http://dx.doi.org/10.15218/zjms.2016.0048

Detection of urinary lactoferrin as an indicator of urinary tract infection in girls

Received: 15/6/2015 Accepted: 7/3/2016

Mohammed Hassan Fatah * Nabeel Elia Waheda * Dik J. Kok *

Abstract

Background and objective: Lactoferrin (LF) is an iron-binding protein that is related in structure to transferrin. It is considered to be a part of the in-nate immune system. This study aimed to assess the role of urinary lactoferrin (LF) as an indicator for diagnosis of urinary tract infection among girls.

Methods: This study was conducted on girls suffering from UTI. Urine samples were tested for Lactoferrin before and after two months of the treatment using ELISA technique.

Results: Urine specimens were collected from 25 girls with mean age ± SD of 6 ± 3 years without UTI as a control group (C) and 25 girls with mean age ± SD of 5.3 ± 3.18 years diagnosed as suffering from UTI (T1) followed by a set of 25 specimens after two months (T2). The mean concentration of urinary LF ± SD was 670 ± 319 ng/ml in the specimens of control group whereas it was 1387 ± 509 in the specimens of girls with UTI during the infection (T1) and 885 ± 268 after two months (T2). The mean concentrations of urinary LF during the infection (T1) were significantly (P <0.001) increased compared with controls (C) and significantly (P = 0.003) decreased after two months (T2), that may refer to a role of urinary LF in UTI. There was no significant (P = 0.089) difference between the mean concentration of urinary LF after two months (T2) compared with controls (C) that may indicate to the normalization of LF concentration after the treatment synchronously with disappearing of UTI symptoms and significantly reduction of positive urinalysis results.

Conclusion: The results of this study indicate the elevation of urinary Lactoferrin (LF) in girls suffering from UTI and probably being a good indicator for diagnosis of UTI.

Keywords: Urinary tract infection; Lactoferrin; Girls.

Introduction

Urinary tract infection (UTI) is a common disease in pediatric practice. Early diagnosis and prompt treatment can reduce the risk of renal scarring and its long-term sequelae such as hypertension and end stage renal failure.[1] Lactoferrin (LF) is an iron-binding protein that is related in structure to transferrin. It was first isolated from human breast milk[2] and was found later in several other organs, such as kidney, gallbladder, lung, pancreas, prostate, seminal vesicle, gut, and liver.[3,4] Lactoferrin is considered to be a part of the in-nate immune system. Due to its strategic position on the mucosal surface, lacto-ferrin represents one of the first defense systems against microbial agents invading the organism mostly via mucosal tissues.[5] Due to the increase in its concentration during most inflammatory reactions and some viral in-fections, several authors classify lactoferrin as an acute-phase protein.[6] The major function of LF probably resides in its bacteriocidal effects, either by sequestering free iron which is one of the elements essential for the growth of bacteria[7] or by the effects of lactoferricin, an antibacterial peptide generated by proteolytic cleavage of LF.[8] Lactoferrin is secreted by kidney cells and is found in neutrophil granules thus it could be involved in combating UTI.[9] Nowadays methods for diagnosis of UTI depends on

* Department of Medical Lab Technology, Kalar Technical Institute, Polytechnique University, Sulaimani, Iraq.
* Department of Microbiology, College of Medicine, Hawler Medical University, Erbil, Iraq.
* Department of Urology, Erasmus MC, Rotterdam, Netherlands.

http://dx.doi.org/10.15218/zjms.2016.0048

detection of pyuria microscopically that require some skills by the microscopist or detection of bacteruria by culturing, that it is a time-consuming procedure (24-48 hours), so rapid and accurate diagnosis of UTI would be useful to the physician and patients to use treatment or not. The aim of this study was to assess the role of urinary lactoferrin (LF) as an indicator in the diagnosis of urinary tract infection among girls under 16 years old as a minimum legally allowed age for finishing girl period and getting marriage in Kurdistan.

Methods

This study was performed during April till July 2013 in General Hospital, Kalar city, Garmiyan district, Kurdistan Region, Iraq.

Subjects:

The study group was composed of 25 girls (< 16 years old) with complaints consistent of urinary tract infections compared before and after two months of the treatment with 25 girls without complaints consistent of urinary tract infections.

Exclusion criteria:

Anatomical abnormality of the urinary tracts, immunodeficiency, diabetes mellitus, menopause subjects and who used antibiotics during the past 48 hours was excluded from the study through direct interviewing with the subjects and filling down a specific questionnaire form containing questions about this information as came in the proposal of this study.

Urine specimens and UTI criteria:

The clean-catch midstream urine specimens were obtained as described by Karlowsky, *et al*.[10] The dipstick technique for nitrite and leukocyte estrase of uncentrifuged urine specimens were carried out according to the method described in the leaflet provided by the supplying company. A part of the urine specimen was centrifuged (8000 rpm/m) and the supernatants were distributed in 4 repeated (1.5ml) size aliquot tube, coded and preserved in a deep freezer (-80˚C). These were then analyzed for urinary lactoferrin using a sandwich ELISA. The microscopic examination of urine specimen precipitants and the calibrated-loop technique for bacterial culture of un centrifuged urine specimens were carried out according to the method described by Smith et al.[11] The guidelines to establish a test result as UTI positive for the purpose of this study were dysuria, frequency, urgency, PMNs at 10 cells at high power field (HPF) and a bacterial count of 10^5CFU/ml of at least one clearly predominating organism.

Estimation of Urinary Lactoferrin (LF):

The frozen urine specimen supernatants were analyzed for LF concentrations by enzyme-linked immunosorbent assay (MyBioSource- Cat no. MBS564053. USA). Different provided concentrations of standard LF were added to the first six wells and urine supernatants to other 90 wells of the microplate reader and allowed to react for 90 minutes at 37 ˚C. Biotinylated Detection Ab was added and allowed to react for 1 hour at 37C. After the wells were washed, Horseradish peroxidase (HRP) Conjugate was added and incubated for 30 minutes at 37˚C. After another washing, Substrate Reagent was added and incubated for 15 minutes at 37˚C. The reactions were stopped by addition of stop solution, and the absorbance of each well was read at 450nm immediately. The results of the (LF) concentration in urine supernatants were calculated in pg/ml from the standard curve drawn between the optical density (OD) values against the different standard concentrations of (LF).

Statistical analysis:

The SPSS software (Statistical Package for the Social Sciences, version PASW 18) was used for the analyses. The data were expressed as mean ± standard deviation. Because the Kolmogorov –Simirnov test resulted in abnormal distribution (P <0.05) of the data, comparisons between patients and controls were done using the Mann-Whitney test and Wilcoxon signed rank was used to make comparisons between patients after and before the

http://dx.doi.org/10.15218/zjms.2016.0048

treatment. Asymptotic McNemar's test was used to make comparisons between the results of the urinalysis test. A *P* value of ≤0.05 was considered statistically significant.

Results

Twenty-five urine samples were collected from girls with mean age ± SD of 6±3 years without UTI as control group (C) and 25 urine samples from girls with mean age ± SD of 5.3 ± 3.18 years with UTI before the treatment (T1) followed by 25 urine samples after two months of the treatment (T2). Symptom analysis showed that mean 24-hour frequency of urination was 9.75 and 5.3 for the patients and control groups, respectively. 88% of (T1) patients had urgency symptoms, and 86% complained of dysuria symptoms while just one case showed symptoms of urgency and dysuria after the treatment (T2). The percentages of nitrite, leukocyte, bacteriuria and pyuria in patients before the treatment (T1) were 48%, 84%, 100% and 88%, respectively. A significant difference (*P* <0.001) in each of pyuria, leukocyte, nitrite, and bacteruria tests was found before (T1) and after the treatment (T2) as shown in Tables 1, 2, 3 and 4, respectively.

Table 1: Results of Pyuria test before (T1) and after (T2) the treatment.

		Pyuria (T2)		Total	*P* value
		Negative	Positive		
Pyuria (T1)	Negative	3	0	3	< 0.001
	Positive	21	1	22	
	Total	24	1	25	

Table 2: Results of Leukocyte test before (T1) and after (T2) the treatment.

		Leukocyte (T2)		Total	*P* value
		Negative	Positive		
Leukocyte (T1)	Negative	4	0	4	< 0.001
	Positive	19	2	21	
	Total	23	2	25	

Table 3: Results of Nitrite test before (T1) and after (T2) the treatment.

		Nitrite (T2)		Total	*P* value
		Negative	Positive		
Nitrite (T1)	Negative	13	0	13	< 0.001
	Positive	12	0	12	
	Total	25	0	25	

Table 4: Results of Bacteruria test before (T1) and after (T2) the treatment.

		Bacteruria (T2)		Total	*P* value
		Negative	Positive		
Bacteruria (T1)	Negative	0	0	0	< 0.001
	Positive	17	8	25	
	Total	17	8	25	

http://dx.doi.org/10.15218/zjms.2016.0048

The quantitative assessment of Lactoferrin (LF)

To quantitatively assess the detectable range of (LF), standard solutions of (LF) at concentrations from 0.625 ng/ml to 20 ng/ml were examined. A well-fitted standard curve was obtained as shown in Figure 1.

The mean concentration of urinary lactoferrin was raised in patients compared to controls (P <0.001) and decreased compared to its concentration after two months (P = 0.003) approaching the controls (P = 0.089) as shown in Table 5.

Discussion

In the current study, urinary tract infection (UTI) was confirmed by symptoms, urinalysis results and culture (>100,000 colony forming unit /ml) results. Bell *et al*.[12] reported that the diagnosis of UTI begins with the screening of patients with symptoms suggestive of UTI and abnormal micturition frequency, dysuria and urgency indicate a bladder irritation[13] that makes these symptoms highly predictive for diagnosis of UTI.[14] Bakker *et al*.[15] concluded that the dipsticks that detect the presence of white blood cells (leukocyte esterase), or the production of nitrite are highly recommended in the preliminary diagnosis of UTI with high specify and low rate of false positive results and now are used in 92% of consultations for UTI.[14] Braunwald et al.[16] reported that the detection of bacteria by urinary microscopy confirms evidence of UTI infection and pyuria is a highly sensitive indicator of UTI in symptomatic patients. The significant increasing of urinary Lactoferrin (LF) during the infection (T1) compared to controls (C) as shown in table (5) are similar to the results of study done by Arao *et al*.[17] who reported that the level of urinary LF was high in patients with UTI and they indicated that urine LF measurement provides a useful tool for the simple, rapid and sensitive marker for the diagnosis of UTI caused by inflammatory pathogens.

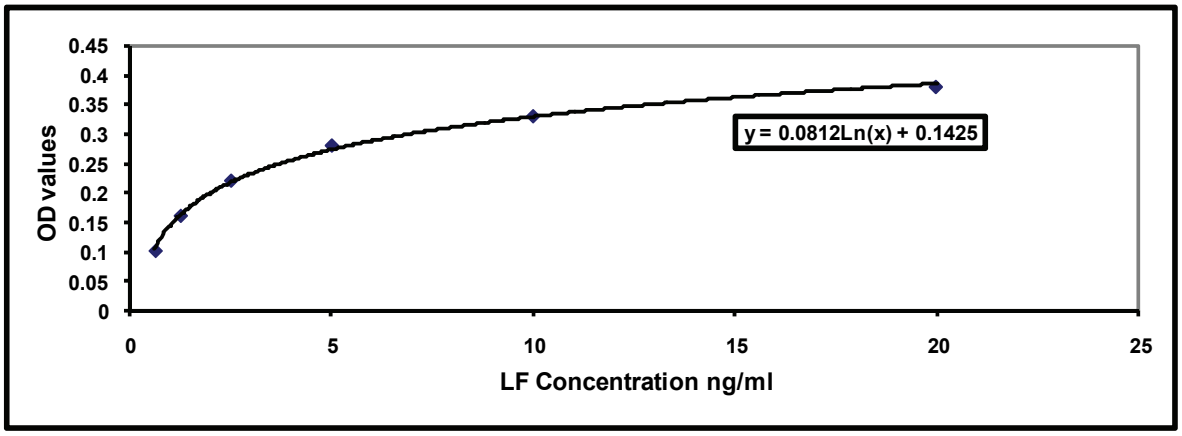

Figure 1: Standard lactoferrin curve.

Table 5: Mean concentrations of LF (Pg/ml) in urine.

	Range of concentrations	Mean of concentrations	SD	*P* value
T1	(790-2200)	1387	509	T1-C < 0.001
T2	(500-1380)	885	268	T1-T2 0.003
C	(300-1150)	670	319	T2-C 0.089

http://dx.doi.org/10.15218/zjms.2016.0048

The significant decreasing of urinary (LF) after (T2) compared to its concentration before the treatment (T1), disappearing of UTI symptoms and significant reduction of positive urinalysis results after treatment as shown in tables (1, 2, 3, 4) supports this viewpoint. The production of lactoferrin by human kidneys has important functions in both the immune defense of the urinary tract and in general iron metabolism.[9] Gill et al.[18] mentioned that LF is a strong candidate for replacing urine dipstick analysis and point-of-contact urinary microscopy with a potential for promoting a considerable improvement in patient diagnosis and management.

Conclusion

The level of lactoferrin (LF) was significantly elevated in the urine of UTI patients compared to the control and significantly decreased after two months of treatment synchronously with disappearing of UTI symptoms and significantly reduction of positive urinalysis results that make a urinary (LF) a possible candidate's marker for detecting UTI.

Conflicts of interest

The authors report no conflicts of interest.

References

1. Hellersteim S. Urinary tract infection. Old and new concepts. Pediatr Clin North Am 1995; 42 (6):1433-57.
2. Neville M, Zhang P. Lactoferrin secretion into milk: Comparison between ruminant, murine, and human milk. J him Sci 2000; 78(3):26-35.
3. Lechene D, Figarella C, Sarles H. Immunocytochemical localization of lactoferrin in human pancreas. Hoppe Seylers Physiol Chem 1981; 362:1293–6.
4. Wichmann L, Vaalasti A, Vaalasti T, Tuohimaa P. Localization of lactoferrin in the male reproductive tract. Int J Androl 1989; 12:179– 86.
5. Legrand D, Elass E, Carpentier M, Mazurier J. Lactoferrin: a modulator of immune and inflammatory responses. Cellular and Molecular Life Sciences 2005; 62: 2549–59.
6. Kanyshkova T, Buneva V, Nevinsky G. Lactoferrin and its biological functions. Biochemistry 2001; 66:1-7.
7. Ellison R. The effects of lactoferrin on gram-negative bacteria. Adv Exp Med Biol 1994; 3575: 71–90.
8. Bellamy W, Takase M, Wakabayashi H, Kawase K, Tomita M. Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. J Appl Bacteriol 1992; 73:472–9.
9. Abrink M, Larsson E, Gobl A, Hellman L. Expression of lactoferrin in the kidney: implications for innate immunity and iron metabolism. Kidney Int 2000; 57(5): 2004-10.
10. Karlowsky J, Hoban D, Decorby R, Laing M, Zhanel G. Fluoroquinolone resistant urinary isolates of Escherichia coli from outpatients are frequently multi-drug: Results from the North American urinary tract infection collaborative alliance-quinolone resistance study. Antimicrobial Agents and Chemotherapy 2006; 50: 2251-4.
11. Smith, PJ, Morris, AJ, Reller, LB. Predicting urine culture results by dipstick testing and phase contrast microscopy. Pathology 2003; 35: 161-5.
12. Bell JM, Paton JC, Turnidge J. Emergence of vancomycin - resistant Enterococci in ustralia: phenotypic and genotypic characteristics of isolate. J Clin Microbiol 1998; 36: 2187-90.
13. Kurowski K. The woman with dysuria. Am Fam Physician 1998; 57(9):2155-64.
14. Hummers-Pradier E, Ohse AM, Koch M, Heizmann WR, Kochen MM. Management of urinary tract infections in female general practice patients. Fam Pract 2005; 22(1):71-7.
1. Bakker E, Van Sprundel M, Van der Aurwera J, Van Gool D, Wyndaele J. Voiding habits and wetting in a population of 4332 Belgian schoolchildren aged between 10 and 14 years. Scand J Urol Nephrol 2002; 36(5):354-62.
1. Braunwald E, Fauci A, Kasper D, Hauser S, Longo D, Jameson J. Harrison's 15th ed. Vol. 2. Principles of Internal Medicine, New York: McGraw-Hill; 2001. P. 1620-5.
17. Arao S, Matsuura S, Nonomura M, Miki K, Kabasawa K, Nakanishi H. Measurement of urinary lactoferrin as a marker of urinary tract infection. J Clin Microbiol 1999; 37: 553 -7.
18. Gill K, Malone-Lee J, Brenton T, Kupelian A, Horsley H, Sathiananthamoorthy S, et al. Urinary Lactoferrin as a promising, new, improved marker for urinary tract infection. Intern Urogynecol J 2012; 23: 164-5.