ORIGINAL ARTICLE

Netrin-1 and Cyclophilin A (CypA): biomarkers of inflammation and kidney injury in Alport syndrome: a pilot study

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ABSTRACT

Background: Netrin-1 and Cyclophilin A (CypA) participate in the pathological processes of inflammation, oxidative stress and apoptosis. Our objective was to determine the level of serum Netrin-1 & Cyclophilin A (CypA) in Alport syndrome.
Materials and Methods: The study included 10 Alport syndrome patients, their age ranged from 5-13 years (8.50±2.87) (mean age ±SD), they were 7 females and 3 males (ratio of 1:0.43) and 10 age and sex matched healthy children. Serum Netrin-1 and Cyclophilin A (CypA) were measured using ELISA kits.
Results: All patients presented with haematuria, while 3 cases had mild proteinuria. The renal pathology revealed features like thin basement membrane disease. Sensorineural hearing loss was present in 8 patients and no patients had retinitis pigmentosa. The serum levels of Netrin-1 were 160.47ng/mL ± 12.833 for controls and 141.91ng/mL ±16.953 for patients ($p < 0.01$), for CypA was 7.55ng/mL ±0.74 in patients compared to 6.27ng/mL ±0.37 in controls ($p<0.001$).
Conclusion: To the best of our knowledge, this is the first research to investigate the levels of Netrin-1 and Cyclophilin A in Alport syndrome patients.
Keywords: Alport syndrome, Cyclophilin A (CypA), Netrin-1, inflammation.

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INTRODUCTION

Alport syndrome is a rare genetic disorder of kidney inflammation affecting around 1 in 5,000-10,000 children distinguished by bilateral sensorineural hearing loss, progressive kidney disease and eye anomalies (1). The hallmark of this disease is the haematuria (presence of blood in the urine) early in life, proteinuria, with kidney inefficiency (progressive deterioration in kidney function) that eventually leads to kidney failure due to mutations in *COL4A3, COL4A4*, or *COL4A5* genes *(2-5)*. The disorder is set on by abnormalities in the genes that produce a connective tissue protein, one of the several collagen subunits.

Defects of glomerular basement membrane (GBM) is necessary for kidney filtration and is a key constituent of the glomerular capillary wall associated with Alport syndrome (6).

Netrin-1 is expressed in numerous tissues comprising renal tissues. Kidney is the highest netrin-1-expressing among studied organs. Furthermore, decrease in the Netrin-1 level compared to control was reported in chronic kidney diseases suggesting Netrin-1 as an early biomarker for chronic kidney disease. Netrin-1 represents an endogenous anti-inflammatory pathway and regulator of apoptosis that is widely expressed in the kidney (7,8). Netrin-1 plays a role in the migration of vascular endothelial cells and accelerating angiogenesis (9) that participates in the growth and development of non-neural tissue and controls the development of malignancies, cardiovascular disorders, kidney diseases, growth and regulation of inflammation (10,11). After ischemia, dysregulation of Netrin-1 contributes to the progress of renal failure. Netrin-1 is also known to control inflammatory cell migration and their functions in various diseases and overwhelm acute kidney injury (12).

Cyclophilin A (CypA) concentrations have been inspected as prospective biomarkers of kidney injury. Increased urine and serum CypA levels associated with subsequent development of acute kidney injury (13). CypA production is stimulated by oxidative stress and inflammation, working as a mediator of tissue injury. CypA is synthesized by normal kidney, so with any damage to the kidney, CypA concentration will increase. CypA is an indirect matrix metalloproteinase inducer and is increased in patients who had impaired kidney function (11). In previous studies, CypA level was a hopeful biomarker for the early detection of chronic kidney disease (12).

The aim of this study was to determine whether Netrin-1 and Cyclophilin A (CypA) contribute to inflammation and kidney injury in Alport syndrome.

MATERIALS AND METHODS

Patients with suspected Alport syndrome after clinical, laboratory and pathologic examination were recruited from the clinics of National Research Centre, Giza, Egypt. The cohort included 10 Alport syndrome patients, their age ranged from 5-13 years (mean age ±SD) 8.50±2.87. They were 7 females and 3 males (ratio of 1:0.43) and 10 age and sex matched healthy children. Detailed family history was collected from the patients and/or their parents, all patients were evaluated for ocular lesions, hearing loss and renal pathology. Serum CypA and Netrin-1 levels were determined by ELISA (R&D Systems) according to the manufacturer's instructions.

The study was approved by the Ethical Committee of the National Research Centre in accordance with the Declaration of Helsinki protocols. In addition, informed written consents were signed by the parents or legal guardians of the studied patients and controls.

Statistical analyses

The data were analysed by using the SPSS 15.0 (SPSS, Chicago, IL, USA) software and are expressed as mean ± standard deviation (SD). The results were considered significant at $P < 0.05$. Student's t-test was used for the analysis of data. A two-sided p value <0.05 was used to define statistical significance.

RESULTS

All patients had haematuria, while 3 cases had mild proteinuria. The renal pathology revealed thin basement membrane disease with irregular thinning and splitting of basement membranes due to the inherited abnormality in collagen tissue. Sensorineural hearing loss was present in 8 patients detected by auditory brain response test (ABR), while no patients had retinitis pigmentosa (Table 1).

Serum levels of Netrin-1 (Table 2) were 160.47ng/mL ±12.83 for controls and 141.91ng/mL ±16.95 for patients ($p=0.01$), while CypA was 7.55ng/mL ±0.74 in patients, compared to 6.27 ng/mL ±0.37 in controls ($p=0.001$).

DISCUSSION

All our Alport syndrome patients had haematuria, some had proteinuria, but none had hypertension, this is similar to the findings of previous studies (13). The renal pathology revealed mesangio proliferative glomerulonephritis in all our patients. A previous study (14) noted that the pathological findings in renal biopsy of Alport syndrome patients are mesangio proliferative glomerulonephritis which occurs in 80-99% of patients.

Sensorineural hearing loss was present in 8 patients (80%), previous studies (15,16) reported hearing loss in 52% of his cohort. Retinitis pigmentosa was not detected in all our patients, this contrasts with the findings of another study (17) which described inner retinal changes in all his patients and that the affection of the eye may occur later in life (16).

Netrin-1 serum level was 141.91ng/mL±16.95 in patients compared to controls 160.47ng/mL±12.83, with a decrease in the Netrin-1 level compared to controls was reported in chronic kidney diseases suggesting Netrin-1 as an early biomarker for chronic kidney disease (8).

The Cyclophilin A (CypA) level was 7.55ng/mL ±0.74 compared to controls 6.27ng/ml =L±0.37. Our findings are consistent with studies showing that CypA level will be augmented with any damage to the kidney (14,20).

Since Netrin-1 and CypA are major regulators of inflammation and apoptosis in chronic kidney disease, they may be useful therapeutic molecules for treating chronic kidney diseases. Nevertheless, it is worth mentioning that more studies regarding the role of Netrin-1 and CypA in Alport syndrome are recommended. Limitation of the study is the small sample size and further studies should be more extensively investigated and larger sample size studies are necessary.

Table 1. The clinical characteristics of Alport syndrome patients

Age (years)	Gender	Consanguinity	Similarly affected members	Haematuria	Proteinuria	Auditory Brain Response (ABR)	Retinitis pigmentosa
5	F		+	+	-	+	-
5	F	+	+	+	-	+	-
6	F	+	+	+	-	+	-
7	F		+	+	-	+	-
8	M	+	-	+	-	+	-
8	F	+	-	+	-	-	-
10	M	+	-	+	-	-	-
11	M	+	+	+	+	+	-
12	F	-	+	+	+	+	-
13	M	-	+	+	+	+	-

Table 2. Mean levels of Netrin-1 and Cyclophilin A in Alport syndrome patients & controls

Biomarker	Patients	Controls	*P*-value
Netrin-1 (ng/mL)	141.91 ± 16.95	160.47 ± 12.83	0.01
Cyclophilin A (ng/mL)	7.55 ± 0.74	6.27 ± 0.37	0.001

CONCLUSION

The challenge is to translate these findings into disease treatment targets or new drug development. To the best of our knowledge, this is the first study to investigate the levels of Netrin-1 and CypA in Alport syndrome patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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