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LETTERS TO THE EDITOR

Phase contrast microscopic examination of hematuria to localize the source of bleeding

Hematuria is a common diagnostic problem in clinical practice. It could be of renal origin or due to conditions affecting the lower urinary tract. Extrarenal diseases could also lead to hematuria. It is mandatory to investigate every case of hematuria to localize the site of the bleed as the protocol for the investigations and management for patients with glomerular hematuria is different from that of non-glomerular hematuria. In a developing country like ours, the high cost of hospital stay and invasive or expensive procedures are not preferred. Thus, it is vital to have a simple, sensitive, preferably non-invasive screening test like examination of the urinary sediment which would separate these two groups of patients.

Presence of dysmorphic red blood cells (more than 20 percent) in cases of significant hematuria has been accepted as an indicator for the hematuria of glomerular origin[1]. In 1979 Birch and Fairley reported that the source of bleeding in the urinary tract could be determined by phase contrast microscopic examination of urine from patients with hematuria[2]. Identification of the source of bleeding was based on the morphological appearances of the red blood cells which were round in shape (isomorphic) if bleeding was from the lower urinary tract and irregular in shape (dysmorphic) if the bleeding was glomerular in origin. Subsequent papers confirmed the results[3 - 7] while others disputed the reliability of this approach[8-9]. Microscopic examination of fresh unstained urine specimens is a simple, noninvasive technique which can be performed in the outpatient clinic; the result can be obtained within a few minutes. If phase contrast microscopy proves a reliable method of detecting glomerular bleeding it will greatly assist the patient's management. We, therefore, reviewed this approach to the identification of the bleeding site to determine its value in clinical practice.

The study was carried out during the period June 2006 to June 2007 on 50 patients with significant hematuria more than 5 red blood cells per high power field of the standard urinary sediment[3,4]. The ages of the patients ranged from 1-70 years. Fresh midstream urine samples (10-20 ml) were obtained from patients attending the vasculitis, urology, and cystoscopy clinics and from patients admitted to the urology wards. The age, sex and relevant medical history were recorded for each patient. Multistix test papers were used for detecting the presence of protein (albumin) and hemoglobin. The change in the color was noted and compared with the standard provided. The urine samples were prepared for light microscopy as follows: 10-20 ml of urine was centrifuged for 5 min at 400 x g in an IEC Centra-7R centrifuge. The urine was prepared for phase contrast microscopy as follows: in cases of macroscopic hematuria one drop of sediment urine was transferred to a labeled glass slide. A cover slip was placed on the specimen.

Hematuria was considered to be present when one red blood cell per two high power fields was seen. The morphology of red blood cells was classified as either dysmorphic or isomorphic. Dysmorphic red blood cells had irregular outline, membrane protrusions, areas of loss of the membrane, irregular deposits of dense cytoplasmic material around the cell membrane, and variation in size. Isomorphic red blood cells had a smooth or crenated outline. If more than 20% of the red blood cells were dysmorphic and less than 80% were isomorphic glomerulopathy was diagnosed. If less than 20% of red blood cells were dysmorphic and more than 80% were isomorphic, glomerulopathy was excluded.

Red blood cell casts and hemoglobin casts were identified by their red color, and intact red blood cells were seen at the cytological results were correlated with the clinical history of the patients.

Twenty cases of glomerulonephritis with hematuria were studied (Table I). Acute glomerulonephritis was diagnosed according to traditional clinical and laboratory criteria, the other glomerulopathies were all confirmed by kidney biopsy and 25 cases with hematuria resulting from non-glomerular causes were also included in the study. Five patients were unknown diagnosis. Twenty of the 50 patients has dysmorphic red blood cells of more than 20% and isomorphic red blood cells of less than 80%. 25 patients had dysmorphic red blood cells of less than 20% and isomorphic red blood cells of more than 80%. In the 20 patients with more than 20% dysmorphic red

Table I: Causes of hematuria

Cause	Total cases
Glomerular	
Minimal change nephritic syndrome with mild mesangial matrix increase	1
Acute glomerulonephritis	8
Focal proliferative glomerulonephritis	2
Chronic glomerulonephritis	2
Crescentic glomerulonephrits	1
Mesangioproliferative glomerulonephritis	3
Membranous glomerulonephritis	1
Systemic lupus erythematosus	2
Non-glomerular	
Post-operative	8
Benign prostatic hypertrophy	2
Urinary tract infection	13
Transitional cells carcinoma	2

blood cells glomerulopathy was confirmed by biopsy in 18 cases (Table II). In the remaining 2 patients no histological diagnosis was available.

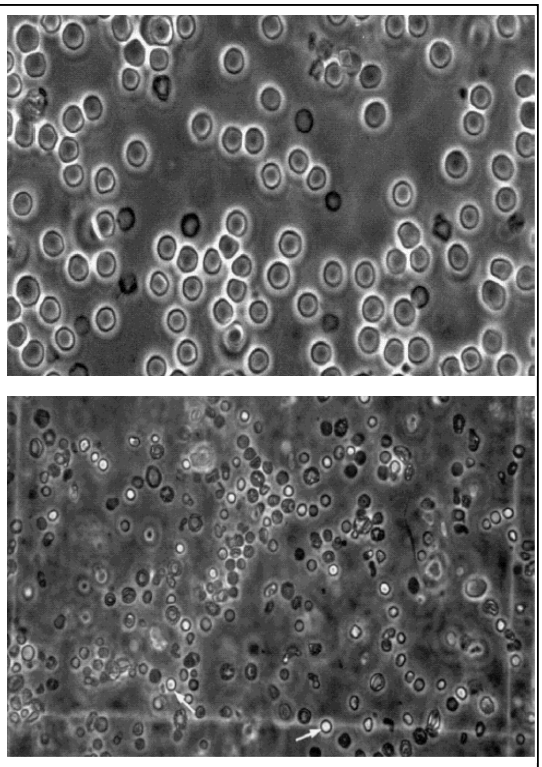

Figure 1: a) Typical nonglomerular erythrocytes that are uniform in size and shape but show 2 populations of cells because a small number have lost their hemoglobin pigment; b) Typical appearance of erythrocytes in glomerular hematuria in which cells are small and vary in size, shape, and hemoglobin content

Correlation of clinical/or histological findings with phase contrast microscopy in the 32 patients with less than 20% dysmorphic red blood cells confirmed the presence of a nonglomerular bleeding site in the urinary tract lesion in 25 of the remaining 7 patients, 2 had glomerulopathy and in 5 a definitive diagnosis was not made. This technique provides a sensitivity of 90% and specificity of 100% for the diagnosis of glomerular lesions.

Two patients with TCC had hematuria on phase contrast microscopy with less than 20% dysmorphic red blood cells.

This study showed that glomerular and non-glomerular bleeding can be differentiated with a high degree of accuracy by phase contrast microscopy of red blood cells. The presence of more than 20% dysmorphic red blood cells was diagnostic of a glomerular origin for the bleeding; if less than 20% dysmorphic red blood cells were present a non-glomerular origin for the bleeding should be suspected. A sensitivity of 90% and a specificity of 100% was achieved for this approach.

Table II: Correlation of phase contrast microscopy of urine sediment with clinical outcome

Disease	Findings of phase contrast microscopy	
	>20% dysmorphic RBC	<20% dysmorphi RBC
Histologically confirmed glomerulopathy	18	2
Non-glomerular lesion	0	25
Diagnosis unknown	0	5

Abdurrahman et al reported a sensitivity of 93% and specificity of 100%[3]. The slight improvement in sensitivity recorded by Abdurrahman et al may have been due to the fact that this group included a borderline category which they applied to those cases where the percentage of dysmorphic red blood cells was 15%-19%. Pillsworth et al found this technique slightly less specific than we did (94% and 100%)[4]. Their margin of decision was selected at more than 14% dysmorphic red blood cells which is slightly different from the one used in this study.

In a study performed by Fassett et at glomerulopathy was diagnosed only when dysmorphic red blood cells of more than 80% were found[8]. Thus a large group of patients with mixed dysmorphic and isomorphic red blood cells were excluded from the diagnostic process. This study included 18 patients with a mixture of dysmorphic

and isomorphic red blood cells. The range of dysmorphic red blood cells was from 20-50% in 8 patients and from 50-80% in 10 patients. In all 10 patients biopsy confirmed the presence of glomerulopathy. Theories have been proposed to explain the change in the morphology of the red blood cells in glomerular diseases. Dysmorphic changes may be due to mechanical deformation of red blood cells on passage through altered glomerular capillary clefts. They may be caused by pathological changes in osmotic pressure which modify the red blood cells membrane. The presence of toxic enzymes resulting from inflammatory processes has also been cited as a cause of dysmorphic red blood cells[10].

Pellet et all[10] used a red cell analyzer to determine the site of bleeding in patients with hematuria. The distinction was based on red cell volume and the results were compared with phase contrast microscopy. They found that the site of bleeding in patients with microscopic hematuria was more accurately identified by phase contrast microscopy than by the red cell analyzer[10]. We have noticed that the technique has the potential for the detection of dysmorphic red cells carred out at an early stage of investigation will help in avoiding unnecessary radiologic and urologic investigations in cases of hematuria of glomerular origin [8, 9, 10]. This may be the way forward for the wider application of this technique in clinical practice.

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Use of anthropometric indicators for predicting risk of delivering low birth weight babies

The birth weight of newborns is an important factor that affects the future survival and quality of life[1]. The importance of predicting the risk of delivering low birth weight (LBW) babies during pregnancy arises from the consistent relationship found between LBW and higher risk of mortality, morbidity, malnutrition and suboptimal growth and development[2]. In Bangladesh, incidence of LBW is unacceptably high[3]. To reduce the incidence of LBW in the country, a search for indicators of high risks of delivering LBW baby should be an important part of the antenatal care. From different studies, it is consistently observed that maternal anthropometric indicators are the established risk indicators for delivering LBW babies[4]. Early prediction of LBW babies from maternal anthropometric indicators will help policy makers, planners, program managers and service providers adopt appropriate intervention strategy for its (LBW) reduction. Thus, the objectives of the present study is to: (i) identify the most suitable anthropometric indicator(s) of risk of delivering LBW babies and (ii) select appropriate cut off point of the indicator(s).

This was an analytical cross-sectional study, conducted in a Government Maternity Hospital at Dhaka from January to May 1999. Anthropo-metric measurements [height, weight and mid upper arm circumference (MUAC) for mothers and birth weight for newborns] of 316 pregnant women and their newborns (singleton) were recorded according to standardized technique[5]. The detailed methodology is described elsewhere[6]. Chi-square test was under taken to observe the association between discrete variables and LBW (data not shown). Multiple regression equation was carried