Urinary microRNAs response in adults exposed chronically to fluoride in drinking water

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Introduction

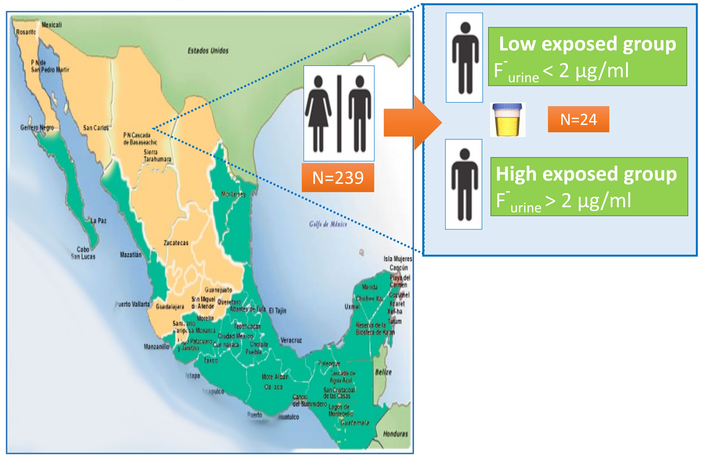
Fluoride in drinking water exceeds safe limits for human consumption in various regions of the world (> 1.5 mg/l). Kidney is the major excretory organ for fluoride, through the urine. Proximal convoluted tubule is main damage area due to ions absorption. different process occurs by fluoride exposure like EMT, hypoxia, apoptosis and inflammation; all these processes are accompanied by dysregulation in gene expression. miRNAs are actively involved in the physiology maintenance of the kidney during life, so alterations in miRNAs profile can evidence damage of the cells.

Objective

In this study, we aimed to identify urinary miRNAs associated with early kidney damage by fluoride exposure. To evaluate in a human population exposure to fluoride in drinking water

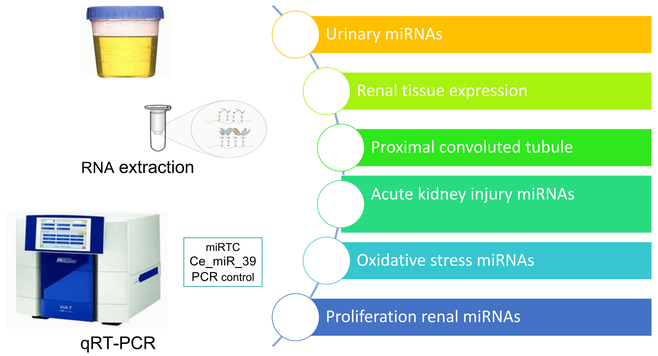
Methods

I. Study Design



The study was approved by the Ethics Committee of the CINVESTAV (COBISH) and the acceptance of each of the participants, by signing a consent form.

II. miRNA selection and extraction



*Only mature and experimentally validated miRNAs with a change in expression of 1.5 times (up or down expression) were selected.

Results

I. Population characteristics

Table 1. General characteristics study population exposed to fluoride

	n	mean ± SD	median	Min- Max	*p value*[2]
Fluoride urine[1] (µg/ml)					
Low Exposed	24	1.34 ± 0.52	1.36	0.47 - 1.96	**0.000**
High Exposed	24	4.84 ± 2.83	4.04	2.77 - 14.56	
Age (years)					
Low Exposed	24	47.41 ± 9.89	49	24 - 60	0.305
High Exposed	24	44.12 ± 12.00	44.5	21 - 61	
Glycemia (mg/dl)					
Low Exposed	24	105.66 ± 22.81	98.5	75 - 173	0.371
High Exposed	24	99 ± 28.08	93	54 - 208	
GFR (ml/min/1.73m²)					
Low Exposed	24	64.05 ± 6.76	64.21	51.81 - 75. 32	0.179
High Exposed	24	61.09 ± 8.22	62.43	48.23 - 76.42	
Cystatin C[1] (µg/ml)					
Low Exposed	24	176.66 ± 113.20	158.68	31.35 - 415.93	**0.020**
High Exposed	24	276.85 ± 170.65	247.47	29.44 - 656.57	
KIM-1 (ng/ml)					
Low Exposed	23	0.87 ± 0.56	0.70	0.22 - 0.2.3	0.261
High Exposed	24	1.11 ± 0.81	0.73	0.78 - 2.65	

GFR: Glomerular filtration rate
SD: standard deviation, [1]Values normalized by urine density, [2]T-student p <0.05

References:
Chung 2013 (DOI: 10.2147/IJNRD.S37885), Weber 2010 (DOI: 10.1373/clinchem.2010.147405), Kito 2015 (DOI:10.1155/2015/465479), Ramachdran 2013 (DOI: 10.1373/clinchem.2013.210245), Aguado-Fraile 2013 (DOI: 10.3265/Nefrologia.pre2013.Aug.12198), Hagiwara 2013 (DOI: /10.1155/2013/173783)

II. Panel miRNAs modified by chronic fluoride exposure

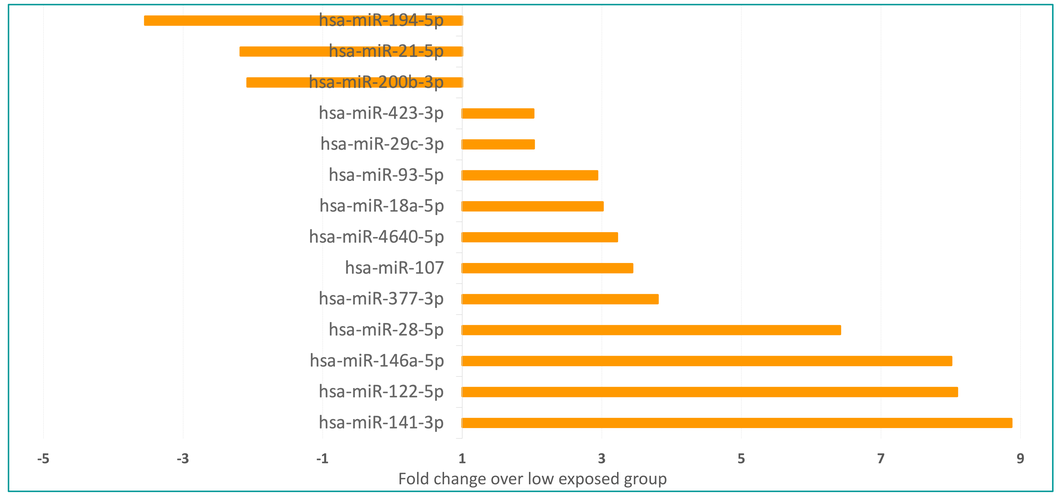


Figure1. A panel of the top 19 urinary miRNAs modified by fluoride exposure (melting curve >30 Ct).Data were obtained from two group samples (n=4) Higher exposure group (4.23 ±2.68 µg/ml F^-_{urine}) and low exposure group (1.05 ±0.04 µg/ml F^-_{urine}), both groups with a mean age of 47.75 ± 6.67 years. SD <1.5 fold, t-student (*p<0.05*).

Table 2. miRNAs valídate targets

miRNA	Validated target genes[1]	Associated Process
hsa-miR-194-5p	GIGYF1 CDH2 IGF1R FZD6	cell adhesion, focal adhesion, adherent junctions, cell carcinoma
hsa-miR-21-5p	PTEN PDCD4 RPS7 RECK BCL2 TPM1 SERPINB5	apoptosis, focal adhesion, p53 signaling pathway
hsa-miR-200b-3p	ZEB1 ZEB2 ANKRD33B CREB1 RND3	oxidative stress induced gene expression via Nrf2, focal adhesion
hsa-miR-423-3p	VEGFA PTMA PABPC3 CDKN1A	VEGF signaling pathway, focal adhesion, renal cell carcinoma,
hsa-miR-29c-3p	MCL1 CTC1 COL4A1 REST TDG DNMT3A	focal adhesion, ECM receptor interaction
hsa-miR-93-5p	FBXL5 KPNA2 CDKN1A E2F1 LAPTM4A	cell cylce
hsa-miR-18a-5p	ATM GIGYF1 HIF1A CREBL2 ESR1 CTGF	mTOR and p53 signaling pathway, renal cell carcinoma
hsa-miR-4640-5p	ARL5C ABT1 EDEM3 E2F7	alternative splicing, coiled coil
hsa-miR-107	CDK6 PLEKHA1 DICER1 AXIN STK38 FERMT2	cell cicle , angiogenesis, actin cytoskeleton organization
hsa-miR-377-3p	EID1 FAM180B EPHB3 SOD1 ARHGAP12	oxidative stress, GTPase activation
hsa-miR-28-5p	SMYD1 TMED4 E2F6 MAPK1	cell cycle
hsa-miR-146a-5p	IRAK1 TRAF6 EGFR SMAD4 NFKB1	ATP binding, coiled coil, inflamatory response, MAPK signaling
hsa-miR-122-5p	OSBPL10 PIGO ADAM17 CCNG1 SLC7A1 CLIC4	chloride intracellular channel, cyclin G1 regulation
hsa-miR-141-3p	ZEB1 ZEB2 BAP1 YRDC ARL5B	GTP binding, focal adhesion

[1]miRTarBase 2016 (Chou 2016), Validated criteria: reporter assay, western blot, qPCR, Next Generation Sequence (NGS).

Conclusion

RNA extraction from frozen urine was successful, despite the small amount of nucleic acids reported from the literature (<100 ng /ml). We extracted a mean of 12ng/ml for profile of miRNAs expression. Our preliminary results show that chronic exposure to fluoride in drinking water modify expression of some miRNAs, which could be useful as biomarkers for fluoride exposure and early kidney damage.

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