Brief Communication

Endocrine and metabolic disease: Confocal microscopy as a diagnostic aid

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ABSTRACT

Diabetes is a systemic disease associated with many complications. These can be prevented and managed effectively if detected promptly. Confocal microscopy (CFM) is a diagnostic tool which has the potential to help in early detection of disease and timely management. CFM has the potential to serve as an excellent noninvasive modality for *in vivo* imaging and morphological analysis, which can aid us in assessing and monitoring various infectious and pathological diseases at the cellular level. Besides ophthalmological indications, CFM has shown good sensitivity and specificity for identifying those at risk of neuropathy and foot ulceration, monitoring evolution and therapeutic response in a wide range of neuropathies apart from diabetic neuropathy. Through this communication, we aim to sensitize the endocrinologists towards cerebral cavernous malformation as a biomarker to evaluate potential outcomes and therapies in human diabetic neuropathy.

Key words: Diabetic neuropathy, early diagnosis of diabetes, ophthalmology

Introduction

Endocrine and metabolic diseases including diabetes are associated with complications, including neural damage. Early diagnosis, monitoring and follow up of these confounding complications pose a major challenge.[1] Confocal microscopy (CFM) is a diagnostic tool which has the potential to help in early detection of disease and timely management.

Concept of confocal microscopy

The basic principle of a confocal microscope is that a single point can be illuminated by light source and simultaneously imaged by a camera in the same plane that is, it is "confocal". This produces an image with a very high resolution, but it has virtually no field of view due to a single point of illumination and detection. To solve this problem, the instrument instantaneously illuminates and synchronously images, that is, it scans a small region of tissue with thousands of tiny spots of light, which are reconstructed to create a usable field of view with high resolution and magnification. By excluding most of the light from the specimen that is not from the microscope's focal plane it creates sharp images that would otherwise appear blurred when viewed with a conventional microscope. To add to it, it enables the reconstruction of three dimensional structures from the obtained images.[2]

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Confocal microscopy and ocular disease

Confocal microscopy has the potential to serve as an excellent noninvasive modality for *in vivo* imaging and morphological analysis of all the layers of the cornea. This can aid us in assessing and monitoring various infectious and pathological corneal diseases at the cellular level. This was earlier possible only to invasive modalities like biopsy. It holds considerable promise in assessing tissue repair following surgery or injury and in conditions like infectious keratitis. It has the ability to differentiate between corneal edema due to corneal graft rejection and endothelial decomposition.[3]

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Studies have shown that corneal small nerve fiber damage can be detected earlier to the electrophysiological changes and abnormalities in the quantitative nerve testing using this technique.[4] It gives a noninvasive measure of the thickness of the cornea after laser *in situ* keratomileusis (LASIK). It has a wide range of applicability such as in the detection and management of corneal dystrophies and ecstasies, monitoring contact lens induced damages and penetrating keratoplasty as well as evaluation of pre- and post-surgical (photorefractive keratectomy, LASIK, radial keratotomy and flap evaluations) changes in cornea. An *in vivo* study of cellular detail, microorganisms, and fibrosis, including Langerhans cells may complement our perception of the basic pathological mechanisms of corneal damage and immune response to tissue injury [Table 1].[5]

Confocal microscopy and systemic disease

Confocal microscopy is a useful diagnostic modality for metabolic or neurological disease as well. CFM has shown good sensitivity and specificity for identifying those at risk of neuropathy and foot ulceration, monitoring evolution and therapeutic response in a wide range of neuropathies apart from diabetic neuropathy.[6] CFM is a surrogate for assessment of peripheral nerve damage.[3] The accuracy of corneal CFM in assessing small nerve fiber damage is equivalent to skin biopsy in the assessment of intraepidermal nerve fiber loss.[7] Thus, the detection of small nerve fiber damage, which was earlier amenable to only invasive procedures like Sural nerve biopsy or skin punch biopsy can now be done accurately by a noninvasive corneal CFM. It has been recently shown to detect early nerve fiber repair following pancreas transplantation,[8] nerve fiber damage in patients with Fabry disease[9] and idiopathic small nerve neuropathy.[6] Very recently, it has been shown to detect the neuropathy in individuals with Impaired Glucose Tolerance[10] and early nerve fibre damage in those with recently diagnosed type 2 diabetes.[11] It may have a future as a stand-in marker for endothelial abnormalities as it can be used to inspect cell densities in different layers from epithelium to the endothelium, including immune cell densities [Table 2].

Confocal microscopy limitations

As with any other procedure, CFM also has certain limitations. Only a limited number of excitation wavelengths are available with common lasers. These are clustered in very narrow bands as it is expensive to produce in the ultraviolet region. The harmful nature of the high-intensity irradiation to cells and tissues, photobleaching and phototoxicity of the fluorescent probes, and the chromatic and spherical aberrations which were limitations of in-vitro confocal microscopy have been overcome in clinical in-vivo confocal microscopy.[12] Their implementation in smaller laboratories is limited by the high cost of purchasing and operating multi-user CFM when compared to par wide field microscopes. This has been taken care of to some extent by the introduction of low-end personal confocal systems that comes at an affordable price for individual users.

With the recent advances in the digital systems that enable data compression and data storage the limitation of acquiring high quality and reproducible images have been promisingly overcome.[12]

Another clinical limitation of using CFM for neuropathy screening in Type 2 diabetes is due to higher prevalence of neuropathy and early nerve fibre damage. This is in contrast to Type 1 diabetes where it has been shown as an excellent diagnostic tool for neuropathy screening.[13]

Conclusion

Through this communication, we aim to sensitize the endocrinologists toward Corneal Confocal Microscopy (CCM) as a biomarker to evaluate potential outcomes and therapies in human diabetic neuropathy. Some key advantages of CCM include a noninvasive technique, reproducibility, quantitative, small nerve fiber assessment. However, still this modality demands considerable research before actual practical application. Studies need to be performed to determine if improvements in CCM parameters accurately coincide with improvements

Table 1: Ocular indications for CFM[5]

Detection and management	In conditions like dystrophy, ecstasies, graft rejection, endothelial decomposition
	Assessing tissue repair following surgery/injury, infections
	Assessing corneal nerve fibre damage
Monitoring and evaluation	Contact lens induced damage
	Postpenetrating keratoplasty evaluation
	Noninvasive measure of thickness of the cornea following LASIK and monitoring pathology at cellular levels

CFM: Confocal microscopy, LASIK: Laser *in-situ* keratomileusis

Table 2: Indications of CFM

Indications	Conditions[6-11]
Identification	Metabolic, neurological disease
	Peripheral nerve damage
Detection	
Nerve fiber repair	Following pancreas transplantation
Nerve fiber damage	Fabry disease, idiopathic small nerve neuropathy
Marker	Endothelial abnormalities for inspecting cell densities
Risk stratification and monitoring	Neuropathy
	Foot ulceration
	Neuropathies including diabetes mellitus

CFM: Confocal microscopy

in traditional neuropathy outcomes as well as patient-centric outcomes-pain, disability, and quality of life. There have been few studies relating CFM and Type 2 diabetes mellitus, and this demands more attention. Finally, CCM has to be related to neuropathy outcomes and their measurements using a wider cohort of subjects. Thus, this might be seen as a robust tool to assess neuropathy in near future.

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