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ORIGINAL ARTICLE

Heparin-Binding Protein as an Immunological Marker in Children Urinary Tract Infection

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ABSTRACT

Background: *Urinary tract infections (UTI) are revealed to be one of the most important causes of morbidity and mortality in the world. Its diagnosis is often difficult, mainly in children with unclear and vague symptoms. The rapid tests for diagnosis of UTI have insufficient significance, and there is a prerequisite for additional trustworthy investigative tests. Heparin-binding protein (HBP) secreted from human neutrophils, has been investigated in different bacterial diseases and its role in diagnosis and prognosis has be proven but, little is known about its diagnostic value in UTI.* ***Methods:*** *The study includes 90 children from Mansoura University Children Hospital diagnosed as UTI by urine culture and 50 children with negative urine cultures as a control group. Urinary HBP (U-HBP) is investigated as a diagnostic marker in UTI. Also, the levels of urinary white blood cells (U-WBC), urinary interleukin-6 (U IL-6) and urinary nitrite (U-nitrite) were also measured and matched with U-HBP at the time of diagnosis and after a period of treatment.* ***Results:*** *The sensitivity and specificity for U-HBP in the diagnosis of UTI were 95.5% and 96% respectively. U-HBP had the highest sensitivity and specificity in comparison to U IL-6 and U-nitrite in diagnosis of UTI. Also, U-HBP was superior in differentiating pyelonephritis from cystitis, compared with the previous markers and can play a role in the follow up of patients after treatment.****Conclusions:*** *A high level of U-HBP is related to UTI and is useful as a prognostic marker to follow up children with UTI after treatment.*

INTRODUCTION

Urinary tract infection (UTI) is considered as one of the most common causes of infections in the world associated with the increased antibiotic-resistant organisms[1].

UTI are mostly caused by *Escherichia coli, Staphylococcus* species, *Proteus and Enterococcus faecalis*. Other causative organisms include *Klebsiella, group B streptococci, Pseudomonas aeruginosa* and *Citrobacter*. They commonly get up from peri-urethral contamination found in faecal flora which then ascends into the bladder via the urethra. Further movement of the organisms from the bladder via the ureters into the kidneys will leads to pyelonephritis[2].

UTI are categorized as either uncomplicated or complicated. Uncomplicated UTI are subdivided into cystitis and pyelonephritis. Patients with cystitis are presented with dysuria, frequency of micturition, suprapubic pain and may be hematuria while patients with pyelonephritis are presented with fever, nausea, vomiting and flank pain, in addition to the previous symptoms. Children are presented with atypical symptoms such as abdominal pain, vomiting, and irritability and may be without urinary tract symptoms [3].

Complicated UTI occur when there is abnormality in the urinary system or host immunity is suppressed. These patients are mainly predisposed to repeated UTI, renal failure, urosepsis and death[4].

The decision of UTI is sometimes built on the present of certain clinical pictures, high number of urinary white blood cells (U-WBC) and positive urinary nitrite (U-nitrite) test done by the fast dipstick assessment. The later test indicates the presence of bacteria in urine. However, this diagnosis has a limited sensitivity and specificity compared with the golden standard test, which is urine culture[5].

The low sensitivity and specificity of these methods leads to unnecessary antibiotics used, leading to a lot of side effects and great cost in therapy [6].

To enhance diagnosis, other tests have been studied. Many studies reported that in response to infection, leukocytes and epithelial cells of the urinary tract produce interleukin-6 (IL-6) and heparin-binding protein (HBP) which is a 37 kDa protein secreted from human neutrophils which act as a chemoattractant and activator of monocytes and play a role in microbial clearance by opsonization [7].

In different bacterial infections, HBP has been evaluated. Different studies shown increased levels of HBP in skin biopsies, plasma, and cerebrospinal fluid have been associated with skin infection, severe sepsis, and bacterial meningitis correspondingly [8,9,10].

In this study, urinary HBP (U-HBP) in children were assessed as a diagnostic tool for UTI and matched

with U-nitrite test observed by the dipstick test, U-IL-6 and U-WBC results.

METHODOLOGY

Study Population

Urine samples were collected from 140 children admitted to Mansoura University Children Hospital between November 2016 and June 2017. Inclusion criteria include age between 4 and 12 years. Exclusion criteria include: urinary system abnormality, suppressed immune system and urosepsesis cases.

Ninety children cases diagnosed as UTI by symptoms such as: frequency of micturition, dysuria, urgency, suprapubic pain, hematuria and flank pain, in addition to the positive urine cultures.

Fifty children as a control group without underlying urological abnormality, symptoms or signs of UTI during the last 2 months and negative urine cultures.This study protocol was approved by the ethical committee in faculty of medicine, Mansoura University and an informed consent was obtained from all children parents.

This study was a case-control observational study.

Patient Characteristics

Urinary tract infections children were categorized into 2 groups (probable UTI and definite UTI) according to the clinical pictures, and the bacterial count and types present in the culture.

According to the European Confederation of Laboratory Medicine guidelines[11], patients with definite UTI existed with typical clinical pictures and count of the primary organism (*Staphylococcus* or *Escherichia coli*) equal or more than10^4 colony-forming units (CFU)/mL or count of a secondary organism equal or more than10^5 CFU/mL detected after doing urine cultures to the midstream urine. While, patients with probable UTI were presented with typical symptoms for UTI, with less count of bacteria in urine (primary organism less than 10^4 CFU/mL, secondary organism less than10^5 CFU/mL) or, negative urine culture, but with elevated numbers of U-WBC or and positive U-nitrite test.

Also, children with UTI were categorized as either pyelonephritis or cystitis. Pyelonephritis children had UTI clinical symptoms, associated with a body temperature higher than 37.5°C and elevated plasma C-reactive protein (CRP) associated to UTI.

According to the measures of International Society of Nephrology [12] children were divided into 2 dissimilar groups based on calculation of glomerular filtration rate (GFR):

1. No kidney disease (NKD): GFR equal or more than 60 mL/min per 1.73 m^2
2. Acute kidney disease (AKD) or Chronic kidney disease (CKD): GFR less than 60 mL/min per 1.73 m^2

Urine Samples

Urine samples were collected in sterile container. Part of urine was centrifuged 3000 rpm for 10 minutes, and the supernatant was stored at -70°C until tested. The other part of urine (non centrifuged) was evaluated with a dipstick test (Combur 10 Test, Roche, Swiss) and culture of bacteria was done at the Microbiology Diagnostics and Infection Control Unit in Mansoura university.

Laboratory Analysis

Urine samples before testing were diluted to a ratio one to twenty. The concentration of U-HBP and U-IL-6 were analyzed by enzyme linked immunosorbent assay (ELISA); MyBioSource, Inc.San Diego, USA and R&D Systems, Inc.Minneapolis, USA respectively. Results of CRP and creatinine were obtained from children laboratory data which were done in clinical laboratory of Mansoura University Children Hospital.

Statistical Analysis

Sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs) were calculated. P value was considered significant if less than 0.05 and highly significant, if less than 0.001. These tests were done using the Statistical Package for Social scientists (SPSS) *(SPSS Inc., Chicago, IL, USA).*

RESULTS

Table 1: Background parameters of both control and UTI children

	Definite Cystitis (n = 33)	*Definite Pyelonephritis (n = 27)*	*Probable Cystitis (n = 20)*	*Probable Pyelonephritis (n = 10)*	*Control (n = 50)*	*P value (UTI and control)*
Mean Age (years)	7.2	6.9	8.1	8.3	7.7	--
Gender (Male/female)	13/20	16/11	11/9	4/6	23/27	--
Temperature (°C)	36.9 (36.7–37.2)	37.8 (37.5–38.7)	37 (36.8–37.2)	37.9 (37.5–38.4)	37.0 (36.8–37.2)	--
CRP (ng/mL)	4 (2-7)	44 (21-66)	3 (1-5)	34 (11-59)	2 (1–4)	0.04
U-HBP (ng/mL)	188(43–389)	233(79–432)	47(18–231)	87(25–655)	5 (3–12)	0.01
U-IL-6 (pg/mL)	33(1–278)	189(6–549)	107(4–439)	112(37–760)	1 (1–8)	0.02
U-Nitrite (negative/ positive)	0.8 (0–1)	0.7 (0–1)	0.9 (0–1)	0.6 (0–1)	0 (0–0)	0.03
U-WBC (WBC/hpf)	≥ 100	≥ 100	≥ 100	≥ 100	2 (2-7)	0.03
Positive Urine culture	33	27	20	10	0	--

This table shows higher U-HBP and U-IL-6 with higher percentage of positive U-Nitrite in UTI children than the healthy children group with a significant P values (<0.05). The values of U-HBP were greater in children with definite UTI characterized by higher bacterial concentrations than in children with probable UTI. However, there were no significant difference between U-HBP in definite UTI and probable UTI (P value < 0.17).

NB1: CRP were considered positive above 6 (ng/mL) and the cutoff level for HBP, U- IL-6 and U-WBC were 30 ng/mL, 30 pg/mL and 5 (WBC/hpf) respectively.

NB2: Positive U-nitrite=1. Negative nitrite= 0.

Our results show that U-HBP results were not affected by the level of GFR (insignificant P value: 0.4 between GFR≥ 60 mL/min per 1.73 m and <60 mL/min per 1.73 m). Also, it shows that U-IL-6 and U- nitrite showed non significant difference in relation to definite UTI characterized by higher bacterial concentrations than in children with probable UTI.

Table 2: Causative organisms of UTI

Causative organisms	*Definite Cystitis (n = 33)*	*Definite Pyelonephritis (n = 27)*	*Probable Cystitis (n = 20)*	*Probable Pyelonephritis (n = 10)*
Escherichia coli	30	25	16	6
Proteus	0	1	2	1
Klebsiella	0	0	0	1
Staph aureus	1	1	2	2
Pseudomonas aeruginosa	1	0	0	0
Citrobacter	1	0	0	0

This table shows that *Escherichia coli* is the most common organism in UTI cases either cystitis or pyelonephritis.

Table 3: Specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) for U-nitrite, U- IL-6, and U-HBP in UTI children

	Sensitivity (%)	*Specificity (%)*	*PPV (%)*	*NPV (%)*
HBP	95.5	96	97.7	92.3
U-IL-6	84.4	88	92.7	75.9
U-Nitrite	76.7	82	88.5	66.1

This table shows higher sensitivity, specificity, PPV, and NPV for U-HBP over U-nitrite and U- IL-6 in UTI children.

Table 4: Sensitivity of U-nitrite, U- IL-6, and U-HBP in either cystitis or pyelonephritis

	Type of UTI	*Sensitivity (%)*
HBP	Cystitis	94.3
	Pyelonephritis	97.3
U-IL-6	Cystitis	81.1
	pyelonephritis	89.2
U-Nitrite	Cystitis	83.1
	pyelonephritis	64.9

This table shows that, higher sensitivity of U-HBP in identifying pyelonephritis (97.3%), than in identifying cystitis (94.3%). Also, the sensitivity of IL-6 was higher between children with pyelonephritis: 89.2% than for patients with cystitis:81.1%. However, U-nitrite sensitivity was higher in cystitis (83.1%) than pyelonephritis (64.9%).

Table 5: U-Nitrite, U- IL-6 and U-HBP values in relation to the bacterial identified

	U-HBP (ng/mL) Mean	*U-IL-6 (pg/mL) Mean*	*U-Nitrite Mean*
Escherichia coli	152	125	0.9
Proteus	204	143	0.5
Klebsiella spp.	159	134	1.0
Staph aureus	161	286	0
Pseudomonas aeruginosa	147	165	1.0
Citrobacter	172	119	0

This table shows no differences between U-HBP, and U-IL-6 among the different bacterial species found while; U-nitrite shows a high positive percentage among *Escherichia coli*, *Klebsiella spp.* and *Pseudomonas* infections.

Table 6: U-Nitrite, U- IL-6 and U-HBP levels in relation to improvement after treatment

		Definite Cystitis	*Definite Pyelonephritis*	*Probable Cystitis*	*Probable Pyelonephritis*
Improved children assessed after 1 week of treatment	**67**	**(n = 25)**	**(n = 20)**	**(n = 15)**	**(n = 7)**
U-HBP (ng/mL)	**Before Treatment**	180 (43–389)	238 (81–432)	44 (21–231)	84 (25–655)
	After 1 week of treatment	18 (4-35)	20 (6-36)	6 (2-19)	8 (3-43)
P value (Before and after treatment)		< 0.001	< 0.01	0.02	< 0.01
U-IL-6 (pg/mL)	**Before Treatment**	35(4–278)	182 (9–549)	111 (4–431)	110(37–760)
	After 1 week of treatment	4(2-11)	8(1-67)	6(5-32)	4(3-49)
P value (Before and after treatment)		0.02	0.01	0.03	0.01
U-Nitrite (negative/positive)	**Before Treatment**	0.9 (0–1)	0.7 (0–1)	0.8(0–1)	0.7 (0–1)
	After 1 week of treatment	0.3 (0-1)	0.2 (0-1)	0 (0-1)	0(0-1)
P value (Before and after treatment)		0.03	0.04	0.03	0.05

This table shows a significant reduction in U-HBP, U-nitrite, and U- IL-6 values in 67 children, improved and available for assessment after 1 week of treatment.

DISCUSSION

Diagnosis and management of upper and lower UTI is a huge problem in clinical practice due to their high proportion, recurrence, and universal increase of antibiotic resistance. Nowadays, the analysis of UTI is built on the presence of certain clinical pictures in combination with the results of nitrite strip assessment in urine and semi-quantitative measurement of U-WBC. Although urine culture is the golden standard in UTI but it is time consuming with a high cost [13].

Heparin-binding protein was recently offered as a marker for diagnosing bacterial meningitis [8], predictor of severe infection with organ dysfunction[14] and a marker to follow up treatment [15]. However, little is known about its role in UTI.

In this study we try to assess U-HPB in correlation to traditional methods and U-IL-6.

Our study revealed that U-HBP, have higher sensitivity and specificity for diagnosing UTI, than U-IL-6 and U-nitrite. This can be explained by that a stronger inflammatory stimulus is needed for IL-6 to be released than is required for the secretion of the stored HBP from human neutrophils. Also, nitrite test give a positive results in association to infection by gram negative organism, mostly *Escherichia coli, Klebsiella* and *Pseudomonas*.

Also, this study shows higher sensitivity of U-HBP in diagnosing pyelonephritis than in diagnosing cystitis. Urinary IL-6 give had similar results; the sensitivity of IL-6 was higher among children with pyelonephritis than among children with cystitis. However, U-nitrite sensitivity was higher in cystitis than pyelonephritis, which is explained by that positive nitrite test results related mainly to the percentage of the gram negative organisms mainly *Escherichia coli*.

Similar to the present study, *Gürgoze et al* showed that serum IL-6 was elevated in the pyelonephritis group [mean: 59 pg/ml (range 0–357.2)] more than the cystitis group [mean: 10 pg/ml (range 0–64)] with a highly significant P value (< 0.001) [16].

This also agrees with ***Kjölvmark et al*** study[17] in children, showing that U-HBP had a high sensitivity to diagnose UTI. Also, it shows that U-HBP had a greater sensitivity and specificity for detecting UTI in contrast to the U-WBC, U- IL-6 levels and the U- nitrite test. In adults ***Kjölvmark et al***., study [18] shows similar results.

Other studies have reported lower levels of U-IL-6 among patients with cystitis than among patients with pyelonephritis[19] and lower levels of U-HBP in patients with cystitis with than pyelonephritis [18]. In the current study, U-HBP was superior than U-IL-6 in differentiating cystitis from pyelonephritis and this agree with *Kjölvmark et al*., study[18].

In the current study, the higher count of bacteria in urine characterize definite UTI, where associated with higher levels of U-HBP. This observation comes in agreement with other studies showing the relation of the bacterial count on the secretion of HBP [20].

However, among different bacterial species isolated, there were no variances among U-HBP levels. Also, U–IL-6 shows no significant difference in relation to the bacterial types.

Urine HBP levels were not affected by the level of GFR, indicating that the use of U-HBP in detecting UTI

is convenient regardless of the kidney functions. This reaches agreement with *Kjölvmark et al*., study[18].

Our results, shows UTI children with elevated levels of U-HBP had a higher number of U-WBC. This association can be explained by that HBP is secreted from neutrophils.

Our study shows a significant reduction in U-HBP in relation to treatment in 67 UTI children receiving treatment and this agree with *Kjölvmark et al*., study [18]. So, U-HBP can be used as a marker to follow up antibiotic treatment.

In conclusion, our study noticed that U-HBP was the greatest indicator for diagnosis of UTI. In addition, U-HBP can distinguish between cystitis and pyelonephritis and can play a role in follow up of UTI patients

Further studies on the role of HBP in UTI should be extended to patient groups with asymptomatic presentation and those with complicated UTI. Other markers as elastase alpha 1 proteinase inhibitor, lactofferin, soluble triggering receptor expressed on myeloid cells-1, α-1 microglobulin (α1Mg) and tetrazolium nitroblue test (TNB) should be studied.

REFERENCES

1. Global Burden of Disease Study 2013 Collaborators (2015): Global, regional and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990- 2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet, 22; 386 (9995):743-800.
2. Lee UJ (2015): Urinary tract infection in women. London: BMJ Publishing Group Ltd.
3. Flores-Mireles AL, Walker JN, Caparon M, and Hultgren SJ (2015): Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol, 13(5):269-284.
4. Foxman B (2014): Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin North Am, 28: 1-13.
5. Knottnerus BJ, Geerlings SE, Moll van Charante EP, and Ter Riet G (2013): Toward a simple diagnostic index for acute uncomplicated urinary tract infections. Ann Fam Med, 11:442-451.
6. Gillings MR. (2013): Evolutionary consequences of antibiotic use for the resistome, mobilome and microbial pangenome. Front Microbiol, 4:4.
7. Linder A, Soehnlein O and Åkesson P (2010): Roles of heparin-binding protein in bacterial infections. J Innate Immun, 2:431-438.
8. Linder A, Åkesson P, Brink M, Studahl M, Björck L, and Christensson B (2011): Heparin-binding protein: a diagnostic marker of acute bacterial meningitis. Crit Care Med, 39:812–817.
9. Bentzer P, Fisher J, Kong H, Mörgelin M , Boyd J and Walley K et al. (2016): Heparin-binding protein is important for vascular leak in sepsis. Intensive Care Medicine Experimental; 4:33.
10. Jane F, James R, Peter B, Devyn P, Matthias S and Keith W, et al. (2017): Heparin-Binding Protein (HBP): A Causative Marker and Potential Target for Heparin Treatment of Human Sepsis-Induced Acute Kidney Injury. Shock, doi: 10.1097/SHK.0000000000000862.
11. European Confederation of Laboratory M (2000): European urinalysis guidelines. Scand J Clin Lab Invest Suppl, 231:1–86.
12. Kidney International Supplements (2012): 2:19-36. Section 2: AKI Definition.
13. Masajtis-Zagajewska A and Nowicki M (2017): Clin Chim Acta; 471:286-291. doi: 10.1016/j.cca.2017.06.003.
14. Linder A, Arnold R, Boyd J, Lange A, Zindovic I and Christensson B: (2015): Heparin-binding protein measurement improves the prediction of severe infection with organ dysfunction in the emergency department. Crit Care Med, 43(11): 2378-2386.
15. Linder A, Christensson B, Herwald H, Bjorck L, and Akesson P (2009): Heparin-binding protein: an early marker of circulatory failure in sepsis. Clin Infect Dis, 49: 1044-1050.
16. Gurgoze MK, Akarsu S, and Yilmaz E (2005): Proinflammatory cytokines and procalcitonin in children with acute pyelonephritis. Pediatric Nephrology (Berlin, Germany), 20(10):1445-1448.
17. Kjölvmark C, Åkesson P, and Linder A (2012): Elevated urine levels of heparin-binding protein in children with urinary tract infection, Pediatr Nephrol, 27: 1301-1308.
18. Kjölvmark C, Påhlman L, Åkesson Pand Linder A(2014): Heparin-Binding Protein: A Diagnostic Biomarker of Urinary Tract Infection in Adults , Infect Dis (1):DOI: https://doi.org/10.1093/ofid/ofu004.
19. Nanda N, Juthani-Mehta M. (2009): Novel biomarkers for the diagnosis of urinary tract infection-a systematic review, Biomark Insights, 4: 111-121.
20. Herwald H, Cramer H, Mörgelin M, Russell W, Sollenberg U, and Norrby-Teglund A, et al., (2004): M protein, a classical bacterial virulence determinant, forms complexes with fibrinogen that induce vascular leakage. Cell, 116:367–379.