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Infrared Microspectroscopy using Synchrotron Radiation (Sr Mftir) and Infrared Microspectroscopy as New Tools for Rapid Detection of Ectopic Calcifications Associated with Peritoneal Dialysis

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Abstract

The cardiovascular calcifications (CVC) represent a central complication of chronic kidney disease (CKD) responsible of high cardiovascular mortality particularly in patients on peritoneal dialysis. Unfortunately, electron beam and multislice computed tomography, planar X-ray, ultrasonography or cardiac echocardiography methods are not accurate to detect calcium phosphate microcrystals since of its small size. We investigated the ectopic calcifications by Von Kossa staining and infrared microspectroscopy using synchrotron radiation (SR μ FTIR) and infrared microspectroscopy in formalin-fixed peritoneal tissues from 3 cases.

Von Kossa staining allowed us to detect vascular calcifications only in one of 3 studied peritoneal biopsies. Vascular calcifications contained mainly carboxylate according to the presence of the IR absorption bands positioned at 1030 cm^{-1} (ν_3), 960 cm^{-1} (ν_1). In all studied biopsy, we found several tissue microcrystals also composed by carboxylate.

To our knowledge, we report for the first time the usefulness of infrared microscopy and SR μ FTIR for the assessment of calcium phosphate microcrystals and identification of biochemical composition of VC associated with CKD in peritoneal membrane from patients on peritoneal dialysis. SR μ FTIR technique and infrared microspectroscopy are new, remarkable, and rapid tools for detection of early stage of ectopic calcifications associated with peritoneal dialysis. Investigation of calcium phosphate microcrystals by both methods might improve our understanding of early stage of CVC pathophysiology.

Keywords: Cardiovascular calcifications; Hyperphosphatemia; Ultrasonography; Microspectroscopy; Echocardiography

Introduction

The cardiovascular calcifications (CVC) represent a central complication of chronic kidney disease (CKD) responsible of high cardiovascular mortality particularly in patients on peritoneal dialysis. Hyperphosphatemia is a leading mediator [1]. Experimental data suggest that the true culprit of phosphate toxicity may be mediated by calcifying nanoparticles (CNP) containing calcium phosphate microcrystals and some protein. Indeed, increased level of CNP is independently associated with CVC in CKD patients [2,3]. Unfortunately, electron beam and multislice computed tomography, planar X-ray, ultrasonography or cardiac echocardiography methods are not accurate to detect calcium phosphate microcrystals since of its small size [1].

Materials and Methods

As ectopic calcifications have been reported in peritoneal tissue in patients on peritoneal dialysis, [4,5] we investigated the ectopic calcifications by Von Kossa staining and infrared microspectroscopy using synchrotron radiation (SR μ FTIR) and infrared microspectroscopy in formalin-fixed peritoneal tissues. To do this, paraffin blocks from 3 cases of EPS available in the archives of pathology department were used. Tissue sections (4 μm) were treated with a silver nitrate solution. Silver ions substitute the calcium ions bounded to phosphates and are subsequently visualized by hydroquinone reduction to metallic silver producing a brown-black staining (von Kossa reaction) as previously reported.

Thereafter, the biochemical composition was determined by infrared microspectroscopy using synchrotron radiation in collaboration with Condensed Material Paris, National Center for Scientific Research (CNRS), Collège de France, Paris, France as follow (chemical composition of pathological microcalcifications can be accurately identified by FTIR spectroscopy through their IR absorption bands. The peritoneal tissue microcrystals were approached by infrared microspectroscopy as detailed previously [6]. This study was evaluated and approved by the local Ethic Committee (Erasme hospital No.: P2014/184) [7].

Results

Von Kossa staining allowed us to detect vascular calcifications only in one of 3 studied peritoneal biopsies but this technique is not able

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to determine significantly their chemical composition. Interestingly, this biopsy provided from patient with the largest number of bacterial peritonitis (8 episodes) and peritoneal dialysis vintage (Figure 1) [8]. Apart from vascular areas no black staining was found in all three analyzed tissues. Vascular Calcifications composition contained mainly carapatite accordingly to the presence of the IR absorption bands positionned at 1030 cm^{-1} (ν_3), 960 cm^{-1} (ν_1) (Figure 2). In all studied biopsy, we found several tissue microcrystals also composed by carapatite (Figure 2).

Discussion

To our knowledge, we report for the first time the usefulness of infrared microscopy and SR μ FTIR for the assessment of calcium phosphate microcrystals and identification of biochemical composition of VC associated with CKD in peritoneal membrane from patients on peritoneal dialysis. The von Kossa staining considered as specific for calcium although was not accurate for specify types of calcifications. Infrared microspectroscopy using synchrotron radiation and infrared microspectroscopy were more adapted as informs precisely

that respectively vascular calcification and tissue microcrystals are composed by calcium phosphate (carapatite) independently of its sizes.

Calcium phosphate microcrystals precipitate in a passive way secondary of phosphate and/or of calcium super-saturation. This funding emphasizes once again, the crucial importance of strict control of phospho-calcium parameters in PD patients [2]. Indeed, carapatite may reflects too origins: 1) the metabolic disorders, mainly hyperphosphatemia and secondary hyperparathyroidism related to lost of renal function and 2) iatrogenic component related to treatment of above mentioned metabolic complications by high calcium load (calcium based phosphate binders) and/or supplementation of vitamin D [1,9].

Importantly calcium phosphate microcrystals but not soluble phosphate have been proposed to play a role in the local genesis of osteo-chondrogenetic transformation in cultured smooth muscle cells [10].

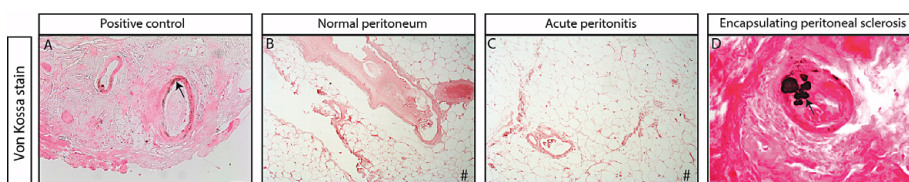


Figure 1: Photomicrographs demonstrating histopathological findings in parietal peritoneal biopsy reported the presence of tissue calcifications. (A) Positive control of Von Kossa staining (internal controls) (A), control case: normal peritoneum (B), case of acute peritonitis (Case control N° 3; C) and Case of encapsulating peritoneal sclerosis (EPS) (A) Abnormal calcifications in vessel walls corresponded to intimal calcinosis. (B-C) Absence of vascular calcifications. (D) Von Kossa coloration demonstrating calcifications in media of vessels; in this method tissue section were treated with a silver nitrate solution and the silver is deposited by replacing the calcium reduced by the strong light, and thereby visualized as metallic (black) silver. (A-D) Von Kossa staining. Original magnifications :A-B-C: 20X et D : 40x.

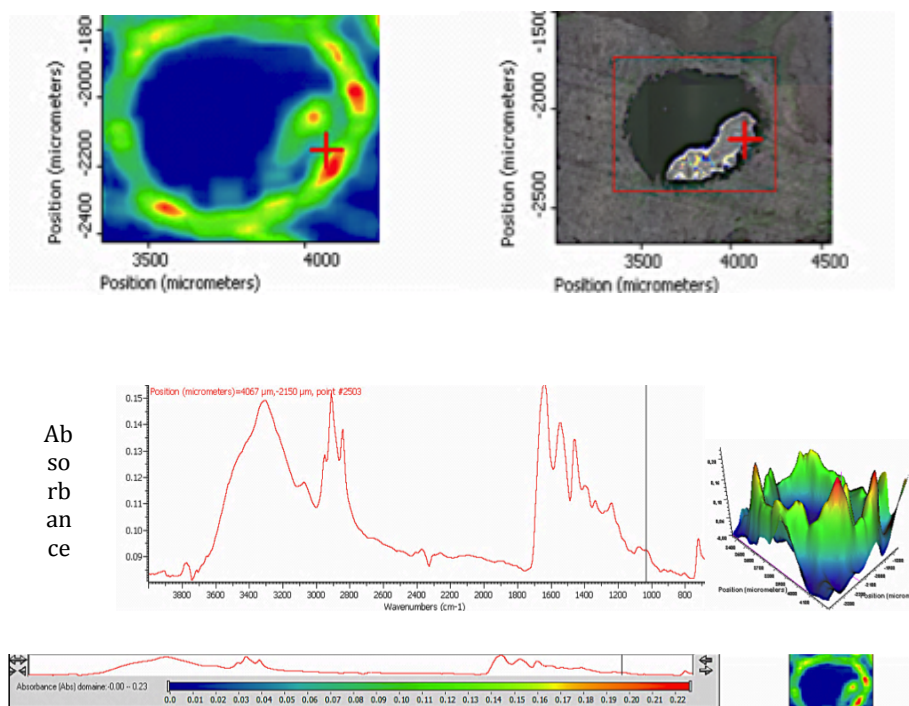


Figure 2: Photomicrographs demonstrating technic applied for analysis of peritoneal tissue vascular calcifications.

Nano-sized complexes of calcium phosphate microcrystals serve as mineral chaperones for further calcification and have been proposed as a very early step of vascular calcification process. Moreover *in vitro*, calcium phosphate microcrystals induce apoptosis of smooth muscle cells, inflammation in the arterial intima such and increase in mineralized multicellular nodules [3]. The identification of carapatite in our peritoneal biopsy samples is of great importance because it is formed preferentially within alkaline pH [2], frequently seen during bacterial infections. Therefore, this finding raises the issue of relation between the previous bacterial peritonitis and peritoneal vasculopathy in EPS.

Conclusion

In conclusion, SR μ FTIR technique and infrared microspectroscopy are new, remarkable, and rapid tools for detection of early stage of ectopic calcifications associated with peritoneal dialysis. Investigation of calcium phosphate microcrystals by this method might improve our understanding of early stage of CVC physiopathology.

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