Diagnostic value of platelet parameters versus interleukin-6 in children with urinary tract infection

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KEYWORDS
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MPV;
PDW;
Interleukin-6

Abstract  Background: Urinary tract infection (UTI) is a common problem that is frequently encountered by pediatric healthcare providers. Diagnosis of UTI is often difficult, particularly in critically ill patient and in patients with nonspecific and mild symptoms. Testing for platelet parameters is inexpensive, easily accessible and routinely performed that had been recognized as a hallmark in the diagnosis of platelet activation during infection and inflammatory disorders.

Aim: To evaluate the value of platelet parameters in the diagnosis of UTI in children in comparison to interleukin-6.

Method: This case–control study included 88 children; 44 of them had culture proved UTI. In addition 44 age and sex matched healthy children served as a control group. Complete blood picture with emphasis on platelet parameters (mean platelet volume (MPV), platelet distribution width (PDW) and platelet count), C-reactive protein, erythrocyte sedimentation rate and interleukin-6 serum levels were done for both groups. We investigated the correlation between platelet parameters, inflammatory biomarkers and the type of organism in children with UTI.

Results: Platelet parameters were significantly higher in children with UTI in comparison to those of the control group. There was a significant positive correlation between platelet parameters, ESR, CRP and interleukin-6 in children with UTI. MPV & PDW have 90.1% & 88.6% sensitivity and 86.3% & 84.1% specificity respectively for diagnosis of UTI. Platelet parameters are significantly higher in children with gram positive infection.

Conclusion: Platelet indices could be used as an indicator of UTI in children. It could predict the type of causative organism. Further researches are needed to evaluate its role for detection of recurrence of UTI in high risk children.

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Introduction

Urinary tract infections (UTI) are a common and a serious clinical problem in children. About 8% of girls and 2% of boys develop at least one episode of acute UTI during childhood. UTI has been increasingly recognized as an occult cause of febrile illness in infants and young children. Upper urinary tract infections (i.e., pyelonephritis) may lead to renal scarring, hypertension, and end-stage renal disease.

The diagnosis of febrile urinary tract infection (UTI) is often difficult in young children who are not capable of accurately describing their physical problems. In young children; UTI presented with nonspecific symptoms include discomfort, fever, poor oral intake, malaise and vomiting. Early diagnosis and prompt antimicrobial treatment are required to minimize renal scarring and progressive kidney damage.

The issue of appropriate urine sampling techniques is of particular concern in young children, where the collection of a sterile, midstream sample can be problematic. Different methods of urine sampling may be differently susceptible to contamination and hence to false positive results. Suprapubic aspiration has been regarded as the reference standard collection method which is an invasive procedure that may require the use of ultrasound guidance. The reference standard for diagnosis of UTI in children is any bacterial growth of a urine culture that had been obtained by suprapubic aspiration.

Although urine culture is regarded as the gold standard of diagnosis, it takes at least 48 h to obtain confirmative results. Although MPV & PDW are not generally taken into consideration by clinicians; they are valuable markers of platelet activation. A higher MPV value is indicative of increased platelet activity reflecting more intense inflammation. Platelet indices had been studied as an inflammatory marker in various diseases but there are no sufficient data about its role in the diagnosis of UTI in children.

IL-6 is a multifunctional cytokine with proinflammatory and immunoregulatory functions. It is a key activator of the acute-phase response and acts on the hypothalamic temperature regulatory center. IL-6 concentrations are considered as useful diagnostic tools increase early in children with different forms of UTI and are higher in patients with acute pyelonephritis than in those with asymptomatic bacteriuria.

On the basis of these previous data, we conducted the present study to assess whether platelet indices are useful markers for diagnosis of UTI in children in comparison to acute phase reactant (ESR & CRP) and IL-6 serum levels. Our hypothesis is that alteration of the platelet morphology can be a reliable index for early diagnosis and management of UTI without waiting for blood culture results. We try to demonstrate a link between the platelet indices and the type of organism, whether gram positive or gram negative to allow proper selection of the empirical antibiotic regimen.

Patients and methods

This prospective case–control study was conducted in pediatric inpatients and outpatient clinics of Al Hussein, Bab El Sharia and AL Zahraa university hospitals, AL-Azhar University, Cairo, Egypt during the period from January 2015 to July 2015. The study included 44 children with culture proved UTI and 44 age and sex matched healthy children as a control group. Informed consent was obtained from the participating children’s parents in adherence to the guidelines of the ethics committee of AL-Azhar University, Cairo, Egypt.

All children were subjected to the following: Detailed history and thorough clinical examination. The relevant clinical history includes demographic data, presenting symptoms, any previous surgery, and current medications. The findings of systemic and abdominal examinations were recorded in detail. The assessment of growth by anthropometric measurements was represented by weight and length/height, which were plotted on Egyptian growth Charts 2002 to detect weight for age and height/length for age percentiles. Systolic and diastolic blood pressure was assessed in all studied children and plotted on blood pressure chart for age to detect any deviation from normal expected values for age.

UTI was diagnosed according to clinical symptoms (fever, vomiting, anorexia, dysuria, frequency of micturition, abdominal or flank pain) and confirmed by abnormal urine analysis and positive urine culture.

The following criteria were included in our study: age from 2 years up to 6 years, positive urine culture (urine culture reviled more than 105 colonies/ml of a single pathogen in a midstream urine sample). On the other hand we excluded children with any hematological disorders affecting the platelet (e.g. Hemolytic anemia, iron deficiency anemia, thrombocytopenia, and leukemia), those with any concomitant acute or chronic illness (e.g. diabetes, obesity, hypothyroidism, autoimmune diseases as ankylosing spondylitis, rheumatoid arthritis, inflammatory diseases as acute appendicitis, ulcerative colitis, chronic infectious diseases, chronic renal, cardiac, hepatic...
Laboratory investigations

Laboratory investigations

Urine analysis. Random urine samples were obtained by either mid-stream technique or by using sterile urine bag after washing and cleaning the genitalia and examined within one hour (physically, chemically aided by Medi Test urine strips and microscopically).

Urine culture. Another clean urine sample was obtained as before. Cultures were done on Cled plate, Blood agar plate, MacConkey and Sabaroud Plates (Oxoid). Yielding plates were subjected to gram stained film, colony count and API for identification. Sensitivity was done on Muller Hinton agar media.

Blood samples

Five ml of venous blood sample was drawn under complete aseptic conditions (alcohol swab 70%) in different BD vacutainer’s, (K2 EDTA for CBC, Na Citrate for ESR and plain gel separator vacutainer for CRP and IL-6).

The plain gel separator vacutainers were kept in a water bath at 37°C for 30 min then centrifuged at 3500 rpm for 10 min. Serum was aspirated in 2 Eppendorf, one for CRP bath at 37°C and the other was stored at -25°C then centrifuged at 3500 rpm for 20 min. 50 ul of stop solution was added to each well including the first plate well. The plate was covered by new supplied adhesive sheet and placed in closed dark cabinet for 20 min. 50 ul of stop solution was added to each well including the first plate well. The plate was gently shaken to homogenize the content. Reading was done by using ELISA Tecan sunrise reader adjusted at 540 nm and 570 nm for background. The first plate’s well reading was subtracted from all other readings. The readings (OD) of the calibrators are plotted on the X axis while the values are plotted on the Y axis. The concentrations were obtained by interpolation (reference range < 12.5 pg/ml).

Statistical analysis

Statistical analyses were performed using SPSS version 15 for Windows software (SPSS Inc., Chicago, IL, USA). The demographic and clinical properties of patients are expressed using mean ± SD and percentage values. Differences between groups were analyzed with the Mann–Whitney U test and paired t-test. Non parametric data were compared by chi square test. Correlations between groups were performed using Spearman correlation coefficients. Receiver operating characteristic curves (ROC) were used to identify sensitivity, specificity and determine optimal cut-off points of platelet indices & IL-6 for prediction of UTI. A P-value < 0.05 was considered to be statistically significant.

Results

This study included 44 children with culture proved UTI (16 male and 28 female). In addition, 44 healthy children were enrolled as a control group (21 male, 23 female). Their age ranged between 2 and 6 years. By urinary culture Gram +ve organisms were detected in 11/44 (25%) of children with UTI; which included Enterococci (5 cases) and Staphylococcus aureus (6 cases), while Gram -ve organisms were detected in 33/44 (%75) of children with UTI; which included Escherichia coli (24 case), Proteus (7 cases) and Klebsiella (2 cases).

Table 1 shows the general characteristics, clinical data of the studied children. No age and gender differences were found among children involved in the study. As regards hematological data, children with UTI had significantly higher WBCs, neutrophil% and inflammatory biomarker (ESR, CRP, IL-6) levels than healthy children. The clinical manifestations of UTI among our studied children are shown in Fig. 1.

Table 2 shows a significant increase in platelet count and indices (MPV & PDW) in children with UTI in comparison with healthy children. While Table 3 shows a significant increase in platelet indices (MPV & PDW) but not platelet counts in children with gram positive UTI in comparison to those with gram negative infection.

Table 3 shows that platelet parameters (MPV & PDW) but not platelet counts are significantly higher in those with gram positive than those with gram negative urinary tract infection. Pearson correlation analysis showed a significant positive correlation between platelet count & parameters (MPV &
and inflammatory markers (ESR, CRP, IL-6) in children with UTI. Additionally, platelet parameters (MPV & PDW) had significant positive correlations with WBCs count and neutrophil% in children with UTI ($p < 0.05$) as demonstrated in Table 4.

Table 5 shows cut off points, sensitivity and specificity of platelet count & parameters (MPV & PDW) and inflammatory markers (ESR, CRP, IL-6) for diagnosis of UTI in children.

**Discussion**

Rapid assessment is important for proper management decisions in young children with suspected UTI. Platelets, as a part of the natural immune system, can be elevated as a part of acute phase reaction during the inflammatory process. This increase in platelets can reflect the bone marrow cell activity in response to proinflammatory interleukins, such as IL-1 and IL-6.25,26 As platelets are natural sources of growth factors including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1) and transforming growth factor β (TGF-β), they have an important role in inflammation, angiogenesis, repair and regeneration of tissues.27

Activation of the platelets causes some morphological alterations: the activated platelets seem larger by becoming spherical in shape and forming pseudopodia. Platelet activation leads to changes in platelet shape with an increase in platelet swelling leading to an increase in MPV and PDW.28

Our study demonstrated that children with UTI had a significantly higher platelet count and indices (MPV & PDW) than healthy children. Additionally, there was a significant increase in platelet indices (MPV & PDW) but not platelet count in children with gram positive UTI in comparison to those with gram negative infection.

This is in accordance with Catal et al.29 who demonstrated that the platelet counts were higher in patients with upper UTI.

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**Table 1** Comparison of clinical and laboratory data of the studied children.

<table>
<thead>
<tr>
<th></th>
<th>Children with UTI ($N = 44$)</th>
<th>Control group ($N = 44$)</th>
<th>Mann–Whitney/Chi-square test</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6.100 ± 2.300</td>
<td>6.401 ± 3.100</td>
<td>−0.962</td>
<td>0.337</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>16/28</td>
<td>21/23</td>
<td>0.681</td>
<td>0.409</td>
</tr>
<tr>
<td>Weight (z-score)</td>
<td>0.241 ± 0.852</td>
<td>0.240 ± 1.091</td>
<td>−0.031</td>
<td>0.976</td>
</tr>
<tr>
<td>Length (z-score)</td>
<td>0.213 ± 1.044</td>
<td>0.214 ± 0.923</td>
<td>0.008</td>
<td>0.994</td>
</tr>
<tr>
<td>Systolic Blood pressure (mmHg)</td>
<td>75.332 ± 11.993</td>
<td>71.562 ± 7.520</td>
<td>0.161</td>
<td>0.873</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>59.783 ± 9.174</td>
<td>57.113 ± 8.281</td>
<td>0.962</td>
<td>0.337</td>
</tr>
<tr>
<td>CBC:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>11.283 ± 1.342</td>
<td>11.482 ± 1.382</td>
<td>−0.411</td>
<td>0.683</td>
</tr>
<tr>
<td>RBCs ($\times 10^{12}$/mm$^3$)</td>
<td>4.321 ± 1.301</td>
<td>4.103 ± 2.214</td>
<td>1.135</td>
<td>0.259</td>
</tr>
<tr>
<td>WBCs ($\times 10^{12}$/mm$^3$)</td>
<td>15.502 ± 3.021</td>
<td>9.403 ± 0.143</td>
<td>5.069</td>
<td>0.000*</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>65.970 ± 14.101</td>
<td>45.285 ± 3.403</td>
<td>2.302</td>
<td>0.021*</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>24 ± 19.651</td>
<td>4 ± 1.801</td>
<td>9.552</td>
<td>0.000*</td>
</tr>
<tr>
<td>ESR (1st hour)</td>
<td>35 ± 22.352</td>
<td>12 ± 2.803</td>
<td>8.225</td>
<td>0.000*</td>
</tr>
<tr>
<td>IL-6 (ng/dl)</td>
<td>22 ± 7.301</td>
<td>19 ± 7.600</td>
<td>2.297</td>
<td>0.023*</td>
</tr>
<tr>
<td>Urine analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pus cells</td>
<td>41.818 ± 16.603</td>
<td>3.863 ± 1.440</td>
<td>15.106</td>
<td>0.000*</td>
</tr>
<tr>
<td>RBCS</td>
<td>7.886 ± 3.895</td>
<td>1.545 ± 1.109</td>
<td>10.046</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

UTI: urinary tract infection; CBC: complete blood count; HB: hemoglobin; RBCs: red blood cells; WBCs: white blood cells; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL-6: interleukin-6.

*Significant.
and MPV values were higher in Gram-positive infections compared with Gram-negative infections.

Tekin et al.\(^{30}\) had reported thrombocytosis and elevated MPV & PDW in patients with UTI in comparison to healthy controls. The platelet counts were similar in children with upper and lower UTI with no statistically significant difference in MPV values between those with Gram-positive and Gram-negative infections.

Experimental studies showed that early circulatory responses to a bolus injection of endotoxin have been correlated with the release of thromboxane, which incites platelet aggregation.\(^{31,32}\) Both platelet hyperaggregability and peripheral damage/consumption could eventually over 24 h lead to positive feedback to the bone marrow, resulting in the production of larger and more active platelets.\(^{33}\)

On the other hand, many studies revealed that platelet count is greatly decreased in severe sepsis (thrombocytopenia) but this occurred mainly in late stages of sepsis or severe septicemia as in septic shock due to severe exhaustion of bone marrow (bone marrow failure).\(^{34}\)

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**Table 2** Comparison of platelet count and indices between children with and without UTI.

<table>
<thead>
<tr>
<th></th>
<th>Children with UTI (N = 44)</th>
<th>Control group (N = 44)</th>
<th>Mann–Whitney test/Independent T test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelet count (×10³/mm²)</td>
<td>489.911 ± 106.320</td>
<td>0.760</td>
</tr>
<tr>
<td></td>
<td>Mean platelet volume (ml)</td>
<td>7.804 ± 0.914</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Platelet distribution width (%)</td>
<td>17.231 ± 6.572</td>
<td>0.018</td>
</tr>
</tbody>
</table>

**Table 3** Comparison of platelet count and indices between children with gram positive and gram negative UTI.

<table>
<thead>
<tr>
<th></th>
<th>Children with gram positive infection (N = 11)</th>
<th>Children with gram negative infection (N = 33)</th>
<th>Mann–Whitney test/Independent T test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelet count (×10³/mm²)</td>
<td>512.551 ± 125.298</td>
<td>0.793</td>
</tr>
<tr>
<td></td>
<td>Mean platelet volume (ml)</td>
<td>8.454 ± 0.902</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>Platelet distribution width (%)</td>
<td>19.536 ± 4.561</td>
<td>0.039</td>
</tr>
</tbody>
</table>

**Table 4** Correlation between platelet parameters and inflammatory markers in children with UTI.

<table>
<thead>
<tr>
<th></th>
<th>MPV</th>
<th>PDW</th>
<th>Platelet count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P-value</td>
<td>r</td>
</tr>
<tr>
<td>WBCs (×10³/mm³)</td>
<td>0.340</td>
<td>0.022</td>
<td>0.376</td>
</tr>
<tr>
<td>Neutrophil%</td>
<td>0.393</td>
<td>0.008</td>
<td>0.436</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.415</td>
<td>0.005</td>
<td>0.358</td>
</tr>
<tr>
<td>ESR (1st hour)</td>
<td>0.484</td>
<td>0.001</td>
<td>0.491</td>
</tr>
<tr>
<td>IL-6 (ng/dl)</td>
<td>0.485</td>
<td>0.001</td>
<td>0.650</td>
</tr>
</tbody>
</table>

UTI: urinary tract infection; WBCs: white blood cells; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL-6: interleukin-6. *Significant.

**Table 5** Sensitivity, specificity, PPV, NPV of variables for diagnosis of UTI in children.

<table>
<thead>
<tr>
<th></th>
<th>Cutoff point</th>
<th>AUC</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (×10³/mm³)</td>
<td>385.8</td>
<td>0.895</td>
<td>86.3</td>
<td>81.8</td>
<td>82.6</td>
<td>85.7</td>
</tr>
<tr>
<td>MPV (ml)</td>
<td>7.1</td>
<td>0.930</td>
<td>90.1</td>
<td>86.3</td>
<td>86.9</td>
<td>90.4</td>
</tr>
<tr>
<td>PDW (%)</td>
<td>16.42</td>
<td>0.863</td>
<td>88.6</td>
<td>84.1</td>
<td>84.8</td>
<td>88.1</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>4.74</td>
<td>0.594</td>
<td>77.2</td>
<td>68.1</td>
<td>70.8</td>
<td>75</td>
</tr>
<tr>
<td>ESR (1st hour)</td>
<td>12.82</td>
<td>0.465</td>
<td>70.4</td>
<td>59.1</td>
<td>63.2</td>
<td>66.7</td>
</tr>
<tr>
<td>IL-6 (ng/dl)</td>
<td>25.35</td>
<td>0.640</td>
<td>79.5</td>
<td>72.7</td>
<td>74.5</td>
<td>78</td>
</tr>
</tbody>
</table>

UTI: urinary tract infection; MPV: mean platelet volume; PDW: platelet distribution width; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL-6: interleukin-6; AUC: area under curve; PPV: positive predictive value; NPV: negative predictive value.
As a response to infection, leukocytes and epithelial cells in the urinary tract produce interleukin IL-6. It has been shown that measurement of IL-6 has a diagnostic value with an ability to discriminate between cystitis and pyelonephritis. IL-6 is an important pro-inflammatory cytokine which could induce thrombocytosis and affect platelet volume.

Regarding IL-6 our results show a significant increase in the UTI group in comparison with the control group. This is in agreement with Benson et al. who revealed that infections of the urinary tract activate an IL-6 response which vary with the severity of infection in children and that cytokine activation is influenced by a variety of host and bacterial variables.

Reactive thrombocytosis is usually mediated by an increased release of numerous cytokines in response to infections, inflammation, vasculitis, tissue trauma, and other factors. Thrombopoietin (TPO), the primary cytokine for platelet production and maturation, and interleukin (IL)-6 levels are usually initially elevated in response to the primary events mentioned earlier; they stimulate an increase in platelet production. However, serum or plasma levels of these cytokines do not seem to be correlated with degree of thrombocytosis.

Our study has demonstrated a significant positive correlation between platelet count & parameters (MPV & PDW) and inflammatory markers (ESR, CRP, IL-6) in children with UTI. Previous studies had reported a direct relationship between platelet count and CRP as well as IL-6 during acute inflammation and infections.

The size of the platelet is correlated with the activity and the function of the platelet; larger platelets are more active than smaller ones. PDW is an indicator of variation in platelet size, which can be a sign of active platelet release. MPV has been reported to be an indirect sign of disturbances in platelet production and activity of bone marrow response to infection in both children and adult studies.

One of the limitations of our study is the small number of patients. Also, we did not differentiate between upper and lower UTI. Although dimercaptosuccinic acid (DMSA) renal scan is the gold standard to differentiate upper from lower UTI, this method is costly and exposes children to radioactivity.

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Conflict of interest

Authors declare no competing financial interests in relation to this work.

References


