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ORIGINAL ARTICLE

Urinary Heparin Binding Protein: an Effective Marker in Assessing the Severity of UTI Compared to Microscopic Urinalysis

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SUMMARY

Background: **Proper and fast diagnosis of urinary tract infections (UTIs) is essential for suitable treatment strategy. Urine culture is the mainstay of diagnostic approaches, but it could be time consuming. Relying on clinical features alone may be misleading and may affect the precision of antibiotic regime. That is why utilizing specific biomarkers such as urinary heparin binding protein (UHBP) may support a more accurate diagnosis and thus a suitable antibiotic choice. In the current work, we aimed to assess the validity of UHBP in distinguishing UTI and whether it is suitable for determining the different severity levels of UTI.**

Methods: **Eighty-seven individuals (71 patients, 16 controls) with different ages were recruited in the current study. Participants were attending the urology outpatient clinic in Al-Rabie hospital with suspected UTI based on clinical features, microscopic, and culture analysis. Urine samples were collected and inspected for UHBP by enzyme-linked immunosorbent assay (ELISA) kit. Microscopic analysis involved red blood cells (RBC), epithelial cells (EC), pus cells (PC), white blood cells (WBC), or leukocytes counts.**

Results: **UHBP showed a significantly high level in UTI patients compared to controls, which parallels the microscopic findings. Categorization of individuals' UHBP results according to the different values of microscopic analysis revealed strong association with the level of UHBP. Based on clinical features, UHBP levels varied significantly with UTI severity, showing significantly elevated UHBP levels (432.4 ± 82.8 pg/mL) in the severe group compared to both the moderate (252.8 ± 79.9 pg/mL, $p < 0.001$) and mild (55.2 ± 39.3 pg/mL, $p < 0.001$) groups. Additionally, the moderate group exhibited significantly higher UHBP levels compared to the mild group ($p < 0.001$). Receiver-operating characteristics (ROC) analysis indicated the power of UHBP in distinguishing the levels of severity of UTI with an area under the curve (AUC) of 0.98 ($p < 0.001$).**

Conclusions: **UHBP is a valid biomarker in distinguishing UTI individuals. It also emerged as an effective marker in detecting the severity of UTI, which could help in distinguishing upper and lower UTIs and supports a more accurate diagnostic scheme.**

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KEYWORDS

UTI, UHBP, microscopic urinalysis, biomarker, urine culture

INTRODUCTION

Urinary tract infections (UTIs) are a common clinical danger that affects worldwide population of all ages [1]. The high incidence rate and the difficult diagnostic scheme have UTIs listed among the challenging illnesses in clinical practice [2,3]. Clinical pictures may

range from asymptomatic bacteriuria to the mostly non-confirming signs of infection in cystitis or the more complicated pyelonephritis, which could be misleading. These may involve pain in urination, incontinence, and hematuria in lower UTI to the more serious fever, flank pain, body aches, and vomiting mostly encountered in upper UTI [4,5]. That is why precise diagnosis is important to differentiate UTIs from other infections with parallel presenting symptoms [6]. Therapeutic options basically involve antibiotics coverage to limit bacterial growth and related complications. However, the choice of antibiotic regime is critical and needs thorough assessment in order to isolate the causative agent and identify the proper medication [7]. These measures are essential to minimize the risk of multi-drug resistant bacterial strains evolving out of improper antibiotic choice or dosage [8]. Currently, microbiologic urinalysis along with culture and sensitivity are the main tools to confirm the diagnosis [9]. Urine culture and sensitivity are the gold standard for diagnosis, which often takes 2 - 3 days to confirm the results [10]. Examining other markers such as interleukin 6 (IL-6), C reactive protein (CRP), and leukocyte esterase may differentially support the diagnosis. However, these may lack specificity and sensitivity, which is why the demand for more reliable biomarkers in UTI is high [11]. Alternative markers in urine that may reflect more accurate results and thus provide evidence to aid in the diagnosis and minimize adverse treatment strategy were investigated [12]. Several markers were identified, both in the serum or urine, to have the potential in recognizing the presence of UTI and aid in the preferential diagnosis. Most of these markers are released in response to inflammation or tissue damage that normally accompanies the bacterial invasion [13,14]. Among these, heparin binding protein (HBP) is an inflammatory mediator that is increased in response to inflammation and infection and is considered a valuable diagnostic aid in certain diseases. It is a multifunctional inflammatory mediator protein that is released by activated neutrophils and contributes to the inflammatory process by increasing vascular permeability [15,16]. Urinary levels of heparin binding protein (UHBP) have shown a strong association with UTI, based on recent studies, showing this biomarker to be a promising aid in the assessment and diagnosis of UTI [13,17]. In the current work, we wanted to highlight the role of UHBP as a marker in reflecting the variable level of severity of UTI. The traditional microscopic urinalysis and urine culture were compared with the measured UHBP levels to assess the correlation between these markers. Also, the type of the bacterial culture obtained was evaluated together with the level of urine HBP to explore any potential association between bacteria and UHBP.

MATERIALS AND METHODS

Study population and design

A total of 149 individuals (122 patients, 27 controls) were screened to be enrolled in the current study. All study participants were attending urology outpatient clinic in Al-Rabie hospital with suspected UTI based on presenting one or more symptoms such as dysuria, supra pubic pain, lower back pain, and fever. The hospital covers a populated area of around 800,000 individuals in the north region of the east coast of Mosul. Out of the total number of screened individuals, only 87 individuals (71 patients, 16 controls) with varying ages (11 - 80 years) were enrolled. The rest of the participants were excluded from the study as they did not meet the inclusion criteria for the current study (including the presence of mixed infections, history of renal impairment, and asymptomatic bacteriuria). Other subjects were excluded owing to faults in samples (such as mixed fungal and bacterial culture) or sample collection errors as many patients failed to follow the mid-stream urine collection requirement. A written consent form was obtained from all participants explaining the purpose of study, individuals' rights, and withdrawal terms. For children, a parent consent form was signed by the person in charge. A questionnaire was collected from each subject to involve age and gender in addition to a brief description of the chief complaints and medical history.

The primary outcome of the current study was to examine the valid use of UHBP as a biomarker for reflecting several levels of severity in UTIs. This was measured through the correlation between UHBP levels and clinical severity indicators, such as symptoms, and microscopic urine analysis including urine culture. In order to achieve this goal, we went through certain exploratory (secondary) outcomes, such as the comparison of UHBP levels between UTI patients and controls, to determine whether UHBP levels could effectively differentiate between individuals with and without UTIs. In addition, we evaluated the relationship of UHBP levels to patient outcomes involving symptoms to assess its role as a prognostic tool for predicting the progression of UTIs. We also utilized the ROC analysis to define cutoff values of UHBP, and we correlated this to the available clinical findings and microscopic analysis.

Exclusion criteria

Individuals with a history of renal failure and diabetes mellitus were excluded from the study. Also, any subject receiving antibiotic treatment in the meantime of collection or recently was not included in the current study. Apparently healthy individuals of varying ages were assigned as controls based on urine analysis and culture results. Accordingly, any individual with positive urine culture but without presenting evident signs and symptoms, clinically diagnosed as asymptomatic bacteriuria, was excluded from the study.

Sample collection

A total of 10 mL mid-stream urine sample was collected from each participant in sterilized plain tubes and centrifuged at 3,000 rpm. Individuals were advised on the proper sample collection by lab professionals. Each collected urine sample was aliquoted into 3 Eppendorf tubes containing 3 mL each. Two of the aliquoted samples were utilized to perform general urine analysis, including chemical and microscopic analysis, and urine culture test. The third aliquot was frozen at -20°C for later detection of HBP level.

Laboratory analysis

Collected samples were immediately inspected for physical and chemical analysis (turbidity, pH, and specific gravity). Microscopic analysis was performed using Olympus light microscope (x 40) to inspect samples for evidence of red blood cells (RBCs), bacteria, leukocytes, and pus cells. The rest of the sample was implicated to urine culture analysis to detect the amount of microbial colony growth. Laboratory analysis procedures were conducted according to the standards of antimicrobial susceptibility testing issued by the Clinical and Laboratory Standards Institute (CLSI).

UHBP testing

The levels of UHBP were assessed by enzyme-linked immunosorbent assay (ELISA) kit supplied by Cusabio, USA (cat. no. CSB-E09698h). The kit supplies a pre-coated microplate with antibodies specific for human HBP, which will ensure the biding of HBP present in samples with the immobilized antibodies. Experimental work and techniques were performed according to manufacturer`s standards and assay protocol provided with the kit. Frozen urine samples were thawed at room temperature along with all kit reagents; urine samples were centrifuged again before assay. Then, 100 µL of samples were added into the pre-coated microplate wells, sealed, and incubated at 37°C for 2 hours. Next, samples were removed from the microplate wells and 100 µL of biotin antibody (specific for HBP) was added to each sample well, sealed, and incubated for 1 hour at 37°C. Sample wells were aspirated and washed 3 times with 200 µL wash buffer. Thereafter, 100 µL of avidin conjugated horseradish peroxidase (HRP) was added to each well and incubated for 1 hour at 37°C. A 5 times washing was then followed to remove any unbound avidin-enzyme reagents. A 90 µL substrate solution (TMB-substrate) was added to the wells and incubated for 30 minutes at 37°C protected from light; color developed in proportion to the amount of HBP present. The color development was stopped by adding 50 µL stop solution, and the intensity of the color was measured by microplate reader at 450 nm wavelength.

Statistics

Data were collected and analyzed with SPSS (version 21), and Microsoft Excel (365th edition). Descriptive statistics were performed to reveal parametric scheme and demographic characteristics showing mean and standard deviation (SD). Student´s *t*-test, Pearson chi-squared test, and Fisher's exact test were utilized to compare data. UHBP and microscopic data-based grouping were compared with Student´s *t*-test and ANOVA. For ANOVA, post-hoc analysis was conducted using Tukey's test. Data correlation was determined using Pearson correlation and Spearman's rho. Data were considered significant at $p \leq 0.05$.

RESULTS

A total of 87 participants were the final recruitment in this study, including 16 healthy controls and 71 patients diagnosed with UTI and confirmed by a positive urine culture. The control group has a mean age of 51.3 ± 12.5 years (range 21 - 67) and an equal gender distribution (50% male, 50% female). In the UTI group, the mean age was 44.2 ± 21.7 years (range 11 - 80 years), with 44 (62%) female and 27 (38%) male participants. Notably, both groups had a comparable gender distribution, and there was no significant difference in age (p-value > 0.05) (Table).

The control group exhibited normal findings on microscopic urinalysis. Additionally, the urine cultures from all control group participants yielded negative results, indicating the absence of bacterial growth. In contrast, the UTI group presented with varying degrees of severity, manifested through a spectrum of symptoms and diverse findings on urine analysis and urine culture (Table). The level of UHBP in the control group was 17.8 ± 0.8 pg/mL, and no significant correlation was observed with age or gender within this group (Table 2). In contrast, the UTI group exhibited markedly elevated UHBP levels, with a mean of 176.9 ± 162.8 pg/mL, which was found to be significantly higher compared to the control group ($p < 0.001$) (Table 1).

To investigate the relationship between UHBP and microscopic findings in UTI patients, the participants of this group were categorized on the basis of their urinalysis results (Table). UTI patients with RBC count ≥ 3/HPF exhibited significantly elevated levels of UHBP (274.9 ± 152.7 pg/mL) compared to those with RBC count < 3/HPF (81.6 ± 106.9 pg/mL) (Table 3, Figure 1a). Conversely, no significant difference in UHBP levels was observed when the participants were stratified by EC count to ≤ 5/HPF and > 5/HPF (Table 3, Figure 1b). The UTI group was also categorized, based on WBC and pus cell counts per HPF, into three groups: low (≤ 20 cells/HPF), medium (21 - 50 cells /HPF), and high (> 50 cells/HPF). Stratification by WBC count revealed significantly higher UHBP levels in the medium (256.2 ± 127.5 pg/mL) and high WBC count (332.3 ± 138.6 pg/mL) groups compared to the low count group (62.8 ± 79.4 pg/mL), with no further difference observed between the medium and high groups (Table 3, Figure 1c). Interestingly, categorizing by pus cell count revealed a more pronounced pattern.

Table 1. Demographic characteristics and measurements for the control and UTI groups.

	Control (n = 16)	UTI (n = 71)	p-value
Gender, n (%)			
Female	**8 (50 %)**	**44 (62 %)**	**> 0.05**
Male	**8 (50 %)**	**27 (38 %)**	
Age (years)			
Mean ± SD	**51.3 ± 12.5**	**44.2±21.7**	**> 0.05**
Range	**(21 - 67)**	**(11- 80)**	
UHBP (pg/mL)			
Mean ± SD	**17.8 ± 0.8**	**176.9 ± 162.8**	**< 0.001**
Range	**16.0 - 19.3**	**20.4 -558.4**	
Microscopic urinalysis			
WBC (≥ 5 /HPF)	**0 (0 %)**	**58 (82 %)**	**< 0.001**
Pus cells (≥ 5 /HPF)	**0 (0 %)**	**67 (94 %)**	
RBC (≥ 3 /HPF)	**0 (0 %)**	**35 (49 %)**	
EC (> 5 /HPF)	**0 (0 %)**	**37 (52 %)**	
Bacteria (≥ 5/HPF)	**0 (0 %)**	**59 (83 %)**	
Urine culture and microbial isolates			
Culture-positive	**0 (0 %)**	**71 (100 %)**	**< 0.001**
Escherichia coli	**-**	**31 (43.7 %)**	
Staphylococcus aureus	**-**	**21 (29.6 %)**	
B Streptococcus	**-**	**10 (14.1 %)**	
Enterococcus faecalis	**-**	**4 (5.6 %)**	
Pseudomonas aeruginosa	**-**	**2 (2.8 %)**	

*** p-values were analyzed with Student´s *t*-test, Pearson´s chi-squared test, and Fisher's exact test.**
*** n - number of participants, SD - standard deviation, RBC - red blood cells, WBC - white blood cells, EC - endothelial cells, HPF - high power field.**

Table 2. Correlation between UHBP and demographic characteristics.

Parameter	Correlation coefficient	p-value
Age	**0.349**	**0.19**
Gender	**0.028**	**0.92**

*** The p-value was determined using Pearson´s correlation analysis for age and Spearman's rho for gender (n = 16).**

The high pus cell group exhibited significantly elevated UHBP levels (360.4 ± 104.4 pg/mL) compared to both the medium (106.9 ± 48.9 pg/mL) and low (31.9 ± 10.1 pg/mL) groups. Additionally, the medium pus cell group had significantly higher UHBP levels compared to the low group (Table 3, Figure 1d). Moreover, the correlation between UHBP and pus cell count was found to be remarkably strong ($r = 0.9$, $p < 0.001$), as shown in Figure 2.

The UTI group was also stratified by bacterial count per HPF. As the majority (97.2%) had counts below 50 cells/HPF, with only two samples (2.8%) exhibiting counts > 100 cells/HPF, the UTI group was categorized into ≤ 10 cells/HPF, 11 - 20 cells/HPF, and > 20 cells/HPF. Participants with bacterial count > 20 cells/HPF exhibited significantly higher UHBP levels (329.0 ± 146.7 pg/mL) compared to those in other groups (11 - 20 cells/HPF: 141.7 ± 155.9 pg/mL and > 20 cells/HPF:

Table 3. Comparison of UHBP levels across microscopic urinalysis and urine culture groups.

Microscopic urinalysis and urine culture groups	n	UHBP (mean ± SD)	Test statistic	p-value
RBC count/HPF			**6.2**	
Low < 3/HPF	**36**	**81.6 ± 106.9**		**< 0.001**
High ≥ 3/HPF	**35**	**274.9 ± 152.7**		
EC count/HPF			**0.4**	
Low ≤ 5/HPF	**34**	**167.1 ± 160.3**		**0.632**
High > 5/HPF	**37**	**185.8 ± 166.8**		
Leukocyte count/HPF			**44.5**	**< 0.001**
Low ≤ 20 cells/HPF	**37**	**62.8 ± 79.4**		**low vs. medium < 0.001**
Medium 21 - 50 cells/HPF	**14**	**256.2 ± 127.5**		**medium vs. high 0.118**
High > 50 cells/HPF	**20**	**332.3 ± 138.6**		**high vs. low < 0.001**
Pus cell count/HPF			**157.9**	**< 0.001**
Low ≤ 20 cells/HPF	**25**	**31.9 ± 10.1**		**low vs. medium 0.002**
Medium 21-50 cells/HPF	**19**	**106.9 ± 48.9**		**medium vs. high < 0.001**
High > 50 cells/HPF	**27**	**360.4 ± 104.4**		**high vs. low < 0.001**
Bacterial count/HPF			**11.9**	**< 0.001**
Low ≤ 10 cells/HPF	**28**	**123.9 ± 124.4**		**low vs. medium 0.89**
Medium 11 - 20 cells/HPF	**27**	**141.7 ± 155.9**		**medium vs. high < 0.001**
High > 20 cells/HPF	**16**	**329.0 ± 146.7**		**high vs. low < 0.001**
Culture isolates				**0.001**
E-coli group	**34**	**220.4 ± 155.2**		**E-coli vs. Sta/Str 0.007**
Sta/Str group	**31**	**104.3 ± 139.5**		**Sta/Str vs. Ent/Ps 0.01**
Ent/Ps group	**6**	**305.7 ± 173.2**		**Ent/Ps vs. E-coli 0.41**

*** n - number of participants, SD - standard deviation, EC - endothelial cells, RBC - red blood cells.**
*** p-values were determined by Student´s *t*-test or ANOVA test as appropriate. For ANOVA, post-hoc analysis was conducted using Tukey's test.**
*** The test statistics are t value for the *t*-test and F value for ANOVA test.**
*** Sta/Str - Staphylococcus aureus and B Streptococcus group, Ent/Ps - Enterococcus faecalis and Pseudomonas aeruginosa group.**

123.9 ± 124.4 pg/mL) (Table 3, Figure 1e). Furthermore, the UTI group was stratified by bacterial isolates (culture results) into Escherichia coli (E. coli), Staphylococcus aureus/B Streptococcus, and Enterococcus faecalis/Pseudomonas aeruginosa groups (Table). Mean UHBP levels were 220.4 ± 155.2 pg/mL, 104.3 ± 139.5 pg/mL, and 305.7 ± 173.2 pg/mL, respectively. UHBP levels were significantly lower in the Staphylococcus aureus/B Streptococcus group compared to the others, while no significant differences were observed between the E. coli and Enterococcus faecalis/Pseudomonas aeruginosa groups (Table 3, Figure 1f). The small sample size in the Enterococcus faecalis/Pseudomonas aeruginosa group (n = 6) may has limited statistical power. Categorizing UHBP based on microscopic findings and urine culture suggests a potential link between UHBP levels and UTI severity. The UTI patients of this study had non-complicated infections with symptoms ranging from mild to severe, while the asymptomatic cases were excluded. Stratifying the participant by clinical presentation allows for examination of UHBP levels across each severity group.

The UTI patients were divided into three groups: mild (n = 40, primarily experiencing dysuria and mild suprapubic pain), moderate (n = 17, showing dysuria, incontinence, suprapubic pain, and sometimes hematuria), and severe (n = 14, displaying fever, body aches, and back or groin pain, in addition to other UTI symptoms). UHBP levels varied significantly with UTI severity. Participants in the severe group exhibited significantly elevated UHBP levels (432.4 ± 82.8 pg/mL) compared to both the moderate (252.8 ± 79.9 pg/mL, $p < 0.001$) and mild (55.2 ± 39.3 pg/mL, $p < 0.001$) groups. Additionally, the moderate group exhibited significantly higher UHBP levels compared to the mild group ($p < 0.001$) (Figure 3).

Receiver-operating characteristics analysis (ROC) was used to determine cutoff values of UHBP distinguishing

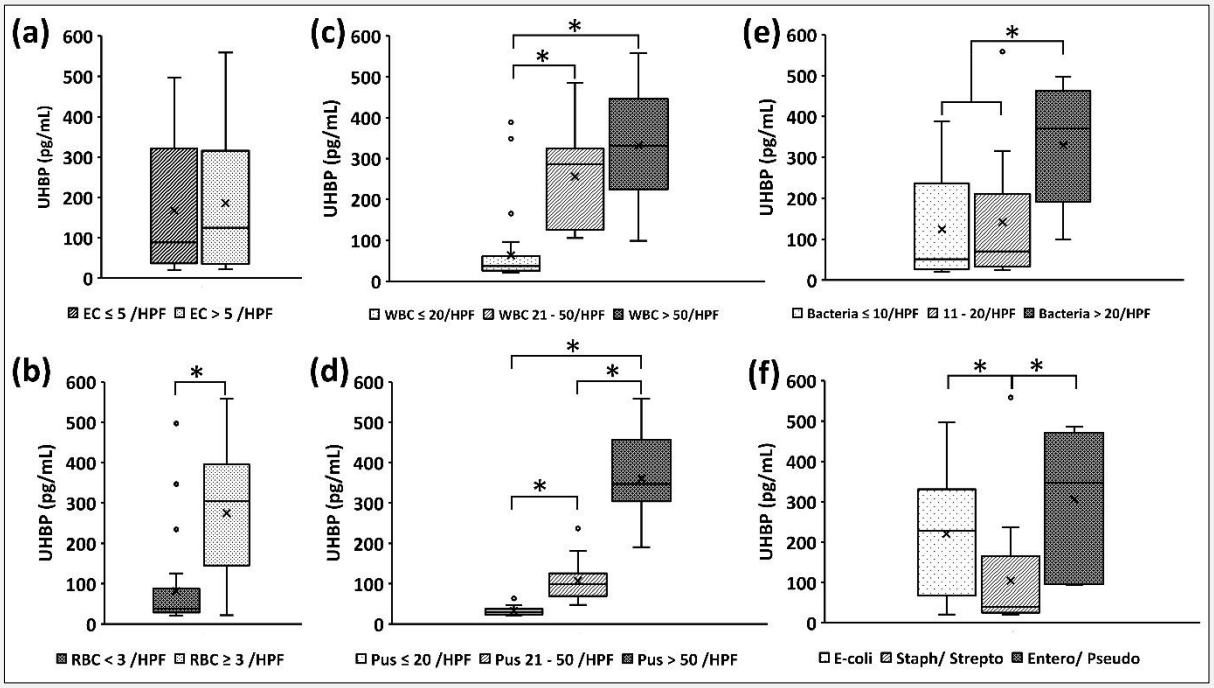

Figure 1. Box plots of UHBP levels (pg/mL) in UTI group stratified by microscopic urinalysis parameters and urine culture isolates.

(a) RBC count, (b) epithelial cells, (c) WBC count, (d) pus cells, (e) bacteria, and (f) bacterial isolates. The box represents the interquartile range, the line through the box represents the median level, and × is the mean. The whisker represents the values up to 1.5 times the interquartile range, while the (•) are the outliers above the upper limit of the whisker.

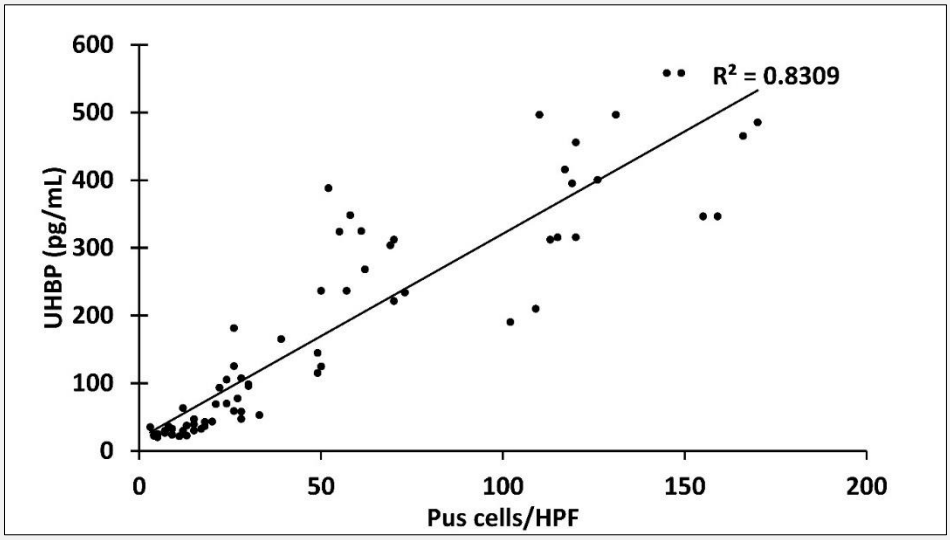

Figure 2. Correlation between pus cell count/HPF and UHBP (pg/mL).

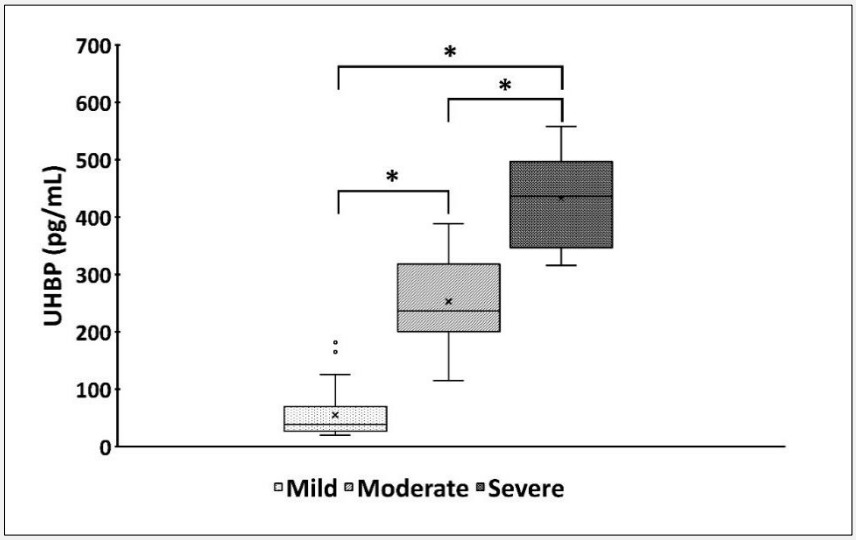

Figure 3. Box plot of the UHBP levels (pg/mL) among the UTI groups stratified by symptoms.

The box represents the interquartile range, the line through the box represents the median level, and × is the mean. The whisker represents the values up to 1.5 times the interquartile range, while the (•) are the outliers above the upper limit of the whisker. Asterisks indicate significant levels at ≤ 0.05.

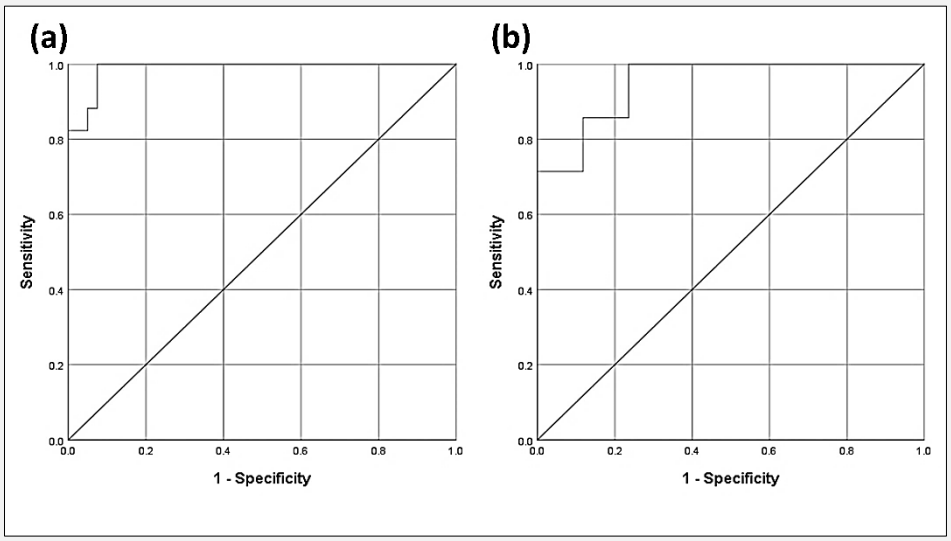

Figure 4. ROC curve analysis of heparin-binding proteins for differentiating UTI different severity levels based on symptoms.

(a) Between mild and moderate level of severity, with AUC of 0.98 ($p < 0.001$); (b) between moderate and severe levels of severity, with AUC of 0.95 ($p < 0.001$).

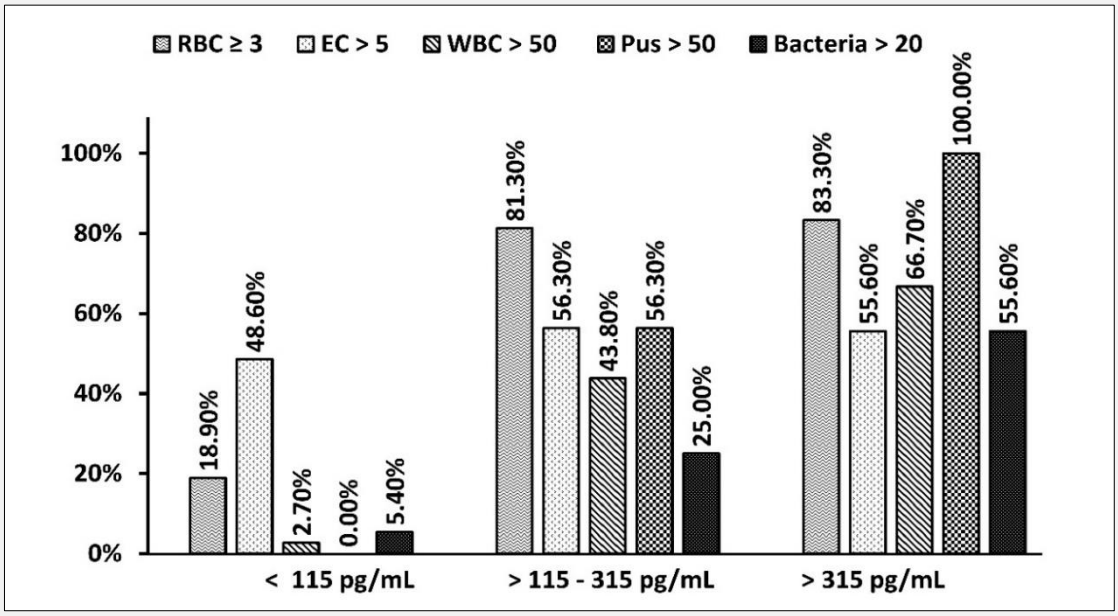

Figure 5. Distribution of urinanalysis findings in UTI patients based on UHBP cutoff values.

Each bar corresponds to the percentage of cases reflected per each microscopic parameter categorized according to the identified levels of UHBP.

between the UTI groups stratified by clinical presentation. The ROC curve yielded an area under the curve (AUC) of 0.98 ($p < 0.001$) between the mild and moderate group, and a UHBP level of 115 pg/mL was chosen as the cutoff point, with sensitivity of 100% and specificity of 99.9%. Similarly, for distinguishing between the moderate and severe groups, an AUC of 0.95 ($p < 0.001$) was obtained, with a cutoff UHBP level of 315 pg/mL, and sensitivity and specificity both at 100% and 97.6%, respectively (Figure 4).

Interestingly, all participants with UHBP > 315 pg/mL displayed a high pus cell count (> 50) in their urinalysis, while none of those with UHBP levels below 115 pg/mL showed such high counts. Additionally, there is a noticeable trend of increased percentages of samples with elevated WBC counts (> 50) and elevated bacterial counts (> 20) as UHBP levels rise from < 115 pg/mL (2.7%, 5.4%) to 115 - 315 pg/mL (43.8%, 25%), with a further increase in the > 315 pg/mL (66%, 55%) category, as shown in Figure 4. The percentage of EC > 5 remained comparable across the UHBP groups (Figure 5).

DISCUSSION

Individuals with UTI recruited in the present study presented a plethora of symptoms, which may reflect their clinical conditions at variable grades. This was shown at the minimum level with only dysuria up to fever and body aches, which could refer to a more complicated infection. Sometimes, the clinical picture could be misleading, as certain UTI patients could be asymptomatic [18]. Although HBP proved to be an effective marker in diagnosing bacterial from non-bacterial infection, the accuracy of UHBP in distinguishing asymptomatic from symptomatic bacteriuria was low [19]. That is why we engaged only individuals with confirmed UTI to assess their UHBP levels based on their microscopic findings and clinical conditions. The levels of UHBP in the UTI group showed significant elevations with wide range (20.4 - 558.4 pg/mL), which may imply a positive correlation with the multiple grades of UTI patients included. Also, the microscopic indices in the UTI group indicated positive values with significant increase in all individuals compared to the negative controls. These findings were in agreement with several studies that highlighted the elevation of UHBP in UTI patients compared to healthy individuals and indicated its validity as a diagnostic marker in UTI [13,20]. The UTI group in the current study has been confirmed with positive urine

culture compared to controls. Culture findings revealed a high percentage of E. coli (43.7%), followed by Staphylococcus aureus (29%), and lower percentages of other bacteria. This may indicate that our findings reflect normal bacterial isolates that are commonly encountered in UTI patients. Our culture findings were in agreement with previous studies indicating E. coli as the predominantly isolated bacteria from urine culture [21, 22]. The results were also in accordance with findings from Bi et al. and Tessema et al. in revealing that S. aureus was the 2nd abundant strain after E. coli isolated in urine culture [23,24]. However, some reports suggested that Klebsiella pneumonia accounts for the 2nd most abundant bacterial isolate in urine, which disagrees with the current findings [25,26]. This is probably because Klebsiella pneumonia is mostly isolated in hospitalized patients and those with poor immunity, which were not included in our study [27].

As we were focusing on the validity of UHBP in reflecting the multiple severity of UTI, the present microscopic and culture findings were grouped based on their recognized positive/negative ranges to show the level of UHBP in each group (Table 3). Only traces of RBCs normally exist in urine samples, which is below 3 cells/HPF; RBC levels above this may be considered abnormal [28]. Shedding of epithelial cell lining of the urinary tract is normally recognized by only small amounts of ECs in urine (below 2 - 3 cells/HPF); when increased, it reflects irritation, infection, or inflammation of the cell lining [29]. That is why both RBCs and ECs were classified into two groups to indicate low or high values which mostly appeared in infection. The mean UHBP level was significantly elevated in individuals with high RBC counts, while no significant difference was observed in UHBP levels between individuals with low or high EC counts. The presence of RBCs in urine may indicate inflammation or irritation of the tissue lining of urethra, bladder, ureters, or glomerulus, which could be a consequence of UTI [20,30]. However, high RBCs in urine may refer to other conditions such as glomerulonephritis or the presence of elevated urate crystals or kidney stone, which may interferes with UTI assessments [31,32]. Leukocytes, pus cells, and bacteria in urine were categorized into 3 groups based on their count/HPF, featuring low, medium, and high. The elevated counts of both leukocytes and pus cells are indicative of inflammation and immunological reaction, which could possibly be associated with variable levels of UTI [29]. The current results revealed a significant increase in UHBP when both leukocytes and pus cells are markedly elevated in urine. A leukocyte's sharp elevation ($p < 0.001$) was reported from the low to the medium range, which then plateaued at the high cell count range ($p = 0.118$). Pus cells present a better statistical strength as they reflect significantly ($p \leq 0.02$) elevated UHBP levels between the three groups, steeping at a mean level of 360 pg/mL when the cells were over 50/HPF. This finding was strengthened by the strong correlation between UHBP level and pus cell count (Figure 2). These findings were in accordance with El-Refaey et al. in revealing strong association between pus cell count and UHBP level [33]. Kjölvmark et al. inspected UHBP levels in children with UTI and indicated that increased levels of UHBP are in accordance with white blood cell (WBC) count in urine, which also is in agreement with the current results [34].

Only individuals with high bacterial count of over 20 bacteria/HPF showed significant UHBP levels compared to medium and low count groups. Interestingly, individuals with low bacterial count of less than 10 bacteria/HPF showed UHBP levels of over 100 pg/mL, which may highlight the validity of this marker to differentiate UTI from non-UTI subjects. The different types of bacterial culture may suggest a potential association with UHBP level as indicated in the comparison between UHBP and different bacterial isolates (Table 3, Figure 1f). A significantly high UHBP was noticed for the E. coli and Enterococcus/Pseudomonas culture group compared to the Staphylococcus/Streptococcus culture group. The Enterococcus/Pseudomonas culture group featured the highest UHBP mean level compared to all bacterial isolates. However, Enterococcus/Pseudomonas culture indicated no significant UHBP level compared to the E. coli group, which may be explained by the few numbers of cases identified with Enterococcus faecalis and the even less identified cases with the Pseudomonas aeruginosa (only 2 cases). We could not find any evidence to support our findings in associating the type of urine bacterial culture obtained to the level of UHBP. As general knowledge, HBP is considered an inflammatory marker that is elevated in response to inframammary reaction or any infection. With this in mind, HBP may be elevated in any type of bacterial infection, with no specific rule to suggest a link between the level and the type of bacteria. However, in a study by Snäll et al. to assess the neutrophil response in bacterial infection and its association with the release of markers involving HBP and resistin, a correlation was suggested between the level of biomarker and the type of bacterial sepsis. The same study declared the strongest release of HBP with Streptococcal strains compared to staphylococcal and E. coli strains [35]. Although the study event and circumstances may not correlate well with the current study design, it provides evidence of a possible association of this biomarker with specific types of bacteria. More studies with a larger sample size are recommended in this field to inspect this hypothesis.

Findings from microscopic urinalysis and UHBP suggest a strong association between the severity of UTI and the level of UHBP. As such, we sought to categorize the participants based on their clinical presentation and severity into mild, moderate, and severe groups. These comparisons came out in support of our previous findings as they revealed significantly increased UHBP ($p < 0.01$), which was in line with the severity of symptoms presented by each group. In light of this, UHBP levels may reflect multiple grades of severity of UTI. This could be helpful considering that these levels

emerged to differentiate between conditions such as cystitis and pyelonephritis at different magnitudes. With this in mind, ROC analysis strengthens the diagnostic power of UHBP and was utilized to obtain cutoff values for UHBP that correlate with different levels of severity. As such, our results revealed a UHBP level of < 115 pg/mL to reflect mild cases of UTI, while > 312 pg/mL indicated sever cases. The levels between (or equal to) 115 and 312 were assumed to reveal moderate cases, in parallel to microscopic findings and symptoms. As we wanted to ascertain the effectiveness of UHBP in showcasing UTI at multiple grades, we assigned the high levels of the microscopic findings in order to compare the percentage of cases based on the obtained cutoff UHBP levels. In this case, the percentage of individuals reflecting WBC and PC counts > 50, bacteria > 20, RBC ≥ 3, and EC > 5/HPF were compared. Results indicated strong association between the level of UHBP and the results from microscopic analysis, showing higher values when the UHBP levels were above 312 pg/mL. PCs were one of the significant findings, as 100% of cases of over 50 PC/HPF scored more than 312 pg/mL of UHBP level. The percentage of individuals showing high values of WBCs and bacterial count analysis was also progressively increased with the level of UHBP. Over 80% of cases showed elevated RBC count when the level of UHBP was above 115 pg/mL. This is reasonable considering that the level of HBP will increase when there is enhanced inflammation and tissue irritation. When the level of UHBP is below 115 pg/mL, we can see that only a small percentage of cases revealed higher microscopic cell counts. However, up to 48% of individuals were showing elevated EC counts of over 5/HPF when the UHBP was below 115 pg/mL. These results may prove that UHBP is an effective biomarker in determining the magnitude of severity of UTI to a certain limit. The UTI group of individuals presenting severe symptoms, such as fever, groin, and body aches, are mostly referring to upper UTI, based on the general knowledge of clinical presentation for UTI [27]. Subjects presenting with less severe symptoms without fever, categorized as mild and moderate, may reflect lower UTI at multiple grades. This may indicate the validity of UHBP to differentiate between cystitis and pyelonephritis. This was in accordance with a review study by Horváth et al. to define specific biomarkers in the diagnosis of UTI suggesting the role of UHBP in distinguishing upper from lower UTI [13]. Kjölvmark et al. evaluated the level of UHBP in 390 adults categorized according to symptoms and microbiological tests into cystitis and pyelonephritis. The study showed the diagnostic accuracy of UHBP in UTI and its effective role in distinguishing cystitis from pyelonephritis, which was in agreement with the current study [36]. In an earlier study by Kjölvmark et al., the level of UHBP was compared with dipstick analysis and interleukin-6 (IL-6) in urine. The study also indicated the high specificity of UHBP in diagnosing UTI compared to other parameters, including WBC and IL-6, which was in accordance with the present study [34]. In the current study, the role of UHBP as a diagnostic marker for UTI has been further highlighted in alignment with previous findings, with additional focus on revealing the validity of this marker in revealing various levels of severity. Based on the present findings, certain levels of UHBP may be valuable in order to define whether the current condition is either mild, moderate, or severe. Our study may also highlight evidence of association between UHBP level and the type of bacteria indicated in urine culture, which may require a future investigation.

The current study may be subject to some limitations that we want to outline. Although our data was confirmed by urine culture and microbiologic analysis, we believe that utilizing computed tomography (CT) scan or ultrasound (US) imaging as added diagnostic evidence would support our results. In addition, we believe that a larger sample size may support more accurate findings. For our study, however, the strict exclusion criteria for the control subjects in regard to sample collection, e.g. no history of recent infections or other comorbidities that could influence urinary HBP levels, made this challenging. Despite the limited sample size, our study could serve as pilot research for investigating the use of the new biomarker, which is UHBP, for UTI severity assessment. Future studies utilizing larger populations will be required to confirm the current findings. With that being said, we believe that our study strengthens the limited data available in showing the validity of UHBP in diagnosing UTI. Our study also detailed the correlation of microbiological and culture analysis with UHBP levels to reflect its strong association with UTI severity. This could further enhance the current knowledge in the diagnosis of UTI, serve the medical practitioners in evaluating the clinical conditions, and potentiate decision making.

CONCLUSION

Finding a suitable, less invasive, reliable, and time-saving biomarker to assess the diagnosis of UTI will aid in providing the appropriate therapeutic regime. UHBP has been observed to be a promising biomarker to assist in the diagnosis of UTI. The current study indicated the valid role of UHBP as a biomarker in distinguishing UTI individuals. The study also revealed a strong association between UHBP levels and microbiological urinalysis. The UHBP levels were also positively correlated with symptoms, making it an effective marker in detecting severity of UTI, which could help in distinguishing upper and lower UTIs. The study found evidence of a possible association between UHBP levels and the type of bacterial infection in UTI. This is an interesting outcome that needs to be further examined in future studies.

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