The Concordance of Dysmorphic Erythrocyte and Cast Erythrocyte Examination using Flowcitometry, Low Condenser Light Microscope, and Phase Contrast Microscope in Children with Glomerular Hematuria

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Abstract

This was an observational analytic research with cross sectional design carried out at the Clinical Pathology Laboratory and Department of Child health of the Dr. Soetomo Hospital Surabaya in April - November 2020. Urine sediment of glomerular hematuria pediatric patient examined using flowcytometry, low condenser light microscope (LLCM), phase contrast microscope (PCM). The concordance of the results of the examination of dysmorphic erythrocytes and erythrocyte cylinders using flowcytometry with PCM was determined by Cohen's Kappa Coefficient (κ) and Bland Altman. There were 40 urine samples of children with glomerular hematuria with many diagnose was lupus nephritis of 60%. The analysis of dysmorphic erythrocyte examination from LLCM and PCM had a very strong kappa agreement κ 0.828 ($p < 0.05$); The concordance analysis of LLCM and PCM cast erythrocyte had a strong agreement, κ 0.625 ($p < 0.05$) and the concordance analysis between Flowcitometry and PCM dysmorphic erythrocyte flagging had a weak agreement, κ 0.302 ($p > 0.05$). LLCM can be considered to replace PCM to determine the origin of hematuria because it is very effective in detecting dysmorphic erythrocytes in patients with glomerular hematuria. The use of automatic tools is useful for pathological urine samples, but it is advisable to review them using a manual macroscope.

Keywords: *Dysmorphic erythrocytes, erythrocyte cylinders, phase contrast microscopy, light microscopy, flowcytometry*

Introduction

Glomerular hematuria is classified according to the number of erythrocytes found in urine into microhematuria and macrohematuria. The causes of glomerular hematuria in children are IgA nephropathy, post streptococcal acute glomerulonephritis (GNAPS), primary glomerulonephritis, systemic lupus erythematosus (SLE), henoch-schonlein purpura (HSP), membranoproliferative glomerulonephritis (MPGN), rapidly progressive glomerulonephritis (RPGN)[1]. The diagnosis of hematuria in children is more difficult than in adults because the symptoms are non specific and often without symptoms. Invasive methods such as kidney biopsy is the gold standard to establish the diagnosis. Birch and Fairy recommend examining the urine sediment using a phase contrast microscope to determine the origin of glomerular or non-glomerular hematuria by assessing the size and formation of erythrocytes in urine[2]. The incidence of asymptomatic isolated hematuria in children ranges from 0.5% and 1%, mostly due to glomerular abnormalities and most often in boys than girls, regardless of age[1,3,4].

Urine sediment examination is a test that can be used to diagnose glomerular hematuria. Dysmorphic erythrocytes and erythrocyte cylinders found in urine are one of the markers of glomerular damage[5]. Examination

of urine sediment with manual microscopy recommended by several international guidelines is a phase contrast microscope (PCM). PCM is considered to be better at identifying cells and formations found in urine compared to low light condenser microscopy[6]. PCM as a reference method has several limitations, namely it is expensive so that it is not available in all laboratories, time consuming and requires reading expertise[7,8].

Study by Barthe et. al, 1986 used a non staining light microscope to evaluate dysmorphic erythrocytes, and the results were similar to studies using PCM[9]. Different results were found in the study by Ince et. al. which states that light microscopy is inadequate for the detection of bacteria, erythrocytes and hyaline cylinders. Flowcitometry is a method used in examining urine sediment with an automatic analyzer. Several journals report the use of flowcitometry can overcome the limitations of PCM because the results are more standardized and the operation is fast, thus saving time and effort, however, a scientist stated that flowcitometry was inadequate in identifying particles such as cylinders and crystals in pathological urine samples so that a manual microscope review is still needed[8].

Examination with a low condenser light microscope and flowcitometry, if it gives the appropriate results as obtained at PCM, can be used as a substitute for examining dysmorphic erythrocytes and erythrocyte cylinders. This prompted researchers to examine the agreement of the results of the examination of dysmorphic erythrocytes and erythrocyte cylinders using flowcytometry, low light condenser microscopy (LLCM), and phase contrast microscopy in children with glomerular hematuria.

Materials and Methods

This study was an observational analytic with a cross sectional design which was carried out in the Laboratory of Clinical Pathology and the Department of Children Heatlh of Dr. Soetomo Hospital Surabaya, Indonesia. Samples were pediatric patients who had been diagnosed with glomerular hematuria by the clinician of the Nephrology Division of Pediatrics, who met the inclusion and exclusion criteria. Samples were collected from April-November 2020.

The first urine sample in the morning from a patient with glomerular hematuria was examined for urine sediment. The volume of urine collected is 10-12 mL. The urine was initially examined with Flowcitometry method by Sysmex UF 5000 automatic tool. Then, the urine was centrifuged at 2000 rpm for 5 minutes, the supernatant was removed by decantation. The remaining sediment was then resuspended with 0.3-0.5 mL of the remaining supernatant. 1 drop of urine was placed between a slide and cover slip then examined under a phase contrast microscope and a low light condenser microscope. The data from the automatic tool was in the form of dysmorphic erythrocyte flagging and the number of cylinders/μL, while PCM and LLCM were the number of dysmorphic erythrocytes and erythrocyte cylinders in 10 fields of view.

The concordance for the examination of dysmorphic erythrocytes and erythrocyte cylinders using flowcytometry with phase contrast microscopy was determined using the Cohen's Kappa Coefficient (κ) and The concordance of the examination of dysmorphic erythrocytes and erythrocyte cylinders using a low condenser light microscope with a phase contrast microscope was determined by Bland Altman.

Results and Discussion

There were a total of 40 study patients who met the inclusion and exclusion criteria consisting of 23 male patients (57.7%) and 17 female patients (42.5%). The mean age of patients in this study was 11.92 ± 3.323 and most diagnosis was lupus nephritis (Table 1).

Table 1. Characteristics.

Characteristics		
Age (year)		
Mean±SD	11.92 ± 3.323	
Median (min-max)	12,5 (5 – 17)	
Sex	n	%
Boy	23	57.5
Girl	17	42.5
Diagnosis		
Lupus Nephritis	24	60
GNAPS+RPGN	2	5.0
IgA Nephropathy	2	5.0
Nephrotic Syndrome	1	2.5
Nephrotic Nephritic Syndrome	6	15
RPGN+CKD	5	12.5

The results of dysmorphic erythrocytes examined using LLCM showed dysmorphic erythrocytes in 32 samples (80%) and using PCM 34 samples (85%) of the 40 samples in this study. For erythrocyte cylinders, 4 samples (10%) and 5 samples (12.5%) were obtained from the entire study sample using LLCM and PCM. Dysmorphic erythrocyte flagging was released by the Sysmex UF 5000 as many as 24 samples (60%). Evaluation by LLCM and PCM showed dysmorphic erythrocytes in 32 samples (94.1%). The Kappa result between LLCM and PCM dysmorphic erythrocytes obtained a Kappa coefficient of 0.828 with $p < 0.001$ (Table 2).

Table 2. Agreement of LLCM and PCM dysmorphic erythrocytes.

Dysmorphic Erythrocyte PCM	**Dysmorphic Erythrocyte LLCM**		**Total**
	Yes	**No**	
Yes	32 (94.1%)	2 (5.9%)	34 (100%)
No	0 (0%)	6 (100%)	6 (100%)
Total	32 (80%)	8 (20%)	40 (100%)

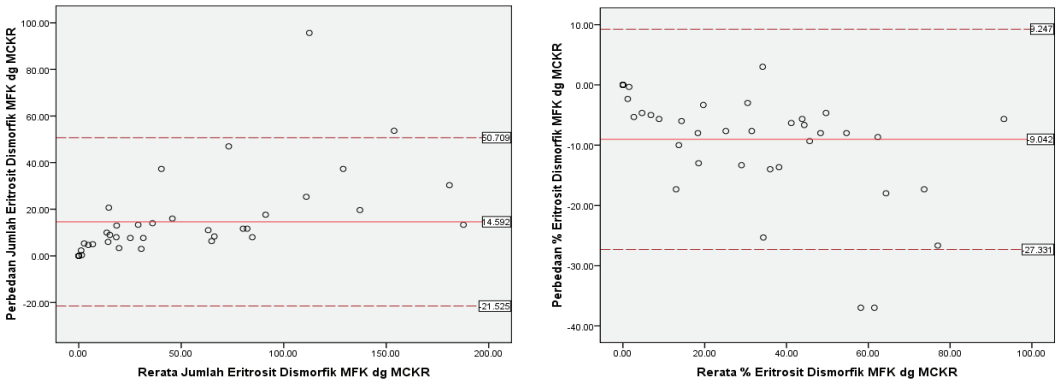

Figure 1. Agreement of Dysmorphic Erythrocyte Amount and Percentage between LLCM and PCM.

The results of the Bland Altman test show that there is an agreement in the number and percentage of dysmorphic erythrocytes between LLCM and PCM (Figure 1). Evaluation by LLCM and PCM obtained erythrocyte cylinders in 3 samples (60%). The Kappa result between LLCM and PCM dysmorphic erythrocytes obtained a Kappa coefficient of 0.625 with p <0.001 (Table 3).

Table 3. Agreement of LLCM Erythrocyte Cylinders with PCM.

Erythrocyte Cylinders PCM	Erythrocyte Cylinders LLCM		Total
	Yes	No	
Yes	3 (60 %)	2 (40 %)	5 (100%)
Ada	1 (2.9%)	34 (97.1%)	35 (100%)
Total	4 (10%)	36 (90%)	40 (100%)

Evaluation dysmorphic erythrocyte by PCM and Flagging on flowcitometry were obtained in 23 samples (60%). The Kappa result between PCM with flowcytometry flagging obtained a Kappa coefficient of 0.302 with p 0.019 (table 4).

Table 4. Agreement of PCM Dysmorphic Erythrocytes with Flowcytometry Dysmorphic Erythrocyte Flagging.

Dysmorphic Erythrocyte PCM	Flagging Flowcytometry		Total
	Yes	No	
Yes	23 (67.6%)	11(32.4%)	34 (100%)
No	1 (16.7%)	5 (83.3%)	6 (100%)
Total	24 (60%)	16 (60%)	40 (100%)

The study subjects consisted of 40 pediatric patients who had been diagnosed with suspected glomerular hematuria. Most of the diagnosis were lupus nephritis. Study by Viteri *et al*. The most common cause of nephritis is GNAPS[10]. Study by Moustafa *et al*. in Egypt the most common cause of glomerular hematuria in children is Alport Syndrome[11]. Current data in Dr. Soetomo Hospital Surabaya, Indonesia in the period from January 1 2020 to December 31 2020, the most common diagnosis for the causes of glomerular hematuria in children were SLE and Nephrotic Syndrome. The difference in diagnosis that is often found as a cause of glomerular hematuria is probably due to differences in patient selection criteria for each study.

The age range of the entire study sample group was 5-17 years with a mean of 11.92 years, with the largest age group being 12-17 years. These results are the similar as studies by Pardede *et al*. 2005 that Acute glomerulonephritis in children is most often found is 6-11 years old and Huang *et al*. 2010, where in the population of children with SLE - LN, the mean age at diagnosis was 8.6-13.5 years[12,13]. Most of the subjects of this study were men (57.5%). These results are the similar as studies by Moustafa *et al*. that male subjects were 60%[11] and Pardede *et al*. also found male subjects were 58%[12]. A literature states that glomerular hematuria is more common in men than in women, regardless of age[1].

Urine sediment examination both microscopic and automatic analyzer can quantitatively describe the number of elements formed in urine[14]. Microscopic examination of urine sediment can improve diagnostic efficiency when performed early in the evaluation of a disease[15]. Dysmorphic erythrocytes are found in the urine if the glomerular filtration barrier (GFB) is impaired. These erythrocytes are smaller in size and have smaller cytoplasmic protrusions or cell fragments[1,15,16,17]. From LLCM examination result by 3 readers, dysmorphic erythrocytes were positive in 32 samples (80%) and negative in 8 samples (20%), while the dysmorphic erythrocytes from PCM was slightly more, positive in 34 samples (85%) and negative in 6 sample (15%). This result was in accordance with previous study by Da Silva et. al. where there was no significant difference in the number of dysmorphic erythrocytes found in low-light condenser microscopes and contrast-phase microscopes in glomerular and non-glomerular hematuria patients[9].

Damage of the GFB can also cause protein and erythrocytes to appear in the urine. Erythrocytes that enter the tubule will be trapped in uromodulin (Tamm-Horsfall protein) to form a cast (erythrocyte cylinder)[18]. The results of this study showed that erythrocyte cylinders in LLCM were negative in 36 samples (90%) and positive in 4 samples (10%). The results were not much different from the review with negative erythrocyte cylinder PCM in 35 samples (87.5%), positive in 5 samples (12.5%). Previous study by Ringsrud *et al*. in patients with interstitial nephritis, cylindrical erythocytes were only found in 4 patients[19]. This result is in accordance with previous study. One literature states that erythrocyte cylinders are rare but highly pathognomic and have a high specificity of 97% for detecting glomerular hematuria[20].

Examination of urine sediment using a conventional microscope, although considered a reference method, has several limitations in its operation. To increase the accuracy and precision of urine sediment examination, several studies have been conducted to compare automatic instruments with manual examination using a microscope[21,22,23]. In addition, agreement analysis of dysmorphic erythrocyte count from LLCM and PCM was carried out on 40 child participants in this study. Dysmorphic erythrocytes were positive in 32 samples (94.1%) and 6 samples (100%) negative from both microscopes. Two samples (5.9%) were positive for dysmorphic erythrocytes in PCM but negative in LLCM (Table 1). The kappa test result was 0.828 ($p<0.01$), it suggest that there is a very strong agreement for the results of dysmorphic erythrocytes in LLCM and PCM. These results are consistent with studies by Barros Silva et. al. PCM has a higher sensitivity than LLCM, both methods have the same accuracy and can be used for examination and a study by Chu shu et al. that LLCM have the same accuracy to recognized isomorfik and

dysmorphic erythrocyte[9,24]. The Kappa coefficient of 0.625 was obtained in the test results between LLCM and PCM erythrocyte cylinders which showed a strong agreement ($p < 0.05$). Furthermore, LLCM and PCM have the same ability to detect dysmorphic and isomorphic erythrocytes. LLCM ability on urine sediment examination was good enough, similar to PCM.

Conclusion

There is a strong agreement for examination of dysmorphic erythrocytes and erythrocyte cylinders using a low light condenser microscope and a contrast phase microscope in children with glomerular hematuria with kappa coefficients of 0.828 and 0.625. With the results, LLCM can be used to replace PCM. The weak agreement between flowcitometry and PCM shows that for pathological urine samples using an automatic flowcitometry method it is recommended to do a review using a conventional microscope.

Conflict of Interest: The author declare that they have no conflict of interest.

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Ethical Approval

This study was approved by Health Research Ethics Committee of Dr. Soetomo Surabaya, Indonesia (approval number: 1810/KEPK/II/2020).

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