

Original Article

Relationship between Pathologic and Laboratory Data of Children Suffering from Hemolytic Uremic Syndrome (HUS): A Center study

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ABSTRACT

Background and objective: Hemolytic uremic syndrome (HUS) is the most prevalent cause of children renal insufficiency which in many cases (90%) occurs following diarrhea. Hemolytic microangiopathic anemia, thrombocytopenia, and renal insufficiency are main symptoms of hemolytic uremic syndrome. This study aims to consider the relationship between pathologic data of nephro-biopsy and laboratory data of children suffering from the disease.

Material and Methods: This study has been carried out in retrospective, cross-sectional and descriptive procedures. For this purpose, 28 patients with an average age of 6 years suffering from uremic hemolytic syndrome referred to Ali Asghar Hospital over the last 10 years. Light microscopic data of glomeruli, arterioles, arteries, interstitial tissue, medullary vessels and tubules were evaluated. Laboratory data including hematology, biochemistry, and urinary tests were extracted from patients' files. Data were analyzed using SPSS software.

Results: The most prevalent damages in glomeruli were decreased capillary lumen and thickening of its wall and in arterioles were mild decrease of lumen and in artery thickening of intima and mild infiltration of inflammatory cells and mild edema in interstitial and hyperemia in vaso recta and the most prevalent pathology in tubules was the existence of cast. Significant relationship was found out between time of recovery of hematological disorders and medullary vessels congestion and reduplication of arterial inner elastic lamina and also improvement of biochemistry changes with glomerulus necrosis and leucocytes assembly in vaso recta. Arteriolar rate with creatinine serum level at discharge time was related and tubular rate with platelet count at discharging time was also related.

Conclusion: Biopsy is an important tool for prognosis and determination of disease intensity. There was valuable statistical relationship between some laboratory data at the time of referral and pathological data which even could influence intensity or prognosis of disease.

Key words: Hemolytic Uremic Syndrome, Histopathology, Laboratory

Received: 20 April 2007

Accepted: 15 June 2007

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Introduction

Hemolytic uremic syndrome (HUS) is the most prevalent cause of children renal insufficiency. Hemolytic microangiopathic anemia, thrombocytopenia, and renal insufficiency are main symptoms of hemolytic uremia syndrome. About 90 percent of cases occur following diarrhea which in 75% of these patients *E. coli* is entero-pathogen of diarrhea. In spite of some epidemic cases, most of cases of the syndromes are as sporadic (1). Endothelium damage of small vessels was observed after shiga toxin attachment and thrombotic microangiopathy of kidney, brain, liver, pancreas and heart and disease of other organs (2). Atypical HUS occurs usually following other basis illnesses and some genetic damages (3). In general view, renal damages is divided into variations of necrosis of cortex of renal, glomerular involvement, and arterial involvement. However, there is usually a spectrum of all damages, but this division is important because of its importance to disease prognosis. Arterial and arteriolar damages have more effect on renal consequence than glomerular damages (4). The clearest anomaly of kidney is edema of capillary endothelium which results in decreased capillary lumen. The basement membrane gets multilayered and there are usually fibrin thromboses (5). In arterioles, there are thrombosis, endothelium inflammation and edema and necrosis and wall mucoid changes. Thickening of arterial intima, necrosis, and thrombosis are also observed in medium-sized arteries (6).

In this study, we considered histopathologic data of involvement of different parts of kidney and compared them with laboratory data. For this purpose, histopathologic data were completely and separately evaluated (27 cases) and compared with laboratory data (7). In this respect, by considering biopsy of HUS patients, each pathologic data finds special meaning and could be used for making decision for treatment and prognosis.

Materials and Methods

This study was carried out based on retrospective, cross-sectional and descriptive procedure. Names and file numbers of all patients who underwent nephro-

biopsy in Ali Asghar hospital over the last 10 years were drawn out from hospital archives. Thus, the study was carried out on 28 patients. Nephro-biopsy criteria in HUS were: renal insufficiency lasting for 2 or more weeks, presence of anuria, atypical HUS cases and relapse cases. Slides and blocks of these patients were isolated from files of Pathology Department and examined by light microscopy for considering histopathologic changes (biopsy taken early in the course of the disease shows fibrinoid necrosis, intimal and subintimal fibrin deposits with red cell insudation, thrombosis, and endothelial cell proliferation in small arteries and arterioles). The glomeruli show acute ischemic changes and some may be infarcted. There is also mesangial endothelial swelling with mild, irregular cell proliferation accompanied by crescent formation. As the lesion progresses, there is an intense basophilic intimal thickening in the small arteries and arterioles that greatly restricts the vascular lumina. These mucoid intimal changes usually develop over several weeks, although they may be seen very early in the course of the disease. Aneurysmal dilatation accompanied by proliferation of some arterioles, particularly at the hilus of the glomerulos is a typical finding. For each patient, changes of glomerular damages (including sclerosis, decreased capillary lumen, mesangial fibrillation, thickening of capillary wall, thrombosis, necrosis, hyperemia, crescent and segmental solidification), arterial damages (thickening of media, thickening of arteries intima, thrombosis, necrosis, lipid intima, doubling of inner elastic layer, arterioles (thrombosis, necrosis, hyperplastic thickening, and decreased lumen), interstitial damages (acute and chronic inflammation, fibrosis), medullary vessels (hyperemia and leucocytes assembly in vaso recta), and tubular damages (necrosis, cast, atrophy) were separately considered (Table 1). All information including, age, gender, symptoms and laboratory data were drawn out from records and assigned into special tables of SPSS. Then, data were analyzed by the same software and frequency and mean comparison were determined using T-test, chi 2, Pearson correlation, and stepwise linear regression for various cases.

Table 1. Histopathologic renal damage intensity in HUS patients

Histopathologic damage types	Mean	S.D
Glomerular score	11.04	3.76
Glomerular sclerosis	0.64	1.16
Glomerular mesangial fibrillarity	1.43	0.50
Glomerular thrombosis	0.68	0.67
Glomerular cellular proliferation	1.61	0.79
Glomerular reduction in cap lumen	1.75	0.75
Glomerular capillary loop thickness	1.61	0.69
glomerular necrosis	0.25	0.44
Glomerular congestion	1.07	0.72
Segmental solidification	1.11	0.88
Glomerular urinary space	1.00	1.91
Glomerular crescent	0.25	0.70
Arteriolar lesion	3.21	2.60
Arterial thrombosis	0.61	0.88
Arteriolar hyperplastic thickening	1.25	0.97
Arteriolar necrosis	0.36	0.73
Arteriolar reduction in luminal diameter	1.79	2.35
Arterial thickening	2.46	2.38
Arterial medial thickening	0.68	0.67
Arterial thrombi	0.39	0.69
Arterial intimal lipid	0.18	0.48
Arterial intimal thickening	0.93	0.60
Arterial necrosis	0.29	0.66
Arterial reduplication inner	0.36	0.62
Interstitium	1.75	1.53
Interstitial chronic inflammation	0.64	0.78
Interstitial fibrosis	0.39	0.63
Interstitial edema	0.71	0.85
Medullary vessels	1.14	1.04
Leukocytes accumulation in vasa recta	0.25	0.44
Medullary vessel congestion	0.89	0.83
Tubules	2.43	1.07
Tubular necrosis	0.57	0.63
Tubular casts	1.14	0.59
Tubular atrophy	0.71	0.53
Final score	22.86	9.38

Results

In this study, 28 nephro-biopsy samples (20 boys and 8 girls) were isolated from 102 children suffering from HUS who hospitalized in Ali Asghar Hospital. The mean age was 6 years old (± 4.68). In this regard, 20 children were boys (71.4%) and 8 children were girls (28.4%). Altogether, 5 patients recovered after less than 7 days, 4 patients between 7-14 days, 4 patients after less than 30 days and 8 patients more than 30 days and 7 patients remained without recovery and affected with chronic renal insufficiency or death (Table 2). We can note these points as significant issue changes in pathology of renal damage: the most prevalent damage of glomeruli was the decrease of capillary lumen and thickening of capillary wall (92.9%). The most prevalent damage of arterioles was mild decrease of lumen (39.3%) and the most prevalent damage of arteries was thickening of arterial intima (78.6%). Mild infiltratiFon of inflammatory cells and intermediate edema in interstitial tissue were both mild with the same degree (50%). Medullary vessels hyperemia was also noted (67.9%). The most prevalent pathology in tubules was the existence of cast (92.8%). Hematological indexes of paraclinical findings were as follows: white cell count = 11241 (± 5358) with 56% polymorphonuclear and 39% lymphocytes, the average of hemoglobin = 8.5 g/dl ($\pm 2/17$), MCV = 84 (± 11) fL, platelet count = 71308 (± 68347), and reticulocyte count = 6% ($\pm 5/7$) and blood sedimentation rate = 48/8 (± 35.3) mm/hour. Tests related to renal functions including average of creatinine at the beginning of hospitalization was 3.8 mg/dl (± 2.5) and BUN = 60 mg/dl (± 46). Meanwhile, 26 patients had microscopic hematuria and in 15 cases, specific-gravity of urine was over 1015. Eleven cases had pyuria. Urine pH in 6 cases was over 6 and in 5 cases was 5.5-6. In addition, 26 patients had proteinuria with different intensities. Average excretion of protein was 1390.7 (± 997). Average level of serum Na = 129.5 (± 15) meq/l, K= 4.2 (± 0.95) meq/l, Ca = 835 (± 0.6) mg/dl, P=5.78 ($\pm 1/8$) mg/dl, alkaline phosphatase = 628 (± 1160) U/l, blood glucose = 110 ($\pm 42/8$) mg/dl, uric acid = 8.76 (± 4.12) mg/dl, SGOT=59.617 (± 38.34) U/l, SGPT = 109.6 (± 318) U/l, serum total protein = 5.78 (± 1.14) g/dl, serum albumin = 3.43 (± 0.7) g/dl, and LDH = 2006.8 (± 1723) U/l was also measured.

Table 2. Time of recovery from clinical and laboratory symptoms along hospitalization in HUS patients

Untreated	>30Day	15- 30Days	7- 14 Days	<7Day	
7	8	4	4	5	Clinical symptoms
6	8	4	3	7	Hematological changes
6	12	4	5	1	Biochemical changes

Significant statistical relationship was found out between glomerular rate with intensity of proteinuria (direct relationship, $p = 0.04$), with intensity of hematuria (negative relationship, $p = 0.013$), level of serum Ca (negative relationship, $p = 0.041$), and blood sedimentation (positive relationship = 0.009). Arteriolar rate related to intensity of hematuria (negative relationship, $p = 0.043$) and blood Na (negative relationship, $p = 0.011$). Arterial rate only related to intensity of pyuria (negative relationship, $p = 0.011$). Interstitial rate had no significant relationship with none of the variables. Significant statistical relationship was found out between tubular rate and level of serum Ca (negative relationship, $p = 0.049$) and blood sedimentation (positive relationship = 0.014). Medullary vessel rate related to white cells counts (positive relationship = 0.04), intensity of hematuria (negative relationship, $p = 0.049$) and serum albumin (negative relationship, $p = 0.029$). Final rate had significant relationship with urine density (negative relationship, $p = 0.033$), intensity of hematuria (negative relationship, $p = 0.031$) and blood sedimentation (positive relationship = 0.019). Using stepwise linear regression, we found significant and weak relationship between recovery time of hematological disorders and medullary vessels congestion and arterial reduplication of inner elastic lamina ($p = 0.043$, $r_2 = 0.32$). We also found significant and weak relationship between biochemical changes improvement and glomerular necrosis and leucocytes assembly in vaso recta ($p = 0.014$, $r_2 = 0.32$). Using Pearson correlation, we found significant relationship between arteriolar rate and creatinine at the time of discharge (positive relationship, $p = 0.02$), tubular rate and platelet counts at the time of discharge (negative relationship, $p = 0.019$). By multiple stepwise linear regressions, arteriolar thrombosis showed statistical relationship with creatinine at the time of discharge ($r_2 = 0.28$, $p = 0.005$). Using binary logistic regression, we also observed relationship between proteinuria and glomerulosclerosis and also between ESR and tubular atrophy.

Discussion

Just as predicted, by knowing disease physiopathology, the relationship between intensity of glomerular involvement and proteinuria level was proved and especially also the relationship between glomerulosclerosis and proteinuria level was also confirmed. That is, higher the involvement of glomeruli, the more will be the intensity of proteinuria. Glomerular involvement and tubular involvement had diverse relationship with level of serum calcium; this hypocalcaemia is explainable by renal insufficiency. Arteriolar involvement had diverse relationship with level of serum Na and hyponatremia is probably due to dilutional and renal insufficiency. Medullary vessels involvement which was defined by leukocyte assembly and also hyperemia had direct relationship with white cell counts in blood which is completely explainable. Glomerular and tubular involvement and final rate had direct relationship with increased ESR which indicates increased inflammation, anemia, and renal insufficiency. Finally, final rate had negative relationship with urine density which is explainable by tubulo-interstitial involvement. But glomerular, arteriolar, and medullary vessel involvement and also final rate showed negative relationship with hematuria which can not be explained and need more consideration. Beth et al found that if the average counts of white cells at the beginning of admission is 10500, the risk of clinical pathologic damage progression would be 5 times. In this study, 13 patients out of 34 patients with over 13000 white cells suffered from more significant pathologic and clinical damages and only 5 patients with less than 13000 white cells showed progress in damages. In this study, the average of platelets were 42000 (29-122000) and hematocrit was 27% (23-29%) and BUN was over 20 mg/dl and also proteinuria and hematuria were also observed in urine and there was significant and direct relationship between less counts of platelet and HCT and more level of BUN and proteinuria and hematuria with damage intensity (8). Elliott et al observed sever anemia and the counts of

platelets was less than 20×10^9 liter which conformed to the intensity of glomerular involvement and the result of other studies (9). Nakisa et al highlighted direct relationship between white cell counts and clinical implications which resulted in surgical operation (10-17). However, white cell counts were above 20000 and serum albumin was less than 3 g/dl, because of colon and ileum perforation and complete colon gangrene and megacolon toxic, implications and damage intensity were more significant. Laboratory data in these patients included 17420 white cells, 61% PMN, 7.5% hemoglobin, level of serum Ca was 8.3 mg/dl, level of serum K was 4/5 meq/l, serum glucose was 115 mg/dl, ceratinin level was 4 mg/dl and albumin level was 3.4 g/dl. These laboratory changes were also associated with intensifying pathologic damages, especially glomerular damages (10-17). Bailey showed that glomerular changes in histopathology findings were the most significant. The changes ranged from endothelial edema to degeneration of endothelial cells and capillary wall thickening and thrombosis which were observed in most glomerules but not all of them and in those patients whom these changes were resistant to treatment or lasted for along time, next biopsy showed glomerulosclerosis. In these patients with glomerular damages, there were decreased counts of platelets, sever anemia, normal or increased white cells counts, high reticulocyte, schistocyte, Bur and Helmet cell in PBS, low serum Na level , high serum K level, proteinuria, and RBC casts in urine test and negative Coombs test and decreased C3 and C4 complements, decreased albumin and slightly an increase of hepatic enzymes and normal coagulation tests of PT and PTT, and these changes were comparable with pathologic damage severity, especially glomerular and arteriolar damages. In our studies, the most prevalent damages were glomerular damages and ranged from decreased capillary lumen and thickening of capillary wall to increased glomerular cellularity and fewer crescents formations. In our studies, we also observed increased white cell counts, decreased Hb, and platelets counts, increased reticulocytes, decreased serum Na level and increased serum K level and 2-3 times increased hepatic enzymes. We also observed microscopic hematuria and proteinuria in urine test. Glomerular damages and arteriolar thrombosis had positive relationship with arteriolar thrombosis and creatinine level of serum. There was also significant statistical relation between glomerular damages and proteinuria as well as hematuria severity. In our studies, infiltration

of mononuclear (MN) and polymorphonuclear (PMN) inflated cells was also clearly observed in interstitial tissue and interstitial tissue rate had no significant relationship with none of the variables (11). But Van Setten in contrast to our results observed infiltration of mononuclear (MN) and polymorphonuclear (PMN) inflated cells in glomerules and even considered it as a primarily damage and noted a relation between level of infiltration of these cells with chemokine level of MCP-1 and IL8. Chemokines level which is released from different renal cells including endothelial, mesangial, proximal tubular epithelial cells, and fibroblasts has direct relationship with level of infiltration of inflated MN and PMN cells, especially MN in glomrules and consequently causing severer pathologic damages (12). These different results need more consideration. In most studies such as Elliott et al and Bailey, coagulation tests were normal and had no relation with pathologic damages (9, 11). In previous studies as well, such as Bailey et al, Coombs test was negative and had no relation with pathologic damages (11-15). Elliott et al observed increase of serum indirect billirubin in the absence of haptoglobin and high LDH in serum which is not only an indicator of degradation of RBC but also an indicator of histological damages severity and tissue ischemic damages (9). Unfortunately, in our study, haptoglobin was not measured but conforming to that study, LDH was increased in serum with the average of 2006.8 (\pm 1723) and was proportional to histological damages severity, especially glomerular damages. An interesting point which was noted by Ishikawa et al was that increase of polymorph nuclear inflated cells and plasma level of granulocyte elastase- α 1-proteinase inhibitor complex or GEPIC was associated with increase of endothelial damages in biopsy and HUS severity (13-16); however, unfortunately, it was impossible for us to measure GEPIC and in our study mean percent of PMN was 56% and we found no relationship between that and histological damages and endothelial damages. Another interesting point which was noted by Chandler et al is that they observed increased plasma level of prothrombin fragment and T-PA and D-Dimer and these abnormalities were associated with increased histological damages severity, azothemia and severer thrombocytopeni (14) and in this study, these coagulation products were considered as causes of damage to endothelium.

Conclusion

Biopsy could be a valuable element to determine degree and prognosis of disease and relationship between histological and laboratory data could be used for the treatment and prediction of degree of involvement of components and determining prognosis.

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