Evaluation of Urine Phase Contrast Microscopy and Renal Biopsy in Differentiating Glomerular and Non-Glomerular Haematuria in Patients With Kidney Disease

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ABSTRACT

Introduction: Haematuria is one of the most common presentations of renal disease Urinary sediment examination by urine phase contrast microscopy (PCM) is a useful diagnostic marker for glomerular bleeding if correctly interpreted and used. Although PCM is simple and cost effective the percentage of dysmorphic red cells regarded as diagnostic of glomerular haematuria is controversial and varied from (10-90)% cases in different series. This study is done with the aim to evaluate urine phase contrast microscopy as a tool in differentiating glomerular haematuria in patients with glomerulonephritis confirmed by renal biopsy and non-glomerular haematuria in patients with renal stone disease.

Materials and Methods: In this study, 175 patients with haematuria were taken and were divided into two groups; Group I with diagnosed cases of glomerulonephritis with haematuria confirmed with renal biopsy and Group II with patients of renal stone disease with haematuria. After diagnosing haematuria, all patients were undergone for urine phase contrast microscopy. Renal biopsy was done in patients suspected for glomerulonephritis.

Results: This study showed that the mean percentage of dysmorphic RBCs in group I by urine PCM was (35.8%) which was significantly higher than in group II (6.8%). A comparison was done between the different cut off values for percentages of dysmorphic RBCs to differentiate glomerular from non-glomerular haematuria. For a cut off value of 20%, the present study showed the most agreeable sensitivity 80.7% and specificity 90.6%.Receiver-operator characteristic curve for percentage of dysmorphic RBCs, area under the curve was 0.934, which gave an optimal sensitivity 80.7% and specificity 90.6% for a decision level

cut off of 20% dysmorphic RBCs. It was found that patients of group I had higher serum creatinine level (mean 1.6 mg/dl) in comparison to group II (mean 1.1mg/dl). Similarly patient of group I had higher level of proteinuria in comparison to group II. It was also ovbserved that patients with proliferative glomerulonephritis had higher percentage of dysmorphic RBCs in comparison to non-proliferative type of glomerulonephritis. **Conclusion:** Urine phase contrast microscopy is a simple, cost effective, non invasive and reliable investigation. Patients with proliferative glomrulonephritis may have a higher percentage of dysmorphic RBCs in comparison to those with non proliferative glomerulonephrits.

Key words:
Dysmorphic Red Blood Cells, Haematuria, Urine phase contrast microscopy .

INTRODUCTION

The patterns of renal diseases are different in different countries of the world, as it is influenced by geographical, environmental and socioeconomic factors. Haematuria may result from various renal, urologic, or systemic processes and requires an immediate initiation of diagnostic procedures to identify the location of hemorrhage. The ability to differentiate glomerular bleeding from non-glomerular bleeding can help in the initial choice of diagnostic tests and minimize the expense and discomfort to the patient. Differentiation between glomerular and non- glomerular haematuria by observation of the erythrocyte morphology using phase contrast microscopy has been well established for almost 20 years, after the initial report by Birch and Fairley. Glomerular haematuria is due to the glomerular injury and is characterized by dysmorphic RBCs whereas non-glomerular haematuria is not due to the glomerular injury and is characterized by isomorphic erythrocytes. One of the study shows 25% of patient could have spared from extensive urological investigation, if the examination of urinary sediment would have been performed at the start of evaluation.

Distinguishing a glomerular from a non-glomerular site of bleeding by microscopic examination of erythrocyte morphology in urine sediment using phase-contrast microscopy is a well-established technique erythrocytes from non-glomerular sites appear normal, whereas those originating from glomerular sites have dysmorphic characteristics. PCM is a method first developed in 1934 by Dutch Physician Frits Zernike and was awarded a Nobel Prize for this discovery. Since examination of the urinary sediment can help to differentiate between glomerular and non-glomerular forms of haematuria, it is also an important tool in patients with asymptomatic haematuria.Birch and Fairley were the first to show that examination of the urinary sediment by urine phase-contrast microscopy can help in the discrimination between glomerular and non-glomerular forms of haematuria[1]. After ensuring thepresence of red blood cells in urine sedimentby routine examination, next step is to determine the morphology of red cells by urine PCM. PCM is an accepted technique for evaluation of urinary red cell morphology due to > 90% sensitivity and specificity and most of the previous studies have also reported sensitivity in the range of 89.8 % to 100 % and specificity of 90 % to 100 %for cut off value of 20 % for dysmorphic red cells by PCM[2].

Renal biopsy is the ultimate step in the diagnosis of glomerular disease. A renal biopsy helps to establish and confirm a suspected diagnosis and may aid in the determining the mechanism of disease, whether antibody or complement mediated and thereby assist in determining disease specific therapy[3].

The main objectives will be to assess the patients presenting with haematuria by easier as well as economic techniques in the diagnosis of glomerular diseases. PCM is a simple, cost effective andnon invasive technique which can be performed in all patients. PCM does not lead to a definite diagnosis, but enable the selection of the most appropriate test and thus avoid unnecessary, often invasive diagnostic procedure [4].

MATERIALS AND METHODS

It was an observational cross-sectional study and was taken place in the Department of Nephrology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, which was conducted from July 2015 to June 2016 for a period of one year. 175 patients with haematuria were taken and were divided into two groups; one group with cases of glomerulonephritis with haematuria and another group with renal stone disease with haematuria.
Written informed consent was taken from all

patients presenting with haematuria. Patient's urine was collected and sent for urine routine microscopy. After diagnosing haematuria, all patient was undergone for urine phase contrast microscopy within one hour of sample collection those fulfilling the inclusion criteria before doing renal biopsy. Urinary sediment was analyzed for the presence and number of red blood cells and no of dysmorphic red cells. Those with haematuria were then counted for percentage of dysmorphic erythrocytes. After proper counseling and both verbal and written consent were taken before renal biopsy and biopsies were done by tru-cut solid organ biopsy needle and spring loaded gun biopsy needle then renal tissues were sent for histopathological examination. All diagnostic tests, including renal ultrasonography, IVU, CT Scan etc were done for non glomerular causes to find out the cause of haematuria. Demographic data on the patient characteristics i.e. Age, gender, race, diabetes status, history of hypertension, congestive heart failure (CHF), and chronic kidney disease (CKD), history of body swelling and oliguria were collected.

Pertinent laboratory data collected included urine routine examination, serum creatinine concentration,UTP/UTV, PCM, USG abdomen, CBC, coagulation profile, blood grouping, & histopathological examination of renal tissue. These findings were recorded on a data collection form. Histopathological examinations were carefully done and recorded. Only renal stone diseases were taken for the uniformity of study and other non-glomerular causes were excluded. Categorical variables were compared via analyses, and continuous variables were compared with student unpaired t tests. Statistical analysis was performed using SPSS ® version 22 (SPSS Inc., Chicago, IL). Results were presented by tables and figures. Data were featured as mean and SD if needed. . P value was considered as significant when it was less than 0.05.

RESULTS

Table 1. Baseline characteristics of the patients in the two groups shows most of the patients were male in both the groups. Hypertension and oliguria was found more in patients of group I

Table 2. Urinalysis of the patients in the two groups showed hematuria , proteinuria and dysmorphic RBCs in group I.

Table 3. shows percentage of dysmorphic red cells of the patients n the two groups, it was observed that 39(33.9%) patients had dysmorphic red cells in group I and 2(3.3%) in group II belonged to 21 to 30 percent. The mean 35.8 percentage of dysmorphic red cells was found in in group I with range from 2 to 90 percent and 6.8 percentages of dysmorphic red cells in group II with range from 0 to 45 percent. The difference was statistically significant ($p<0.05$) between two groups.

Table 4: shows Dysmorphic red cells for prediction of glomerular and non glomerular Haematuria. It was observed that majority 98.6%Sensitivity where as 40.7%Specificity was observed at cut of value for 10% dysmorphic red cells**.** At cut of value of 20%, it was observed that 80.7% Sensitivity and more than 90% Specificity was found. After than specificity was gradually increased to 96.7%, 98.0%, and 100.0% at cut of value 30%, 40%, and 60% respectively.

Table 5. shows distribution of the patients by renal histopathology in group I, it was observed that 40(34.8%) patients found to be MesPGN , 33(28.7%) patients IgA nephropathy, 9(7.8%) patients Lupus nephritis, 6(5.2%) patients Crescentic GN, 5(4.3%) patients MN and 3(2.6%) patients FSGS.

Table 1. Baseline characteristics of the patients in the two groups.

	Group-I	Group-II
No of Patients	115	60
Sex		
Male	61(53.0%)	41(68.3%)
Female	54(47.0%)	19(31.7%)
Age	34.6±13.2	38.5±12.8
Hypertension (no of patients)	66 (57.3%)	16 (26.6%)
Oliguria	72 (62.6 %)	2 (3.0 %)
Mean S. creatinine (mg/dl)	1.6	1.1

Table 2. Urinalysis of the patients in the two groups.

Urinalysis	Group I	Group II
Proteinuria		
Nil	5 (4.3%)	39 (65.0 %)
1+	28 (24.3%)	20 (33.3%)
2+	33 (28.6 %)	1 (1.66%)
3+	49 (42.6 %)	0 (0.0 %)
Hematuria		
Mean RBC (No/ HPF)	34.4	20.2
PH	6.0(5-8)	6.0(5-8)
Dysmorphic RBCs(%)	35.8	6.8

Table 3: Frequency of patients with different percentage of dysmorphic red cells in the two groups (n=175)

Dysmorphic red cells (%)	GroupI (n=115)		GroupII (n=60)		P value
	n	%	n	%	
<10	7	6.1	52	86.7	
10-20	23	20.0	4	6.7	
21-30	39	33.9	2	3.3	
31-40	11	9.6	0	0.0	
41-50	14	12.2	2	3.3	
51-60	8	7.0	0	0.0	
60-70	2	1.7	0	0.0	
70-80	9	7.8	0	0.0	
>80	2	1.7	0	0.0	
Mean	35.8		6.8		0.001[s]
Range (min to max)	2to 90		0 to 45		

Table 4: Sensitivity and Specificity of different values of dysmorphic RBCs within the Receiver Operator characteristic curve.

Cut off value of dysmorphic RBCs (%)	Sensitivity	Specificity	Area under the ROC curve	95% Confidence interval (CI)	
				Lower boundary	Upper boundary
10.0	98.6	40.7	0.934	0.892	0.976
20.0	80.7	90.6	0.934	0.892	0.976
30.0	46.7	96.7	0.934	0.892	0.976
40.0	30.4	98.0	0.934	0.892	0.976
50.0	18.3	100	0.934	0.892	0.976
60.0	17.4	100	0.934	0.892	0.976

Table 5: Distribution of the study patients by renal histopathology in group I (n=115).

Renal histopathology	Number of patients	Percentage
MesPGN	40	34.8
MCGN	33	28.7
IgA nephropathy	16	13.9
Lupus nephritis	9	7.8
Crescentic GN	6	5.2
MN	5	4.3
FSGS	3	2.6
Not done	3	2.6

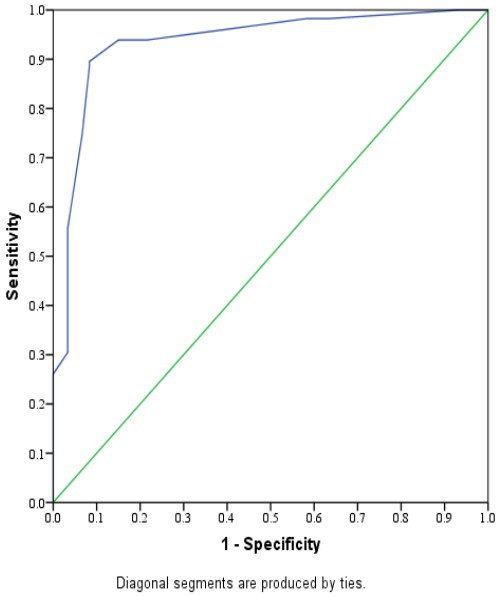

Figure 1: Receiver-operator characteristic curves for percentage of dysmorphic RBCs by urine PCM for diagnosis of glomerular haematuria

DISCUSSION

This prospective observational study was carried out in the department of Nephrology, BSMMU with the aim to evaluate urine phase contrast microscopy as an investigation to differentiate glomerular

haematuria and non-glomerular haematuria and to identify the cut off values of percentage of dysmorphic red blood cells for this differentiation. Analysis of demographic data indicated that there was no significant difference between group I (mean age 34.6 ±± 13.2) and group II (mean age 38.5 ± 12.8) regarding age of the patients. Similar findings were observed by other studies (Swaminathan et al[5]; Bottini et al[6]).

In this present study, number of RBCs per high power field was significantly higher in group I in comparison to group II. The current study showed that the mean percentage of dysmorphic red cells in group I by urine phase contrast microscopy was 35.8% which was significantly higher than in the group II (6.8%). The result shows that most of the patients in group I had percentage of dysmorphic red cells > 20% whereas it was < 20%in group II. Comparable figures were found by Dinda et al[2], who conducted a study in All India Institute of medical Sciences, New Delhi. They showed that in group I the mean percentage of dysmorphic red cells was 46.4 ± 6.2% and in group II it was 8.2 ± 2.4%, which was statistically significant. In the study of Abolfathi et al[4], they reported findings similar to the present study in which percentage of dysmorphic RBC were 48.7 ± 18.4 in group I and 8.3 ± 6.6in group II.

In this study, a comparison was done between the different cut off values for percentage of dysmorphic RBCs to differentiate glomerular from non-glomerular haematuria and it showed that for the 10% cut off value, sensitivity was 98.6% and specificity 40.7% for a cut off value of 20 %, the present study showed the most agreeable sensitivity 80.7% and specificity 90.6%. Similarly at higher cut off values of 30%, 40% and 50%, the specificity gradually increased to 96.7%, 98.0% and 100% respectively. Based on the receiver-operator characteristic (ROC) curves for percentage of dysmorphic red cells, area under the curve was 0.934, which gave an optimal sensitivity 80.7% and specificity 90.6% at a decision level of 20 % dysmorphic RBCs. Similar study done by Mohammad et al[7], reported 90% sensitivity and 100% specificity for detecting the glomerular source of bleeding. They showed the presence of more than 20% dysmorphic red cells was diagnostic of glomerular bleeding which was also consistent with the current study.

Patients presenting with RPGN, Isolated haematuria and Nephritic syndrome had higher percentages of dysmorphic red cells i.e. 62.0%, 50.0% and 38.3 % respectively whereas patients with Nephrotic and Nephrito- nephrotic presentation had lower percentages of mean dysmorphic red cells i.e. 33.2% & 35.5% respectively.

In our study, percentage of dysmorphic RBCs found in urine of different histological types of glomerulonephritis were compared with each other and it was observed that patients with proliferative glomerulonephritis had higher percentages of dysmorphic RBCs in comparison to non-proliferative types of glomerulonephritis. Of these, Crescentic GN, MCGN, MesPGN, IgA nephropathy, Lupus Nephritis had higher percentages of dysmorphic RBCs i.e 62%, 37.2%, 31.4%, and 29% respectively in comparison to FSGS and MN. FSGS and MN had 22.7% and 12.4% respectively. Pollock et al[8] had performed a similar study and graded different histological types of glomerulopathy like IgA nephropathy, MesPGN, MN, FSGS, Focal necrotising GN, Post infectious GN, transplant rejection and Thin basement membrane disease into groups of mild, moderate and severe but there was no difference in degree of urine RBC dysmorphism among the three groups with mild moderate and severe groups having 72%, 75% and 78% RBCs being dysmorphic respectively.

CONCLUSION

Urine phase contrast microscopy is found to be simple, cost effective, non invasive and reliable investigation to diagnose glomerular haematuria which has been proven by renal histopatology reports. Patients with proliferative glomrulonephritis has a higher percentage of dysmorphic RBCs in comparison to those with non proliferative glomerulonephrits.

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