7 and AKR1B8 in the absence of AR.

Conclusions

The findings of this study highlight the influences of AR gene deletion on glucose and galactose metabolism as well as gene expression profiles in Schwann cells. 1970C3 and IKARS1 cells will be useful tools to help elucidate physiological and pathological roles of AR in the nervous system.



[Figure 1] Contents of sorbitol, fructose, and galactitol in 1970C3 and IKARS1 cells. Values represent means±SD. *P<0.05, **P<0.001.

[P17] ADJUVANT EFFECTS OF DIABETES ON HYPERTENSIVE NEUROPATHY IN SPONTANEOUSLY HYPERTENSIVE RATS

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Objectives

Hypertension causes peripheral neuropathy in spontaneous hypertensive rats (SHR), and has been speculated to contribute to initiation or worsening of diabetic neuropathy (Gregory *et al.* Acta Neuropathol 2012, Nukada, *et al.*, Muscle Nerve 2016). However, it is not known whether diabetes contributes to worsening of hypertensive neuropathy. We studied the potential impact of diabetes upon existing hypertension for the development of neuropathy using SHR and neonatal STZ-induced diabetes.

Methods

Following 5 groups of rats have been used: SHR complicated with diabetes (SHR+DM), hypertension only (SHR), diabetes only in Wistar rats (DM), and two controls; Wistar Kyoto rats (WKY) and Wistar rats (Wistar). In SHR+DM group, diabetes was induced in SHR by injecting STZ within 48 hours after their birth. Because STZ-induced hyperglycaemia in WKY was transient, DM in Wistar rats was added. Blood pressure, blood glucose, and motor and sensory nerve conduction velocity (MCV, SCV) of sciatic-tibial and sural nerves were monitored every 8–12 weeks. Morphology of sciatic, tibial and sural nerves was assessed at 48 weeks of age. A life span of SHR+DM rats is less than 50–60 weeks.

Results

Blood pressure was significantly increased in SHR+DM and SHR, though there was no significant difference between SHR+DM and SHR. Blood glucose was significantly greater in SHR+DM and DM than non-diabetic groups. MCV and SCV were significantly delayed in SHR+DM, DM, and SHR in that order compared to WKY and Wistar. MCV and SCV in SHR+DM and DM were significantly slower than in SHR. Furthermore, SCV in SHR+DM was significantly slower than in DM at age of 16 and 24 weeks, but not at 44 weeks. MCV in SHR+DM was not significantly different from those in DM. Morphologically, axonal atrophy of myelinated nerve fibres was prominent in tibial and sural nerves of SHR+DM, SHR and DM, and confirmed morphometrically. Microvascular basement membrane thickening was observed in SHR+DM and SHR. In SHR+DM, focal ischaemic lesions of sural nerves were found, but not in other groups.

Conclusions

SHR complicated with diabetes showed early sensory impairment, and more severe neuropathy electrophysiologically and histologically when compared with SHR or DM rats. These findings suggest that diabetes contributes to worsening of hypertensive neuropathy.

[P18] ROLE OF DESCENDING PAIN PATHWAY IN DIABETIC PERIPHERAL NEUROPATHY**Premkumar L. S.**

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Most common long-term complication with diabetes is the diabetic peripheral neuropathy (DPN). By the time diabetic peripheral neuropathy (DPN) is diagnosed, significant small fiber nerve terminal degeneration has already occurred. In the initial stages of hyperglycemia, mild oxidative stress increases the expression of Transient Receptor Potential Vanilloid 1 (TRPV1), an ion channel responsible for inflammatory thermal hypersensitivity. Since TRPV1 channels are a highly calcium permeable, excessive calcium influx combined with uncontrolled hyperglycemia leads to nerve terminal degeneration. The delay in DPN diagnosis occurs because, although TRPV1 expression is enhanced in the periphery and spinal cord that constitute the ascending pain pathway, pain is not perceived because it is compensated by increased expression of TRPV1 in periaqueductal gray (PAG) and rostral ventral medulla (RVM) structures that are in the descending pain pathway, which mediates antinociception via GABAergic-Opioidergic pathway. Neuropathic/spontaneous pain is perceived only at a later stage when descending antinociceptive control is impaired due to reduced expression of TRPV1 in PAG and RVM. This results in the paradoxical clinical finding that spontaneous pain persists although the thermal sensation is lost in the periphery. In animal models of DPN, this problem is further compounded by solely relying on reflexive behavior to test pain sensitivity. At this point in time, treatment options include only symptomatic relief of pain rather than reversal of the condition. It is proposed that interfering with the process well before peripheral nerve degeneration occurs by strict glycemic control or drugs that can reduce oxidative stress or blocking calcium influx by TRPV1 antagonists may prevent, halt, or delay the disease process.

[P19] PERIPHERAL NEUROPATHY IN PATIENTS WITH SARCOPENIA AND TYPE 2 DIABETES MELLITUS

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Objective

Purpose of this pilot cross-sectional study is to evaluate clinical and instrumental features of peripheral neuropathy in patients with sarcopenia and type 2 diabetes *mellitus* (T2DM) in hospital settings.

Material and methods

Sixty-six patients with T2DM over 50 years old were examined. Clinical and antropometric characteristics were evaluated. Muscle strength was measured with carpal dynamometry, muscle function was evaluated with short physical performance battery (SPPB) tests. The SPPB includes standing balance, gait speed, timed chair stands test.

Skeletal muscle mass index was evaluated with bioimpedance testing using ABC-01 MEDASS analyser (Russia). Patients with decrease of skeletal muscle mass index and/or muscle strength and/or muscle function were diagnosed as sarcopenic – «S+» group and non-sarcopenic – «S-» group.

Peripheral neuropathy was studied with calculation of TSS, NDS and NIS-LL scales.

Results

Sarcopenia revealed in 11 patients (17%) aged 69,3±7,3 years and diabetes duration of 11,1±9,1 years; HbA1c – 9,1±2,3%; 55 patients aged 64,4±7,4 years and diabetes duration of 12,8±6,4 years, HbA1c – 9,1±3% were not sarcopenic. «S+» patients demonstrated lower composites of SPPB, such as lower walking speed in 81,8% and lower ability to hold balance in 90,9% of cases. Lower indices of dynamometry demonstrated 72.7% of «S+» patients. «S+» patients were more symptomatic (TSS 5,89±3,07) in comparison with «S-» patients (TSS 3,95±2,82, p=0,048). Severe diabetic neuropathy (NISLL>10) was noted in 63,6% of «S+» patients and in 25,4% of «S-» patients. «S+» patients had worst vibration score vs «S-» patients (3,30±1,64 vs 4,92±1,4, p=0,001). Absence of pressure perception to monofilament SW5.07 revealed in 45% of «S+» patients in comparison with 9% of «S-» patients ($\chi^2=9,43$, p=0,002).

History of fractures occurred more often in «S+» patients (45%) in comparison with «S-» group- (25%). Pain, temperature, position sense and reflexes scores did not differ between groups.

Patients with sarcopenia characterized with lower BMI (26,4±5,4 vs 32,8±5,3 kg/m², p<0,001); smaller waist and neck circumference (94,2±14,7 vs 106,9±12,6cm, p=0,002; 36,1±2,7 vs 40±2,9cm, p<0,001).

Interestingly that «S+» patients received metformin less frequently (36%) than «S-» patients (78%) ($\chi^2=7,82$; p=0,005).

Conclusion

1) Sarcopenia evaluated in 17% of T2DM patients over 50 years old in hospital settings. 2) Sarcopenia is easily recognised with bioimpedance testing, SPPB test and carpal dynamometry. 3) Large fiber function loss based on evaluation of NDS and NIS-LL scales is more common in T2DM patients with sarcopenia.

[P20] METAL LEVELS ARE NOT DYSREGULATED IN THE SCIATIC NERVE AND DORSAL ROOT GANGLIA OF DIABETIC RATS

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Objectives

Transition metals play pivotal roles in tissue homeostasis and cellular processes including enzymatic activities, antioxidant defence and immune function. Copper (II) toxicity in diabetes contributes to oxidative stress and advanced glycation endproduct accumulation in several diabetic complications. Here we measure the levels of thirteen metals in peripheral nervous system tissue (sciatic nerve and dorsal root ganglia (DRG)) of streptozotocin (STZ)-induced diabetic rats to investigate whether their levels alter in diabetes and also determine whether the copper chelator triethylenetetramine (TETA) is protective.

Methods

Thirteen metals (Sodium, Magnesium, Aluminium, Potassium, Calcium, Chromium, Manganese, Iron, Cobalt, Copper, Zinc, Selenium, and Molybdenum) were quantified using inductively coupled-mass spectrometry (ICP-MS) in lumbar 4/5 DRG and sciatic nerve samples (40 ± 4 mg wet weight) from adult male Wistar rats with 4, 8, 12 and 16 weeks of STZ-induced diabetes (including one diabetic group treated with TETA (34 mg/day)) and age-matched controls. Liquid (LC) and gas chromatography (GC)-mass spectrometry (MS) studies were performed on the sciatic nerve and DRG of diabetic ($n=6$) and diabetic-TETA treated rats ($n=6$; 12 weeks post-STZ), followed by univariate analysis of data.

Results

No significant changes were found in metal concentrations in the sciatic nerve of rats 12 (control $n=8$; diabetic $n=16$) or 16 weeks (control $n=12$; diabetic $n=13$; TETA-treated $n=9$) post-STZ compared to controls, nor were metal level altered in the DRG at any timepoint studied. TETA afforded some protection against nerve conduction velocity (NCV) deficits, but not intraepidermal nerve fibre loss. 55 metabolites were identified and measured with GC-MS but no significant changes were found in the sciatic nerve of TETA-treated diabetic rats compared to diabetic-untreated rats. Of the 52 metabolites identified in the DRG, only one (eicosanoic acid) showed a significant upregulation in TETA-treated diabetic rats. LC-MS data similarly revealed no significant changes in metabolites between the two groups.

Conclusions

Our data reveals, for the first time, untargeted metal level analysis of the peripheral nervous system of control and STZ-diabetic rats. Interestingly, whilst TETA treatment ameliorated NCV deficits, metabolite and copper levels were unaltered in the sciatic nerve in diabetes. This suggests dysregulation of metal concentrations does not play a role in the pathophysiology of diabetic neuropathy at these timepoints.

[P21] DIABETIC PERIPHERAL NEUROPATHY IN SDT FATTY RAT, A NEW ANIMAL MODEL OF OBESE TYPE 2 DIABETES

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Objectives

To characterize the diabetic peripheral neuropathy (DPN) in Spontaneously Diabetic Torii (SDT) fatty rat, a new obese type2 diabetic animal model with severe diabetic complications, we conducted functional and pathophysiological evaluations. Phlorizin, a sodium-glucose co-transporter inhibitor, was used to control the blood glucose levels.

Methods

Male SDT fatty rats were treated the vehicle or phlorizin (100 to 150 mg/kg/day) from 5 to 16 weeks (n=6). Sprague-Dawley (SD) rats were used as an age-matched control (n=6). Body weights and biochemical parameters were periodically measured. The sensory and motor nerve conduction velocity (SNCV and MNCV) of the sciatic nerve, blood pressure, pupil size, and electrocardiogram were measured. At 16 weeks, rats were sacrificed, and sural nerve and intraepidermal nerve were sampled for the histological studies, transmission electron microscopic (TEM) analysis and determination of nerve fiber density.

Results

SNCV and MNCV were delayed in the SDT fatty rats compared with the control SD rats (SNCV; SD 59.1±4.5 m/sec, SDT fatty 39.8±5.4 m/sec, MNCV; SD 49.3±13.0 m/sec, SDT fatty 41.2±8.5 m/sec). Intraepidermal nerve fiber density (IENFD) slightly decreased in the SDT fatty rats (SD 29.7±3.9 fiber/mm, SDT fatty 22.8±2.0 fiber/mm). In the TEM evaluation, the mitochondrial abnormalities and thinning of myelin sheath in small myelinated fibers, and vacuolation and mitochondrial swelling in unmyelinated fiber were found in the SDT fatty rats. Blood pressure increased (SD 112.7±8.8 mmHg, SDT fatty 142.2±15.2 mmHg) and the coefficient of variance of R-R (CV_{R-R}) intervals tended to decrease in SDT fatty rats (SD 2.6±1.3%, SDT fatty 1.3±0.3%). The maximum pupil diameter responded to the mydriatic drugs in SDT fatty rats was smaller than that of SD rats (SD 5.3±0.2 mm, SDT fatty 4.8±0.3 mm). These changes, except the thinning of myelin sheath, were prevented by controlling blood glucose level with phlorizin treatment.

Conclusions

Functional and pathological abnormalities in somatic and autonomic nerves were observed in male SDT fatty rats and were dependent on hyperglycemia. SDT fatty rats will be useful animal model in studies of the pathogenesis of DPN in type2 diabetes.

[P22] RISK FACTORS FOR COGNITIVE IMPAIRMENTS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background and aims

Diabetes *mellitus* is associated with increased risk of cognitive impairments and dementia, either Alzheimer disease or vascular one. However, the factors associated with an increased risk of cognitive deterioration in patients with diabetes are not fully characterized. Therefore, the aim of this study was to investigate the factors associated with the worsening of cognitive functioning in patients with type 2 diabetes *mellitus*.

Materials and Methods

We examined 196 patients with type 2 diabetes *mellitus*, 121 (61.7%) women and 75 (38.3%) men (mean age – 61.85±7.04 years, years of education – 14.59±2.62, diabetes duration 10.86±8.29 years, BMI – 31.48±5.15 kg/m², HbA1c – 8.73±1.48%, FPG 9.84±3.35 mmol/l). All subjects did not have a history of cerebrovascular accidents. Cognitive functioning was assessed by Clock drawing test, Frontal Assessment Battery (FAB), Mini-Mental State Examination (MMSE), test «<5 words>», Schulte Table. We also screened patients for depression using self-questionnaire Centre for Epidemiologic Studies Depression Scale (CES-D). There were no episodes of hyperglycemia or hypoglycemia immediately before assessment of cognitive functioning. Regression tests were performed adjusted for education, age. The statistical analysis was performed using SPSS-23.

Results

We found statistically significant positive correlations between FAB and vibration sensation threshold (B (95% CI) = 0.318 (0.286/0.676)); p<0.0001, negative correlation between FAB and HbA1c (B (95% CI) = -0.213 (-0.794/-0.164)); p=0.003, FAB and FPG (B (95% CI) = -0.166 (-0.304/-0.026)); p=0.02, between FAB and CES-D (B (95% CI) = -0.176 (-0.102/-0.014)); p=0.011). Also, it was statistically significant positive correlation between MMSE and vibration sensation threshold (B (95% CI) = 0.185 (0.067/0.421)); p=0.007, negative correlation between MMSE and HbA1c (B (95% CI) = -0.229 (-0.724/-0.175)); p=0.001, MMSE and FPG (B (95% CI) = -0.148 (-0.251/-0.007)); p=0.039, between MMSE and CES-D (B (95% CI) = -0.221 (-0.102/-0.025)); p=0.001). There was no significant correlation between the results of Clock drawing test, test «<5 words>», Schulte Table and characteristics of disease.

Conclusions

We may conclude that poor metabolic control reflected by higher HbA1c and FPG levels, diabetic peripheral neuropathy and the signs of depression contribute to the worsening of cognitive function in patient with type 2 diabetes *mellitus*.

[P23] EFFICACY OF ADMINISTRATION FOR TWO YEARS OF ACE-INHIBITION ON DIABETIC PERIPHERAL AND AUTONOMIC NEUROPATHY IN PATIENTS WITH DIABETES MELLITUS

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Aim

The present prospective, open, parallel and placebo controlled study was undertaken to investigate the effect of the angiotensin converting enzyme inhibitor Quinapril (20 mg/day) on definite Diabetic Autonomic Neuropathy (DAN), as assessed by cardiovascular reflex tests (CRT) and Peripheral Neuropathy (DPN) over a period of 24 months.

Patients – Methods

Sixty three consecutive patients (27 DMT1) 34 women with mean age 52 years (range 22 to 65 years) were studied. Patients had definite diabetic autonomic (2 of the following 4 tests abnormal) and peripheral neuropathy. Patients randomized in two groups: group A=31 patients received Quinapril and group B=32 patients placebo. All patients were well characterized and highly selected without hypertension and coronary artery disease (based on a normal scintigraphy test). The following methods for detecting DPN and DAN used: Michigan Neuropathy Screening Instrument Questionnaire and Examination (MNSIQ and MNSIE), measurement of vibration perception threshold with biothesiometer (BIO) and CRT: R-R variation during deep breathing [assessed by expiration/inspiration ratio (E/I), mean circular resultant (MCR) and standard deviation (SD)], Valsalva maneuver (Vals), 30:15 ratio and blood pressure response to standing (OH)].

Results

The following indices increased significantly in group A (basal vs final): E/I 1.11+0.06 vs 1.23+0.12, MCR 18.1+6.2 vs 38.7+20.5, SD 31.1+11.9 vs 56.6+23.0, 1 (in all $p<0.05$). We did not observe a significant change in all other indices: vals 1.48+0.28 vs 1.56+0.33, 30:15 1.15+0.12 vs 1.18+0.12, OH 16+11.8 vs 10.4+6.1, MNSIQ, MNSIE and Bio 23+8 vs 20+7. In group B: all CRT indices, except Vals, deteriorated significantly. MNSIQ, MNSIE and Bio did not change.

Conclusions

In present study DAN (mainly parasympathetic dysfunction) was improved after two years of treatment with ACE Inhibition. Improved autonomic balance may be of clinical importance in long-term prognosis of DM patients.

[P24] TREATMENT OF NEUROPATHY IN TYPE 2 DIABETIC MICE: MENHADEN OIL VS RESOLVIN D1, E1 OR D1 OR D2 METHYL ESTERS

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Objective

This study sought to determine whether methyl esters of resolvins D1 or D2, presumably due to a longer half-life *in vivo*, are more effective than menhaden oil or resolvins D1 or E1 on diabetic neuropathy.

Methods

Type 2 diabetic mice after 8 weeks of hyperglycemia were treated for 8 weeks with menhaden oil or with daily injections (1 ng/kg) resolvins D1, E1 or the methyl esters of resolvins D1 or D2. The endpoints evaluated included motor and sensory nerve conduction velocity, thermal and mechanical sensitivity and innervation of sensory nerves in the cornea and skin.

Results

Our findings show that menhaden oil or resolvins did not improve hyperglycemia. Untreated diabetic mice were thermal hypoalgesic, had mechanical allodynia, reduced motor and sensory nerve conduction velocities and decrease innervation of the cornea and skin. These endpoints were generally improved with menhaden oil or resolvin treatment (see Table below). However, the methyl esters of resolvin D1 or D2 were less potent than menhaden oil or resolvins D1 or E1.

Conclusions

These studies further support n-3 polyunsaturated fatty acids derived from fish oil via in part due to their metabolites could be an effective treatment for diabetic neuropathy.

Determination	Control (12)	Diabetic (12)	Diabetic + Menhaden Oil (12)	Diabetic + Res D1 (12)	Diabetic + Res E1 (11)	Diabetic + Res D1 ME (11)	Diabetic + Res D2 ME (11)
MNCV (m/sec)	38.2±1.9	23.3±1.0 ^a	33.7±1.0 ^b	33.0±1.3 ^{ab}	30.7±1.3 ^{ab}	30.1±1.6 ^{ab}	31.0±1.3 ^{ab}
SNCV (m/sec)	22.1±0.5	18.1±0.6 ^a	21.1±0.5 ^b	22.0±0.6 ^b	20.4±0.6 ^b	19.4±0.6 ^a	19.6±0.4 ^a
IENF (profiles/mm)	25.4±0.5	16.2±0.3 ^a	21.3±0.5 ^{ab}	20.0±0.5 ^{ab}	19.7±0.6 ^{ab}	18.7±0.4 ^{ab}	20.7±0.5 ^{ab}
Corneal nerve fiber length (mm/mm ²)	1.83±0.14	0.80±0.06 ^a	2.08±0.16 ^b	1.95±0.20 ^b	1.69±0.17	1.42±0.15	1.37±0.08
Thermal nociception (sec)	5.6±0.1	9.4±0.3 ^a	6.8±0.3 ^{ab}	7.2±0.2 ^{ab}	7.0±0.3 ^{ab}	8.2±0.3 ^{ab}	8.1±0.2 ^{ab}
Mechanical allodynia (g)	2.78±0.10	1.23±0.06 ^a	2.00±0.12 ^{ab}	2.13±0.07 ^{ab}	2.00±0.07 ^{ab}	1.62±0.12 ^a	1.93±0.11 ^{ab}

Data are presented as the mean±S.E.M. a) p<0.05 compared to control; b) p<0.05 compared to diabetic. Parentheses indicate the number of experimental animals.

[P25] EFFECT OF DIETARY OILS ON PERIPHERAL NEUROPATHY RELATED ENDPOINTS IN DIETARY OBESE SPRAGUE–DAWLEY RATS

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Objective

Determine the effect of dietary oils enriched in different mono- or poly-unsaturated fatty acids (olive (18:1, oleic acid), safflower (18:2 n-6, linoleic acid), flaxseed (18:3 n-3, alpha-linolenic acid), evening primrose (18:3 n-6, gamma-linolenic acid) or menhaden (20:5/22:6 n-3 eicosapentaenoic/docosahexaenoic acids) on peripheral neuropathy in diet-induced obese Sprague-Dawley rats.

Methods

Rats were fed a high fat diet (45% kcal primarily lard) for 16 weeks. Afterwards the rats were fed diets with 50% of the kcal of fat derived from lard replaced by the different dietary oils. In addition, a control group fed a standard diet (4% kcal fat) and a high fat fed group (45% kcal primarily lard) was maintained. The treatment period was 32 weeks. The endpoints evaluated included motor and sensory nerve conduction velocity, thermal sensitivity and innervation of sensory nerves in the cornea and skin.

Results

Our findings show (see Table below) that menhaden oil provided the greatest benefit for preventing peripheral nerve damage caused by dietary obesity. Similar results were obtained when we examined acetylcholine-mediated vascular relaxation of epineurial arterioles of the sciatic nerve. Vascular relaxation as were neural deficits was nearly fully protected when the high fat diet was enriched with menhaden oil. Enriching the diets with fatty acids derived from the other oils provided none to partial improvements.

Conclusions

These studies further support n-3 polyunsaturated fatty acids derived from fish oil could be an effective treatment for peripheral neuropathy.

Determination	Control (9)	DIO (11)	DIO + Olive Oil (9)	DIO + Safflower Oil (9)	DIO + Flaxseed Oil (9)	DIO + Evening Primrose Oil (8)	DIO + Menhaden Oil (9)
MNCV (m/sec)	60.0±4.3	38.5± 1.5 ^a	39.4± 1.6 ^a	45.0± 2.7 ^a	47.4±1.1 ^{ab}	51.4± 2.1 ^b	58.9± 2.1 ^b
SNCV (m/sec)	33.5± 1.0	24.2± 0.7 ^a	24.7±0.4 ^a	27.9±0.6 ^{ab}	26.0± 1.0 ^a	27.3± 0.7 ^a	30.9± 1.5 ^b
IENF (profiles/mm)	20.1± 0.5	11.9± 0.5 ^a	12.7± 0.9 ^a	17.3± 1.1 ^b	14.4± 0.4 ^a	14.4± 0.7 ^a	16.9± 0.6 ^b
Corneal nerve fiber length (mm/mm ²)	8.8± 0.5	5.5± 0.4 ^a	4.8± 0.4 ^a	5.3± 0.5 ^a	7.7± 0.7	7.4± 0.9	9.2± 0.6 ^b
Thermal nociception (sec)	11.1± 0.4	20.1± 1.3 ^a	21.8± 0.9 ^a	21.5± 1.0 ^a	19.5± 1.3 ^a	19.0± 1.0 ^a	14.4± 1.5 ^b

Data are presented as mean±SEM. a) p<0.05 compared to control; b) p<0.05 compared to DIO. Parenthesis indicate the number of experimental animals.

[P26] COMPARISON OF EFFICACY AND TOXICITY OF DIODE LASER AND CAPSAICIN ON PIGSKIN INNERVATION AS A POTENTIAL TREATMENT FOR PAINFUL DIABETIC NEUROPATHY

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Most therapeutics used to treat painful diabetic neuropathy (PDN) are assumed to target CNS pain mechanisms. However, fewer than 1/3rd of patients achieve at best 50% relief while the vast majority suffer debilitating side effects. Therefore, therapeutics are being sought that could target peripheral mechanisms implicated in diabetic pain. One such target is pathologically hyperactive, mechanically-insensitive C-fiber that express TRPV1 and that terminate in the epidermis and upper dermis of the skin. Acting as a toxic agonist, 8% capsaicin patch has been shown to reduce these TRPV1 endings, thereby, providing partial relief. However, capsaicin's tissue half-life of ~ 24 hours leads to prolonged inflammatory pain that limits the patch application. Herein, we test and compare the effectiveness of 980 nm diode laser (DL) light that we have previously documented as a potent, presumed non-contact thermal agonist for TRPV1^{1,2}. We hypothesized that minimally invasive DL radiation may more effectively reduce TRPV1 innervation, which may provide more sustained relief of PDN. Herein, the 8% capsaicin patch and DL were tested on pig skin which is one of the closest surrogates of human skin. The effects on innervation density in pig skin biopsies were assessed seven days after a 1 hour 8% capsaicin patch treatment or four different doses of laser irradiation that induced peak temperature up to 55°C. Pigs were habituated for 2–3 days and trained to respond to painful mechanical stimulation by neurological pins and DL C-fiber selective stimulation. Behavioral tests conducted one day before and 6 days after the treatments showed a significant increase of response latency to the C fiber stimulation of the irradiated versus untreated skin areas. PGP9.5 quantification of biopsies demonstrated a significant depletion of cutaneous innervation with 10 DL pulses similar to or greater than that produced by the patch. Examination of alternating sections stained for H&E revealed no obvious damage from the capsaicin or DL treatments with 10 or 20 pulses, but minor damage with 40 pulses. Therefore, the impact of a one-hour capsaicin treatment efficacy could be achieved in less than 2 minutes by non-contact DL treatment with no adverse effects.

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[P27] N-3 POLYUNSATURATED FATTY ACIDS PROMOTE NEURITE OUTGROWTH VIA PI3K AND JNK-MEDIATED SIGNALING PATHWAYS IN NEURO2a CELLS

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Objectives

We previously demonstrated that n-3 polyunsaturated fatty acids (n-3 PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) induced the anti-oxidative enzyme, heme oxygenase-1 (HO-1) through the nuclear factor erythroid 2-related factor 2 (Nrf2) and protected immortalized adult mouse Schwann (IMS32) cells from oxidative stress. It has been reported that DHA has cerebral neuroprotective effects and it may contribute to the prevention and treatment of dementia. In this study, we investigated the effects of n-3 PUFAs on neurite outgrowth of murine neuroblastoma Neuro2a cells which were derived from sympathetic cells in peripheral nervous system.

Methods

1) Neuro2a cells were incubated with DHA, EPA or 4-hydroxy hexenal (4-HHE) for 72 hours. 2) Cells were labelled with a neuronal specific mouse anti β -tubulin III antibody by immunofluorescent staining and the level of neurite outgrowth was quantified by the imaging cytometer (IN Cell Analyzer 2000). 3) Cells were treated with PI3K inhibitor (LY294002), JNK inhibitor (SP600125), MEK inhibitor (PD98059) or p38 inhibitor (SB203580) for 72 hours.

Results

1) DHA or EPA significantly promoted neurite outgrowth compared with control. Furthermore, 4-HHE, an end-product of n-3 PUFAs peroxidation, also enhanced neurite outgrowth. 2) LY294002 or SP600125 inhibited neurite outgrowth promoted by n-3 PUFAs, while PD98059 or SB203580 did not affect neurite outgrowth induced by n-3 PUFAs.

Conclusions

These results suggest that n-3 PUFAs not only protect Schwann cells from oxidative stress, but also promote neurite outgrowth in neural cells via PI3K and JNK pathway and that n-3 PUFAs might be beneficial in the treatment of diabetic neuropathy.

[P28] EFFECTS OF GAMMA LINOLEIC ACID IN PEOPLE WITH DIABETIC PERIPHERAL NEUROPATHY

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Background

Several classes of medications such as alpha lipoic acid (ALA), tricyclic antidepressants, anticonvulsants, narcotic analgesics, and $\alpha 2$ - δ ligands have been reported to be efficacious in the treatment of diabetic peripheral neuropathy (DPN). However, no large double-blind, placebo-controlled trials have been reported that evaluated the efficacy of gamma linoleic acid (GLA) for the treatment of DPN.

Objectives:

The aim of this study was to evaluate the efficacy and tolerability of GLA in treatment of DPN.

Methods

This was a double-blind, parallel-group study in which patients were randomized in a 1:1 ratio and treated with either flexible-dose GLA 320 mg/d, or corresponding flexible-dose ALA 600 mg for 12 weeks. The primary efficacy end point was change in the mean pain visual analog scale (VAS) score and total symptom score (TSS) based on a daily neuropathic pain in DPN patients. Secondary end points included Michigan neuropathy screening instrument (MNSI), Modified brief pain inventory for diabetic peripheral neuropathy (BPI-DPN), EQ5D, Current perception threshold (CPT). Adverse events and physical and laboratory examination results were also collected.

Results

GLA (N=26) and ALA (N=31) treatment groups were well-matched in terms of demographic and patient characteristics. On the primary outcome, end point change in mean TSS and VAS score, treatment with GLA and ALA group resulted in significant improvement compared with baseline ($p = 0.001$). There were no significant differences between GLA and ALA group regarding TSS and VAS score. Treatment with GLA and ALA group also resulted in significant efficacy compared with baseline on secondary measures, including MNSI ($p = 0.01$), Modified BPI-DPN ($p = 0.001$) except EQ5D and CPT. There were no significant differences between GLA and ALA group regarding MNSI and Modified BPI-DPN. There were no differences between the both groups regarding the rates of adverse events.

Conclusions

We suggest that GLA in daily doses of 320 mg/d was effective and well tolerated in people with DPN, indicated through improved TSS and VAS score.

[P29] THE COMPARISON OF NEUROPROTECTIVE EFFECT BETWEEN GLUCOSE CONTROL AND ALPHA LIPOIC ACID IN THE STZ-INDUCED DIABETIC RATS

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Objectives

Strict glucose control is well-proven therapeutic approach for diabetic peripheral neuropathy (DPN) patients although the clinical efficacy is dependent on the type of diabetes. Alpha lipoic acid (ALA) has been accepted as one of pathogenic therapeutic agent for DPN. However, it is not clear how much potent it is in the DPN prevention or treatment compared to the benefit from glucose control. In this study, we investigated the neuroprotective effect of glucose control and ALA, and compared potency.

Methods

Animals were divided into 6 groups (n = 6-8) according to the intervention as follows: Normal (G1), diabetes (DM, G2), DM + once daily insulin glargine (G3), DM + once daily insulin glargine with twice daily insulin glulisine (G4), DM + ALA (G5), DM + Racemic ALA (G6). DM was induced by intraperitoneal STZ injection (60mg/kg) and both ALA and R-ALA were administered orally (100 mg/kg). Sensory tests were assessed and immunohistochemistry of peripheral nerves from skin was performed at 24 wks.

Results

Response threshold for von frey monofilament was most lower in DM group as compared with normal group. Intervention blunted this decrease in G4 and G6 ($p < 0.05$) and not observed in G3 and G5. Randall Selitto response threshold was lower in DM group. Both of glucose control and ALA treatment significantly blunted these decrease in four treatment groups ($p < 0.05$), but there was no significant difference. Quantitative comparisons of peripheral nerve by intraepidermal nerve fiber density (IENFD) were decreased in DM group compared to normal group (11.5 ± 1.2 vs 15.9 ± 0.9 , $p < 0.05$) and it was preserved in G5 and G6 compared to DM group (17.9 ± 0.7 vs 18.4 ± 0.9 , $p < 0.05$). However, there was no significant difference between G5 and G6. Similarly, IENFD was preserved after insulin treatment in G3 and G4, but this trend did not reach to the significant difference (13.8 ± 0.8 , 14.3 ± 0.9 , $p > 0.05$) and did not differ in between G3 and G4. IENFD was more preserved in both type of ALA group compared to both of insulin group ($p < 0.05$).

Conclusions

Both of insulin and ALA treatment demonstrated little neuroprotective effect in experimental diabetes regardless of insulin and ALA type. In terms of IENFD, both type of ALA treatment had a mildly beneficial effect compared to insulin treated group in this study. Blood glucose control should be more stringent to obtain a therapeutic benefit in DPN. ALA alone is also difficult to expect therapeutic effect in the DPN.

The background features a complex network of white lines and circles on a solid red field. The lines intersect to form various geometric shapes, including triangles and polygons. Three prominent white circles are positioned at key nodes: one in the top-left corner, one in the upper-middle section, and one in the lower-left section. The text 'AUTONOMIC NEUROPATHY' is printed in a bold, white, sans-serif font, centered horizontally and partially overlapping the lower-left circle.

AUTONOMIC NEUROPATHY

[P30] ROLE OF AUTONOMIC ACTIVITY IN HYPERTENSION AND LEFT VENTRICLE FUNCTION IN OBESE PATIENTS

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Aim

We previously reported that alterations of cardiac autonomic reflex tests were associated with a greater prevalence of hypertension in diabetic patients. Changes in autonomic activity might be involved in hypertension and changes in cardiac output often present in obese patients. The aim was here to examine the central and peripheral hemodynamic changes according to cardiac autonomic nervous system activity in obese or overweight patients with normal (NGT), impaired glucose tolerance (IGT) or type 2 diabetes (T2D).

Patients and methods

We included 81 patients (38 NGTs, 28 IGTs and 15 well-controlled T2Ds), 25 of them with well-controlled hypertension and all free of cardiovascular history. During 6 minutes at a controlled breathing rate, we evaluated cardiac vagal activity (HF-HR), sympathetic activity (LF-HR) and sympatho-vagal balance (LF/HF-HR) by spectral analysis of heart rate variations (Task Force Monitor® digital plethysmography), and we measured stroke volume (SV), cardiac output (CO), cardiac index (CI) and thoracic fluid content (TFC, indicative of blood volume) by thoracic impedance. Radial and central blood pressure and carotid-to-femoral pulse wave velocity (PWV) were measured by tonometry (Sphygmocor®) and cutaneous blood flow (CBF) by laser doppler flowmetry (Periflux®). Endothelial function was evaluated by CBF response to acetylcholine and reactive hyperemia index (RHI, Itamar®).

Results

We separated the patients in two groups according to HF level. Compared to patients with HF>median value, patients with HF<median value were older (48±13 vs 39±10 years, p<0.01), with lower BMI (34.9±5.9 vs 37.5±5.3 kg/m²), they did not differ for glucose status and had similar HbA1c levels (5.8±0.9 vs 5.6±0.9%), they had lower LF-HR but higher LF/HF-HR ratio (p<0.001). They had higher heart rate, more prevalent hypertension (42% vs 20%, p<0.04) but similar blood pressure levels, similar PWV and TFC, lower SV (55±14 vs 76±19 ml), CO (3.9±0.9 vs 5.3±1.5 l/min) and CI (1.9±0.4 vs 2.4±0.6 l/min.m²) (p<0.01 to p<0.0001). All these differences remained significant after adjustment for age, BMI and glucose status. Mean CBF, CBF response to acetylcholine and RHI were similar in the two groups.

Conclusion

The present data show that in obese individuals, a lower vagal activity with relative sympathetic predominance is associated with hypertension and alteration of left ventricular contractility, without difference for neither pre-load or post-load. Impaired autonomic balance seems to be involved in the impairment of left ventricular function and the inadaptation to volume condition.

[P31] THE IMPACT OF OBSTRUCTIVE SLEEP APNOEA ON CARDIAC AUTONOMIC NEUROPATHY IN PATIENTS WITH TYPE 2 DIABETES: A LONGITUDINAL STUDY

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Background and aims

Obstructive Sleep Apnoea (OSA) is known to be associated with sympathetic overactivation but data regarding the impact of OSA on autonomic function in patients with type 2 diabetes (T2DM) are lacking. We have previously shown that OSA was associated with sympathetic and parasympathetic withdraw (based on frequency domain analysis of heart rate variability (HRV)) in patients with T2DM in a cross-sectional study. The aim of this study was to assess the longitudinal impact of OSA on cardiac autonomic neuropathy (CAN) in patients with T2DM.

Methods

Patients with T2DM were recruited from a single tertiary centre in the UK 2009–2011 and were followed up in 2013–2014. CAN was assessed using ANSAR™ technology based on HRV. CAN was present if at least 2 of the standardized tests were abnormal. OSA was diagnosed based on apnoea hypopnea index (AHI) of ≥ 5 events/hour using overnight cardiorespiratory portable monitoring. Patients who were diagnosed with OSA at baseline were started on continuous positive airway pressure (CPAP) if indicated clinically and hence we had 4 groups of patients by the end of follow-up: Group 1: no OSA (n=69); Group 2: mild OSA (n=73); Group 3: moderate to severe OSA not compliant with CPAP (n=25); and Group 4: moderate to severe OSA who were compliant with CPAP (n=15). Compliance with CPAP was defined as CPAP usage of ≥ 4 hours/night based on data downloaded from the CPAP equipment.

Results

182 patients were included in the analysis. OSA and CAN prevalence were 62.1% (n=113) and 43.4% (n=79) respectively. The mean follow-up duration was 4.6 (0.5) years. Progression to CAN was similar in patients without and with OSA (39.1% [n=9] vs 37.5% [n=15]; p=0.9). After adjustment for the baseline value of the outcome measure of interest, ethnicity, gender, age at diagnosis, BMI, baseline eGFR, diabetes duration, Hb1Ac, systolic blood pressure, triglyceride levels, use of insulin and anti-hyperglycaemic agents. CPAP compliance was associated with higher study-end log E/I ratio (B=0.06; p=0.002), study-end log resting LFA (B=0.7; p=0.001), study-end log resting RFA (B=0.6; p=0.006), study-end log deep breathing LFA (B=0.5; p=0.03), study-end log deep breathing RFA (B=0.4; p=0.03) and study-end log standing LFA (B=0.6; p=0.01). There was no effect on LFA/RFA ratios.

Conclusion

In patients with OSA and CAN, CPAP improved autonomic function based in frequency domain analysis of HRV. However, OSA did not affect the progression to CAN during the follow up. This might suggest that OSA may not play a role in CAN development in patients with T2DM but OSA can worsen CAN when it develops. Randomized clinical trials assessing the impact of CPAP on CAN are needed.

[P32] THE IMPACT OF THE INTERACTION BETWEEN OBSTRUCTIVE SLEEP APNOEA AND CARDIAC AUTONOMIC NEUROPATHY ON eGFR DECLINE IN PATIENTS WITH TYPE 2 DIABETES: A LONGITUDINAL STUDY

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Introduction

Diabetes-related chronic kidney disease (CKD) is a leading cause of end-stage renal disease (ESRD). We have previously shown that both obstructive sleep apnoea (OSA) and cardiac autonomic neuropathy (CAN) are independently associated with renal function decline in patients with Type 2 diabetes (T2DM). Hence, in this study we aimed to assess the impact of the interaction between OSA and CAN on renal function longitudinally.

Methods

Patients were recruited from a single tertiary diabetes centre in the UK 2009–2011. Renal function was assessed using MDRD-calculated estimated glomerular filtration rate (eGFR). Rapid eGFR decline was defined as 4% decline of eGFR/year. OSA was diagnosed if the apnoea hypopnea index (AHI) was ≥ 5 events/hour using overnight cardiorespiratory portable monitoring. CAN was assessed based on heart rate variability using ANSAR™ technology. CAN was present if at least 2 of the standardized tests were abnormal. Patients were divided into 4 groups; Group 1: no OSA and no CAN (n=45); Group 2: CAN with no OSA (n=27); Group 3: OSA with no CAN (n=67); and Group 4: OSA and CAN (n=54).

Results

A total of 200 patients were included. The mean follow-up duration was 2.5 (0.7) years. The OSA and CAN prevalence was 63% (n=126) and 39.5% (n=79) respectively. The eGFR decline (defined as % of baseline eGFR) was greater in patients with OSA and CAN [-1.9 (6.5) %; vs -1.4 (11.7) %; vs -3.2 (10.6) %; vs -9.4 (13.2) % for group 1 to 4 respectively; p=0.002]. Rapid eGFR decline was more common in patients with OSA and CAN [21.4% (n=9) vs 25% (n=15) vs 24% (n=6) vs 51% (n=26) for groups 1 to 4 respectively, p=0.005].

After adjusting for baseline eGFR, age, sex, ethnicity, diabetes duration, body mass index, mean arterial pressure, HbA1c, total cholesterol, triglycerides, insulin use, lipid lowering treatment, anti-hypertensive use, anti-platelets, oral anti diabetic agents, and smoking, having OSA and CAN predicted lower study-end eGFR ($R^2=0.9$; $B=-7.2$, p=0.006), and greater drop in eGFR described as eGFR change% ($R^2=0.17$; $B=-0.1$, p=0.005). After similar adjustments, OSA and CAN predicted rapid eGFR decline (OR=3.38; 95% CI 1.08, 9.9; p=0.04).

Conclusion

Detecting OSA and CAN in patients with T2DM identifies a high risk population for eGFR decline. Patients with T2DM and OSA and CAN are at increased risk of greater eGFR decline compared to those with either OSA or CAN. Studies assessing the impact of OSA treatment on eGFR decline are ongoing.

[P33] CARDIOVASCULAR AUTONOMIC NEUROPATHY AND RENAL FUNCTION IN PARTICIPANTS IN THE PREVENTING EARLY RENAL LOSS IN TYPE 1 DIABETES TRIAL

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Objective

Cardiovascular autonomic neuropathy (CAN) may predict progression of diabetic kidney disease (DKD) and more serious cardiovascular outcomes. We analyzed the cross-sectional association between CAN and DN in subjects with type 1 diabetes (T1D) and mild to moderate DKD enrolled in the ongoing PERL trial, which is testing the uric acid lowering effect of allopurinol on kidney function decline.

Methods

Measures of DKD included estimated glomerular filtration rate (eGFR), iothexol plasma disappearance GFR (iGFR), and albumin excretion rates (AER). Measures of CAN included resting heart rate, standard deviation of normal RR interval (SDNN), and QT_i (Bazzett formula) derived from resting ECG recordings and analyzed by ECGSCAN software.

Results

Baseline analyses include 154 T1D PERL participants with valid CAN and DKD data (age 53±11 years, 73% male, 82% Non-Hispanic White, duration 36±13 years, HbA1c 8±1%). Subjects in the lowest tertile of iGFR were older (53±10 vs 47±12 year, p=0.0007), had longer duration of diabetes (39±13 vs 29±10 year, p=0.001), lower diastolic blood pressure (69±11 vs 74±7 mm Hg, p=0.047), elevated serum uric acid level (6.7±1.7 vs 5.5±0.8 mg/dl, p=0.0004) and lower log SDNN (2.5±0.7 vs 3.0±0.8, p=0.0054) as compared to those in the upper tertile of iGFR. No differences in the heart rate and QT_i were observed between groups. Lower SDNN correlated with lower iGFR (r=0.33, p<0.0001), lower eGFR (r=0.27, p=0.0009) and higher AER (r= -0.26, p=0.001). In multiple linear regression analysis there was a positive association between log SDNN (independent variable) and iGFR (dependent variable) ($\beta = 5.9$, SE=1.4, R²=0.31, p=0.0039) independent of age, gender, glycemic control, blood pressure, body mass index, and serum uric acid.

Conclusions

T1D PERL participants with worse renal function present with more advanced measures of CAN. Shared pathophysiological pathways between DKD and CAN are currently evaluated for novel therapy such as serum uric acid lowering therapy to prevent the decline in kidney function and improve cardiac autonomic function.

[P34] IMPAIRED CARDIOVASCULAR AUTONOMIC FUNCTION AND PERIPHERAL SENSORY NERVE FUNCTION ARE PRESENT AMONG SUBJECTS WITH HIGH RISK FOR THE DEVELOPMENT OF TYPE 2 DIABETES MELLITUS SCREENED BY THE FINDRISC QUESTIONNAIRE

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Diabetes *mellitus* and even impaired glucose tolerance are associated with autonomic and sensory nerve dysfunction. The Finnish Diabetes Risk Score (FINDRISC) is a validated and one of the most widely used T2DM risk score questionnaire.

The aim of our study was to compare autonomic and sensory nerve function among patients with higher future T2DM risk (minimum 12 points in the FINDRISC questionnaire) with healthy control subjects.

Our study involved 30 patients with higher future T2DM risk (mean age: 58,3±13, female 12, fasting glucose 5,7±0,4 mmol/l, mean FINDRISC score 18[15; 19]) and 18 healthy control subjects (mean age:52,8±13, female 6, fasting glucose 5,03±0,5 mmol/l, mean FINDRISC score 8[7; 10]). Sensory function was evaluated by Neurometer (Neurotron Inc., Baltimore, United States of America) device, 128 Hz calibrated tuning fork, Semmes-Weinstein monofilament and Q-sense (Medoc Ltd., Yamat Rishai, Israel) device. Neuropathic symptoms was measured by NTSS-6 (Neuropathy Total Symptom Score) questionnaire.

Patients with higher future T2DM risk had significantly higher vibration perception thresholds both on the upper extremities (6,6 vs 7,6, p=0,037) and both on the lower extremities (5,8 vs 7,4, p=0,004) than healthy control subjects. In case of stimulating the median nerve at 2000 Hz (2,99 mA vs 2,65 mA, p=0,014) and at 250Hz (1,36 mA vs 0,86 mA, p=0,0008) the current perception thresholds were significantly higher among patients with higher future T2DM risk compared to controls. Moreover, patients with higher future T2DM risk had significantly higher heat perception thresholds assessed by the Q-Sense device, both on the upper extremities (35,5 °C vs 34 °C, p=0,02) and both on the lower extremities (41,5 °C vs 38 °C, p=0,02) than healthy control subjects. During the determination of cold perception patients with higher future T2DM risk had significantly lower cold perception thresholds in case of the upper (29,3 °C vs 30,5 °C, p=0,042) and the lower extremities (26,9 °C vs 29,5 °C, p=0,019). Using the 10-g monofilament diminished protective sensory function was detected among patients with higher future T2DM risk compared to healthy controls (3,8 vs 4,8, p=0,073). None of the subjects examined had any symptoms of neuropathy. Assessing autonomic function, we detected attenuation of respiratory arrhythmia in the high risk group compared with the healthy group (11 vs 18,4, p=0,001). The total autonomic impairment score (2,67 vs 1; p=0,007) was higher among patients with higher future T2DM risk compared to controls.

Impaired cardiovascular autonomic function and peripheral sensory nerve function might be present among subjects with high risk for the development of type 2 diabetes *mellitus* compared to healthy controls. Our results highlight the importance of early neuropathy assessment among these patients.

[P35] DIABETIC RETINAL SENSORY NEUROPATHY PROGRESSION PREDICTS CARDIOVASCULAR AUTONOMIC NEUROPATHY IN TYPE 1 DIABETES

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Objective

Diabetic retinal sensory neuropathy (DRSN) and cardiovascular autonomic neuropathy (CAN) may result from type 1 diabetes (T1D) -induced dysfunction of unmyelinated sensory neurons. We tested the hypothesis that CAN and DRSN progress in parallel.

Methods

Forty T1D patients without known complications at baseline were included in a longitudinal observational study and phenotyped for several complications. CAN was evaluated with standardized cardiovascular reflex tests [deep breathing test (expiration/inspiration ratio E/I), Valsalva ratio], and heart rate variability [low frequency (LF), high frequency (HF) and their ratio (LF/HF)] at baseline and during 3 years follow-up. Measures of DRSN included dilated ophthalmic examination, frequency doubling perimetry (FDP) using the 24-2 strategy, fundus photographs, and optical coherence tomography (OCT) with retinal layer segmentation. Students t-test was used to identify characteristics that were different between progressors and non-progressors.

Results

Twenty-six T1D subjects (mean age 38±14 years, mean diabetes duration 14±7 years, mean HbA1c 7.9±1%, mean visual acuity 20/20) at baseline, had valid CAN and DRSN data at baseline and follow-up. During follow-up 11 T1D subjects developed mild diabetic retinopathy (DR) and 3 moderate non-proliferative DR. At 3 years FDP threshold sensitivities were unchanged or improved in 81% of T1D participants (nonprogressors); 19% of T1D patients exhibited a > 3 dB sensitivity reduction (progressors). Older age at baseline and greater diabetes duration were associated with progression whereas gender, baseline HbA1c, body mass index, total cholesterol, LDL-c, HDL-c, and triglycerides were not. Mean deep breathing HF, LF/HF, and baseline and 3 year E/I ratios were significantly different between DRSN progressors vs nonprogressors.

Conclusions

These are the first data to suggest a longitudinal correlation between progression of DRSN and CAN in persons with T1D.

[P36] RELATIONSHIP BETWEEN ABNORMAL HANDGRIP TEST RESULTS AND AMBULATORY BLOOD PRESSURE MONITORING (ABPM) PARAMETERS

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Introduction

The handgrip test is no more suggested to be performed for the assessment of cardiovascular autonomic neuropathy among patients with diabetes *mellitus*. In our previous study, an inverse relationship between handgrip test abnormality and the presence of hypertension was established.

Aim of the present study was to characterize more precisely the association between handgrip test and hypertension via performing ambulatory blood pressure monitoring (ABPM) among diabetic patients.

Methods

Seventy patients with diabetes (mean age: 62.4±11.8 years; 58.6% female; 10% with type 1 diabetes; BMI: 30.8±5.2 kg/m²) were studied. Cardiovascular autonomic function was assessed by the standard cardiovascular reflex tests. For normal, borderline and abnormal handgrip test results, the scores 0, 1 and 2 were given. Cardiovascular autonomic neuropathy was diagnosed in patients with ≥2 abnormal CARTs excluding the handgrip test. All patients underwent ABPM as well. Correlations between handgrip test results and ABPM parameters were analyzed using monotony coefficient gamma (γ).

Results

Cardiovascular autonomic neuropathy was diagnosed in 56 patients. Significant negative associations were found between the handgrip test results and the 24-hour mean systolic ($\gamma=-0.355$) and diastolic ($\gamma=-0.245$) blood pressure values ($p<0.05$). An inverse relationship between handgrip scores and systolic ($\gamma=-0.268$) and diastolic ($\gamma=-0.216$) blood pressure load was observed ($p<0.05$). Furthermore, the handgrip test results were inversely associated with higher systolic ($\gamma=-0.254$) and diastolic ($\gamma=-0.240$) hyperbaric impacts ($p<0,05$). Diurnal indices of systolic and diastolic blood pressure, anthropometric parameters and results of the standard cardiovascular reflex tests did not show any associations with handgrip test results.

Conclusion

Higher values of ambulatory blood pressure monitoring parameters are associated with lower impairment scores obtained during isometric handgrip exercise. Our study confirms the inverse association between handgrip test abnormality and hypertension, using ambulatory blood pressure monitoring.

[P37] ASSOCIATION BETWEEN INDICES OF HEART RATE VARIABILITY AND METABOLIC SYNDROME IN AN ADULT CHINESE POPULATION

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Objective

We aimed to evaluate the prevalence of the metabolic syndrome (MetS) in a stable Chinese population, and to assess the association between components of MetS and cardiovascular autonomic neuropathy (CAN).

Methods

We phenotyped a cohort of 4000 subjects from the Pinggu district of Beijing, China with demographic and metabolic data including age, height, weight, systolic and diastolic blood pressure, fasting glucose, triglycerides, total cholesterol, HDL cholesterol (HDLc), and 1-minute ECG recordings. CAN was assessed by time-domain indices of heart rate variability (HRV) including standard deviation of the R-R intervals (SDNN) and root mean square of successive differences (RMSSD) that were derived from resting ECG recordings using ImageJ software. Waist circumference was not available. Presence of the MetS was defined according to the modified NCEP ATPIII criteria for individuals of Asian descent. Healthy controls were those individuals with normal fasting glucose, normal blood pressure and normal HDLc and triglycerides.

Results

Of the 4000 Chinese subjects in the cohort, 892 (22%) were found to meet criteria for MetS. Initial HRV data was available to date for a subset of this cohort that included 59 healthy controls and 162 MetS subjects. In this subset of participants there were no significant differences between the SDNN (25.2±11.8 vs 23.4±12.6 msec, p=0.56) or the RMSSD (22.7±13.2 vs 20.6±12.5 msec, p=0.59) in controls versus MetS subjects. Age and systolic blood pressure were negatively correlated with the SDNN in both healthy control and MetS subjects (Table).

Conclusions

We found a high prevalence of the MetS in the Pinggu region of China, which is similar with prior published observations in other cohorts, although due to the lack of waist circumference data, this prevalence may be an underestimate. In preliminary analyses in a small sample of this cohort, there were no significant differences in either of the time domain HRV indices assessed between the MetS and healthy controls. Analyses of the HRV indices are ongoing in the rest of the cohort to better understand the relationship between CAN and MetS in this population.

Variable	Control (n = 59)				MetS (n = 162)			
	SDNN		RMSSD		SDNN		RMSSD	
	Pearson Coefficient	p-value	Pearson Coefficient	p-value	Pearson Coefficient	p-value	Pearson Coefficient	p-value
Age (yr)	-0.29	0.02*	-0.13	0.31	-0.23	0.00*	-0.23	0.00*
BMI	-0.11	0.40	0.01	0.95	-0.07	0.37	-0.01	0.93
SBP	-0.26	0.04*	-0.17	0.19	-0.19	0.02*	-0.15	0.06
DBP	-0.27	0.04*	-0.23	0.08	-0.07	0.37	-0.01	0.88
TG	0.000	0.99	0.12	0.37	-0.04	0.63	-0.05	0.52
T chol	-0.17	0.21	-0.10	0.44	-0.01	0.87	-0.06	0.47
HDL-c	-0.18	0.17	-0.24	0.07	-0.12	0.14	-0.10	0.21
FBG	0.11	0.41	0.12	0.36	-0.10	0.21	-0.07	0.37

BMI: body-mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglycerides; T chol: total cholesterol; HDL-c: high density lipoprotein; FBG: fasting blood glucose

*Denotes significant correlations

[P38] THE ROLE OF AUTONOMIC NEUROPATHY IN THE PATHOGENESIS OF GLYCEMIC VARIABILITY IN TYPE 1 DIABETES

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Background

A relationship is suspected between autonomic neuropathy (AN) and glucose variability (GV) as variability is associated with oxidative stress responsible for neuronal damage while the impaired autonomic function has a detrimental effect on metabolism.

Objectives

The aim of our study was to find relationship between the parameters of AN and GV in type 1 diabetic patients.

Patients and methods

21 type 1DM patients were involved (age: 39.5±3.4 years, duration of DM: 17.5±2.5 years; HbA1c: 8.1±0.2%, mean±SE). Autonomic neuropathy (AN) was assessed by the cardiovascular reflex tests (CRT). The interstitial glucose levels were determined following insertion of a subcutaneous electrode during the continuous glucose monitoring (CGM) method on 6 consecutive days. GV was characterized by calculation of 4 parameters.

Results

Standard deviation of interstitial glucose values (SD) correlated positively with the overall AN score and the degree of the orthostatic reduction of systolic blood pressure (AN-score-SD $r=0.47$, $p<0.05$; orthostasis-SD: $r=0.51$, $p<0.05$). Mean Absolute Glucose: (MAG) correlated with 3 parameters of AN (AN-score-MAG: $r=0.62$, $p<0.01$; 30/15 ratio-MAG: $r=-0.50$, $p<0.05$; orthostasis-MAG: $r=0.59$, $p<0.01$). The HbA1c also correlated with 2 parameters of GV (HbA1c – Continuous Overlapping Net Glycaemic Action (CONGA): $r=0.56$, $p<0.05$; HbA1c-MAG: $r=0.45$, $p<0.05$). The frequency of hypoglycemia did not exhibit any correlation with characteristics of GV.

Conclusions

Severity of glycemic variability correlates with both parasympathetic and sympathetic dysfunctions in long-standing type 1 diabetes. Higher HbA1c is associated with more severe glucose variability. The relationship of increased glucose variability and autonomic neuropathy might be explained by the higher frequency of hypoglycemia and postprandial hyperglycemia in patients with neuropathy.

[P39] CORRELATION BETWEEN SYMPTOMS OF GASTROPARESIS AND ¹³C-OCTANOIC ACID BREATH TEST IN PATIENTS WITH DIABETES

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Background and aims

Gastroparesis, one of the commonest gastrointestinal (GI) complications of diabetes *mellitus* (DM), produces symptoms of gastric retention in the absence of physical obstruction. The aim of this study was to correlate symptoms using the GCSI with delayed gastric emptying in patients with DM referred for ¹³C – octanoic acid breath test (¹³C-OBT).

Materials and methods

We studied 180 subjects with DM duration of 9.9±6.4 years, mean value of HbA1c was 8.6±1.7% and control group: 30 healthy volunteers. In results of GCSI all diabetics divided into 2 groups: I group consisted of 94 (52.2%) participants (56 male/38 female, mean age was 54.7±6.7 years) without GI symptoms and II group: 86 (47.8%) patients (48 male/38 female, mean age was 59.8±8.3 years) with diabetic gastroparesis (DG). All subjects had negative markers of other disorders of GI tract. GCSI questionnaire for assessment of GI symptom severity which consists of 9 questions answered by use of a six-point Likert response scale, ranging from 0 (none) to 5 (very severe). The severity of DG was assessed by GCSI questionnaire and Gastric emptying rate (GER) with ¹³C-OBT.

Results

From the patients of type 2 DM, all subjects of II group were reported one or more GI symptoms from the GCSI scale: 50% reported fullness and/or early satiety from very mild to severe, 30% reported bloating from very mild to severe, 19% reported vomiting or nausea from very mild to moderate, other participants and volunteers without GI symptoms. GCSI total score in the II group was 16.7±0.7, mean GCSI nausea/vomiting score – 0.4±0.2 and mean GCSI bloating score – 1.1±0.4. Low gastric motility has been diagnosed in patients with the help of ¹³C-OBT: in the II group T_{1/2} – 99.5±13.7 min, but in the I group T_{1/2} – 60.1±5.4 min and control group that result is T_{1/2} – 50.4±6.5 min. There was a strong correlation between ¹³C-OBT and the GCSI total (r=.85, p=0.001), nausea/vomiting (r =.80, p=0.001) and bloating scores (r =.79, p=0.001).

Conclusion

Gastroparesis is quite a common complication of DM and use of the questionnaire GCSI allows to screen patients for DG symptoms which is confirmed by the strong correlation between GER and severity symptoms of questionnaire revealed in our study.

[P40] DIAGNOSIS OF DIABETIC CARDIAC AUTONOMIC NEUROPATHY BY HIGH-FREQUENCY ULTRASONIC DOPPLEROGRAPHY

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Objectives

Autonomic neuropathy of any severity is present in all patients with diabetic sensomotor neuropathy. However in most cases it proceeds asymptotically or its clinical symptoms are not specific. Search of diagnostic methods to screen autonomic neuropathy is of current interest. We decided to estimate method of high frequency Ultrasonic Dopplerography for diagnosis of cardiac autonomic neuropathy (CAN).

Methods

26 patients with type 2 diabetes and sensomotor neuropathy were included in the study. Standard cardiovascular tests (Valsalva's test, dynamometer test, test with passive orthostasis, cold vasoconstriction measured by photoplethysmography) and spontaneous arterial baroreflex have been performed for all patients. Microcirculatory blood flow of skin has been estimated by method of high frequency ultrasonic dopplerography using the "Minimax-Doppler-K" device (LLC JV "Minimax", Russia, St. Petersburg) at rest and during functional tests: cold and occlusive (cuff) ones. Research has been approved by ethical committee, all patients have signed informed consent before study beginning. Data were presented as means±SEM. Mann-Whitney U test and Chi-square statistic were used to compare continuous or categorical variables. A pvalue of less than 0.05 was considered statistically significant.

Results

CAN has been diagnosed for all patients: functional in 38.5 % and organic in 61.5 %. In the group of organic CAN severity of sensomotor polyneuropathy was significantly higher according to NDS, and macrovascular complications of diabetes were met reliably more often. Initial parameters of microcirculatory blood flow have been decreased in all patients and did not differ between groups of the patients ($V_{am} 1.9 \pm 0.22$ and 1.7 ± 0.51 cm/s respectively, $p > 0.10$). During conducting occlusive (cuff) test its positive result (pathologically changed reaction of blood flow) is revealed for 20% patients in the group of functional CAN and for half of patients in the group of organic CAN. The positive result of cold test has been registered in half of patients with the functional CAN and in all patients with organic CAN. We assessed diagnostic value of functional tests and proposed algorithm for detection of severity of CAN. The first stage included conducting cold test (its sensitivity 100%, specificity 50%, positive prognostic value 76%, negative prognostic value 100%). Secondly we suggested to perform occlusive (cuff) one (sensitivity 58%, specificity 80%, positive prognostic value 78%, and negative prognostic value 62%).

Conclusions

Proposed algorithm is sufficiently simple for realization and allows to divide quickly forms of CAN for majority of the patients without additional expensive examination inspection forms that has crucial significance for development of further tactics of treatment and observation of these patients. To eliminate shortcomings of research it is necessary to continue it and to increase number of observations that will increase reliability of the proposed algorithm.

[P41] ASSOCIATION OF CARDIOVASCULAR AUTONOMIC NEUROPATHY AND STRUCTURAL CHARACTERISTICS OF MYOCARDIUM IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Cardiovascular disease are the major cause of high morbidity and mortality in patient with type 2 diabetes. There is growing body of evidence that cardiovascular autonomic neuropathy (CAN) is a grave prognostic factor and represents an important marker for increase mortality from cardiovascular disease. Remodeling of myocardium predisposes to the clinically significant cardiovascular diseases.

Objectives

Investigate the association between signs of CAN and echocardiographic characteristics of myocardium in patients with type 2 diabetes.

Methods

We examined 19 patients with type 2 diabetes *mellitus* (mean age $58,7 \pm 6,2$ years, 11 women and 8 male, mean diabetes duration $7,7 \pm 3,1$ years, mean glycated hemoglobin $7,1 \pm 0,3$ %). All patients with diabetes had the history of arterial hypertension which was controlled by appropriate antihypertensive regimen. Structural characteristics of myocardium were assessed by echocardiography and CAN was diagnosed by the standard battery of Ewing cardiovascular autonomic reflex tests. Types of myocardial remodeling were determined by the size of the cavities and walls of the heart. The correlation between structural characteristics of myocardium and tests for CAN was investigated. Statistical analysis was performed by SPSS, the correlation was determined by the Pearson test.

Results

All patients studied had some signs of CAN which were presented by the decrease of the tone of the both sympathetic and parasympathetic system in 15 patients and in 4 patients the only parasympathetic tone was impaired. All patients had some changes of the structure of the myocardium – in 10 subjects it was concentric remodeling, in 2 – concentric hypertrophy of the left ventricle. There were some correlations between indices of CAN and echocardiographic changes in the studied cohort of patients. We found significant negative correlations between heart rate variability during deep breathing and the size of the left atrium ($r = -0,51$; $p = 0,03$), between increase of blood pressure with prolonged isometric muscular tension (grip test) and mass of myocardium ($r = -0,47$; $p = 0,04$), between heart rate variability during the Valsalva maneuver and size of the left atrium ($r = -0,76$; $p < 0,001$). The orthostatic hypotension was negatively correlated with the left ventricular myocardial mass index ($r = -0,46$; $p = 0,04$). It was the positive correlation between the size of the left atrium and the degree of cardiovascular autonomic neuropathy ($r = 0,52$; $p = 0,03$).

Conclusions

We revealed some association between the signs of CAN and echocardiographic characteristics of myocardium in patients with type 2 diabetes *mellitus* which could indicate the possible causative relationship between structural remodeling of myocardium and CAN in these subjects. However, the question which of those impairments is primary and secondary needs to be further investigated.

[P42] IN PATIENTS WITH TYPE 1 DIABETES AND CARDIAC AUTONOMIC NEUROPATHY THE MICROVESSEL DENSITY IN DERMAL ENDOTHELIAL CELLS IS MARKEDLY INCREASED

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Objectives

The interaction between neuronal and vascular activities is postulated in the pathogenesis of late diabetic complications. Skin biopsy allows new insight into the pathogenesis of diabetic microangiopathy considered as neurovascular unit. The aim of the study was to assess microvascular density (MVD) in skin biopsy in patients with type 1 diabetes (T1DM) and correlate these data with clinical characteristics and the presence of late diabetic complications with special regard to diabetic neuropathy.

Methods

We included 98 patients with T1DM (55 men), median age 41 (31–52) years, diabetes duration 22 (18–30) years. Inclusion criteria were: age > 18 years, duration of diabetes > 5 years, consent to participate in the study. Exclusion criteria were: coagulopathy (APTT > 37s, INR > 1.1, PLT < 100G/mm³ blood), anticoagulant or antiplatelet medication, skin lesions in the biopsy area. Skin biopsy specimen was taken at the distal end of the leg. All samples were exposed to monoclonal mouse anti-human antibody anti-CD34 and anti-CD133 (CD-cluster of differentiation) to assess MVD – averaged number of blood vessels per 1mm², calculated using "hot spots" technique in optical microscope Nikon Eclipse e600. CD34 antigen expression was regarded as a reference marker for all vessels. CD133 is membrane glycoprotein responsible for regulating cell growth and differentiation. CD133 antigen expression is high in early endothelial cell and decreases as they mature. Cardiac autonomic neuropathy (CAN) was diagnosed using ProsciCardIII.

Results

Retinopathy was diagnosed in 53 (54%) patients, diabetic kidney disease in 15 (15%), peripheral neuropathy in 44 (45%), CAN in 14 (14%) patients. 40 (41%) individuals were diagnosed with hypertension and 30 (31%) smoked cigarettes. MVD for anti-CD34 for all tested specimens was 137.5 (112.5–162.5) /mm², CD133 expression was 87.5 (66.7–95.8) /mm². Microvascular density was higher in patients with autonomic neuropathy [for CD34: 162.5 (141.7–175.0) vs 129.2 (112.5–162.5), p=0.01 and for CD133: 93.7 (87.5–104.2) vs 83.3 (66.7–95.8), p=0.05]. In the multivariate logistic regression model, the presence of CAN was associated with CD34 expression (OR 1.02; 95%CI: 1.00–1.03, p=0.04) independently of sex, age, diabetes duration, smoking, HbA1c and LDL cholesterol.

Conclusions

Presented results suggest relationship between cardiac autonomic neuropathy and increased vascularization in type 1 diabetic patients.

[P43] RELATIONSHIP OF HEART RATE VARIABILITY WITH PERIPHERAL ARTERIAL STIFFNESS IN PATIENTS WITH TYPE 2 DIABETES

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Objectives

Cardiovascular autonomic neuropathy is one of common diabetic complications in type 2 diabetes, which leads to the wide range of morbidity from mild orthostatic hypotension to fatal cardiac arrhythmia. Measurement of arterial stiffness is used as for a manifestation of early subclinical atherosclerosis. Spectral analysis of heart rate variability has been suggested to an alternative tool to define cardiovascular autonomic neuropathy. We aimed to investigate the relationship between heart rate variability and peripheral arterial stiffness.

Methods

Total of 105 patients with type 2 diabetes was enrolled in this study. Spectral analysis of resting heart rate variability was measured by using SA-3000P spectral analyzer. Time domain parameters; standard deviation of all the normal heart rate interval (SDNN), root mean square of successive differences (RMSSD) and frequency domain parameters; physical stress index (PSI), total power (TP), very low frequency (VLF), high frequency (HF), low frequency (LF), LF/HF ratio were measured. Arterial stiffness was estimated by using brachial-ankle pulse wave velocity. Fasting blood was withdrawn from patients to measure biochemical parameters such as lipid profile, aminotransferase, alanine aminotransferase, gamma glutamyl transferase, blood urea nitrogen, creatinine etc.

Results

Mean brachial-ankle pulse wave velocity was significantly associated with age, height, body weight, body mass index, duration of diabetes, systolic blood pressure, and fasting plasma glucose. Mean brachial-ankle pulse wave velocity was also significantly associated with physical stress index, very low frequency, low frequency, and high frequency among heart rate variability variables. In multiple regression analysis, among heart rate variability variables, VLF was significantly associated with mean brachial-ankle pulse wave velocity. Duration of diabetes, albumin creatinine ratio and serum creatinine in first quartile of VLF were significantly smaller compared with fourth quartile of VLF.

Conclusion

Heart rate variability associated with brachial-ankle pulse wave velocity. In particular, low VLF value showed increased arterial stiffness in type 2 diabetes.

[P44] THE ASSOCIATION BETWEEN SERUM 25 (OH) D LEVELS AND CARDIAC AUTONOMIC NEUROPATHY

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Objectives

We investigated the association between serum 25 (OH) D levels and cardiac autonomic neuropathy in patients with type 2 diabetes.

Methods

A total of 171 patients with type 2 diabetes were recruited from the tertiary hospital. A cardiovascular autonomic function test was performed to diagnose cardiovascular autonomic neuropathy (CAN) using heart rate variability parameters. The time and frequency domains of the heart rate variability were evaluated. Serum 25 (OH) D levels was measured. Data was analyzed according to tertile of 25 (OH) D levels.

Results

Among all participants, 59 subjects (34.5%) had CAN. There were no differences in baseline characteristics, such as age, sex, and duration of diabetes, according to tertile of 25 (OH) D levels. Standard deviation of normal-to-normal RR intervals was tended to decrease as 25 (OH) D levels decreased. Low frequency in the supine position was significantly lower in the subjects with the lowest tertile of 25 (OH) D, compared to the subjects with the highest tertile of 25 (OH) D. Among five tests of CAN, only BP responses to handgrip test showed significantly different prevalence according to 25 (OH) D levels. 25 (OH) D levels were positively associated with standard deviation of normal-to-normal RR intervals and low frequency in the supine position. However, on multiple logistic analysis, only age showed significant association with the presence of CAN.

Conclusions

Although serum 25 (OH) D levels was correlated with heart rate variability parameters, there was no association between serum 25 (OH) D levels and CAN after adjustment confounding factors.

[P45] RELATIONSHIP BETWEEN HYPOGLYCEMIA UNAWARENESS, DRIVING AND COGNITIVE FUNCTION IN T1DM PATIENTS WITH DIABETIC AUTONOMIC NEUROPATHY

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Objectives

The aim of this study was to investigate the association between hypoglycemia unawareness (HU), cognitive disorders and diabetic autonomic neuropathy (DAN) in T1DM drivers.

Methods

The 80 T1DM patients (35±1,3 y.o.) were submitted to continuous glucose monitoring (previous version of IPro2) up to 7 days. DAN was assessed by Ewing's cardiovascular autonomic tests. Cognitive function was estimated using neuropsychological tests in 41 patients: working memory, perceptual reasoning, divided and sustained attention. Twenty (25%) T1DM drivers were completed a special questionnaire to assess their awareness of hypoglycemia, which was based on the ability to recognize hypoglycemia.

Results

Patients were divided into 3 groups: 1st – with hypoglycemia awareness (42,5%), 2nd – with 1-2 episodes of HU (35,0%), 3rd – with 3 or more episodes of HU (22,5%). The 91,25% of patients had abnormal autonomic tests. Autonomic failures were differed between 1st and 2 (3) groups ($p<0,01$). Relation between HU and DAN was found ($r=0,76$, $p<0,001$). Drivers of 1st group (35%) were characterized by hypoglycemia awareness; drivers of 2nd (25%) and 3rd (40%) groups were characterized by long episodes of HU during CGM-time in comparison with 1st group (68,0±12,1 vs 90,3±0,9 vs 107,6±12,3 min, $p<0,001$). Driving accidents were reported in 6 (75%) of T1DM drivers of 3rd group. The presence of impaired awareness of hypoglycemia was found in patients with HU in comparison with patients of 1st group (19,8% vs 10,6%; $\chi^2=10,3$, $p<0,001$). Cognitive disorders were differed between 1st and 2 (3) groups ($p<0,05$) and were most pronounced in drivers of 2nd and 3rd groups. Twelve (60%) of twenty drivers did not measure blood glucose before driving.

Conclusions

Patients with HU were characterized by more significant autonomic failures. Drivers of 2nd and 3rd groups had impaired awareness of hypoglycemia, pronounced cognitive dysfunction and increased risk of accidents during driving.

[P46] DIAGNOSTIC VALUE OF DIFFERENT AUTONOMIC SYMPTOMS ASSESSED BY COMPASS 31 FOR CARDIOVASCULAR AUTONOMIC NEUROPATHY AND DIABETIC POLYNEUROPATHY

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Objectives

We recently validated the questionnaire Composite Autonomic Symptom Score (COMPASS) 31 for autonomic symptoms of diabetic neuropathy. In a wider population from the same diabetes centre, we aimed to further investigate the diagnostic performance of different autonomic symptoms (i.e., orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor domains of COMPASS 31) for diabetic cardiovascular autonomic neuropathy (CAN) and diabetic polyneuropathy (DPN).

Materials and methods

A total of 93 participants with diabetes (age 54±14 years, diabetes duration 13±10 years) completed the COMPASS 31 questionnaire before undergoing cardiovascular reflex tests and assessment of neuropathic symptoms (using the Michigan Neuropathy Screening Instrument Questionnaire), signs (using the Michigan Diabetic Neuropathy Score), vibration, and thermal thresholds.

Results

As expected, the COMPASS 31 total weighted score was higher in the presence of CAN (26.7±19.5 vs 12.5±14.3; p=0.0044) and DPN (26.9±17.8 vs 12.5±11.3; p=0.0000). Among the 6 domains of COMPASS 31, the highest differences were seen in gastrointestinal (p=0.0004) and orthostatic intolerance weighted scores (p=0.0188) according to the presence of CAN, and in secretomotor (p=0.0000), gastrointestinal (p=0.0000), pupillomotor (p=0.0001), and orthostatic intolerance weighted scores (p=0.0005) according to the presence of DPN. Receiver-operating curve analysis confirmed a fair diagnostic accuracy of total weighted score for CAN [area under the curve (AUC) 0.685±0.067, 95% CI 0.583–0.782] and DPN (AUC: 0.755±0.050, 95% CI 0.652–0.836). Among the six COMPASS 31 domains, a fair diagnostic accuracy for CAN was reached only by gastrointestinal domain (AUC: 0.728±0.066, 95% CI 0.630–0.821), whereas it was achieved for DPN by secretomotor (AUC: 0.758±0.050, 95% CI 0.652–0.836), gastrointestinal (AUC: 0.728±0.066, 95% CI 0.630–0.821) and pupillomotor domains (AUC: 0.705±0.056, 95% CI 0.606–0.799). Secretomotor weighted score at the cut-off of 4.28 had a sensitivity of 61.5% (95% CI 46.3–76.8) and a specificity of 81.5% (95% CI 71.1–91.8) for DPN. Vasomotor and bladder domains showed the worst diagnostic performance.

Conclusions

Among autonomic symptoms as assessed using COMPASS 31, the best diagnostic performances were provided by the secretomotor domain (exploring sweating changes, dry eyes and dry mouth) for DPN and by the gastrointestinal domain for both CAN and DPN. These findings expand previous observations, give insights into the relationship between autonomic and sensorimotor neuropathy, and further support the inclusion of COMPASS 31 in a comprehensive evaluation of diabetic neuropathy.

[P47] CHANGES IN THYREOTROPIC AXIS ARE NOT ASSOCIATED WITH CARDIAC AUTONOMIC DYSFUNCTION IN THE OBESE POPULATION

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Rationale and aim

Cardiac autonomic dysfunction (CAD) is a common disorder in obese patients and was shown to be associated with a more severe insulin resistance and metabolic disorders. An increase in leptin levels may enhance sympathetic activity and has been suggested to contribute to changes in thyreotropic axis. The present study aimed to investigate in a large euthyroid obese population whether metabolic disorders associated with CAD might be related to an underlying thyroid disturbance.

Patients and methods

A total of 1165 obese patients (BMI 37.2 ± 6.8 kg/m², 82% female, 39.2 ± 13.6 years) without known diabetes or dysthyroidism were included. Three CAD function tests were performed: deep breathing, lying-to-standing and Valsalva, and interpreted by taking age into account. Serum thyroid stimulating hormone (TSH) and free thyroxine (FT4) concentrations were measured. Plasma glucose and insulin (at fasting and 2-hour 75-g post-load), glycated hemoglobin, fructosamine and lipid parameters were measured, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated.

Results

Mean values of TSH and FT4 were 2.1 ± 0.9 mU/l and 14.8 ± 2.4 pmol/l, respectively. TSH correlated with age ($r = -0.107$, $p < 0.0001$) and HOMA-IR ($r = 0.079$, $p = 0.008$). FT4 correlated with age ($r = -0.134$, $p < 0.0001$), BMI ($r = -0.073$, $p = 0.012$), fructosamine ($r = 0.101$, $p < 0.0001$), HOMA-IR ($r = -0.07$, $p = 0.02$) and triglycerides ($r = -0.115$, $p < 0.0001$). CAD was assessed in 292 patients. CAD as defined by one or more abnormal tests was present in 135 patients. Patients with CAD were older (38.7 ± 14.5 vs 35.6 ± 12.9 years, $p = 0.046$), had a higher waist-to-hip ratio (WHR) (0.91 ± 0.11 vs 0.89 ± 0.09 , $p = 0.055$) and higher post-load plasma glucose (6.6 ± 2.2 vs 6.5 ± 0.2 mmol/l, $p = 0.042$) than patients without CAD. Since FT4 and CAD were associated with age we reanalyzed the data in patients younger than 60 years: most of the correlations of FT4, TSH and CAD with metabolic parameters were observed. However TSH and FT4 did not differ significantly in the patients with or without CAD.

Conclusion

These data suggest that in this large euthyroid obese population cardiac autonomic dysfunction and related metabolic changes are not associated with changes in thyreotropic axis.

The background features a complex network of white lines and circles on a solid red field. The lines intersect at various points, some of which are marked with white circles of varying sizes. The overall effect is that of a stylized, interconnected web or a molecular structure.

**DIAGNOSIS AND
SMALL FIBERS**

[P48] SMALL FIBRE NEUROPATHY IN OBESE NON-DIABETIC PATIENTS: RELATIONSHIP TO ABNORMALITIES IN LIPOPROTEINS AND INFLAMMATION

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Objectives

Obesity has been implicated as a cause of peripheral neuropathy and experimental studies have suggested that abnormalities in lipoproteins and inflammation may play a role.

Methods

31 non-diabetic morbidly obese subjects and 41 age-matched controls underwent detailed neuropathy phenotyping and assessment of lipoproteins, HDL-functionality and systemic inflammation.

Results

HDL-C (1.0 ± 0.0 v 1.5 ± 0.1 , $p < 0.0001$), apo-lipoproteins A1 (139.2 ± 4.6 v 164.6 ± 6.2 , $p = 0.002$) and B (76.5 ± 4.5 v 89.8 ± 4.0 , $p = 0.006$), Paroxonase-1 (62.7 ± 11.8 v 200.8 ± 19.1 , $p < 0.0001$), serum amyloid A (102.2 ± 7.2 v 46.1 ± 8.8 , $p < 0.0001$), TNF- α (38.1 ± 7.4 v 17.5 ± 3.2 , $p = 0.008$) and cholesterol efflux (14.0 ± 1.1 v 17.9 ± 1.0 , $p = 0.028$) were abnormal in obese subjects. Obese subjects had evidence of a small fibre neuropathy with a lower cold perception threshold (25.6 ± 1.1 v 28.2 ± 0.5 , $p = 0.02$), higher warm perception threshold (40.4 ± 0.6 v 37.1 ± 0.5 , $p < 0.0001$), lower heart rate variability (23.7 ± 3.2 v 43.3 ± 19.8 , $p = 0.03$) and lower corneal nerve fibre density (25.5 ± 1.2 v 33.5 ± 1.0 , $p < 0.0001$), branch density (30.9 ± 3.1 v 47.7 ± 3.3 , $p = 0.001$) and fibre length (CNFL) (14.6 ± 0.6 v 19.4 ± 0.5 , $p < 0.0001$). They also had a higher neuropathy symptom profile (4.3 ± 1.1 v 0.5 ± 0.2 , $p < 0.0001$) and lower sural (11.3 ± 2.1 v 23.2 ± 1.9 , $p < 0.0001$) and peroneal nerve amplitudes (3.8 ± 0.5 v 5.7 ± 0.4 , $p = 0.006$), but preserved nerve conduction velocities, compared to control subjects. BMI was found to independently predict CNFL ($F = 6.4$, $p = 0.01$, $R^2 = 0.3$, $R^2_{Adjusted} = 0.3$, $p = 0.002$). Obese subjects with small fibre neuropathy (SFN) had higher triglycerides (1.7 ± 0.3 v 1.1 ± 0.2 , $p = 0.02$), 3-NT (108.7 ± 5.2 v 83.2 ± 6.2 , $p = 0.01$) and myeloperoxidase mass (1115.6 ± 154.4 v 667.7 ± 138.5 , $p = 0.04$), with a lower PON-1 activity (36.4 ± 12.7 v 83.2 ± 20.5 , $p = 0.02$). 3-NT independently predicted CNFL ($F = 7.4$, $p = 0.017$, $R^2 = 0.83$, $R^2_{Adjusted} = 0.72$, $p = 0.02$).

Conclusion

Small fibre neuropathy occurs in morbidly obese subjects without diabetes and HDL functionality and 3-NT may be potential targets to prevent or reverse this neuropathy.

[P49] NEW INSIGHTS INTO DIABETIC NEUROPATHY PROVIDED BY NOCICEPTOR FIBER-SELECTIVE STIMULATION

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Painful diabetic neuropathy is a paradox in that patients experience pain within an area of pain sensory loss. Epidermal denervation is thought to be responsible for loss of evoked pain sensitivity; however, no correlation has been found between denervation and spontaneous neuropathic pain. Similarly, unlike inflammatory pain, where C-fiber spontaneous activity is thought to result in sensitization and lowered pain thresholds, patients with painful diabetic neuropathy (PDN), who also have high spontaneous C-fiber activity do not demonstrate lowered evoked pain thresholds.

We have developed a fiber selective method (laser fiber selective stimulation; DLss) that is noninvasive, reproducible, and is simple to apply in the clinic. DLss is based on the temperature of activation of TRPV1 channels and tissue heating by infrared laser light absorption of the water of the skin. Both the TRPV1 activation temperature and laser light absorption are consistent across different species; therefore the method has been uniquely translatable.

We hypothesized that non-epidermal C fibers in PDN patients may be sensitized to heat, which means the loss of evoked pain sensitivity may be due to an inability to access these fibers using traditional stimulation methods. Therefore, stimuli that may selectively assess subtypes of C and A delta nociceptive fibers may allow assessment of sensitized fibers and solve the neuropathic pain paradox. To test this hypothesis, we measured responses of PDN patients to DLss.

We found that:

a) A subtype of C fibers, C mechano-insensitive fibers (CMi), which are normally "silent", are both abnormally spontaneously active and are sensitized to heat, b) DLss C selective protocol (C-DLss) allows for selective stimulation of silent CMi fibers in healthy subjects, c) Using DLss, the expected loss of evoked pain sensitivity was not found in PDN in response to selective activation of C fibers but was demonstrated in response to the stimulation of A delta fibers.

C-fiber pain sensitivity was not decreased because, despite denervation, the assessed CMi fibers were sensitized and DLss provides uniform epidermal and dermal heating that allows for the assessment of C fibers with the lowest thresholds regardless of the depth of their location. However, response of A delta fibers demonstrated substantial loss of pain sensitivity. Therefore, the DLss quantitative sensory test (QST) may serve as a quantitative biomarker of painful peripheral neuropathy.

[P50] ASSESSMENT OF DIABETIC POLYNEUROPATHY IN ZANZIBAR: COMPARISON BETWEEN TRADITIONAL METHODS AND A POINT-OF-CARE NERVE CONDUCTION DEVICE (NC-STAT DPNCHECK)

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Objectives

In tandem with increasing diabetes (DM) prevalence, dire endpoints such as ulcers and amputations are major socioeconomic challenges in sub-Saharan Africa. Scant information is available about the prevalence of neuropathy, as well as the applicability of screening tools in this population. Hence, we aimed to investigate these issues in Zanzibar (Tanzania).

Methods

We included 100 consecutive DM patients attending the diabetes clinic at Mnazi Mmoja Hospital in Zanzibar. Signs of neuropathy were investigated by the following methods: a) self-reported numbness of the lower limbs (yes/no), b) ten-point monofilament test (\geq four missed point indicating neuropathy), c) the Sibbald 60-second Tool (a standardized foot tool) and d) nerve conduction studies (NCS, using an automated handheld point-of-care device, the NC-Stat DPNCheck, Neurometrix Inc.).

Results

Fifty-one male and 49 female patients were included. Mean age 54 years (29–85) *, body mass index 26 kg/m² (17–43), 10 type one DM, the rest type two. DM duration was 9 years (0–30). Blood pressure 155/88 mmHg (81–233/51–127). HbA1c was 8.5% (3.7–22.9). For the neuropathy screening, 62% reported numbness, 61% had positive monofilament whereas the Sibbald tool was positive in 87% of cases. NCS defined neuropathy in 45% of the patients (in total 15% were classified as mild, 20% moderate and 10% severe). Table and Figure one describe the degree of agreement between the methods.

*All clinical characteristics given as mean (range).

Table 1. Diagnostic accuracy and agreement of neuropathy screening methods compared to NCS

(PPV/NPV= positive/negative predictive value)

Method	Sensitivity	Specificity	PPV	NPV	Cohen's kappa of agreement
Numbness	74%	49%	54%	69%	0.22
Monofilament	86%	59%	63%	83%	0.43
Sibbald	100%	21%	51%	100%	0.19

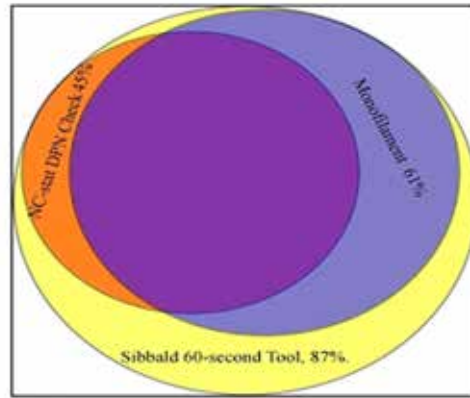


Figure 1. Venn diagram comparing the methods

Conclusions

The patient population was characterised by poor glycaemic control and hypertension. In line with this, neuropathy was rampant. The monofilament test tended to define more cases of probable neuropathy than the NCS, however specificity was low. Palmar skin thickening may have led to false positives in this population. The point-of-care NCS device was useful in a low-resource research setting.

[P51] UTILITY OF *IN-VIVO* CORNEAL CONFOCAL MICROSCOPY IN COMPARISON WITH OTHER MEASURES OF SMALL FIBER FUNCTION IN SUBJECTS WITH TYPE-2 DIABETES

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Objective

In vivo Corneal Confocal Microscopy (IVCCM), a non-invasive tool for detecting morphological alterations of small nerve fibers in the cornea has been proposed as a diagnostic tool for the detection of diabetic peripheral neuropathy (DPN). However, quantification of different morphologic parameters of nerve fibers comprising IVCCM have not been extensively examined in comparison to other measures of small fiber function. The aim of this study was to evaluate the diagnostic utility of IVCCM in comparison to other small fiber structural and functional measures of DPN in subjects with type 2 diabetes (T2DM)

Methods

Subjects with T2DM underwent: bilateral eye IVCCM examination of the corneal sub-basal nerve plexus using the Rostock cornea module of the Heidelberg Tomograph III; sudomotor function using Sudoscan™ that measures electrochemical skin conductance (ESC) of hands and feet; cardiac autonomic reflex tests (CARTs) with time and frequency domain analysis of heart rate variability (HRV); Neuropathy Impairment Scores of lower limbs (NIS-LL); Utah Early Neuropathy Scale (UENS); quantitative sensory testing for vibration (VDT), cold (CDT) and warm detection thresholds (WDT); and nerve conduction studies (NCS) of peroneal and sural nerves. Manual image analysis was used to quantify corneal nerve fiber density (CNFD), branch density (CNBD) and length (CNFL). DPN in T2DM subjects was defined according to published clinical and electrophysiological criteria (Toronto Consensus Panel).

Results

We included 105 T2DM subjects. IVCCM nerve fiber quantifications were assessed by 2 independent observers. ICCs were calculated for all measures (CNFD=0.82, CNBD=0.79, and CNFL=0.86). CNFD (17.69 ± 0.70 vs 21.58 ± 1.69 $p=0.03$), CNBD (35.73 ± 3.19 vs 55.77 ± 6.60 , $p=0.01$) and CNFL (15.04 ± 0.81 vs 19.42 ± 1.46 , $p=0.02$) were significantly lower in T2DM patients with DPN vs T2DM patients without DPN. CNBD had the highest area under ROC curve (AUC) at 0.71 followed by CNFL at 0.68 (Optimal threshold of 15.7 mm/mm² with 56% sensitivity and 80% specificity). Similar results were observed for neuropathy scores, QSTs, and sudomotor function. Of all the measures assessed, CDT, WDT, UENS and feet ESC had the highest AUC (0.75, 0.79, 0.78 and 0.71, with sensitivities and specificities of 63/66, 79/64, 84/65 and 72/68%, respectively).

Conclusion

IVCCM showed good repeatability with moderate sensitivity and good specificity, which was comparable to other measures of small fibre dysfunction, for diagnosing DPN in patients with T2DM, despite DPN being defined utilizing predominantly large fiber dysfunction. Further studies establishing the relationship between IVCCM and functional measures of small fiber neuropathy are warranted.

[P52] CORNEAL CONFOCAL MICROSCOPY DETECTS SEVERE SMALL FIBRE NEUROPATHY IN DIABETIC PATIENTS WITH CHARCOT FOOT

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Objectives

The pathogenesis of the Charcot foot remains unclear. Small nerve fibers play an important role in blood flow and inflammation and therefore may play an important role in Charcot. We have undertaken corneal confocal microscopy (CCM) to identify the extent of small fibre neuropathy in patients with Charcot.

Methods

20 patients with a chronic Charcot foot were compared to 20 age and diabetes duration matched patients with Type 2 diabetes *mellitus* (T2DM) and 20 age-matched healthy controls. Patients underwent CCM and quantification of corneal nerve fibre density (CNFD), corneal nerve branch density (CNBD) and corneal nerve fibre length (CNFL); electrochemical skin conductance (ESC) to assess sudomotor function and vibration perception threshold (VPT).

Results

In patients with Charcot compared to T2DM and controls there was a significant reduction in CNFD (14.94±8.23 vs 23.86±7.71, p=0.004 vs 34.84±9.13, p<0.001), CNBD (18.61±16.7 vs 41.62±22.67, p=0.032 vs 76.47±38.44, p<0.001) and CNFL (8.40±4.83 vs 14.87±4.76, p=0.001 vs 21.24±6.48, p<0.001), ESC in the feet (20.57±13.99 vs 61.50±22.26, p<0.001 vs 76.23±12.01, p<0.001) and hands (30.86±18.10 vs 61.13±19.14, p=0.001 vs 68.31±11.96, p<0.001) and vibration perception in the feet was significantly higher (38.46±15.10 vs 14.15±10.25, p<0.001 vs 7.75±4.01, p<0.001).

Conclusions

Diabetic patients with a Charcot foot have evidence of a very severe large and particularly small fiber neuropathy.

[P53] DECLINE IN CMAP AMPLITUDE OF THE TIBIALIS ANTERIOR MUSCLE INDICATES ADVANCED DIABETIC POLYNEUROPATHY

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Objective

Foot drop is an important sign of neuropathic muscle weakness. We measured CMAP amplitude of the tibialis anterior (TA) muscle, the main muscle for foot dorsiflexion, to examine if a fall in TA amplitude is a simple indicator of advanced diabetic distal symmetric neuropathy (polyneuropathy).

Methods

In addition to the routine nerve conduction study of the lower limb with standard methodology (the peroneal nerve motor conduction study using the extensor digitorum brevis (EDB) muscle recording, the tibial nerve motor conduction study with the abductor hallucis (AH) muscle recording, and the sural sensory nerve conduction study in the distal leg), we recorded TA-CMAP by peroneal nerve stimulation at the fibra-head in 96 type-2 diabetic patients aged 32-69years (mean 59years). As a neurological control, similar recording was performed in 40 non-diabetic patients with carpal tunnel syndrome aged 31-70years (mean 59years).

Results

The mean±standard deviation of the TA-CMAP of DM group was $3.8\pm 1.1\text{mV}$ (range 1.7-10.5mV), which was significantly lower than the control value of $4.6\pm 1.3\text{mV}$ (range 3.1-8.9). In 24 diabetic patients with TA-CMAP less than 3.1mV (the lowest value of the neurological control) (lower TA-CMAP group), the sural SNAP and the tibial CMAP were $1.7\pm 2.0\mu\text{V}$ and $4.7\pm 2.7\text{mV}$ respectively, either of which were about a half of the mean value in 72 diabetic patients with TA-CMAP more than 3.1mV (normal TA-CMAP group). Among the 24 patients of lower TA-CMAP group, 23 had lower SNAP less than 5 μV , and 17 showed lower tibial AH-CMAP than normal range. When TA-CMAP was less than 2.5mV, no patients had tibial AH-CMAP more than 5.5mV. In short, low sural SNAP and/or low tibial AH-CMAP was rare finding in patients with normal TA-CMAP more than 3mV, while tibial AH-CMAP was low in almost all patients with TA-CMAP less than 2.5mV.

Discussion

In the standard peroneal NCS, fall in CMAP amplitude of the EDB muscle is one of the earliest findings indicating the presence of diabetic polyneuropathy, which is followed by fall in the sural SNAP amplitude: Fall in amplitude of AH-CMAP is then added later during further advanced stage of the diabetic polyneuropathy. The present data of TA muscle recording indicate that decline in TA-CMAP happens in the most advanced stage of the polyneuropathy of diabetes.

Conclusion

The TA-CMAP is a simple indicator of severely-advanced diabetic polyneuropathy.

[P54] ADAPTATION AND VALIDATION OF THE NEUROPATHY-FOOT ULCER SPECIFIC QUALITY OF LIFE INSTRUMENT (NEURQOL) FOR THE ARABIC LANGUAGE

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Objectives

To perform the adaptation of the NeuroQol –Neuropathy – and Foot Ulcer – Specific Quality of Life instrument to the Arabic language, and to test the reliability and validity of the Arabic version when used on patients with diabetes *mellitus* (DM) in the presence of diabetic peripheral neuropathy (DPN) and foot ulcers (FU).

Methods

The first step of the study included adaptation of the NeuroQol instrument for the Arabic language. The 2nd step included analysis of the responses of 50 Saudi patients with DM to the adapted NeuroQol instrument and to the Arabic version of (SF-36) instrument, through individual interviews. The NeuroQol instrument included 6 domains: pain (P), loss/reduction in sensitivity (LS), diffuse sensory-motor symptoms (DSMS), limitations in daily activities (LDA), interpersonal problems (IP) and emotional distress (ED). Informed consent obtained. The study protocol was approved by the Hospital's local Ethics committee. Confirmed generalized DPN was assessed using neuropathy symptom score (NSS), neuropathy disability score (NDS), nerve conduction studies and electrochemical skin conductance. Pearson's correlation coefficient was used to test the relationship between variables. Cronbach's alpha was used to assess the reliability. Independent T test was used to compare variables. SPSS version 20 was used for statistical analysis.

Results

Mean age 54.1±8.9 years. History of FU was present in 56%. NSS: 7.16±0.8, NDS: 7.94±3.3. Feet-ESC: 34.0±24.83µs. Mean NeuroQol instrument score was 6.11±2.7. Cronbach's alpha values in the 6 domains of the NeuroQol ranged from 0.645 for (P) to 0.924 for (IP). Internal consistency of the items in each domain of the NeuroQol assessed through Cronbach's alpha coefficient ranged (0.71–0.98), and through Pearson's correlation coefficient ranged (0.688** –0.871**). There was a negative correlation between NeuroQol instrument score and SF-36 instrument score: -0.670 (p<0.0001). Convergent construct validity was verified through correlation between the 6 domains of the NeuroQol scores and the physical and mental components (PC, MC) scores of the SF-36 and showed negative correlation for the PC (P-0.411†, LS -0.612**, DSMS -0.515**, LDA -0.613**, IP-0.694**, ED -0.588**) and for the MC: (P-0.488**, LS -0.665**, DSMS -0.479**, LDA -0.531**, IP -0.666**, ED -0.554**). Discriminant construct validity was verified through comparison of the means of the NeuroQol instrument domains between patients groups without and with FU: p (5.25±3.29, 9.72±3.99 <0.0001), LS (5.2±3.3, 9.8±4.23<0.0001), DSMS (5.0±3.62, 8.6±4.4<0.003), LDA (5.48±3.1, 7.79±3.4<0.018), IP (5.0±3, 8.4±3.3 <0.001), ED (5.6±2.8, 7.2±3.3<0.076).

Conclusion

Results of adaptation and validation show that NeuroQol in the Arabic version has psychometric properties that allow its utilization as a valid and reliable instrument.

[P55] CORRELATIONS BETWEEN SKIN AUTOFLUORESCENCE, DIABETIC NEUROPATHY DIAGNOSTIC SCORES AND QUALITY OF LIFE IN PATIENTS WITH DIABETES

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Background

Skin autofluorescence (SAF) is a surrogate indicator for long term glycemic exposure and advanced glycation end-products in tissues accumulation. It is a relative new simple, noninvasive method for the risk for diabetes chronic complications quantification.

Aim of the study

Was to evaluate associations between and SAF and peripheral diabetic neuropathy (PDN) and quality of life (QoL) in patients with diabetes during a regular office visit.

Method and patients

This is a sub-analysis of a cross-sectional, analytic, multicenter study in which consecutive outpatients with type 1 diabetes *mellitus* (T1DM) or T2DM who attended diabetic units for regular diabetes care were included. The patients had a BMI between 25 and 45 kg/m² and HbA1c \leq 9.5 %. SAF was assessed using the AGE Reader (Groningen, The Netherlands), measurements were performed at the forearm, and results of 3 measurements were averaged. For DNP evaluation the Toronto Clinical Neuropathy Score (TCNS), the Neuropathy Symptoms Score (NSS) and the Neuropathy Disability Score (NDS) were used. The Romanian version of Norfolk QoL-DN was self-administrated by the patients and analyzed as total score and sub-domains scores. We have done descriptive statistics and we used the Sperman's correlation coefficient.

Results

There were 497 patients included in the study, 51.3% females, 93.4% with type 2 diabetes, with a mean (\pm SD) age of 60.54 (9.10) years and a mean (\pm SD) duration of diabetes of 17.36 (9.96) and 9.20 (5.53) years for with T1DM and T2DM respectively. The median (25th-75th percentile) SAF score (arbitrary units) was higher in those with poorer QoL (total score $>$ 5), 2.37 (2.13-2.77) vs 2.53 (2.17-2.87) but without reaching statistical significance ($p=0.178$). SAF (measured in arbitrary units- AU) was significantly higher in subjects with PDN (TCNS $>$ 5, n=237 subjects): 2.59 \pm 0.56 AU compared with patients without PDN (TCNS \leq 5, n=260): 2.45 \pm 0.53 AU, ($p=0.04$) and significantly increased with the severity of PDN. SAF was also significantly higher in those with neuropathic symptoms (NSS $>$ 2, n=410): 2.54 \pm 0.56 AU compared to patients without symptoms (NSS \leq 2, n=87): 2.40 \pm 0.47 AU, ($p=0.022$).

Conclusions

SAF is a relative simple and accesible method for the regular practice. Our results showed a good correlation with a well validated diagnostic score for PDN (TCSS) and some parameters of the QoL in patients

[P56] DIABETIC NEUROPATHY: AN ASSESSMENT OF SCREENING QUANTITATIVE SENSORY TESTING

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Aim

Diabetic Neuropathy (DN), a common cause of morbidity in Diabetic patients (DM) is associated with sensory loss. Quantitative sensory testing (QST) instruments use is limited because there are expensive, not handled and testing can be time consuming. We assessment a fast screening method of QST, for detecting presence and severity of medium and small caliber nerves fibers dysfunction in DM.

Methods

198 DM type 1-2; 55 (12.9) years old and <10 years of DM evolution (A), 54.7 (14.2) years old with > 10 years of DM (B), (no signif.) DM type 1-2 with NDS alteration (C). DM duration Media 14.9 (7.9), all patients were assesment DN underwent NerveCheck software device for methodology of Levels: vibration perception threshold (VPT), cold (CPT), warm (WPT) and methods of Limits for Heat pain test (HPT). NerveCheck complet 4Test (NCK.CT) time consuming about 12 min, it has been evaluating in comparison with VPT and CPT (SC), consuming 6 min. Neuropathy diagnostic (Neurodiab consensus).

Results

NCK.CT vs SC detect 71% of 100% group A 82% and group B 84%.

Conclusion

This findings could suggest that NerveCheck QST screening test allows an accuracy detects nerves fibers subtype damage in patients with clinical or subclinical DN stages.

[P57] PATTERNS OF NERVE CONDUCTION STUDIES IN PATIENTS WITH HYPERESTHESIA AND DIABETIC PERIPHERAL NEUROPATHY

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Objectives

In the early stage of diabetic peripheral neuropathy (DPN), diagnosis is difficult as there are no symptoms and signs. However, hyperesthesia is known as early manifestation of DPN. Although the nerve conduction studies (NCS) have been suggested as a surrogate markers and confirmative methods for diagnosis of DPN, it has a limitation in evaluation of early sensory change and small-fiber neuropathy. Measurement of current perception threshold (CPT) using Neurometer[®] at 2000, 250 and 5 Hz suggested as a comprehensive way of assessing patients' symptoms of DPN. The aim of this study was to compare the parameters of NCS in patients with normal NCS but having hyperesthesia determined by Neurometer[®].

Methods

We retrospectively enrolled the patients with type 2 diabetes who underwent both CPT test and NCS from January 2014 to December 2016. Results of Michigan Neuropathy Screening Instrument (MNSI) were collected to determine a subjective symptom score. CPT using the Neurometer[®] was applied at right index finger (C7 dermatome) and right great toe (L4/5 dermatome). If a study was not testable at the right side, left side was performed. Two hundred forty-two patients with type 2 diabetes were included in our final analysis. DPN diagnosed by symptoms (MNSI score ≥ 3) and/or sign, and NCS.

Results

The grade score of CPT in lower extremities was significantly correlated with the diagnostic stage of DPN. The relationships between the CPT at 5 Hz, 250 Hz, and 2,000 Hz show significant inverse correlations conduction velocities of tibial, peroneal, and sural nerve. Among 242 patients, fifty-three (21.9%; NCS normal and abnormal, 22 and 31, respectively) were found to be hyperesthesia based on grade scores of CPT. All measures of CPT at 5 Hz, 250 Hz, and 2,000 Hz showed significantly lower thresholds in hyperesthetic patients compared to asymptomatic patients. Parameters attributed to conduction velocities were not different between asymptomatic and hyperesthetic patients with normal NCS. However, ulnar sensory nerve action potential and sural nerve amplitude were significantly increased and decreased, respectively, in hyperesthetic patients compared to asymptomatic patients.

Conclusion

The CPT is a useful instrument to detect hyperesthetic patient in the course of DPN. And sensory action potential or amplitude of extremities in NCS could be complementary to detect minimal changes in these patients even in normal NCS results. The results of the current study may prove useful in monitoring of patients in the course of DPN

[P58] FIVE YEAR PROSPECTIVE STUDY ON THE PROGRESSION OF DIABETIC POLYNEUROPATHY IN JAPANESE PATIENTS WITH EARLY TYPE 2 DIABETES–MULTICENTER STUDY

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Objective

Natural progression of diabetic polyneuropathy (DPN) in type 2 diabetes is yet to be fully clear probably due to its diversity. We attempted to follow the progression of DPN in Japanese patients with type 2 diabetes and explored the factors that may have influenced the progression.

Methods

We prospectively followed up the neuropathy status and clinical profile in 244 patients with type 2 diabetes (average age 59.5) recruited from 20 institutions all over the Japan. Diagnosis and clinical stage of DPN were determined by the consensus criteria for DPN by Japanese study group of DPN; diagnosis of clinical neuropathy was made when 2 or more of subjective symptoms of DPN, loss or decrease in ankle jerk, and increased threshold of vibration perception (≤ 10 second, C128 tuning fork) and clinical staging was defined as stage I (no neuropathy), II (asymptomatic neuropathy), III (symptomatic neuropathy with decreased sensation), IV (III with decreased sensation and autonomic symptoms), and V (IV with motor deficits). At baseline, nerve conduction studies were conducted to confirm the severity of neuropathy. The neuropathic profile of the above was examined annually in each patient and factors that affected the progression of DPN were evaluated.

Results

Average HbA1c levels were 7.7% at baseline and 7.1% at end and there were no significant changes in BMI (around 25), blood glucose and lipid values, and blood pressures over the observation period. The nerve conduction data well correlated with clinical stage of DPN. Over 5 years, prevalence of clinical neuropathy was gradually increased from 27% to 34%. The increase in clinical neuropathy was mostly based on the new onset of abnormal ankle jerks which frequency step-wisely increased from 40% to 59%. In contrast, subjective symptoms of DPN and VPT abnormality were consistent during 5 years. Other signs of DPN such as decreased sensation or autonomic symptoms were not predictive for the staging and the number of patients with motor deficits was too small for analysis. In this cohort, initial age at baseline, smoking and high BMI were found to be a risk for the progression of DPN.

Conclusions

This prospective study demonstrated that the prevalence of DPN was gradually increased for 5 years, mostly ascribed to the new onset of abnormal ankle jerk.

[P59] MORE BRANCHING OF CORNEAL NERVE FIBRES IN PATIENTS WITH PAINFUL COMPARED TO PAINLESS DIABETIC POLYNEUROPATHY

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Objectives

The determinants and mechanisms contributing to the development of diabetic sensorimotor polyneuropathy (DSPN) as a painful (+p) or painless (-p) clinical entity remain unclear. We aimed to determine whether DSPN+p is characterized by a predominant small-fibre damage by assessing the corneal subbasal nerve plexus using confocal corneal microscopy (CCM).

Methods

Included were 53 participants of the PROPANE study with DSPN+p and 63 with DSPN-p (DSPN+p/DSPN-p: age [mean±SD]: 67.2±8.5/67.4±9.5 years; sex: 74/90% male; BMI: 32.4±5.8/29.3±5.4 kg/m²; HbA1c 7.48±1.43/7.33±1.14%; type 2 diabetes: 89/70%; diabetes duration: 15.6±10.9/19.6±15.1 years; Neuropathy Disability Score (NDS): 6.47±2.52/6.25±2.87 points) as well as 46 individuals without diabetes (KON: age: 66.0±5.2 years; sex: 80% male; BMI: 27.3±3.2 kg/m²) from the German Diabetes Study. CCM parameters were quantified manually, including corneal nerve fibre length (CNFL), corneal nerve fibre density (CNFL) and corneal nerve branch density (CNBD). DSPN was diagnosed according to modified Toronto Consensus (2011) criteria and a cutpoint of 4 on the 11 point numerical rating pain scale was used to differentiate between DSPN+p and DSPN-p.

Results

After adjustment for age, sex, BMI, and smoking, participants with DSPN+p and DSPN-p showed reduced CNFD and CNFL compared to controls (DSPN+p/DSPN-p vs KON: CNFD: 23.0±8.0/22.2±7.8 vs 29.0±6.1 fibres/mm²; CNFL: 18.6±6.7/16.9±6.3 vs 22.8±4.9 mm/mm²; p<0.05). Participants with DSPN-p showed lower CNBD compared to controls (43.8±28.3 vs 70.5±26.7 branch points/mm²) and, additionally adjusted for diabetes type, diabetes duration, and HbA1c, compared to DSPN+p (55.8±29.9 branch points/mm²) (p<0.05). CNFL was abnormal (below 0.05th quantile)¹ in 32% of the DSPN-p group and in 23% of the DSPN+p group, while the corresponding numbers for CNBD were 14% and 6%.

Conclusions

Despite a similarly pronounced nerve fibre loss, corneal nerve branching is higher in patients with painful DSPN than in painless DSPN. These findings indicate that corneal nerve regeneration is maybe preserved, albeit not to a sufficient degree, in painful DSPN.

1. Tavakoli M *et al.* Normative values for corneal nerve morphology assessed using corneal confocal microscopy: a multinational normative data set. *Diabetes Care* 2015; 38:838–843

[P60] PATTERNS OF SMALL AND LARGE FIBER DYSFUNCTION IN PAINFUL AND PAINLESS DIABETIC POLYNEUROPATHY

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Objectives

The distinguishing features contributing to the phenotype of diabetic sensorimotor polyneuropathy (DSPN) as a painful (+p) or painless (-p) entity are poorly understood. We hypothesized that DSPN+p and DSPN-p are characterized by predominant small and large nerve fiber dysfunction, respectively.

Methods

We assessed somatic, cardiac autonomic, and sudomotor function in 332 patients with DSPN from the PROPANE study, 179 of whom had DSPN+p and 153 had DSPN-p and 54 diabetes patients without DSPN (DM) from the German Diabetes Study (DSPN-p/DSPN+p/DM [mean±SD]: age: 68.4±10.4/66.1±10.0/63.2±5.2 years, BMI: 29.5±5.3/30.9±5.6/30.1±4.9 kg/m²; T2D: 83/86/87%, diabetes duration: 16.4±12.4/16.1±12.1/5.1±3.2 years, HbA1c: 7.3±1.1/7.7±3.3/6.8±0.9%). DSPN was diagnosed using modified Toronto Consensus (2011) criteria, while DSPN+p and DSPN-p were stratified using a cutpoint of 4 points on the Likert scale for chronic pain (>1 year) in the distal lower limbs.

Results

After adjustment for sex, age, BMI, smoking, diabetes type, diabetes duration, and HbA1c, compared to patients with DSPN-p those with DSPN+p showed impaired warm thresholds (foot: 46.0±4.4 vs 45.3±4.4 °C), Coefficient of R-R interval variation during deep breathing (5.18±3.95 vs 6.71±4.86%), and electrochemical skin conductance (ESC: hand: 56.1±17.8 vs 60.9±17.3 μS, foot: 57.8±20.7 vs 62.8±19.9 μS) (all p<0.05). Large fiber function tests (nerve conduction, vibration threshold) did not differ between the DSPN groups. ROC analyses showed that both small and large fiber function tests were useful to detect DSPN+p (AUC vs DM: 0.7–0.9) and DSPN-p, albeit to a slightly lesser degree (AUC vs DM: 0.6–0.8).

Conclusions

Compared to painless DSPN, painful DSPN is characterized by more pronounced small fiber dysfunction involving sudomotor, cardiac autonomic, and cutaneous C fibers, while large fiber dysfunction is common to both entities.

[P61] THE ROLE OF SUDOMOTOR FUNCTION TESTING IN THE DIAGNOSTIC PATHWAY OF DIABETIC NEUROPATHY

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Objectives

The recent availability of simple methods for sudomotor function assessment has facilitated the study of the relationship between sudomotor dysfunction and cardiovascular autonomic (CAN) or diabetic polyneuropathy (DPN) but with variable results. This study was aimed at evaluating the diagnostic performance of sudomotor function testing for CAN and DPN in a well-characterized diabetic population, using a comprehensive neurological examination.

Methods

In 61 participants with diabetes (age 56.9 ± 13.0 years, duration 16.9 ± 15.1 years, 21 with type 1), we performed 4 cardiovascular reflex tests (CARTs), assessed autonomic symptoms (by COMPASS 31), symptoms and signs of DPN, and measured vibratory perception threshold (VPT), warm and cold thermal thresholds (TT), and electrochemical sweat conductance (ESC) using SUDOSCAN. We defined early and confirmed CAN in the presence of at least 1 or 2 abnormal CARTs, respectively, and DPN with at least 2 abnormalities among symptoms, signs, VPT and TT.

Results

Abnormality in hands ESC and/or feet ESC was associated with higher CARTs score (2.31 ± 2.17 vs 0.54 ± 1.04 , $p=0.0003$), lower expiration/inspiration ratio ($p<0.0001$), lower 30:15 ratio ($p=0.0056$), lower VPT ($p=0.031$), with the presence of early CAN [$\text{Chi}^2=6.57$, $p=0.0165$, sensitivity 75% (95% C.I. 54–96%), specificity 63% (95% C.I. 48–78%)], confirmed CAN [$\text{Chi}^2=7.27$, $p=0.0089$, sensitivity 100% (95% C.I. 100–100%), specificity 58% (95% C.I. 44–72%)], and DPN [$\text{Chi}^2=7.47$, $p=0.0098$, sensitivity 64% (95% C.I. 47–80%), specificity 71% (95% C.I. 55–88%)]. Feet ESC was related to CARTs score ($\rho=-0.47$, $p=0.0007$), expiration/inspiration ratio ($\rho=0.59$, $p<0.0001$), 30:15 ratio ($\rho=0.33$, $p=0.0179$), VPT ($\rho=-0.26$, $p=0.0449$) and Michigan Diabetes Neuropathy Score ($\rho=-0.32$, $p=0.0117$). Hands ESC was related to CARTs score ($\rho=-0.48$, $p=0.0005$), expiration:inspiration ratio ($\rho=0.65$, $p<0.0001$), 30:15 ratio ($\rho=0.57$, $p<0.0001$), cold TT ($\rho=0.32$, $p=0.0219$), and VPT ($\rho=-0.39$, $p=0.0022$). Abnormality in hands ESC (and not in feet ESC) was marginally associated with higher COMPASS 31 score ($p=0.0244$), and hands ESC was related to the question score about body sweating changes ($\rho=-0.342$, $p=0.0105$). In evaluating the diagnostic accuracy of feet ESC, ROC analysis showed an area under the curve (AUC) of 0.734 ± 0.094 (95% C.I. 0.548–0.919) for early CAN, an AUC of 1.000 ± 0.000 (95% C.I. 1.000–1.000) for confirmed CAN, and an AUC of 0.684 ± 0.068 (95% C.I. 0.551–0.818) for DPN.

Conclusions

Feet and hands ESC are related to CARTs and to a lesser extent to somatic measures of DPN, and show a fair diagnostic accuracy for early CAN and DPN, and an excellent diagnostic accuracy for confirmed CAN with a noteworthy high sensitivity. Assessment of sudomotor function can be a complementary tool in the apparatus of autonomic and peripheral nerve function testing, with the additional advantage of ease of use.

[P62] RELATION OF OXIDATIVE STRESS AND GLYCEMIC VARIABILITY WITH *IN VIVO* CORNEAL CONFOCAL MICROSCOPY PARAMETERS IN TYPE 1 DIABETES MELLITUS

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Objectives

In vivo corneal confocal microscopy (CCM) is a validated screening tool, diagnosis method, and biomarker of diabetic sensorimotor polyneuropathy (DSP), in type 1 diabetes *mellitus* (T1DM). The metabolic activity of the corneal sub-basal nerve plexus is documented by nerve beadings, which represent accumulation of mitochondria along the nerve. However, the impact of oxidative stress and of copper and iron homeostasis on corneal sub-basal nerve plexus parameters is unknown.

Methods

15 T1DM patients with no symptoms/signs of DSP and 15 healthy controls (C) matched for age and gender were studied. All patients underwent CCM, including the number of fibers, the number and density of fiber beadings (rich in mitochondria), the degree of fiber branching, and the degree of fiber tortuosity of corneal sub-basal nerve plexus. Oxidative stress was measured by systemic variations of markers of iron metabolism, such as serum iron, ferritin (ferr), transferrin (Tf) and ceruloplasmin (concentration and activity), and the ratio of ceruloplasmin to transferrin (Cp/Tf) a systemic marker of oxidative stress. All Type 1DM persons underwent a 72-h continuous glucose monitoring (CGM), from which glycemic variability (GV) indexes were calculated.

Results

Cp/Tf was significantly increased and Tf decreased in T1DM group vs C group (11.03 ± 3.32 vs 9.46 ± 1.86 , $p=0.019$; $2.40 \text{ g/L} \pm 0.46$ vs $2.70 \text{ g/L} \pm 0.3$, $p=0.003$, respectively). A positive correlation between density of beadings, of corneal sub-basal nerve plexus and Tf ($r=0.760$, $p=0.003$) and a negative correlation between density of beadings and Cp/Tf ($r=-0.824$, $p=0.001$) were found in T1DM group. A negative correlation between density of beadings and Continuous Overall Net Glycemic Action (CONGA) 1–2–4 h index ($r=-0.755$, $p=0.005$; $r=-0.790$, $p=0.002$; $r=-0.818$, $p=0.001$, respectively) were also observed in T1DM group. However, no correlation was found between glycated hemoglobin and CCM parameters.

Conclusions

The role of mitochondria, contained in the fibers beadings, is critical in controlling nerve function and their morphological and functional anomalies which are involved in development of diabetic neuropathy. The well-know link between mitochondria and oxidative stress is confirmed also from our results, in fact, oxidative stress and iron homeostasis systemic markers, such as GV are associated with density of beadings, representing a metabolic activity parameters of small nerve fibers in T1DM.

[P63] SMOKING INFLUENCES SKIN AUTOFLUORESCENCE IN PATIENTS WITH SENSORIMOTOR DIABETIC NEUROPATHY

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Objectives

Skin autofluorescence (SAF), as a measure of skin advanced glycation endproducts (AGEs) accumulation, represents an integrated parameter of glucose exposure, oxidative stress and smoking over years. We hereby present the results of secondary parameters' analysis of a study which aimed at investigating the relationship between SAF and sensorimotor diabetic neuropathy (SSDNP).

Methods

A multi-centric (8 centers) cross-sectional study was conducted in 2016. Consecutive patients between 30 and 75 years old with type 1 or type 2 diabetes *mellitus* were included. Exclusion criteria were HbA1c level > 9.5%, smoking of more than 15 cigarettes/day, severe pain that required treatment with more than one drug. Assessment of SDNP was performed using the Neuropathy Symptom Score (NSS) and the Neuropathy Disability Score (NDS). The pain scale and SAF (measured with the AGE-Reader, Groningen, The Netherlands) were also recorded. Patients with NDS score ≥ 3 were considered to have SDNP.

Results

A total of 497 patients were investigated. SAF was increased in the presence of SDNP and smoking. Mean SAF was found to be lowest in patients who did not smoke and had no SDNP ($n=198$, 2.42 ± 0.53), and gradually increased in patients who did not smoke and had SDNP ($n=229$, 2.56 ± 0.57), in those who smoked and had no SDNP ($n=37$, 2.61 ± 0.51), and was highest in patients who smoked and had SDNP ($n=26$, 2.82 ± 0.49), $p < 0.001$. Interestingly, patients with SDNP who smoked had lower mean score of pain scale (3.62 ± 2.76) than those who did not smoke (4.14 ± 2.69), $p < 0.001$. Anova two-way analysis revealed that both NDS score ≥ 3 ($p=0.019$) and smoking ($p=0.002$) were significantly associated with SAF, but no significant combined effect was found ($p=0.630$). Similar results were obtained if the NSS score was used for the definition of SDNP.

Conclusions

Smoking and SDNP contribute both to increased SAF, their interplay in influencing SAF warrants further research. A limitation of this study is that we did not include heavy smokers.

[P64] SELECTIVE STIMULATION OF SILENT, MECHANO-INSENSITIVE C NOCICEPTIVE FIBER IN HUMANS

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Abnormal spontaneous activity of C-mechano-insensitive fibers (CMi) is a likely source of spontaneous neuropathic pain. Activation of CMi fibers produces axon-reflex related flare¹. However, CMi activation thresholds are substantially higher compared to C-polymodal CMH fibers which makes these fiber inaccessible for any quantitative sensory or cortical evoked potential tests. These high thresholds are likely due to the deeper location of CMi fibers compared to CMH fibers. Thus, assessment of CMi fibers is impossible for clinic practice, clinical research or analgesic development. We developed a diode laser C-fiber type selective stimulation (C-DLss) technique that may selectively assess CMi fibers². DLss radiation homogeneously heating superficial and subepidermal skin layers, thereby allowing access to deeply located C fibers. A decreased density of intraepidermal fibers and 50% lower density of CMH vs CMi fibers³ as well as sensitization to heat of CMi fibers allows for their selective activation in PPN patients⁴. Here we present a modified method that provides selective activation of CMi vs CMH fibers in healthy subjects. We hypothesized that controlled cooling of skin surface allows to temporarily decrease response from epidermal C fibers mimicking functional denervation, though deeper located dermal CMi fibers would be spared from cooling allowing for study by DLss. Thus, selective stimulation of CMi nociceptors could be possible. The activation of CMi fibers was confirmed by generation of axon-reflex flare. The C-DLss stimuli with duration 1 s were applied to cooled (22 C° +/- 1 C°) and non-cooled (32 C° +/- 1 C°) inner forearm skin of healthy subjects. Sub- and supra pain threshold intensities did not produce warm or hot sensations in cooled skin, but only a single modality step function of pain perception. We found that activation threshold of CMi fibers could be below pain thresholds of CMH fibers when stimulated by C-DLss.

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[P65] ASSOCIATION BETWEEN THE RISK OF CARDIAC NEUROPATHY AND BLOOD PRESSURE IN PATIENTS WITH TYPE 2 DIABETES

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Aim

The purpose of this study was to investigate the association between the risk of cardiac neuropathy given by sudomotor function assesment and systolic (SBP) and diastolic (DBP) blood pressure, antihypertensive treatment in patients with type 2 diabetes.

Material and methods

For this study, 33 patients with type 2 diabetes were selected from the patients of a private podiatry clinic in Cluj-Napoca. They were investigated with SUDOSCAN, which is an medical echipment for assesment of the galvanic response of epidermis. SUDOSCAN echipment had incorporated the calculation of a risk-score for cardiac neuropathy based on the electrochemical sweat conductance of hands and foot, age, glicated hemoglobin and body mass index. Neuropathy Disability Score (NDS) and Neuropathy Symptoms Score (NSS) were apply also. Blood pressure was measured, antihypertensive treatment was noted.

Results

Of the 33 patients, 18 (54.5%) were male, the mean age of the patients was 64.61 ± 15.92 years, the duration of the diabetes was 12.85 ± 9.25 years. Sudoscan identified 25 (75.8%) patients with possible risk ($25 < \text{risk-score} \leq 50$) of cardiac neuropathy, and 7 (21.2%) with increased risk of cardiac neuropathy ($\text{risk-score} > 50$). NDS scores above 2 had 14 (42.4%) patients, of whom 9 (27.3%) had mild periferic neuropathy, 4 (12.1%) patients had moderate periferic neuropathy, 1 (3.0%) patient had severe periferic neuropathy. The risk of cardiac neuropathy was significantly correlated with TAD ($r = 0.35, p=0.04$), NDS score ($r = 0.59, p<0.001$), NSS score ($r = 0.37, p=0.037$). Antihypertensive treatment was significantly different among patients with risk of cardiac neuropathy ≥ 50 (42.9%) and those with risk of cardiac neuropathy < 50 (88.5%), $p=0.023$. The risk of cardiac neuropathy was not significantly correlated with TAS ($r = 0.01, p=0.97$).

Conclusion

The risk of cardiac neuropathy given by the Sudoscan device is associated with TAD, antihypertensive treatment and NDS and NSS scores, but not with TAS.