Original Article

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The influence of diabetes on atherosclerosis and amyloid fibril formation of coronary arteries. A FT-IR spectroscopic study

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Abstract

In the present study the ATR-FT-IR (Attenuated total reflection Fourier transform infrared) spectroscopy was used to investigate 54 biopsies from atheromatic coronary arteries of patients who had elevated serum glucose. Comparison of FT-IR spectra among insulin-dependent patients and patients who were on oral hypoglycemic drugs treatment and patients with normal serum glucose showed that insulin-dependent diabetic patients pronounced changes across the absorption infrared spectral range 4,000-400 cm^{-1}. The increase of the intensity of the characteristic stretching vibrations of antisymmetric ($\upsilon_{as}CH_2$) and symmetric ($\upsilon_{s}CH_2$) methylene groups indicates the increasing of lipophilic environment of the membranes. This finding is attributed to the damage on the membrane induced by free radicals produced during oxidative stress especially in diabetic patients. The new band at 1,744 cm^{-1} is assigned to aldehyde C=O vibration mode and the intensity is related to LDL cholesterol in serum of the patients. The appearance of a new band at 1,728 cm^{-1} only in the spectra of insulin-dependent patients, is attributed to influence of keto groups of cardiolipin due to atherosclerosis.

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Shifts to lower frequencies of the stretching vibration absorptions of amide I and amide II modes of proteins indicate that protein folding changed from a-helix to random coil and β-sheet configuration. The most significant changes were observed in the region 1,200-1,000 cm^{-1}, where the vibration modes of C-O-C groups of the sugars and C-O-P of DNA and phospholipids absorb. SEM analysis showed the production of aggregates as a result of the protein amyloid formation and the increased lipophilic environment, in agreement with FT-IR spectroscopic data. The changes were related to clinical history of the patients and were more pronounced in insulin-dependent diabetic patients. The presence in the FT-IR spectra of cardiolipin vibrations in insulin dependent diabetic patients needs more investigation.

Key words: coronary arteries; atherosclerosis; amyloid proteins; cardiolipin; insulin-dependent patients; Fourier transform infrared spectroscopy; scanning electron microscopy

1. Introduction

Diabetes is a life-long metabolic disease. It is estimated that it affects about 6.1% of the population in Greece.[1] Diabetes is characterized by high blood serum glucose, which disturbs the carbohydrate and lipid metabolism. Diabetes is associated with a number of secondary implications, such as heart attack and stroke. There are many risk factors who promote diabetes development, which include metabolic syndromes, hypercholesterolemia and obesity. Histopathologically the type II diabetes is characterized by the fibril amyloid formation in the islet of Langerhans, but the mechanism and the role of amyloids on the cell's membrane is not yet well understood. The last years the scientists have observed that patients with Alzheimer's disease (AD) and Parkinson (PD) have a higher risk to develop diabetes type II compared to age-related individuals.[2,3] It is known that the neurological diseases AD and PD are defined from their protein misfolding, protein aggregates and the ability of amyloidogenesis. Amyloid is a Greek word amylon (***ἀμυλον***, not-breaking in mill) and was proposed by Rudolf Virchow (1821) to characterize the starch based on iodine-straining technique.[4]

Using infrared spectroscopy it was also observed the amyloid-like protein formation on atherosclerotic carotid and coronary arteries and calcified aortic stenosis during oxidative stress.[5-11]. In recent years[12], there are found increasing data showing that protein aggregates play a toxic role in diabetes progression and insulin resistant patients. There is evidence that protein misfolding and aggregates are involved in many cardiac diseases, including heart failure.[13] The conventional methods used to measure the glucose levels of blood and the diagnostic clinical methods (Ultrasound, CT, MRI) of atherosclerotic plaque in the coronary and carotid arteries development provide information only on the visual image, architecture and histological composition of atheroma in the corresponding arteries. They do not provide any information at an early stage of atheromatic development and processes nor in the mechanism of growth of atherosclerotic plaques and they also do not answer to the question why some of them are hemorrhagic or corrosive and lead to acute cardiovascular events.

Infrared spectroscopy, and especially the ATR-FT-IR (attenuated total reflectance Fourier transform infrared) technique, offers a new powerful technique in clinical practice to identify the atheromatic plaques, with emphasis to amyloid protein formation.[14,15] ATR-FT-IR spectroscopy is a simple, fast and non-destructive technique, which requires small amounts of sample *(μgr* or *μl)*, without any sample preparation and can be applied in many medical fields. Infrared spectroscopy is shown to be a very sensitive method for evaluating the secondary protein structure of the human tissues[5-11,16,17] and other components, including DNA, lipids, phospholipids, in a single spectrum.

Infrared spectroscopic analysis provides characteristic "fingerprint bands" or "marker bands" of the tissue of each patient and can differentiate the normal and malignant tissues as well as the progression of the disease.[5-11,16-18] FT-IR spectroscopy is also one of the few direct methods that gives information about the presence and strength of hydrogen bonds.

In the present work we focused on the influence of serum glucose and how it affects the molecular structure of proteins and the composition of lipid membranes, in order to approach at a molecular level the mechanism of protein misfolding in diabetic patients. The differences between the spectra of atheromatic plaques of insulin-dependent diabetic patients, diabetic patients on oral hypoglycemic drugs treatment and patients with normal glucose levels are emphasized. In addition, SEM-EDX (Scanning electron microscopy- Energy dispersive X-ray) analytical data were used to evaluate the architecture of atherosclerotic plaques and to detect the amyloid protein formation in macroscale. It is of high interest to exploit amyloid protein formation with infrared biomarkers for diabetic patients.

2. Materials and methods

2.1 Patients

54 representative specimens of human coronary arteries biopsies were examined *ex vivo*. The patients, 13 women and 41 men aged 48-83 years old, presented with severe coronary artery stenosis and underwent coronary atherectomy during cardiopulmonary bypass surgery. The risk factors of patients according to their biochemical measurements and clinical history showed diabetes (56%, 7 women, 2 insulin-dependent, 5 on oral hypoglycemic drugs and 23 men, 5 insulin-dependent, 18 on oral hypoglycemic drugs), hyperlipidemia (44%), hypertension (69%), hyperuricemia (31%), smoking (91%) and positive family history of coronary heart disease (61%). The samples were collected soon after the cardiac surgery of the patients according to the Greek ethical rules and the permission of Hospital.

2.2 ATR-FT-IR spectrophotometer

The coronary arteries' specimens were fixed in buffered formaldehyde solution immediately after the removal. The FT-IR spectra were recorded with a Nicolet 6700 thermoscientific spectrometer, equipped with an Attenuated Total Reflection (ATR) accessory.[5-11,16-18] With the ATR-FT-IR technique the samples are not homogenized and this allows us to obtain spectra from different parts of the site of tissue of each patient. Each spectrum consisted of 120 co-added spectra at a spectral resolution of 4 cm^{-1} and the OMNIC 7.2a software was used for data analysis. The spectra for each patient were taken from different parts of the coronary artery plaque specimen in order to see the changes, which are induced from the disease and the influence of glucose. Each infrared spectrum was compared with the corresponding infrared spectra of all other patients, taking into account the concentration of the serum glucose levels, as well as other risk factors.

The tissues were dissolved in hexane, a non-polar solvent, in order to remove the soluble lipophilic organic components. After one hour the tissues were dried under vacuum. In the tissues remained the non-degradable components and the stable products which are produced during the disease development. The non-polar solvent hexane was evaporated and the precipitated molecules were also analyzed. For the isotopic effect studies the tissues were left over night in a desiccator in the presence of deuterated water (D_2O) in order to allow hydrogen-deuterium exchange to take place.

2.3 Scanning Electron Microscope (SEM)

For the study of the surface analysis of coronary arteries it was used SEM (Scanning electron microscopy) analysis, a non-destructive physicochemical method. The samples were used without any preparation[5-11,16,17] and could be used for further analysis, such as FT-IR in special detected regions and XRD (X-ray diffraction). The morphology of the arteries was obtained using the SEM instrument from Fei Co, The Netherlands, which was combined with Energy Dispersive X-Ray (EDX) apparatus for the chemical elemental composition analysis in different sites of the coronary artery's tissue, even of spots. SEM as well as ATR-FT-IR are non-destructive techniques and allow studying the same specimens many times.

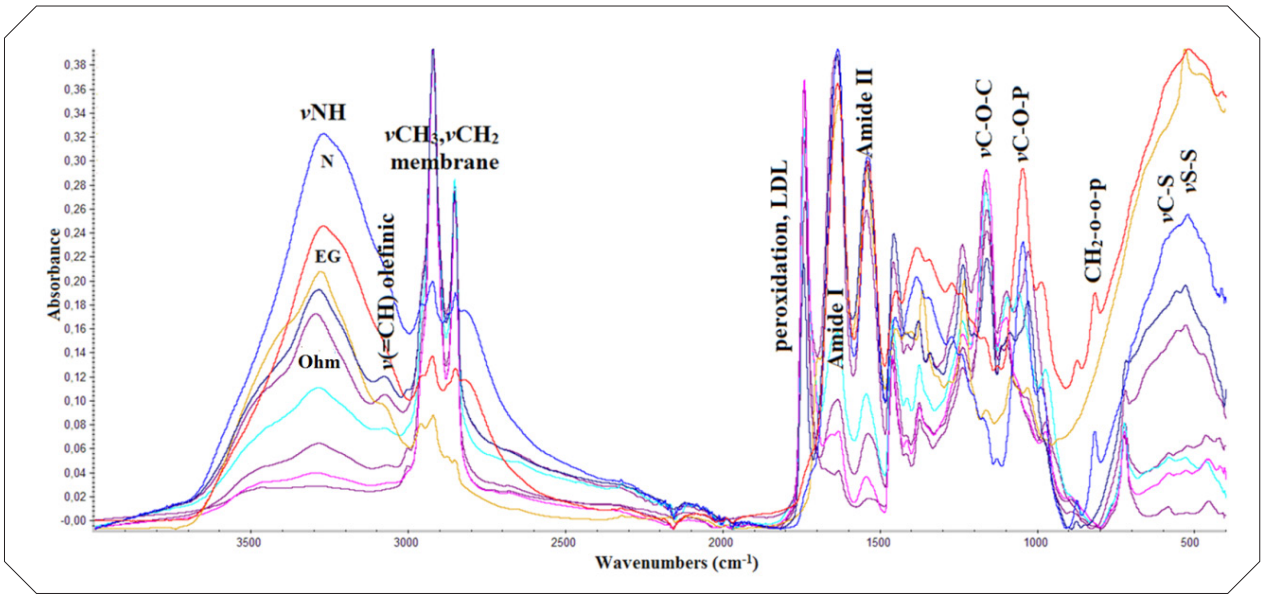


Figure 1. FT-IR spectra of coronary biopsies obtained from various patients with normal serum glucose (N), insulin-dependent-patients (Idep) and (Ohm) patients on oral hypoglycemic drugs treatment

3. Results

3.1 ATR-FT-IR spectra

In **Figure 1** are shown the ATR-FT-IR spectra of coronary arteries obtained from patients on insulin-dependent (Ide), on oral hypoglycemic medication (Ohm) and with normal (N) concentrations of glucose in serum. Comparison between the spectra of the patients shows that some of the spectral regions have the same pattern, while in other regions are shown considerable differences in band absorption intensity, bandwidths, frequency shifts, as well as appearance of new bands in all infrared spectral regions from 4,000-400 cm^{-1}.

In the infrared spectral region between 4,000-3,000 cm^{-1} are located the characteristic stretching vibrational modes of the νNH obtained from proteins.[5-11] Comparison of the intensities of the vibrational band of νNH shows reduction of intensity in the spectra originated from the patients with hypoglycemic medication compared to the spectra originated from patients with normal glucose levels. As it is shown in **Figure 1** the reduction of the intensities of the νNH absorption band is more pronounced in insulin dependent patients and less in those who are under oral hypoglycemic treatment. It is also observed a blue shift to lower frequencies in the νNH absorption band from 3,300 cm^{-1} to 3,200 cm^{-1}. The reduction and the shifts are attributed to the damage of proteins and to the change of intermolecular hydrogen bonding of proteins and ester-phospholipids. Furthermore, the shift to lower frequencies was found to be related to the increase of the lipophilic environment of the membranes. From the shifts it is also suggested that the strength of hydrogen bonds of the peptide bond of proteins changed due to the disease, suggesting that the disease is responsible to the changes of the secondary conformation of proteins.

Important changes are observed in the region 3,000-2,850 cm^{-1}, where are located the absorption bands of antisymmetric and symmetric stretching vibrations of methyl (νCH_3) and methylene (νCH_2) of membranes' lipids and phospholipids.[5-11] The intensities of the bands of antisymmetric and symmetric of methylene groups are higher in the insulin-dependent diabetic patients, followed by oral hypoglycemic treatment patients, while increase of the bands appears also in patients with normal glucose blood levels, comparing with the healthy infrared spectra. Subtraction of an insulin-dependent patient spectra with that of a normal glucose (spectra not shown) shows that the absorption

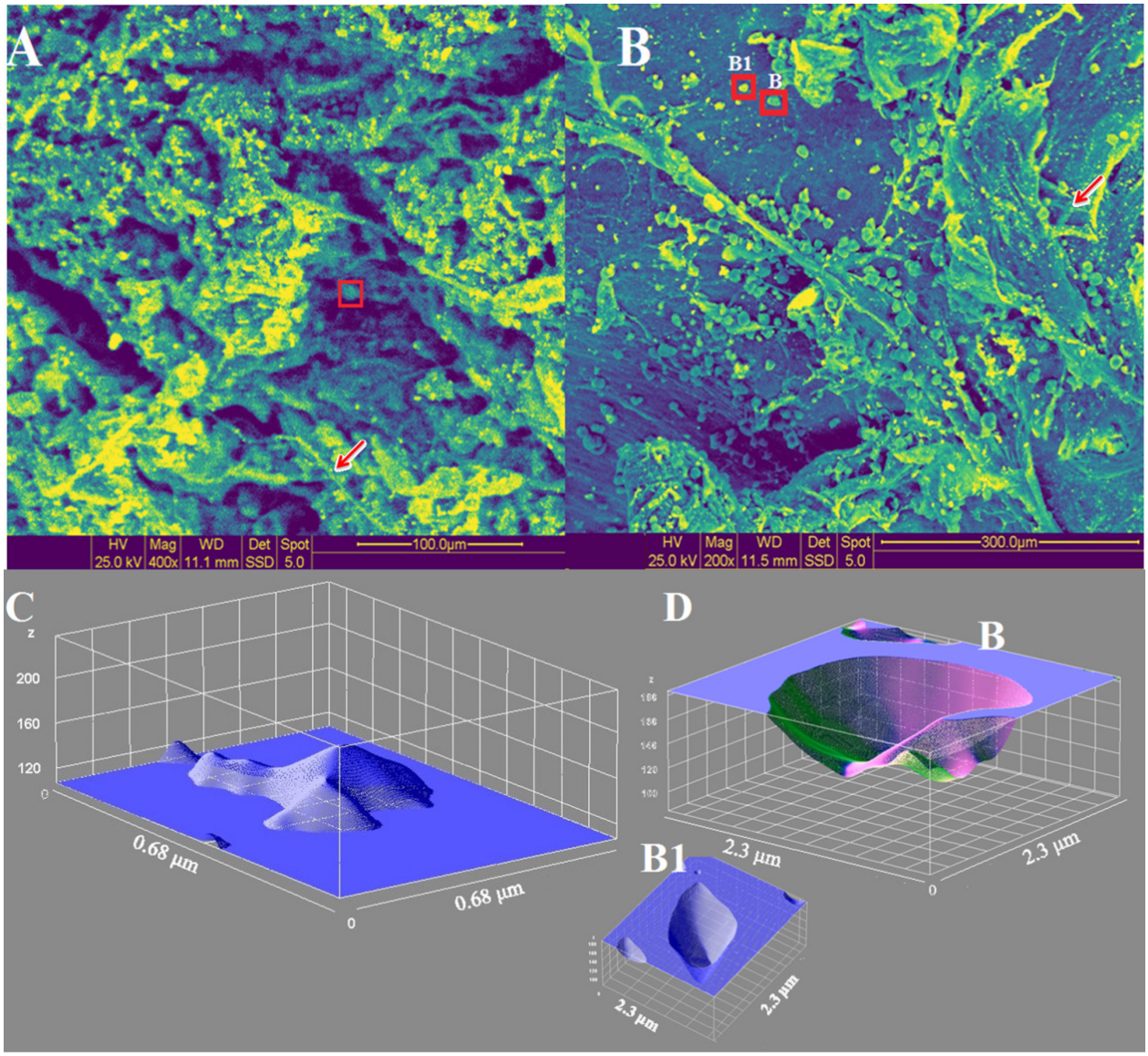


Figure 2. SEM morphology of coronary artery of A: Normal serum glucose (M400x, scale 100 µm), B: diabetic patient (M200x, scale 300 µm). The bright yellow are calcium deposits, while the blue are aggregates. The arrows show parts of polymerized proteins. C and D are the ImageJ analysis of SEM of the square surrounded region of at A and B pictures. The mineralized deposits C, D-B1 appear as bumps (hill-like) and are consistent from calcium carbonate. The aggregate appears as crater D-B

bands of asymmetric $\upsilon_{as}CH_3$ and υ_sCH_3 decreased considerably, suggesting that the lipophilic environment shows higher conformational order.

The high intensity band at 1,744 cm^{-1} is more pronounced in insulin-dependent patients. This aldehydic band at 1,744 cm^{-1} was also observed in carotid arteries atheromatic plaques and in calcified aortic valves[5-11] and can be used as a "marker band", which characterizes the oxidative stress induced damage to cells. A new band at 1,728 cm^{-1} is assigned to keto groups of cardiolipin molecule.[18] This interesting band was observed only in insulin dependent patients and not in the spectra of patients under oral hypoglycemic treatment, indicating that this band could be used as "marker band" for the insulin dependent patients.

Another prominent region in the spectra is that between 1,700 cm^{-1} and 1,500 cm^{-1}. In this infrared spectral region appear the Amide I and Amide II absorption bands of proteins. Both are assigned to

bending δ-NH vibrations in combination with the stretching υC=O of the peptide bonds (CO-NH).[5-11,16-18] The typical vibrational absorption amide I and amide II bands are found at about 1,655 cm^{-1} and 1,550 cm^{-1}, respectively, for the native protein configuration, which is an α-helix. The intensity of these bands is directly related to the protein concentration and conformation. In almost all patients these bands shift to lower wavelength and some new bands appear at the same region. These changes were more distinct to the insulin-dependent patients. Thus the shift of these bands to lower frequencies indicates the changes of secondary structure from α-helix to random coil and finally to β-sheet conformers.

The infrared spectral region 1,300-900 cm^{-1} is of high importance since in this region are located the absorption bands of –C-O-C groups of sugar moieties and C-O-P groups of phospholipids. Substantial differences are found between the spectra of patients with normal glucose in blood serum and the diabetic patients. For insulin-dependent patients the spectra show three bands of high intensity, with a maxima at about 1,167 cm^{-1}, 1,070 cm^{-1} and 1,028 cm^{-1}. The bands at 1,167 cm^{-1}, 1,070 cm^{-1} are assigned to C-O-P groups of phospholipids and sugar-phosphates, respectively, while the band at 1,028 cm^{-1} is attributed to C-O-C stretching vibrations of glycogens. The shape of the spectra of insulin dependent patients is similar to the a-D-glucose.[20]

3.2 SEM analysis

In the last years the scientists noticed that diabetes is a structural related disease and that amyloid-like proteins are produced.[2,3] According to this hypothesis in the lipophilic environment are produced aggregates, as well as fibrils. In order to see if there are any alterations of those lipophilic products in patients' coronary arteries with pathological concentrations of glucose in blood serum, we used SEM microscopy to analyze the architecture and morphology of the surface of the coronary arteries. **Figure 2A** shows the SEM image of a coronary artery architecture of a patient with normal serum glucose (**Figure 2A**) and an insulin-dependent patient (**Figure 2B**).

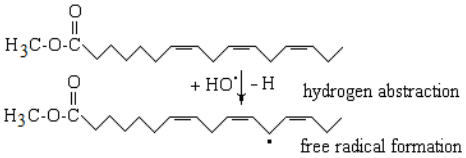
The morphology of the coronary artery shows many spherical particles. The bright yellow color indicates the presence of calcium carbonate and the blue color the aggregates. Comparison between the two tissues shows that in the normal serum glucose patient the calcium carbonates are prevalent, while in the insulin dependent patient the aggregates predominate. ImageJ analysis of SEM of the square surrounded region of at A and B pictures shows that the salt deposits appear as bumps (hill-like), while the aggregates appear as craters (C-B).

4. Discussion

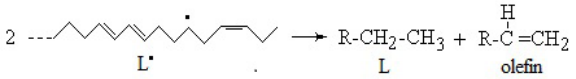
Most of the studies on diabetes are focused on individual proteins and gene expression, which are related to the disease and not to other pathways that could be involved in the pathogenicity. In this study we used infrared spectroscopy, a very sensitive and not destructive physicochemical method to study the differences between the spectra originated from patients who are insulin-dependent or in oral hypoglycemic drug treatment and the infrared spectra of patients with normal blood glucose levels, in order to understand the disease at a molecular level. Our results indicated that infrared spectroscopy can discriminate differences of secondary protein structure between the patients, as well as to find new characteristic bands of the disease and its progression.

In the FT-IR spectral region 3,000-2,850 cm^{-1} are located the stretching vibrations bands of methyl (υCH_3) and methylene (υCH_2) groups of aliphatic chains of lipids and phospholipids. These bands are very sensitive to conformational changes of membrane lipids. It was observed that in insulin-dependent patients the intensity of both antisymmetric and symmetric bands of methyl (υCH_3) groups decrease considerably. On the contrary, the intensity of the symmetric stretching vibration bands of methylene (υCH_2) groups increased considerably. By calculating the intensity ratios of stretching symmetric and antisymmetric vibrations of methylene groups [$\upsilon_s CH_2$]:[$\upsilon_{as} CH_2$] it was found a higher increase in insulin-dependent patients, compared with the other groups of patients. This ratio is of high diagnostic value and provides information about the puckering of long acyl chains

of lipids and phospholipids in the membranes. The increase of the ratio is particular evidence that the disease cause new hydrophobic interactions between the lipids and phospholipids of cell membrane leading to higher order conformation of the acyl chains due to oxidative stress.[10,11,21,22] Recently, it was established that the insulin resistance, in old adults, increases the free fatty acids and inflammatory cytokines.[23,24] As is shown in the spectra (**Figure 1**) by increasing the lipophilic environment increases the band intensity at about 3,080 cm^{-1} (**Figure 1**), which is assigned to stretching vibration of υ=CH terminal olefinic group of aliphatic chains. The formation of the =CH group is based on the fact that the hydroxyl free radicals produced during the oxidative stress, reacted with lipids by hydrogen abstraction leading to lipid free radical (L^*) formation (*reaction 1*):[4]



The formed L^* radicals react with each other following the well-known dismutation reaction (*reaction 2*):



This band was proposed as a "marker band" for the progression of the oxidative stress, which induced damage to coronary arteries and was also observed in infrared spectra of carotid arteries and degenerative calcified aortic valve stenosis.[5-11] The produced L^* radicals are not stable and can also react to produce dimers, or linear, branch and cross-linked polymers. The formation of branch and cross-link polymers is confirmed by SEM analysis in all surface of the coronary arteries. The arrows in **Figure 2** notice some of these products. In the presence of oxygen the lipid radicals are attacked by oxygen forming lipid peroxides, which are not stable and lead to other fragments. All these products change the permeability and mobility of the membranes and indirectly they could induce secondary damage to cells and tissues.

In order to confirm our findings about the lipophilic environment that is formed from the disease, and especially for the insulin-dependent patients, we performed a detailed investigation of the samples, by removing the most soluble components of the coronary arteries. The samples were washed with non-polar organic solvent. In **Figure 3** are presented the FT-IR spectra of an insulin-dependent patient. The spectra a, b and c originate from a region rich in aggregates, a normal-like site and after washing the coronary artery with hexane, respectively.

Comparison between the spectra originated from the rich in fat region (**Figure 3a**) and the normal adjacent region (**Figure 3b**) in the spectral range 4,000-2,850 cm^{-1} shows considerable differences in many absorption bands. From the decrease of the intensities, which correspond to υNH vibrations at the region 4,000-3,000 cm^{-1} it is suggested that the proteins have been damaged and changed their secondary conformation. This is confirmed from the red shift of the band at 3,290 cm^{-1} to 3,420 cm^{-1}, which appears as shoulder in the spectrum and indicates that the intramolecular hydrogen bonding strength is weakened and changed the secondary structure of proteins.[17] This shift is observed only at low pH, and shows that in patients the acidosis is taking place during the development of the disease. This absorption band is almost disappeared after washing and is shown to be related with the increased lipophilic environment of the membranes and the atherosclerotic plaque formation. To identify the weakening of the hydrogen bond we used the isotopic effect. After substitution of hydrogen atoms with deuterium atoms the band of υNH at 3,290 cm^{-1} was shifted to 2,520 cm^{-1} due to υND vibration and the band at 3,420 cm^{-1} is assigned to υNH free from hydrogen bond.

As it is shown in **Figure 3c** the bands in the spectral region 3,000-2,850 cm^{-1} are reduced after the treatment with the organic solvent. Moreover, the intensities of the bands of antisymmetric and symmetric vibrations of methylene groups υCH_2 decreased, reflecting the higher disorder in acyl chain of lipids.[21]

The intense band at 1,744 cm^{-1}, assigned to aldehyde carbonyl groups (RHC=O), confirms the suggestion that hydroperoxidation of lipids is taking place most likely due to the free radical

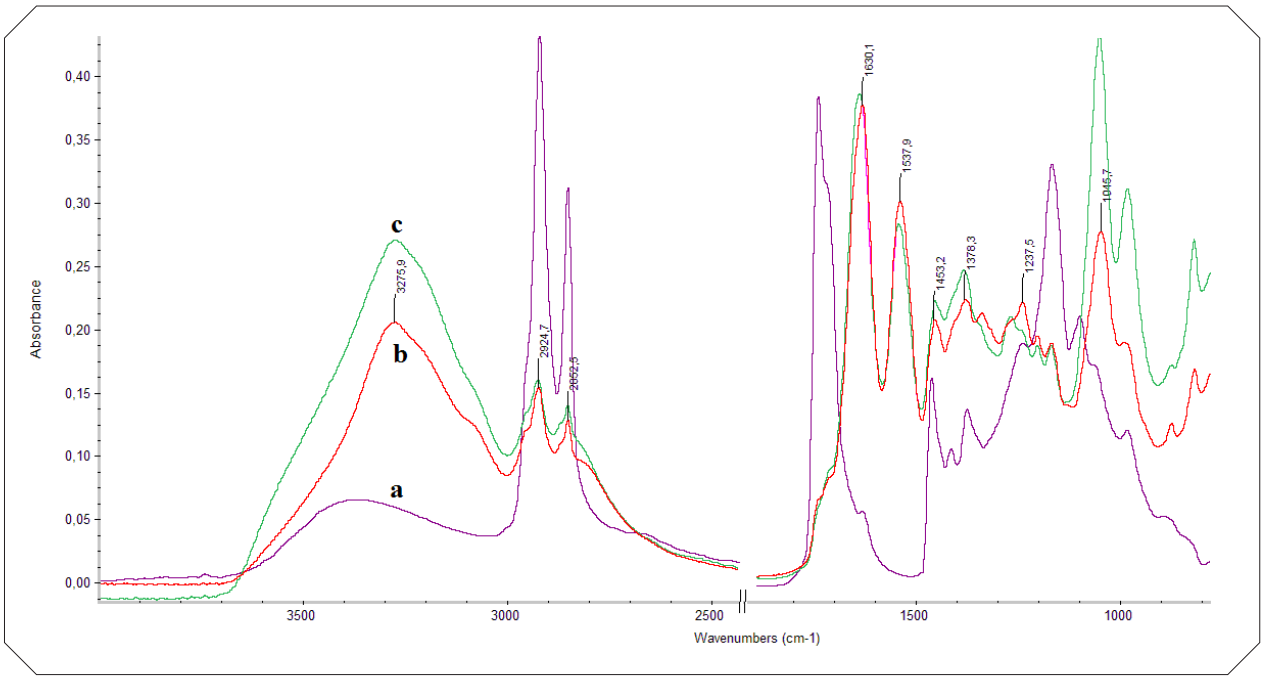


Figure 3. FT-IR spectra of a coronary artery of an insulin-dependent patient**. a:** rich in fat region (detected also by SEM image Fig.2a), **b:** normal-like tissue of the same biopsy and **c:** after washing the sample with non-polar organic solvent

formation.[5-11] These products are produced from lipid peroxidation, especially from arachidonic acid and the intensity of the band is directly related to serum LDL of the patients.[5-11] The band at 1,728 cm^{-1}, which is attributed to keto C=O group (RRC=O) of cardiolipin, is reduced dramatically after washing the coronary artery with hexane. Infrared spectroscopic studies of cardiolipin show that the appearance of the band at 1,728 cm^{-1} indicates that cardiolipin is located in the double-layer membrane of the cell (vesicles) and that the pH in the environment of the patient was acidic.[25-28] Cardiolipin alone at a range pH 7-7.3 gives a band at 1,729 cm^{-1}, while in acidic pH 6-7 the band shifts to lower frequencies at about 1,719 cm^{-1}, indicating a stronger hydrogen bond to C=O group.[25-28]

These data confirm the presence of the lipophilic environment and are in agreement with the reduction of the intensity of the band of C=O of cardiolipin after treatment with non-polar organic solvent. From these results it becomes clear that cardiolipin is oxidized in diabetic patients and this damage alters the atherosclerosis of coronary artery, which leads to the increase of oxidative stress. It is well known that cardiolipin is a polyglycerophospholipid found in bacteria and mitochondria and plays a pivotal role in many biological enzymatic processes and it regulates oxidative stress inducing phosphorylation.[29-32] Cardiolipin is found in the inner mitochondrial membrane and is associated with the proteins involved in catalytic activities of oxidative phosphorylation and respiratory chain, including NADH and ADP-ATP carrier.[33,34] Any damage of the cardiolipin molecule is associated with mitochondrial dysfunction, including ischemia, heart failure and aging. [35-37]

The next intense bands at 1,650 cm^{-1} and 1,550 cm^{-1} for normal tissues are attributed to amide *I* and amide II of proteins, respectively. These bands in both spectra that originated from the healthy-like part of the coronary artery and the alteration by removal of lipophilic components have the same shapes. However, they are broader and shift to lower wavenumbers 1,630 cm^{-1} and 1,540 cm^{-1}, respectively. From these shifts it is suggested that the disease changes the proteins' secondary structure from α-helix to random coil. These changes expose the protein helices to oxidative stress and that the

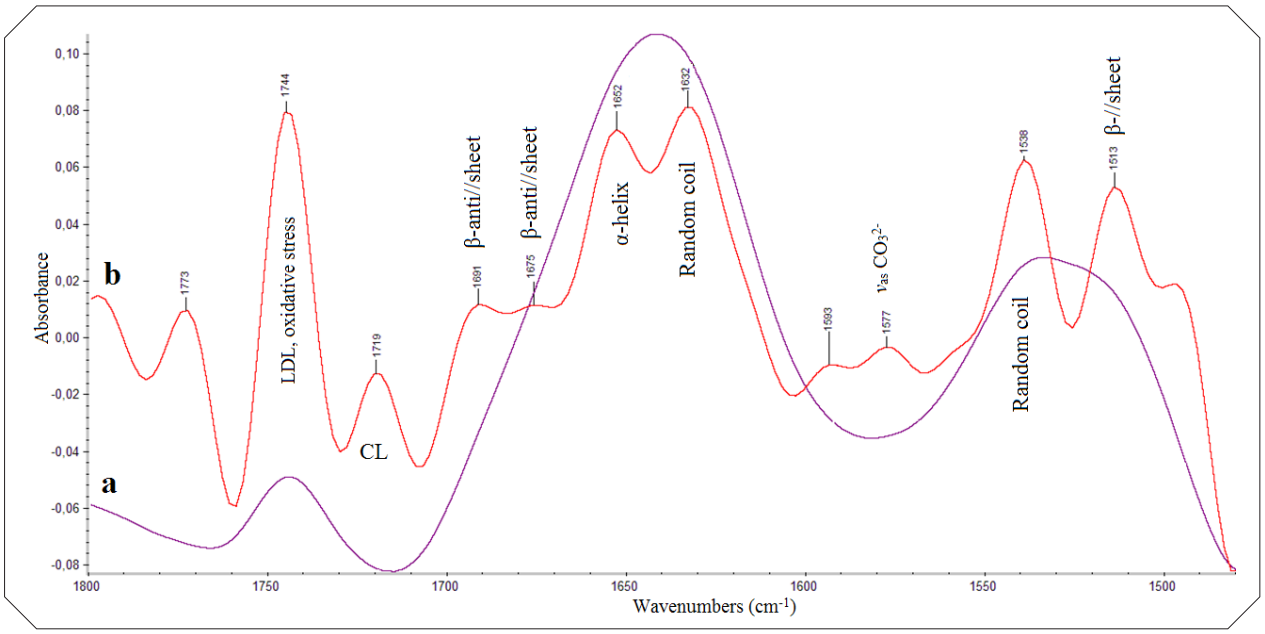


Figure. 4. a: FT-IR spectrum of insulin-dependent patient after treatment with organic solvent. **b:** Deconvolution of spectrum a in the spectral region 1,800-1,500 cm^{-1}

hydrogen bonds change during oxidative stress[38,39] in agreement with the changes observed in the spectral region 3,500-3,100 cm^{-1}.

Deconvolution of these peptide bands shows the formation of new bands at about 1,690 cm^{-1} *(β-antiparallel sheet)* and 1,513 cm^{-1} *(β-parallel sheet)*, which indicates the formation of amyloid-like proteins. The amyloid proteins are not characterized from their non-specific sequence but from their structural folding configuration (**Figure 4**), which is known as cross *β*-structure and form aggregates.

From the deconvolution of the spectra it is found that after the removal of the damaged fragments, which were produced during atherosclerosis a portion of α-helix still remains, suggesting that amide I is less sensitive that amide II native structure. However, amyloid formation was also pronounced in insulin-dependent diabetic patients, in agreement with literature.[40]

Moreover, the band at about 1,577 cm^{-1} in deconvoluted spectra in combination with that at 1,453 cm^{-1} are dominated to asymmetric carbonate anions ($\upsilon_{as}CO_3^{-2}$) and symmetric ($\upsilon_sCO_3^{2-}$) vibration of carbonate anions resulting from calcium carbonate components, of the atheromatic plaque.[5-7,22,41] The symmetric ($\upsilon_sCO_3^{2-}$) vibration of carbonate is not influenced from organic solvents, since they are inorganic salts. The calcium deposits were also detected in atheromatic plaques of coronary arteries. The detection of calcium carbonates indicates alteration of calcium release, which affects the coronary arteries' membranes of the patients. SEM morphology of patients shows the production of aggregates and fibrils (**Figure 2**). Several studies demonstrate that the aggregates tend to organize into oligomers and to amyloid-like proteins, which could be responsible for heart failure.[43]

It seems that in insulin-dependent patients, as it is shown from both FT-IR spectra and SEM, the aggregates play crucial role in the toxicity and calcium deposits of the arteries. Recently, it is described that amylin or IAPP (islet amyloid polypeptide formation), a regulator polypeptide for insulin and glucagon secretion inhibition, has the ability to aggregate into pancreatic islet amyloid deposits, associated with type 2 diabetes of human.[44] IAPP was also found in other tissues, such as neurons (diabetic neuropath) and heart (diabetic cardiomyopathy). IAPP aggregates are toxic for the membrane permeability and enhance the production of membrane pores resulting in leakage followed by Ca^{2+} dysregulation.[12] The produced aggregates have been shown to disrupt

the mitochondrial membrane and this dysfunction is pathogenic and for type II diabetic patients.[12,45] However, our experiments demonstrate that in insulin-dependent patients were produced similar toxic aggregates and other damage products with the type II diabetic patients.

The fingerprint spectral region between 1,300-800 cm^{-1} is very important in order to study the coronary arteries from diabetic patients, because the bands in this region arise from sugar ring, sugar-phosphate and phosphates of phospholipids. As it is shown in **Figure 3** the absorption band at 1,165 cm^{-1}, which is assigned to phospholipids, diminished in intensity. It seems that this band arises from cardiolipin, since only in insulin-dependent patients was observed. The band at 1,165 cm^{-1} corresponds to advanced glycation endproducts (AGEs) and could be used as "marker band" to discriminate insulin-dependent patients and maybe type II diabetic patients from patients with elevated concentrations of glucose, who normalize the glucose by using orally hypoglycemic drug treatment. This band could also be used as an indicator of blood glucose and the extent of proteins' glugosylation.[41] It is found that AGEs are related with diabetes mellitus and play a crucial role in the development of cardiovascular diseases in type II diabetic patients.[46,47] It could also be valuable for the prediction of possible damages of arteries due to atherosclerosis.

From the spectral region 600-400 cm^{-1} (**Figure 1**) it is shown that in insulin-dependent patients both the bands at 557 cm^{-1} and 521 cm^{-1}, which are assigned to υC-S and υS-S frequencies, respectively, are reduced dramatically.[11] These changes lead to the suggestion that glutathione, one of the main endogenous protectors, was damaged irreversibly, increasing thus the LDL peroxidation. This finding is in accordance with literature data, where in experiments with rats it is suggested that reduction of glutathione leads to calcium accumulation in mitochondria.[47] The reduction of glutathione is also related with hypoxia and lower pH (acidosis) in heart tissues. The low pH in patients was also detected, as mentioned above, from the shift of the υNH bands in the region 3,500-3,100 cm^{-1}, due to the change of the strength of hydrogen bonds in patients.

5. Conclusions

In the present manuscript it is shown that FT-IR spectroscopy is a powerful tool to investigate the coronary artery atherosclerosis, amyloid-like protein formation and to discriminate the diabetic patients from their infrared spectra. The characteristic infrared absorption bands in the "fingerprint" regions of methyl (υCH_3) and methylene (υCH_2) demonstrate the changes in the membrane permeability of coronary artery, the formation of aggregates and the implication of cardiolipin molecule in an oxidative damage progression. The cardiolipin molecule is implicated with keto groups in altering the oxidative stress mechanism of oxidation in insulin-dependent patients. The bands at 1,728 cm^{-1} and 1,167 cm^{-1} can be used as "marker bands" to distinguish insulin-dependent patients and maybe type II diabetic patients. The blue shift of the absorption bands of amide I and amide II from 1,650 cm^{-1} and 1,550 cm^{-1}, respectively, is strongly correlated with the amyloid-like protein formation and progression of diabetes. From the shapes and the frequencies of the bands in the region 1,250-950 cm^{-1} it is suggested that in insulin-dependent patients there is a higher glycation and phosphorylation process, due to the disease, in comparison with the patients who receive orally hypoglycemic drugs.

Moreover, our findings strongly support the hypothesis that oxidative stress is involved during the progression of the valve mineralization amyloid protein formation, giving stable final products, such as linear-, branched- and cross-linked co-polymers. It has been found that the most sensitive sites are the sulfur (S) atoms of proteins and the bridges S-S that are formed in proteins.

Conflict of Interest

All authors declare no conflict of interest.

Περίληψη

Η επίδραση του διαβήτη στην αθηροσκλήρωση και τον σχηματισμό αμυλοειδούς τύπου πρωτεϊνών στις στεφανιαίες αρτηρίες. Μελέτη με FT-IR φασματοσκοπία

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Στην παρούσα εργασία χρησιμοποιείται η ATR-FT-IR (αποσβένουσας ολικής ανάκλασης με μετασχηματισμό Fourier υπέρυθρη) φασματοσκοπία για την μελέτη 54 βιοψιών αθηρωματικών στεφανιαίων αρτηριών από ασθενείς με αυξημένη γλυκόζη στο αίμα. Σύγκριση μεταξύ των φασμάτων των ινσουλινο-εξαρτώμενων ασθενών και των ασθενών που λάμβαναν φαρμακευτική αγωγή με αντιδιαβητικά δισκία και εκείνων με φυσιολογικά όρια σακχάρου στο αίμα, έδειξαν σημαντικές διαφορές σε όλη την έκταση του υπέρυθρου φάσματος από 4.000-400 cm^{-1}. Η σημαντική αύξηση των εντάσεων των χαρακτηριστικών ομάδων των συμμετρικών και αντισυμμετρικών δονήσεων των μεθυλεν (υCH_2) ομάδων τονίζει την αύξηση του λιπόφιλου περιβάλλοντος που επικρατεί στις μεμβράνες των ινσουλινο-εξαρτώμενων ασθενών. Η μεταβολή αυτή συνδέεται με την αύξηση της βλάβης που προκαλούν οι ελεύθερες ρίζες, λόγω του οξειδωτικού στρες στους διαβητικούς ασθενείς. Η ταινία στα 1.744 cm^{-1} αποδίδεται στην δόνηση τάσης της καρβονυλικής ομάδας $\nu C=O$ των αλδεϋδών. Η ένταση της ταινίας σχετίσθηκε με την LDL χοληστερόλη των ασθενών και αποτελεί «διαγνωστική ταινία» για το μέγεθος της υπεροξείδωσης των στεφανιαίων αρτηριών. Η νέα ταινία στα 1.728 cm^{-1} που εμφανίζεται μόνο στο φάσμα των ινσουλινο-εξαρτώμενων ασθενών αποδίδεται στις κετο-ομάδες της καρδιολιπίνης και δηλώνουν την επίδρασή της στην αθηροσκλήρωση για τους ασθενείς με εξάρτηση στην ινσουλίνη. Η ταινία αυτή ενδεχομένως να μπορέσει να αποτελέσει «διαγνωστικό δείκτη». Η μετατόπιση των ταινιών των δονήσεων των ομάδων amide I και amide II των πρωτεϊνών προς μικρότερες συχνότητες δείχνουν ότι η δομή των πρωτεϊνών μεταβλήθηκε και από α-έλικα απέκτησαν τυχαία διαμόρφωση και β-επίπεδα. Οι μεταβολές αυτές ήταν πιο εκφρασμένες στους διαβητικούς ασθενείς. Οι μεγαλύτερες μεταβολές παρατηρήθηκαν στην περιοχή του φάσματος 1.200-1.000 cm^{-1}, όπου απορροφούν οι ομάδες O-C-O των σακχάρων και οι ομάδες C-O-P του DNA και των φωσφολιπιδίων. Στους ινσουλινο-εξαρτώμενους ασθενείς διαπιστώθηκαν επί πλέον ταινίες της D-γλυκόζης. Η SEM ανάλυση έδειξε την παραγωγή συσσωματωμάτων, ως αποτέλεσμα του σχηματισμού αμυλοειδών πρωτεϊνών και την αύξηση του λιπόφιλου περιβάλλοντος. Οι μεταβολές ήταν ανάλογες του ιστορικού των ασθενών και περισσότερο εκφρασμένες στους ινσουλινο-εξαρτώμενους ασθενείς.

Η εμφάνιση στο FT-IR φάσμα των δονήσεων της καρδιολιπίνης στους ινσουλινο-εξαρτώμενους ασθενείς χρειάζεται περαιτέρω μελέτη.

Λέξεις ευρετηρίου: στεφανιαίες αρτηρίες, αθηροσκλήρωση, αμυλοειδείς πρωτεΐνες, καρδιολιπίνη, ινσουλινο-εξαρτώμενοι ασθενείς, υπέρυθρη φασματοσκοπία με μετασχηματισμό Fourier, ηλεκτρονικό μικροσκόπιο σάρωσης

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