Mean Urinary Cytokine MCP-1 in Children with Urinary Tract Obstruction and Healthy Children

Simin Sadeghi-Bojd,[1,2,*] Ebrahim Alijani,[3] and Mahdie Sadat Mahdavian[4]

[1]Pediatric Nephrologist, Children & Adolescent Health Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran
[2]Department of Pediaterics, School of Medicine, Zahedan University of Medical Sciences, Zahedan, IR Iran
[3]Clinical Immunology Research Center, Zahedan University of Medical Sciences, Zahedan, IR Iran
[4]Student of School of Medicine, Zahedan University of Medical Sciences, Zahedan, IR Iran

[*] *Corresponding author*: Simin Sadeghi-Bojd, Research Center for Children and Adolescents Health, Zahedan University of Medical Sciences, Zahedan, IR Iran. Tel: +98-9151417880, E-mail: sisadegh@yahoo.com

Abstract

Background and Objectives: Hydronephrosis is defined as varying degrees of dilatation in renal pelvis and calyxes, which leads to renal parenchymal atrophy together with obstruction of urinary flow. The present study was conducted to compare levels of urinary cytokine MCP-1 in children with urinary obstruction and healthy children attending Ali-Asghar hospital of the city of Zahedan.
Methods: The present case-control study recruited 20 children with obstructive hydronephrosis, 20 children with non-obstructive hydronephrosis, and 30 healthy children as the control group that matched in terms of gender and age and were selected by the convenience sampling method. Urine samples were taken from these children, and urinary creatinine and MCP-1 levels were measured. Data were analyzed with the SPSS-21 software using analysis of variance (ANOVA), Kruskal-Wallis, and Mann-Whitney tests.
Results: Mean MCP-1 level was 542.56 ng/L in the obstructive hydronephrosis group, 424.7 ng/L in the non-obstructive hydronephrosis, and 335.97 ng/L in the control group. Mean MCP-1 to creatinine ratio was 58.94 in the obstructive hydronephrosis group, 39.58 in non-obstructive hydronephrosis, and 33.1 in the control group. Mean MCP-1 and MCP-1 to creatinine ratio was significantly higher in the obstructive hydronephrosis group compared with non-obstructive hydronephrosis and control groups (P < 0.05). No significant difference was found in MCP-1 and MCP-1 to creatinine ratio between patients with unilateral and bilateral obstructive hydronephrosis (P < 0.05).
Conclusions: The present study results showed that MCP-1 could be a marker for renal tubular injury in patients with obstructive hydronephrosis.

Keywords: Urinary Tract Obstruction, Cytokine, Monocyte Chemoattractant Protein-1

1. Background

Hydronephrosis, defined as dilatation of renal pelvis and calyxes, is a common embryonic and postnatal problem that is diagnosed by ultrasound. Given the increasing application of ultrasound during the prenatal period and infancy, the rate of early diagnosis of hydronephrosis is increasing, such that its prenatal prevalence is reported 1% to 5%, while some cases present late symptoms, such as flank pain, nausea, vomiting, and abdominal pain (1, 2).

Fetal hydronephrosis can be caused by distal obstruction at the ureteropelvic junction, ureterovesical junction, or at bladder outlet. However, it may also be caused with no obstruction, or due to a reflux. Sometimes, in mild cases, it is regarded as a normal variant. Vesicoureteral reflux, upper or lower urinary tract obstructions and neurogenic bladder are among common causes of neonatal hydronephrosis (2, 3).

Urinary tract obstruction causes hemodynamic changes, impaired intra-tubular pressure, progressive damage to nephrons with tubular atrophy, and fibrosis of interstitial tissue. This process is initiated through free radical species, growth factors, nitric oxide, various cytokines, and prostaglandins (4, 5). The damage presents tubular atrophy, cellular proliferation, apoptosis, fibrosis, scarring, and development, and progression of fibrosis engages various parts of the renal tissue, including interstitial tissue and tubules, glomeruli, and renal vessels (6). Apoptosis of tubular cells releases biomarkers in the urine through epithelial-mesangial trans-differentiation.

Therefore, the non-invasive method of urinary biomarkers can be used to assess progress of nephropathy and predict functional and structural changes (4, 5).

Progressive renal fibrosis is exacerbated by inflammation of renal tissues, for which, different inflammatory

cells, including macrophages and monocytes are responsible. By producing a series of pro-inflammatory cytokines, such as MCP-1, these inflammatory cells activate renal fibroblasts, and subsequently cause renal tissue fibrosis (7).

Monocyte Chemoattractant Protein-1 (MCP-1) is an example of inflammatory mediators from the beta-chemokine family, and according to data obtained from human and animal studies, MCP-1 is highly likely to be responsible for the absorption and accumulation of monocytes in the kidneys. Recent studies have shown that MCP-1 activates monocytes as well as other inflammatory cells, and this may be due to ischemia caused in the renal tubular cells, which leads to the production of various cytokines and inflammatory mediators (4, 5).

Since urinary tract obstruction occurs in early renal development, it may affect renal functional capacity (6). Serum creatinine is not a reliable marker for the assessment of renal damage due to obstruction because its level does not increase until 50% of nephrons are lost. Therefore, to assess severity of obstruction and differential renal function, better alternatives, such as imaging methods, are used, including renal scintigraphy modalities such as ^{99m}Tc- MAG3 mercapto-acetyl-triglycine (MAG3), ^{99m}Tc-DTPA, and ^{99m}Tc-EC scans, which are based on the amount of radioactive substance excretion; if less than 1.2 is excreted in longer than 20 minutes, or kidney does not function, obstruction is suggested. These techniques are expensive and unavailable in some countries and health centers, besides, they are invasive and require injections. There is also a need for frequent sonography and urinary renography in these children, which stresses them in addition to being time-consuming. Moreover, the above methods lacked sufficient sensitivity and specificity for identifying cases requiring treatment (7).

Given the need for a sensitive method to control specific changes in renal function in patients with urinary tract obstruction and to identify MCP-1 as a urinary biomarker (8), the present study was conducted to assess the level of urinary cytokine MCP-1 in children with urinary tract obstruction compared to healthy children.

2. Methods

2.1. Patients

In the present case-control study, 40 children initially diagnosed with asymptomatic hydronephrosis, who were presented to the Nephrology Clinic of Ali-Asghar hospital of Zahedan (Iran) were assessed. All patients were aged between 1 month and 5 years, with unilateral obstruction and no urinary tract infection. Children with renal failure, bilateral obstruction, or posterior urethral valve reflux were excluded.

Renal and urinary tract sonography of all patients was performed by one person. Hydronephrosis was confirmed if hydronephrosis higher than grade 2 was found in ultrasound, and the pelvis diameter was greater than 10 mm.

Renography was carried out in patients with hydronephrosis. Ureteropelvic obstruction was confirmed if the ureter was not visible. Retrograde cystography was performed to rule out urinary reflux.

Patients diagnosed with hydronephrosis were divided to 2 groups:

The first group comprised of patients with obstructive hydronephrosis, and the second group comprised of patients with non-obstructive hydronephrosis. The third group (as controls) comprised of 30 healthy children visiting the nephrology clinic for routine examinations and growth control, and matched the groups of patients in terms of age and gender. Parents' consents were obtained, and the study was approved by the ethics committee of Zahedan University of Medical Sciences.

Urine samples were taken from all children in case and control groups to measure urinary cytokine MCP-1 and Cr levels. Morning urine samples were collected in sterile containers, and then transferred to a -80°C freezer.

Measurement of urinary MCP-1 was performed using relevant Enzyme Linked Immunosorbent Assay (ELISA) kit and according to the manufacturer's instructions (Hangzho Eastbiopharm Co LTD, China), with detection range of 15 ng/L to 1500 ng/L. For standardization of urinary (MCP-1)/Cr ratio, (ng/L)/ (mg/mL) was also calculated. The urinary creatinine level was assessed using the creatinine kit (mg/dL) by the photometric method (Pars Azmoon), according to the manufacturer's instructions.

2.2. Statistical Analysis

Data were entered in the SPSS-21 software. Data were described using central and distribution indices (mean and standard deviation) and descriptive statistical Tables of frequency percentage and graphs. Analysis of variance (ANOVA) test was used to compare variables of normal distribution, and non-parametric Kruskal-Wallis and Mann-Whitney tests were used to compare variables of non-normal distribution between the groups. P values of < 0.05 were considered significant.

3. Results

The present case-control study was conducted to compare levels of urinary MCP-1 in children with urinary tract obstruction and healthy children visiting the Pediatrics Nephrology Clinic of Ali-Asghar hospital in the city of Zahedan. This study assessed 70 children in 3 groups:

the first group included 20 children with obstructive hydronephrosis, the second group comprised 20 children with non-obstructive hydronephrosis, and the third group comprised of 30 healthy children.

According to Table 1, in the group of children with obstructive hydronephrosis, 4 (20%) were female and 16 (80%) were male, in the non-obstructive hydronephrosis group, 5 (25%) were female and 15 (75%) were male, and in the control group, 12 (40%) were female and 18 (16%) were male. The Chi-square test showed no significant differences (P = 0.27).

According to Table 2, mean and standard deviation of age was 23.5 ± 21.79 months in children with obstructive hydronephrosis, 18.35 ± 19.3 months in children with non-obstructive hydronephrosis, and also 25.18 ± 20.22 months in the control group children. Kruskal-Wallis test showed that the difference was not statistically significant (P = 0.25).

According to Table 3, mean and standard deviation of glomerular filtration rate (GFR) was 75.75 ± 21.99 mL/minute in children with obstructive hydronephrosis, 71.23 ± 18.34 mL/minute in children with non-obstructive hydronephrosis, and 84.32 ± 21.17 mL/minute in the control group children. Analysis of variance (ANOVA) test showed that this difference was not significant (P = 0.082).

According to Table 4, mean and standard deviation of urinary MCP-1 was 542.56 ± 56.1 ng/L in children with obstructive hydronephrosis, 424.7 ± 66.25 ng/L in children with non-obstructive hydronephrosis, and 335.97 ± 81.49 ng/L in control group children. The ANOVA test showed that this difference was statistically significant (P < 0.0001).

According to Table 5, mean and standard deviation of MCP-1 to urinary creatinine ratio was 58.94 ± 30.37 in children with obstructive hydronephrosis, 39.58 ± 28.16 in children with non-obstructive hydronephrosis, and also 33.1 ± 18.19 in the control group children. Kruskal-Wallis test showed that this difference was statistically significant (P = 0.001).

Mean MCP-1 to urinary creatinine ratio was higher in children with obstructive hydronephrosis (58.94) than the control group (33.1). Mann-Whitney test showed that this difference was statistically significant (P = 0.001).

Mean MCP-1 to urinary creatinine ratio was higher in children with obstructive hydronephrosis (58.94) compared to children with non-obstructive hydronephrosis (39.58). Mann-Whitney test showed that this difference was statistically significant (P = 0.005).

4. Discussion

Any disruption in normal urinary flow and its subsequent outcomes in children is regarded as urinary ob-struction. The most common obstructive abnormality in neonates is Ureteropelvic obstruction (UPJO), which is often diagnosed through invasive methods, and some patients remain undiagnosed until they experience serious renal dysfunction. Most urologists often follow-up UPJO cases, and perform surgery only in cases with renal dysfunction or clinical symptoms. In a study by Chertin, 20% to 30% of neonates needed surgery (2). Furthermore, in a study conducted in 2015, 200 neonates diagnosed with congenital hydronephrosis were assessed, of whom, 12.5% required surgery (3).

The present study aimed at comparing children with urinary tract obstruction and healthy children visiting the pediatrics nephrology clinic of Ali-Asghar hospital in Zahedan in terms of urinary MCP-1 levels.

Comparing urinary MCP-1 level and MCP-1 to urinary creatinine ratios in the study of children showed that this marker was significantly higher in children with obstructive hydronephrosis compared to children with non-obstructive hydronephrosis and children in the control group. Although mean MCP-1 was significantly higher in children with obstructive hydronephrosis compared to children in the control group, the difference between these 2 groups was not significant in terms of MCP-1/creatinine ratio.

The results of several previous studies proposed monocytes as one of the sources releasing inflammatory factors in interstitial tissue in kidneys with obstruction (9, 10).

Monocyte Chemoattractant Protein-1 is an inflammatory mediator from the family of beta-chemokine, and based on the data obtained from human and animal studies, it is very likely that this inflammatory mediator is responsible for the absorption and accumulation of monocytes in the kidneys (4, 5). Tubular atrophy is a characteristic of damage due to urinary tract obstruction, and can be used as an index in monitoring tubular damage and in adopting measures for treatment of these patients (11).

In their study, Gerber compared patients with UPJO and a control group in terms of NGAL, KIM-1, CD13, CD10, and CD26 urinary biomarkers, and found a significant increase in CD13, CD10, and CD26, and suggested that measurement of these biomarkers in the urinary sample is not different from urinary samples obtained from the obstruction site (12).

In their case-control study in Egypt, Hassan et al. assessed and compared serum MCP-1 level in 50 patients with glomerulonephritis, 20 with nephropathy for reasons other than glomerulonephritis, and 20 healthy individuals. Their results showed a high level of this serum marker in patients with glomerulonephritis compared to the other 2 groups. In their study, high level of serum MCP-1 was proposed as a marker for progress of nephropathy (13).

Table 1. Comparison of Children Based on Gender[a]

Group	Sex		P Value
	Female	Male	
Hydronephrosis without obstruction	4 (20)	16 (80)	
Hydronephrosis with obstruction	5 (25)	15 (75)	0.27
Healthy control	12 (40)	18 (60)	

[a]Values are expressed as No. (%).

Table 2. Comparison of Children Based on Age

Group	Number	Mean ± SD	Median	Mode	P Value
Hydronephrosis without obstruction	20	23.50 ± 21.79	35.75	14	
Hydronephrosis with obstruction	20	18.35 ± 19.30	29.63	12	0.25
Healthy control	30	25.18 ± 20.22	39.25	16.50	

Table 3. Comparison of Mean GFR Difference in Groups with Analysis of Variance

Groups	Number	Mean ± SD	Min	Max	P Value
Hydronephrosis without obstruction	20	75.75 ± 21.99	46.50	114.40	
Hydronephrosis with obstruction	20	71.23 ± 18.34	50.50	105.60	0.082
Healthy control	30	84.32 ± 21.17	46.50	118.80	

Table 4. Comparison of Mean MCP-1 Difference in Groups with Analysis of Variance

Groups	Number	Mean ± SD, ng	Min	Max	P Value
Hydronephrosis without obstruction	20	542.56 ± 56.10	366.10	569.30	
Hydronephrosis with obstruction	20	424.70 ± 66.25	340.20	548.80	< 0.0001
Healthy control	30	335.97 ± 81.49	218	545	

Table 5. Comparison of Mean MCP-1 to Creatinine Difference in Groups with the Kruskal-Wallis Test

Groups	Number	Mean ± SD	Median	Mode	P Value
Hydronephrosis without obstruction	20	58.94 ± 30.37	49.40	48.96	
Hydronephrosis with obstruction	20	39.58 ± 28.16	31.85	31.24	< 0.0001
Healthy control	30	33.10 ± 18.19	28.67	26.06	

The relationship between increased expression of MCP-1 in kidneys with some degrees of tubular damage (10) and reduced expression of MCP-1 after obstruction was (14) indicative of the possible role of MCP-1 in pathogenesis of renal tubular damage.

The present study results confirm those of other studies regarding increased level of urinary MCP-1 in children with urinary obstruction compared to healthy children (15, 16). In a review study, Madsen et al. (2011) investigated urinary biomarkers of infants with previously diagnosed unilateral hydronephrosis and showed that MCP-1 level increases in patients with UPJO (17). In a case-control study, Bartoli et al. (2011) compared 76 children with UPJO and 30 healthy children in terms of urinary cytokines of epidermal growth factor (EGF), beta-2 micro-globulin (β2 mic), and MCP-1. Children with UPJO were assessed in obstructive (12 children), functional (36 children), and surgery (28 children) groups. Their results showed that

urinary β2 mic and MCP-1 significantly increased in children with UPJO compared to the control group. Furthermore, EGF/MCP-1 ratio and EGF/β2 mic ratio were significantly reduced in children with untreated UPJO compared to the control group ([11]). In a cohort study conducted by Taranta-Janusz et al. (2012), urinary cytokines in unilateral fetal hydronephrosis were assessed. The results obtained from 15 children with severe hydronephrosis, 21 children with mild non-obstructive hydronephrosis, and 19 healthy children were compared. The results showed a significant increase in urinary MCP-1 in children with severe hydronephrosis compared to children with mild non-obstructive hydronephrosis and the control group ([18]), which concurs with the present study results.

In a case-control study, Madsen et al. (2013) compared EGF and MCP-1 biomarkers in 28 children with obstructive hydronephrosis and 13 healthy children. Their results showed a significant increase in these 2 biomarkers in children with obstructive hydronephrosis compared to the other children ([17]), and with respect to MCP-1, these results agreed with those of the present study.

In a prospective study conducted by Mohamadjafari et al. (2014) on the role of urinary Endothelin-1 (ET-1), MCP-1, and N-Acetylglocosaminidase (NAG) in predicting severity of obstruction in infants with hydronephrosis, 42 infants with fetal hydronephrosis were assessed to have severe obstruction, requiring surgery (24 infants), and mild obstruction with no dysfunction (18 infants). The severe obstruction group comprised of 21 males and 3 females, and the mild obstruction group comprised of 16 males and 2 females. Their results showed no significant difference between the 2 groups in level of ET-1, MCP-1, NAG, or ET-1, and NAG/creatinine ratio. The MCP-1/creatinine ratio was significantly higher in infants with severe obstruction compared to infants with mild obstruction. It was therefore recommended that MCP-1/creatinine ratio should be used in diagnosis of severe obstructive hydronephrosis (P = 0.012) ([16]). However, some studies only investigated and compared specific groups of patients with urinary obstruction, such as children with UPJO ([10, 11, 17]), treated and untreated cases of obstructive hydronephrosis (10 , 11), severe and mild cases of obstruction ([16, 18]), or unilateral cases only ([17, 18]). Moreover, renal damage depends on the severity and duration of obstruction ([11]), and unfortunately, in clinical conditions, these parameters are different and often unpredictable in different studies.

The present study limitations included lack of distinction between severe and mild cases of obstructive hydronephrosis, which could indirectly confirm presence or absence of MCP-1 relationship with severity of renal damage. Patient follow-up after surgery was beneficial to the value of these markers as a diagnostic method.

4.1. Conclusion

The present study results showed the indirect role of MCP-1 in the progress of tubule-interstitial damage in obstructive and even non-obstructive nephropathy.

This marker can be used in prognosis of tubular damage or for monitoring children with hydronephrosis.

Regarding the present study and other similar studies results showing increased level of MCP-1 in patients with hydronephrosis, especially cases with obstruction, it is recommended that the present study results should be considered when using this marker as an indicator of severity of obstruction, and monitoring and prognosis of disease in these children, such that patient recovery can be achieved through early detection of renal damage and rapid intervention.

Acknowledgments

Footnotes

References

1. Krajewski W, Wojciechowska J, Dembowski J, Zdrojowy R, Szydelko T. Hydronephrosis in the course of ureteropelvic junction obstruction: An underestimated problem? Current opinions on the pathogenesis, diagnosis and treatment. *Adv Clin Exp Med*. 2017;**26**(5):857–64. doi: 10.17219/acem/59509. [PubMed: 29068584].

2. Chertin B, Pollack A, Koulikov D, Rabinowitz R, Hain D, Hadas-Halpren I, et al. Conservative treatment of ureteropelvic junction obstruction in children with antenatal diagnosis of hydronephrosis: lessons learned after 16 years of follow-up. *Eur Urol*. 2006;**49**(4):734–8. doi: 10.1016/j.eururo.2006.01.046. [PubMed: 16504374].

3. Sadeghi-Bojd S, Kajbafzadeh AM, Ansari-Moghadam A, Rashidi S. Postnatal Evaluation and Outcome of Prenatal Hydronephrosis. *Iran J Pediatr*. 2016;**26**(2). e3667. doi: 10.5812/ijp.3667. [PubMed: 27307966].

4. Xu ZM, Li MJ, Tao C. Serum and urinary thioredoxin concentrations are associated with severity of children hydronephrosis. *Clin Chim Acta*. 2017;**466**:127–32. doi: 10.1016/j.cca.2017.01.020. [PubMed: 28111273].

5. Herget-Rosenthal S, Poppen D, Husing J, Marggraf G, Pietruck F, Jakob HG, et al. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. *Clin Chem*. 2004;**50**(3):552–8. doi: 10.1373/clinchem.2003.027763. [PubMed: 14709451].

6. Hashim H, Woodhouse CRJ. Ureteropelvic junction obstruction. *Eur Urol Suppl*. 2012;**11**(2):25–32. doi: 10.1016/j.eursup.2012.01.004.

7. Madsen MG. Urinary biomarkers in hydronephrosis. *Dan Med J*. 2013;**60**(2):B4582. [PubMed: 23461995].

8. Taranta-Janusz K, Zalewska-Szajda B, Chojnowska S, Wasilewska A. Urine exoglycosidases are potential markers of renal tubular injury in children with ureteropelvic junction obstruction. *Acta Paediatr*. 2015;**104**(11):e518–23. doi: 10.1111/apa.13068. [PubMed: 26095925].

9. Harris KP, Schreiner GF, Klahr S. Effect of leukocyte depletion on the function of the postobstructed kidney in the rat. *Kidney Int*. 1989;**36**(2):210–5. doi: 10.1038/ki.1989.181. [PubMed: 2789309].

10. Grandaliano G, Gesualdo L, Bartoli F, Ranieri E, Monno R, Leggio A, et al. MCP-1 and EGF renal expression and urine excretion in human congenital obstructive nephropathy. *Kidney Int*. 2000;**58**(1):182–92. doi: 10.1046/j.1523-1755.2000.00153.x. [PubMed: 10886563].

11. Bartoli F, Penza R, Aceto G, Niglio F, D'Addato O, Pastore V, et al. Urinary epidermal growth factor, monocyte chemotactic protein-1, and beta2-microglobulin in children with ureteropelvic junction obstruction. *J Pediatr Surg*. 2011;**46**(3):530–6. doi: 10.1016/j.jpedsurg.2010.07.057. [PubMed: 21376205].

12. Gerber C, Harel M, Lynch ML, Herbst KW, Ferrer FA, Shapiro LH. Proximal tubule proteins are significantly elevated in bladder urine of patients with ureteropelvic junction obstruction and may represent novel biomarkers: A pilot study. *J Pediatr Urol*. 2016;**12**(2):120 e1–7. doi: 10.1016/j.jpurol.2015.10.008. [PubMed: 26705690].

13. Hassan AM, Nagy H, Mohamed SM. Circulating MCP-1 level and 2518 gene polymorphism as a marker of nephropathy development in Egyptian patients. *Egypt J Med Hum Genet*. 2010;**11**:159–66. doi: 10.1016/j.ejmhg.2010.10.002.

14. Stephan M, Conrad S, Eggert T, Heuer R, Fernandez S, Huland H. Urinary concentration and tissue messenger RNA expression of monocyte chemoattractant protein-1 as an indicator of the degree of hydronephrotic atrophy in partial ureteral obstruction. *J Urol*. 2002;**167**(3):1497–502. doi: 10.1016/S0022-5347(05)65352-9. [PubMed: 11832777].

15. Madsen MG, Norregaard R, Palmfeldt J, Olsen LH, Frokiaer J, Jorgensen TM. Epidermal growth factor and monocyte chemotactic peptide-1: potential biomarkers of urinary tract obstruction in children with hydronephrosis. *J Pediatr Urol*. 2013;**9**(6 Pt A):838–45. doi: 10.1016/j.jpurol.2012.11.011. [PubMed: 23228281].

16. Mohammadjafari H, Rafiei A, Mousavi SA, Alaee A, Yeganeh Y. Role of urinary levels of endothelin-1, monocyte chemotactic peptide-1, and N-acetyl glucosaminidase in predicting the severity of obstruction in hydronephrotic neonates. *Korean J Urol*. 2014;**55**(10):670–6. doi: 10.4111/kju.2014.55.10.670. [PubMed: 25324951].

17. Madsen MG, Norregaard R, Frokiaer J, Jorgensen TM. Urinary biomarkers in prenatally diagnosed unilateral hydronephrosis. *J Pediatr Urol*. 2011;**7**(2):105–12. doi: 10.1016/j.jpurol.2010.12.004. [PubMed: 21220211].

18. Taranta-Janusz K, Wasilewska A, Debek W, Waszkiewicz-Stojda M. Urinary cytokine profiles in unilateral congenital hydronephrosis. *Pediatr Nephrol*. 2012;**27**(11):2107–13. doi: 10.1007/s00467-012-2230-9. [PubMed: 22744767].