CHARACTERIZATION OF miRNA CONTENT IN CIRCULATING MICROVESCICLES DERIVED FROM DIALYTIC PATIENTS

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OBJECTIVES

MicroRNAs are small non-coding RNAs that regulate various biological processes, modulating gene expression at posttranscriptional levels. Circulating miRNA levels in blood have been shown to be critically involved in many physiological and pathologic processes. Microvesicles (MVs) include a heterogeneous population of vesicles released as exosomes from the endosomal compartment or as shedding vesicles from the cell surface of different cell types and play a pivotal role in cell-to-cell communication. MVs may directly stimulate the target cells or may transfer various bioactive molecules including mRNAs and microRNAs (miRNAs) from the cell of origin. We aimed to study the spectrum of miRNA content in plasma circulating microvesicles derived from patients undergoing renal replacement therapy and treated with two different dialytic therapies, Bicarbonate Hemodialysis (BED) and On-line Hemodiafiltration (OL-HDF).

METHODS

A blood sample from patients (n=2 for conditions) was collected at the beginning (10 minutes from the start of the session, pre-dialysis) and at the end of the session (10 minutes before the conclusion of the session, post-dialysis) and MVs were isolated by ultracentrifugation. RNA from microvesicles was isolated and miRNA expression levels were analyzed using Comprehensive coverage of Sanger miRBase v14, TaqMan Array MicroRNA Cards (Card A) for a total of 375 unique assays specific to human mature miRNAs.

Cardio-enriched mature miRNAs (miR-1), (miR-208a), (miR-208b), (miR-133a), (miR-133b), (miR 17-5p), (miR-30b-5p), (miR-92a), (miR-223), (miR-499a-3p), (miR-423-5p), (miR 1249), (miR 454-5p), (miR-483-5p) and (miR-451a) were measured using real time PCR in plasma microvesicles from patients undergoing BED and OL-HDF to make a comparison beetween the two dialytic therapies. Total RNA was extracted from plasma microvescicles using All in One Kit (Norgen, Thorold Ontario, Canada) and cDNA was prepared using the miScript II RT Kit (Qiagen, Hilden, Germany). Quantitative real time polymerase chain reaction (qRT-PCR) was carried out using SYBR Green Master Mix with miScript Universal primer (Qiagen). Thermal cycling consisted of an initial activation step at 95°C for 15 min, followed by 40 cycles of 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s.

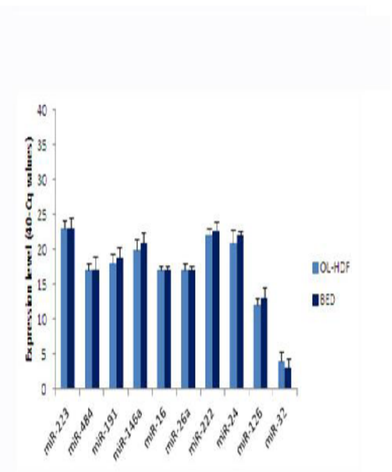


Fig.1 Detection of common miRNAs expressed in plasma microvescicles. Graph showing the more highly characterizing miRNAs in human peripheral blood expressed in plasma microvescicles derived from patients undergoing BED and OL-HDF hemodialysis.

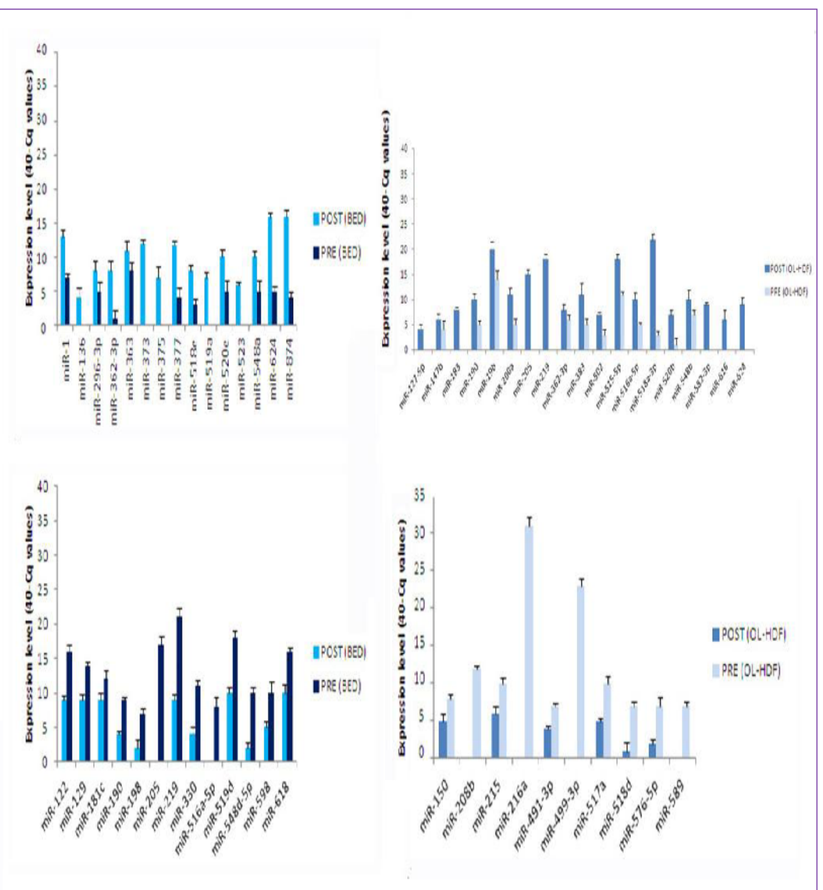
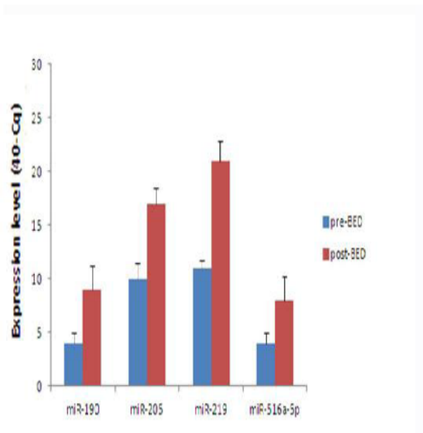


Fig.2 Differential miRNAs expression from beginning and end of dialysis sessions. Graphs showing hierarchical clustering of comparative miRNA expression profile analyses of human mature miRNA in circulating plasma microvescicles indicating that 15 miRNAs were increased in patients undergoing BED (A) and 19 miRNAs in OL-HDF (C) ; while 13 and 10 miRNAs were decreased after BED and OL-HDF respectively **(B-D).**



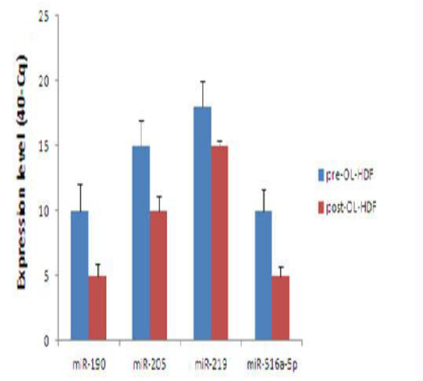


Fig.3 Crossmatch between post-dialysis samples.
Histograms showing the increased expression after BED (A) and the reduction after OL-HDF (B) of 4 miRNAs: miR-190, miR-205, miR-219, miR 516a-5p

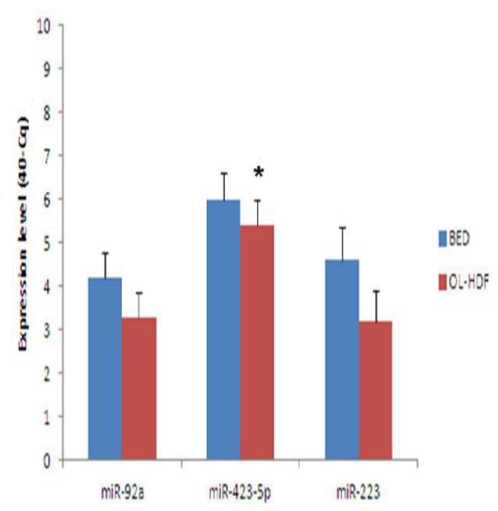


Fig.4 Detection of cardio-enriched miRNAs in plasma microvesicles. qRT-PCR performed on plasma microvesicles derived from patients undergoing BED and patients who switched from BED to OL-HDF. miR-92a, miR-223 and miR-423-5p exhibit a decrease when patients change dialytic therapy from BED to OL-HDF.

RESULTS

Circulating plasma microvescicles derived from patients undergoing BED and OL-HDF renal replacement therapies, were first checked for the expression of the most common miRNAs described in literature for being detected in plasma microvesicles: miR-223, miR-484, miR-191, miR-146a, miR-16, miR-26a, miR-222, miR-24 and miR-126 were all detected, the only exception is represented by miR-32. (Fig.1)

We observed that plasma microvesicles exhibit significant differences in their miRNA content from pre and post dialysis. Hierarchical clustering of the data indicated that 15 miRNAs were increased in patients undergoing BED (Fig. 2A) and 19 miRNAs in OL-HDF (Fig. 2C) moreover, 13 and 10 miRNAs were decreased after BED and OL-HDF respectively (Fig.2B-D). The crossmatch between post dialysis samples showed an increase after BED (Fig. 4A) and a reduction after OL-HDF (Fig. 4B) of 4 miRNAs (miR-190, miR-205, miR-219, miR 516a-5p) involved in the regulation of proliferation, differentiation, angiogenesis and apoptosis .

In hemodialysis patients, structural changes at all levels of the cardiovascular system are common, moreover evidence suggests that cardiac dysfunction may cause renal disfunction and vice versa so heart failure and chronic kidney disease frequently co-exist.

We performed comparative real-time PCR analysis experiments to evaluate the expression of 15 selected miRNAs, known to play a crucial role in the heart health and disease, between circulating plasma microvesicles derived from patients undergoing BED and patients who changed dialysis technique from BED to OL-HDF.

We demonstrate that miR-92a, miR-223 and miR-423-5p exhibit a decrease when patients change dialytic therapy from BED to OL-HDF, while the other 12 screened miRNAs were not detected or show no differences (data not shown).

The levels of miR-423-5p were significantly decreased in patients who switched from BED to OL-HDF treatment. For miR-223 and miR-92a, levels were also reduced in patients who switched from BED to OL-HDF but the difference did not reach statistical significance (Fig. 4).

CONCLUSIONS

Firstly, the results of this study indicate that there are several differences in the miRNA pattern observed between the beginning and the end of the dialysis session suggest that dialysis procedure might influence the circulating plasma microvesicles and that could lead to important implications in the study of the effects of dialysis techniques.

Moreover, also the switch between a dialysis technique (BED) and the other (OL-HDF) could likely result in some changes including mutations in miRNA content of plasma circulating microvesicles. The investigation of several miRNAs implicated in cardiovascular diseases led to the finding of a decreased expression of three miRNAs (miR-92a, miR-223, miR-423-5p) when patients change dialysis therapy.

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Extracorporeal dialysis: techniques and adequacy - A

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