IJKD KIDNEY DISEASES

Correlation Between Serum Level of Fibroblast Growth Factor 2 and Severity of Reflux Nephropathy

Seyed-Javad Nasiri,[1] Nakysa Hooman,[1] Mitra Mehrazma,[2] Mansoor Movahed[3]

[1]Department of Pediatric Surgery, Ali-Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran
[2]Department of Pathology, Ali-Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran
[3]Department of Nuclear Medicine, Hashemi-Nejad Hospital, Iran University of Medical Sciences, Tehran, Iran

Keywords. vesicoureteral reflux, fibroblast growth factor 2, technetium Tc 99m dimercaptosuccinic acid, child

Introduction. Fibroblast growth factor 2 (FGF2) is a potent mitogenic factor of cortical fibroblasts and induces kidney fibrosis. We hypothesized that serum levels of FGF2 has an association with the severity of vesicoureteral reflux (VUR) and renal parenchymal scar.
Materials and Methods. Between 2007 and 2009, a total of 28 children with VUR were enrolled in this study and were compared with 52 healthy children. All children with VUR underwent technetium Tc 99m dimercaptosuccinic acid renal scintigraphy. Fibroblast growth factor 2 was measured in both groups.
Results. The mean level of FGF2 was 65.0 ± 19.0 pg/mL in the VUR group and 62.5 ± 15.3 pg/mL in the control group ($P > .05$). There was no correlation between serum levels of FGF2 and sex, age, or the grade of VUR. Of the 28 children with VUR, 19 had renal parenchymal scar on dimercaptosuccinic acid renal scintigraphy. The mean serum level of FGF2 was not significantly different in the children with and without renal parenchymal scar.
Conclusions. This study showed no correlation between serum FGF2 and renal parenchymal scar or grade of VUR.

IJKD 2015;9:46-9
www.ijkd.org

Original Paper

INTRODUCTION

Vesicoureteral reflux (VUR) is one of the most common malformations of the urinary tract system in children, which may be primary or secondary. The potential of recurrent urinary tract infection (UTI) is relatively high in children with VUR. Recurrent UTI is the main cause of renal parenchymal injury (reflux nephropathy) and its secondary complications such as hypertension and chronic kidney failure, especially in developing countries.[1] To prevent or reduce the frequency of these complications, timely detection and appropriate care and treatment are essential. The main goal of treatment in these patients is prevention of UTI and renal damage. Hence, assessment of the renal damage is essential during the process of the treatment and care. Technetium Tc 99m dimercaptosuccinic acid (^{Tc99m}-DMSA) renal scintigraphy has been adopted for this purpose, but in addition to being costly and time consuming, it is not available everywhere. Therefore, finding another simple, cost benefit, available, and reliable test for prediction of the renal scars may facilitate timely diagnosis and management of the disease and ultimately prevent the complications.

Recently, measurement of the serum level of fibroblast growth factor 2 (FGF2; also known as basic fibroblast growth factor) has been introduced, which is claimed to have independent relationship with the severity of the scar tissue in the kidney.[2] Fibroblast growth factor 2 is a myogenic and chemotaxis factor of the fibroblasts with a molecular weight of 18 kDa. Fibroblast growth factor 2 will increase the growth of the fibroblasts by autocine stimulation, and the fibroblasts will cause renal parenchymal damages.[2-5] In this study, we assessed

the relationship between serum levels of FGF2 and degree of renal parenchymal damage evident by DMSA of children.

MATERIALS AND METHODS

This was a case-control study conducted between 2004 and 2006 in a university hospital. Twenty-eight children (21 girls and 7 boys) with VUR detected by cystography were enrolled in the study. All patients with VUR underwent DMSA scintigraphy. Fifty-two healthy children (23 girls and 29 boys) with no history of UTI and normal renal ultrasonography were considered as controls. Consent was taken from the caregivers or patients. The study was performed in accordance with the ethics standards laid down in the 1964 Declaration of Helsinki revised in Tokyo 2008. The study protocol was approved by the ethics committee of Iran University of Medical Sciences.

All renal parenchymal scans were studied by a specialist in nuclear medicine that was unaware of the patients' history. The results were categorized into normal tissue and inflammation, as previously described.[6]

Serum FGF2 level was measured using an enzyme-linked immunosorbent assay (ELISA) measurement kit (R&D Systems, UK). This 2-site ELISA was performed according to the manufacturer's instructions. Briefly, a capturing anti-FGF2 monoclonal antibody was coated onto the microtiter plate provided in the kit, and serum samples were added together with a horseradish peroxidase-conjugated anti-FGF2 monoclonal antibody. After incubation for 90 minutes at room temperature, the substrate reaction was stopped by the addition of tetramethylbenzidine. Absorbance of the colored reaction product was read with an ELISA microplate reader at 450 nm. Concentrations of circulating FGF2 in serum samples were determined by relating absorbance values to values from a standard curve generated with recombinant human FGF2. The limit of detection of FGF2 using this ELISA assay was 1 pg/mL. All serum samples were assayed in duplicate by one operator to assess interassay precision. The laboratory coefficients of variations were 3.5% to 8.2%.

For comparison of the continuous variable, independent Student *t* test or nonparametric median test were applied. Categorical data were compared using the chi-square test, and for assessment of correlations, the Pearson test was used. *P* values less than .05 were accepted for significance.

RESULTS

Of the 28 children (mean age, 49 ± 34 months) with VUR, 21 had moderate (grade II and III) and 7 had severe VUR. Technetium Tc 99m dimercaptosuccinic acid scintigraphy revealed renal parenchymal scar in19 children (10 were unilateral and 9 bilateral). The control group included 52 children electively admitted to the Department of Surgery for repair of hydrocele, hypospadiasis, anal fissure, cleft palate, foreign body, ambiguous genitalia, laryngomalacia, or undesendent testes. All renal ultrasonography results and kidney function tests were normal in the control group. The mean age was 29.2 ± 37.4 months in the control group (*P* = .03).

Because the two groups were not age- and sex-marched, we first analyzed the differences of FGF2 levels based on age categories and sex distribution before comparing the two groups. Age categories were younger than 24 moths (n = 41), between 24 and 48 months (n = 16), and older than 48 months (n = 23). The mean FGF2 levels were 61.2 ± 17.0 pg/mL, 70.5 ± 17.6 pg/mL, and 62.4 ± 13.8 pg/mL, respectively (*P* = .15). The mean FGF2 level was 62.0 ± 15.6 pg/mL in the girls and 65.3 ± 18.0 pg/mL in the boys (*P* = .40). This analysis revealed the age and sex had no impact on FGF2 level.

The mean level of FGF2 was 65.0 ± 19.0 pg/mL in the VUR group and 62.5 ± 15.3 pg/mL in the control group (*P* > .05). In the VUR group we compared FGF2 levels based on the severity of VUR and renal parenchymal scar. Categorizing the DMSA results, the mean FGF2 were not significantly different in children with no scar as compared with those with unilateral or bilaterial scars (64.7 ± 16.4 pg/mL versus 71.0 ± 25.7 pg/mL for unilateral and 60.0 ± 14.4 pg/mL for bilateral scars; *P* = .49). Moreover, the level of FGF2 was not different in children with moderate and severe VUR.

DISCUSSION

Urinary tract infection is a common infection in children and infants. Vesicouretral reflux predisposes the children to UTI that if treated accordingly or followed up precisely, the risk of renal parenchymal scar will increase.[6] The prescience

of lower urinary tract dysfunction has adverse effects on kidney function.[7] Moreover, there is a major concern about the policy of administration antibiotic prophylaxis to children with VUR or recurrent UTI, due to antibiotic resistance.[8] Some studies have assessed the value of procalcitonin for detection of VUR or its severity in children with UTI. However, the sensitivity and specificity suggested in studies are not uniform and has wide discrepancy.[9,10] Serum levels of FGF2 is another suggested predictive factor, but the literature is not consistent about its usefulness.

There is not enough evidence about the relationship of the serum level of FGF and reflux nephropathy. In an experimental study on rats with VUR, Sencan and coworkers showed that the apoptosis activity was initiated through the Caspass pathway and they found the collagen tissue increase in renal parenchyma of those rats with VUR.[11] Our study revealed that serum level of FGF2 has no correlation with renal scar or the severity of VUR. Fibroblast growth factor 2, a monochain peptide with 18-kDa molecular weight, is a myogenic and chemotactic factor for the fibroblasts. During an inflammation process, accumulation of the lymphocytes, monocytes, and macrophages results in matrix producer cells, specifically fibroblasts which will result in synthesis activation of a lot of extra cellular matrix and fibrosis. It is shown than the severity of the tubulointerstitial fibrosis is in direct relationship with kidney function. It is thought that FGF2 has a significant role in the pathogenesis of the kidney fibrosis, because FGF2 is a strength mitogen for cortical fibroblasts, which by its autocrine stimulation, induces rapid growth of the fibroblasts, and produces renal fibrosis.[12] In a case-control study conducted by Kobayashi and colleagues, higher levels of FGF2 were found in children with VUR when scar is present.[2]

A major limitation of our study was the unmatched groups; however, we assessed the impact of age and sex as confounding factors and did not found any correlation. Okamoto and colleagues determined age-related serum levels of FGF2 and observed that there were no significant differences between children and adults. They added that the value for children had a wider range and suggested the comparison should be done in age-matched controls.[13] In addition, the majority of the cases had moderate grades of VUR that might reduce the possibility of the size or the number of renal scars.

CONCLUSIONS

More studies are needed to evaluate the value of serum FGF2 as a substitute for DMSA renal scintigraphy for diagnosis of renal scar in children with VUR.

ACKNOWLEDGEMENTS

The authors would like to thank Mr Ali Mousavi, MSc, at the Hormone Section of the Laboratory of Ali-Asgar Children, Hospital, for performing the measurement of FGF2. This study received a funding grant from Iran University of Medical Sciences (Number 223, dated 2004).

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Madani K, Otoukesh H, Rastegar A, Van Why S. Chronic renal failure in Iranian children. Pediatr Nephrol. 2001;16:140-4.
2. Kobayashi H, Miyakita H, Yamataka A, Koga H, Lane GJ, Miyano T. Serum basic fibroblast growth factor as a marker of reflux nephropathy. J Pediatr Surg. 2004;39:1853-5.
3. Miyakita H, Puri P, Surana R, Kobayashi H, Reen DJ. Serum intercellular adhesion molecule (ICAM-1), a marker of renal scarring in infants with vesico-ureteric reflux. Br J Urol. 1995;76:249-51
4. Kobayashi H, Wang Y, Puri P. Increased levels of circulating endothelial leukocyte adhesion molecule-1 (ELAM-1) in children with reflux nephropathy. Eur Urol. 1997;31:343-6
5. Kaneyama K, Kobayashi H, Yamataka A, Lane GJ, Miyano T. Serum soluble vascular cell adhesion molecule-1 (VCAM-1) concentrations in children with reflux nephropathy. Pediatr Surg Int. 2005;21:39-42.
6. Sobouti B, Hooman N, Movahed M. The effect of vitamin E or vitamin A on the prevention of renal scarring in children with acute pyelonephritis. Pediatr Nephrol. 2013;28:277-83.
7. Otukesh H, Sharifiaghdas F, Hoseini R, et al. Long-term upper and lower urinary tract functions in children with posterior urethral valves. J Pediatr Urol. 2010;6:143-7.
8. Nateghian AR, Robinson JL, Mohandessi S, Hooman N. Resistance pattern of breakthrough urinary tract infections in children on antibiotic prophylaxis. J Infect Public Health. 2009;2:147-52.
9. Rahimzadeh N, Otukesh H, Hoseini R, Shadani S, Hooman N. Serum procalcitonin level for prediction of high-grade vesicoureteral reflux in urinary tract infection. Iran J Kidney Dis. 2014;8:105-8.

10. Mortazavi F, Ghojazadeh M. Usefulness of serum procalcitonin level for prediction of vesicoureteral reflux in pediatric urinary tract infection. Iran J Kidney Dis. 2014;8:37-41.
11. Sencan A, Vatansever S, Yilmaz O, et al. Early renal parenchymal histological changes in an experimental model of vesico-ureteral reflux and the role of apoptosis. Scand J Urol Nephrol. 2008;42:213-9.
12. Strutz F, Zeisberg M, Hemmerlein B, et al. Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. Kidney Int. 2000;57:1521-38.
13. Okamoto Y, Tanaka M, Miyahara N, et al. Determination of the age-related changes of serum basic fibroblast growth factor (bFGF) level in healthy Japanese subjects. Clin Lab. 2009;55:114-9.

Correspondence to:
Seyed-Javad Nasiri, MD
Department of Pediatric Surgery, Ali-Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran
E-mail: sarveravan@yahoo.com

Received March 2014
Revised June 2014
Accepted July 2014