VDR GENE EXPRESSION AND GLOBAL DNA METHYLATION ARE ASSOCIATED WITH INSULIN RESISTANCE IN ESRD PATIENTS ON CHRONIC HAEMODIALYSIS

D. Kirmizis[1], F. Chatzopoulou[2], D. Chatzidimitriou[2], G. Tzimagiorgis[3], A. Papagianni[1], G. Efstratiadis[1]

[1]Department of Nephrology, Hippokration General Hospital, Aristotle University, Thessaloniki, Greece;
[2]Laboratory of Microbiology, Aristotle University, Thessaloniki, Greece;
[3]Laboratory of Biochemistry, Aristotle University, Thessaloniki, Greece

OBJECTIVES

Evidence suggest a probable role of genetic inactivation of the vitamin D receptor (VDR) gene, and possibly of epigenetic gene modifications, in insulin resistance in uraemia.

The aim of the present study was to investigate the potential genetic and epigenetic associations of insulin resistance, with vitamin D deficiency, VDR gene expression, and DNA methylation, in nondiabetic end-stage renal disease (ESRD) patients on chronic haemodialysis (HD).

METHODS

- Cross-sectional study: 50 non-diabetic ESRD Caucasian patients stabilized on HD (>6 months) (URR ≥0.65; eKt/V ≥1.2)
- Exclusion criteria: diabetes mellitus, pre-diabetes, liver disease, active infection, malnutrition, autoimmune diseases, malignancies, folate supplementation, antibiotics, corticosteroids or cytotoxic drugs.
- Blood samples taken from a peripheral vein under fasting conditions. Biochemistry, plasma 25(OH)D, fasting glucose, insulin, total homocysteine (Hcy), intact parathormone (iPTH), and serum high-sensitivity C-reactive protein (hsCRP) levels were measured by routine techniques.
- Insulin resistance was assessed with the use of the Homeostasis Model Assessment (HOMAIR=[fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/L)]/22.5).
- VDR gene expression (VDRG) (real-time PCR): relative quantification of leukocyte VDR mRNA, reported as 2-ΔΔCt, was performed with the use of calibration curves using GAPDH mRNA as an endogenous control.
- Global DNA methylation (GMeth) status (colorimetry): the final concentration was then calculated by reference to standard curves performed with the corresponding recombinant molecule.

RESULTS

Parameter	1st tertile (n=17) (≤2.31)	2nd tertile (n=18) (2.32-4.57)	3rd quartile (n=15) (>4.57)
Age, years	68 (42-87)	65 (47-85)	74 (43-86)
Gender (male), n(%)	12 (71)	10 (44)*	8 (53)*
HD duration, months	71 (12-291)	45 (12-108)	58 (12-96)
Dialysis method: HD, n (%) Online HDF, n(%)	10 (77) 3 (23)	8 (57) 6 (43)	8 (67) 4 (33)
BMI, kg/m²	22.6±1.9	24.9±2.8	25.6 ±2.3*
Kt/V	1.18±0.3	1.19±0.44	1.21±0.38
Mean BP (mmHg)	104±7	106±9	105±4
CVD, n(%)	8 (62)	8 (57)	6 (50)
Art. hypertension, n(%)	14 (82)	16 (89)	14 (93)
Hb, g/dL	10.8±0.2	11.3±0.4	11.1±0.2
WBC, x10⁹/mL	8.6±0.4	8.2±1.0	7.8±0.5
Glucose, mg/dL	85±12	104±11	112±30
Insuline, μU/mL	9.1 (3.0-38.3)	13.7 (3.4-33.5)	16.7 (3.6-53.6)*
HOMAIR	2.21 (0.58-13.32)	3.48 (0.65-13.80)	4.93 (0.86-18.27)*
hsCRP, mg/L	0.80 (0.22-2.11)	1.79 (1.1-3.9)*	3.26 (2.1-5.9)*
Albumin, g/dL	3.5±0.4	3.4±0.3	3.3±0.2
Urea, mg/dL	146±39	148±37	141±42
Alkaline phosphatase, U/L	101±54	122±98	188±122*
25(OH)D, pg/mL	10.2±6.2	9.1±2.6	6.7±1.8*
iPTH, pg/mL	222±183	369±255	651±460*
Hcy, μmol/L	25.5±11.9	21.7±10.8	16.3±6.7*
GMeth (%)	3.66±1.03	4.28±0.95	4.68±0.84*
VDRG	0.84±0.65	0.66±0.79	0.16±0.38*

Results are expressed as mean ±SD, median (range) or patient number (%), as appropriate. *P<0.05 compared to the respective 1st tertile

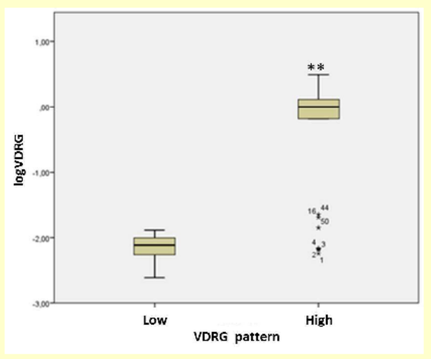
Parameter	Low VDRG (n=20)	High VDRG (n=30)
Age, years	69 (47-90)	67 (42-87)
Gender (male), n(%)	14 (70)	18 (60)*
HD duration, months	49 (6-108)	64 (6-291)*
BMI, kg/m2	25.6±2.7	23.3±2.2*
Glucose, mg/dL	115±24	95±18
Insulin, μU/mL	19.3± 10.7	11.9± 10.4**
HOMAIR	4.22 (0.62-16.31)	2.29 (0.58-18.27)**
hsCRP, mg/L	0.82 (0.11-5.5)	0.7 (0.1-6.2)
Albumin, g/dL	3.4±0.5	3.3±0.3
Urea, mg/dL	134±35	155±36
Alkaline phosphatase, U/L	140±95	121±94
25(OH)D, pg/mL	9.8±4.6	10.1±5.3
iPTH, pg/mL	663±436	295±225*
Hcy, μmol/L	19.1±7.3	26.8±12.8*
GMeth (%)	4.26±0.87	4.24±2.0
VDRG	0.0078±0.0031	1.1±0.92**

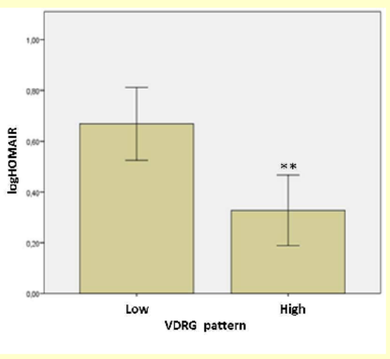
Results are expressed as mean ±SD, median (range) or patient number (%), as appropriate.
* *P*<0.05 compared to low VDRG; ** *P*<0.001 compared to low VDRG

Low VDRG			High VDRG		
Parameters	r	P value	Parameters	r	P value
VDRG	-0.493	0.002	Hcy	-0.419	0.002
GMeth	0,513	0.003	VDRG	-0.398	0.003
iPTH	0.591	0.04	GMeth	0,38	0,05
Out of Model:			*Out of Model:*		
BMI	0.228	0.207	Urea	0.310	0.102
Hcy	-0.215	0.258	iPTH	-0.214	0.264
logCRP	-0,301	0.265	logCRP	-0.197	0.281
25(OH)D	0,17	0,276	25(OH)D	0.172	0.374
Urea	0.094	0.369	Calcitriol	0.144	0.422
Calcitriol	0.144	0.389	BMI	0.115	0.518
HD duration	-0.038	0.464	HD duration	-0.022	0.908

†Adjusted for age.

- HOMAIR tertiles differed significantly in hsCRP, fasting insulin, iPTH, GMeth values, Hcy, 25(OH)D, and VDRG.
- Two discrete patterns of VDRG, a high and a low expression pattern, were noticed. They differed significantly in iPTH (295±225 vs 663±436 pg/ml respectively, P=0.014), HOMAIR (3.14±3.66 vs 5.88±4.27 respectively, P<0.001) and Hcy values (26.75±12.85 vs 19.07±7.27 μmol/L respectively, P=0.02).
- On multivariate analysis, VDRG and GMeth were strong independent correlates of HOMAIR in both VDRG groups, whereas iPTH and Hcy were correlates of HOMAIR in the low and the high VDRG groups, respectively.





CONCLUSIONS

VDR gene expression and DNA methylation are independent correlates of insulin resistance in non-diabetic ESRD patients.

REFERENCES:

1. DeFronzo RA, Alvestrand A, Smith D et al. Insulin resistance in uraemia. J Clin Invest 1981; 67: 563–568
2. Kobayashi S, Maesato K, Moriya H et al. Insulin resistance in patients with chronic kidney disease. Am J Kidney Dis 2005; 45: 275–280
3. Siew ED, Ikizler TA. Insulin resistance and protein energy metabolism in patients with advanced chronic kidney disease. Semin Dial 2010; 23: 378–382
4. Friedman DJ, Bhatt N, Hayman NS, Nichols BJ, Herman M, Nikolaev N, Danziger J. Impact of activated vitamin D on insulin resistance in nondiabetic chronic kidney disease patients. Clin Endocrinol (Oxf) 2012; 77; 56–61
5. Kobayashi S, Maejima S, Ikeda T, Nagase M. Impact of dialysis therapy on insulin resistance in end-stage renal disease: comparison of haemodialysis and continuous ambulatory peritoneal dialysis. Nephrol Dial Transplant 2000; 15: 65–70

254--MP
53 ERA
Chronic Kidney Disease. Pathophysiology, progression & risk factors.
Dimitrios Kirmizis
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA AUSTRIA MAY 21st-24th, 2016
Held jointly with Österreichische Gesellschaft für Nephrologie
PosterSessionOnline.com