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Short communication

Comparative study on secondary structural changes in diabetic and non-diabetic human finger nail specimen by using FTIR spectra

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ABSTRACT

Background: In human anatomy, a nail is a hornlike envelope covering the dorsal aspect of the terminal phalanges of fingers and toes. Nail disorders are most common among the geriatric population. Diabetes mellitus is also supposed to affect the condition of nails. Acceptable differences in infrared (IR) spectra of chronic and acute diabetes mellitus patient fingernail specimens compared to control normal specimens were investigated in this study.
Methods: Using a Nicolet 360 Fourier Transform Infra Red (FTIR) spectrometer, the spectra of the nails of diabetics and normal specimens were recorded.
Result: In the case of non-diabetic patients, the amide I band was observed <1640 cm^{-1} (1626, 1632, and 1638 cm^{-1}). The bands around 1637 cm^{-1}, were attributable to amide I of β sheet structures. Amide II bands were absent in all the non-diabetic patients. Amide III bands around 1250 cm^{-1} were observed both in diabetic and non-diabetic patients. In all the diabetic patients, a peak of <500 cm^{-1}, particularly around 468 cm^{-1}, was observed.
Conclusion: The proteins in the nails of diabetic patients contain α-helical structure, including the presence of amide II bonds. Alkyl thiolated structures are observed. Nails of non-diabetic patients do not have the amide II structures.

1. Introduction

The human nail plate is one of the most impervious of biological structures and the penetration of chemical agents is low [1]. The nail plate is mainly composed of hard α-keratin, which is the substance forming the stratum corneum [2,3]. The changes in mechanical and physical properties and the alteration in water content may be reflected in the molecular structure of nail proteins [4]. The nail keratin belongs to the group of hard keratin, which also includes hair and horn keratin [5]. Hard α-keratin has a high cystine content compared to soft α-keratin. The α-keratin contains α-helical polypeptides, which are organized into intermediate filaments (IFs). The IF polypeptides are richest in those amino acids favoring an α-helix formation, namely lysine, aspartic acid, glutamic acid and leucine, and comparatively poor in half-cystine and proline [2]. Cysteine–cysteine disulfide cross-links and secondary cross-linking mechanisms stabilize the matrix phase. The matrix adheres to the filaments through secondary bonds, including Waals's forces, hydrogen bonds and ionic interaction [5]. The hydration of nails is thought to be the most important factor influencing the physical properties of nail and possibly act through changes in keratin structure. Mavis et al. described a quantitative method for the determination of all the seventeen amino acids in nail hydrolysate and quantitatively gave the data [6]. NIR-FT Raman spectroscopy has been shown to be an excellent method to detect structural changes of water, proteins and lipids in skin, nails and hair. Williams et al. [7] examined nails and the different structures of keratin. Akhtar et al. [8] compared the Raman spectra of different keratotic biopolymers (stratum corneum, human nail, feather, and bull's horn). The study pointed out that mammalian keratin occurs mainly in the K-helical form, and that the C–S–S–C linkage shows the gauche–gauche–gauche conformation. Schrader et al. [9] and Gniadecka et al. [10] found that the internal water of the nail mainly exists in the bound form. Here, we therefore, investigate the time-dependent penetration of water into nails and its influence on protein and water structure [10]. Sonja et al. [11] used NIR-FT spectroscopy to examine the molecular structural changes of moistened nails. They found that protein water interactions could lead to a slight change of the dihedral angle of the C–S–S–C bonds and to geometric changes in cooling behavior of the α helical protein[11]. Normally, hemoglobin A1 (HbA1), glycosylated hemoglobin, has been clinically used as an indicator of long term control of blood glucose in diabetic patients [12]. The reaction involved in the formation of HbA1

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is called the Maillard reaction and occurs between the glucose and proteins including hemoglobin [13]. Oimomi et al. estimated furosine as an indicator of long term blood glucose control in diabetes patients. Furosine is formed due to the binding of the glucose to lysine [14]. The problem is more complex in polymedicated patients and in those showing an increased propensity to develop fungal infection of the nails [15,16]. Overall, clinically abnormal with mycological evidence of fungal disease have been reported in approximately one third of diabetes [17]. The highest prevalence was reported in men with type II diabetes [17–20]. The overall risk ratio of diabetic patients having onychomycosis was estimated as high as 2.77 compared to age- and sex matched non-diabetic subjects [17]. Angiopathy, retinopathy, neuropathy and increasing age are considered to represent predisposing factors for onychomycosis in diabetic patients [18,21]. One study found Terry's nails in 25% of 512 consecutive hospital inpatients, with researchers linking the disorder with adult-onset diabetes mellitus, cirrhosis, and chronic CHF [22]. Terry's nails have been reported in hemodialysis patients and renal transplant recipients [23]. From the above references it was concluded that human nails with mycological evidence of onychomycosis leads to change in nail plates, i.e., change in structural configuration of nail. This nail infection represents a risk factor in the diabetic patients because of possible sequelae [23].

Chemical analysis of nails mainly been performed in forensic medicine [24], in the diagnosis of mucoviscidosis [25], and hepatolenticular degeneration [26]. IR spectroscopy has become a widely used analytical method in biochemical, pharmaceutical, chemical and medical fields [27]. IR spectroscopy has been used for secondary structural analyses of proteins, usually based on the amide I mode, the C O stretching vibrations of the amide groups coupled to in-plane bending of the N–H and stretching of the C–N bonds appeared in the region between approximately 1700 and 1600 cm^{-1} [28].

2. Materials and methods

2.1. Sample

Ten numbers each of diabetic and non-diabetic specimens were selected between age group of 35 and 60 years with the permission of the individuals and also approved by the internal ethical committee of Central Leather Research Institute (CLRI). Using sterile razor, blade nail ends were cut and washed thoroughly with double distilled water. They were then dried at 37 °C. The nail ends were powdered using pestle and mortar and powdered samples were stored in a dry, clean and sterile Eppendorf tubes for further analysis. The spectra of the nails of diabetics and normal specimens were recorded on a Nicolet 360 Fourier Transform Infra Red (FTIR) Spectrometer using KBr pellet containing 2–6 mg of sample; it took 15 min to complete an assay and the scanning numbers are 5.

2.2. Spectral collection and secondary structural analysis

The structural properties of hand nail specimen were studied by FTIR in the range of 400 cm^{-1}–4000 cm^{-1} (~5 mg of the powdered nail sample was mixed with 300 mg of spectral grade KBr followed by pressing of pellet at 12,000 kg cm^{-2}). Fig. 1 shows that the FTIR spectrum of the non-diabetic hand nail specimens, amide I, is observed at <1640 cm^{-1} (1626, 1632 and 1638 cm^{-1}). The band 1637 cm^{-1} is attributable to amide I of the β-sheet structure. Amide II band is absent, amide III bands are around 1250 cm^{-1} in both non-diabetic and diabetic. Fig. 2 shows the FTIR spectrum of the diabetic hand nail specimens; here amide I bands are seen from 1645 to 1649 cm^{-1}. The band around 1650 cm^{-1}, is attributable to amide I of α-helical structures. Amide II bands are also observed around 1540 cm^{-1}. Amide III bands are observed at 1250 cm^{-1}.

Blood glucose fasting and postprandial, $HbA_{1c,}$ total cholesterol and triglyceride concentrations were also estimated for both the normal subjects and diabetic patients. The spectral studies were determined by the Stat Fax 3300 (Awareness Technology Inc, Palm City, FL).

2.3. Blood glucose

Glucose was measured using the glucose oxidase method. The absorbance of samples were determined by using Stat Fax 3300 [32,33]. Blood glucose of the samples was estimated by using an analytical kit supplied by Accurex Biomedical Pvt. Ltd., Mumbai, India and their instructions were followed.

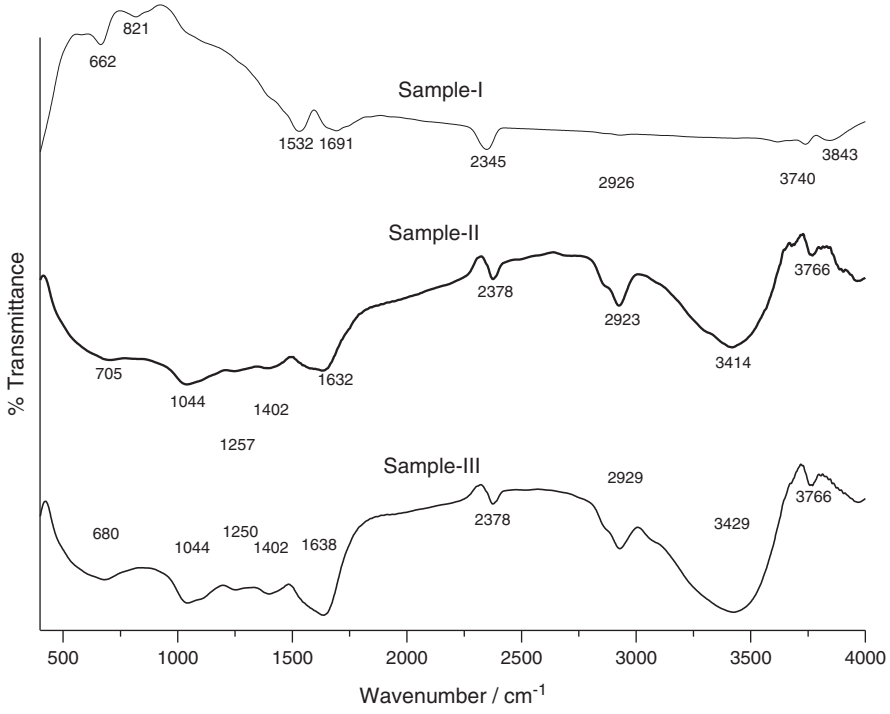

Fig. 1. IR spectra of nails of normal subjects. There is no peak at 468 cm^{-1}.