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Urinary N-acetyl-β-D-glucosaminidase and Malondialdehyde as a Markers of Renal Damage in Burned Patients

This study was aimed to evaluate renal dysfunction during three weeks after the burn injuries in 12 patients admitted to the Hallym University Hankang Medical Center with flame burn injuries (total body surface area, 20-40%). Parameters assessed included 24-hr urine volume, blood urea nitrogen, serum creatinine, creatinine clearance, total urinary protein, urinary microalbumin, 24-hr urinary N-acetyl-β-D-glucosaminidase (NAG) activity, and urinary malondialdehyde (MDA). Statistical analysis was performed using repeated measures ANOVA test. The 24-hr urine volume, creatinine clearance, and urinary protein significantly increased on day 3 post-burn and fell thereafter. The urine microalbumin excretion showed two peak levels on day 0 post-burn and day 3. The 24-hr urinary NAG activity significantly increased to its maximal level on day 7 post-burn and gradually fell thereafter. The urinary MDA progressively increased during 3 weeks after the burn injury. Despite recovery of general renal function through an intensive care of burn injury, renal tubular damage and lipid peroxidation of the renal tissue suggested to persist during three weeks after the burn. Therefore, a close monitoring and intensive management of renal dysfunction is necessary to prevent burn-induced acute renal failure as well as to lower mortality in patients with major burns.

Key Words : *Burns, Wounds and Injuries; Kidney; Blood Urea Nitrogen; Ceatinine Clearance; Acetyl glucosaminidase, Malondialdehyde*

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INTRODUCTION

Extensive burn is not only a skin injury but also a serious systemic illness often accompanied by various complications. Acute renal failure (ARF) is one of the major complications of burns, carrying an extremely high mortality rate (1). Although ARF is not commonly encountered in burned patients, this complication merits a special attention in that its outcome is generally very poor and varies depending on the severity and adequacy management of the burn injury. The quoted incidence of renal failure in burned patients varies widely between 0.5 and 30% and its mortality is as high as between 73 and 100%. The incidence and mortality rate of ARF in burned patients depend on the severity of the burns and on the criteria of renal failure such as free water clearance (greater than -0.5 mL/min), blood urea nitrogen (above 50 mg/dL) and serum creatinine level (above 2.0 mg/dL) (2). These criteria have been used to assess the incidence and as an index in treating ARF in burned patients (3). Increased urinary excretion of protein is one of the most common and easily detected signs of renal pathology. Renal abnormalities may occur both in the quantity and in the composition of urinary proteins (4). Renal pathologies in burns are characterized by the development of extensive inflammation inducing an intensive acute phase response in the kidney. Urinary malondialdehyde (MDA) is a gross indicator of renal lipid peroxidation (5), and has been shown to increase after burns (6). The present study was aimed to estimate the degree of burn-induced renal damage and the recovery and to determine the treatment response in 12 patients with second- or third-degree burns admitted to the burn center at the Hallym University Hankang Medical Center within 12 hr of insult onset.

MATERIALS AND METHODS

Patients

With approvals by the Ethics Committee of the Hallym University Hankang Medical Center and informed consents, 12 patients were selected among flame-burned patients admitted to the burn center at the Hallym University Hankang Medical Center within 12 hr of their injury. The subjects consisted of 10 males and 2 females with an age range from 23 to 65 yr (mean±SD, 37.7±13.6 yr) without chronic illnesses such as hypertension and diabetes mellitus. They had second- or third-degree flame burns covering 20 to 40%

of the total body surface area (TBSA): two with 20%; four with 25%; two with 30%; one with 35%; three with 40% (mean±SD, 29.6±7.5%). Patients were hospitalized at the burn center and were resuscitated according to the Parkland Formula. The burn wounds were thoroughly and gently cleaned by immersing the patients in a whirlpool bath to which sodium hypochlorite had been added (150:1). The wounds were then debrided by a surgical sponge, rinsed, dried, and 1% silver sulfadiazine cream was applied and then covered with dry coarse mesh gauze. This procedure was done daily in patients. The goal of nutritional support was to maintain a positive nitrogen balance by greater than 6 g/day.

Blood sampling and urine collections

Blood was drawn on the admission day (day 0: mean±SD, 7±6 hr post-injury), and at every 6 a.m. thereafter. Several parameters such as 24-hr urine volume, blood urea nitrogen (BUN), serum and urine creatinine, creatinine clearance, total urine protein, urine microalbumin, urinary N-acetyl-β-D-glucosaminidase (NAG) activity, and urinary malondialdehyde (MDA) were measured from day 0 to day 3, and on days 7, 14, and 21.

Analytical methods

Seum creatinine, urine creatinine, and blood urea nitrogen (BUN) were quantified by automated chemistry analyzer (SBA-300, Gilford Co, U.S.A.). Total urine protein was quantified according to the Braford method (7) and urine microalbumin was determined by immunoturbidimetry. Urinary NAG activity was measured by a colorimetric procedure as described by Horak et al. (8). Urinary MDA diethyl-thiobarbituric acid complexes were isolated and quantified according to the method described by Guichardand et al. (9). All samples were analyzed in duplicate.

Statistics

Data are presented as mean±SD. Statistical analysis was performed using repeated measures ANOVA test in SAS PROC GLM program to compare the patients' characteristics and biological values. A *p*-value less than 0.05 were considered statistically significant.

RESULTS

As shown in Table 1, the urine volume was elevated throughout three weeks of post-burn period with a peak level on day 3 post-burn. The BUN and serum creatinine levels were significantly decreased to their nadirs on day 3 post-burn and creatinine clearance was significantly elevated during the 3 days post-burn. The total urinary excretion of protein showed a maximum level on day 3 post-burn, while the mean urinary excretion of microalbumin was at its peak level on admission, and rapidly fell on day 1 post-burn. And then the level increased on days 2 and 3, and was reversed again on day 7 onwards, until on day 21, it was below the level of day 1 (Table 2). By 1 week after the burns the 24-hr urinary NAG activity was progressively elevated to its maximal level and still remained elevated at 5.5-fold level of day 0 on day 21. The 24-hr urinary excretion of MDA was slightly decreased on day 1 and thereafter progressively increased to its maximal level on day 21 (Table 3).

DISCUSSION

Severe burn must be managed not only as a dermal injury but also as a serious systemic illness often accompanied by

Table 1. Various indices of renal function in 12 burned patients during their hospitalization under intensive treatment

Day (post-burn)	0	1	2	3	7	14	21
Urine Volume (dL)	17.8±5.8	25.1±11.8*	32.9±13.2†	33.4±11.2†	26.6±8.4†	26.6±8.4	23.2±8.5
BUN (mg/dL)	12.1±4.9	12.0±4.3	8.8±3.6*	8.2±3.6†	10.1±2.7	8.9±2.3	10.0±3.3
Serum creatinine (mg/dL)	0.86±0.21	0.82±0.21	0.71±0.14*	0.72±0.16*	0.77±0.18	0.77±0.16	0.83±0.32
Creatinine clearance (mL/min)	146.2±85.9	229.0±82.1*	315.8±144.1†	311.2±104.6*	218.4±97.5	144.8±65.4	122.2±49.8

Data are mean±SD (n=12) at the consecutive days after burn injury. The normal references values are as follows; urine volume=0.75-2 L/day, BUN=4-18 mg/dL, serum creatinine=0.5-0.9 mg/dL, creatinine clearance=125±23.6 mL/min. The p-values calculated by repeated measures ANOVA test indicate statistically significant differences compared with day 0 post-burn; *$p<0.05$ and †$p<0.01$.

Table 2. Total 24-hr urinary protein and microalbumin in 12 burned patients during their hospitalization under intensive treatment

Day (post-burn)	0	1	2	3	7	14	21
Total Protein (mg/24 hr)	138.8±65.2	352.2±108.5†	535.6±195.7†	835.2±288.9*	493.7±158.9*	232.0±67.0	200.1±78.2
Microalbumin (mg/24 hr)	65.8±55.2	38.5±25.6*	56.0±44.9	64.0±55.3	29.2±29.8	29.6±50.9	20.7±17.8*

Legends are the same as in Table 1

Table 3. 24-hr urinary NAG activity and MDA excretion in 12 burned patients during their hospitalization under intensive treatment

Day (post-burn)	0	1	2	3	7	14	21
NAG (U/mg of urine creatinine)	15.8±9.5	29.7±16.7*	41.3±29.0*	68.3±26.9†	111.7±59.0†	105.0±50.0†	87.8±40.5†
MDA (μmol/24 hrs)	309.2±69.8	226.9±53.7	261.8±50.5	260.8±65.9	271.1±37.2	382.9±74.5‡	424.8±80.5‡

Data are mean±SD (n=12) at the consecutive days after burn injury. The *p*-values calculated by repeated measures ANOVA test indicate statistically significant differences compared with day 0 post-burn (*$p<0.05$ and †$p<0.01$) in NAG activity and day 1 post-burn (‡$p<0.05$) in MDA excretion.

various complications (1). ARF is one of the major complications of burns, carrying an extremely high mortality rate. The reported incidence and mortality rate of ARF in burned patients vary depending on the severity of the burn injury. ARF occurs either immediately after burn or at a later stage, most often in the third week or later. Although the early form of ARF has become less frequent than before with current aggressive fluid resuscitation to maintain an adequate cardiac output and urine output, it is still a life-threatening complication, particularly in patients with extensive third-degree burns (10). In this study, the average concentrations of BUN and serum creatinine slightly increased only during the first 24 hr after the burn injury and thereafter were maintained within normal ranges during the 3-weeks post-burn period. The average 24-hr urine volume seemed to increase, but it was not assumed to represent acute and severe renal pathology such as oliguric and polyuric renal failure.

A useful indicator for glomerular filtration rate is endogenous creatinine clearance. In this study, average value of endogenous creatinine clearance markedly increased during the first 48 hr after the burn injury and thereafter gradually decreased to the normal level. Collectively, these data showed that our patients underwent and recovered from a transient ARF after the burn injury. Proteinuria following a renal damage has been studied most intensively and is still regarded as one of the most sensitive markers for the pathologic conditions of the kidney (11). The normal excretion rate of urinary protein is less than 150 mg per 24 hr for adults, but values as high as 300 mg per 24 hr may be encountered in apparently healthy adolescents. The normal composition of urinary protein is about 40% of albumin, 40% of tissue proteins originating from renal and other urogenital tissues, 15% of immunoglobulins and their fragments, and remaining 5% of other plasma proteins (4). Healthy individuals are known to excrete protein in their urine. The daily amount of excretion is relatively constant. Abnormalities may occur both in the quantity and in the composition of urinary proteins. Stress such as burn and heavy physical exercise can cause moderate to severe proteinuria (12). It is now recognized that the kidney plays an important role in catabolism of low molecular weight proteins. Low molecular weight proteins readily pass through the glomerular membrane and are largely reabsorbed and catabolized in the renal tubular cells (13). Plasma proteins passing through the glomerular membrane constitute a large proportion of normal urinary proteins, being the albumin the predominant. The glomerular capillary wall restricts the passage of plasma proteins according to their surface charge and more specially their size. A number of systemic and primary renal diseases may affect one or more glomerular structures and thereby increase the effective permeability of the glomeruar capillary wall to proteins. Under these pathologic conditions, the degree of proteinuria may range from 0.2 to greater than 20 grams per 24 hr. Proteinuria that exceeds 3 to 5 grams per 24 hr provides a direct evidence of an increased effective permeability of the glomerular capillary wall, since that amount is greater than that may be filtered by the normal glomerulus and reabsorbed by the renal tubules (4). Increased albumin excretion usually reflects a glomerular disease but when the quantity is small it may be due to an impaired tubular reabsorption. Thus some albuminuria is commonly found in patients with renal tubular disorders (14). This study showed that the urinary protein excretion in patients sustaining moderate burns was greater than normal with a peak level day 3 post-burn, and then fell to the normal level.

Increased excretion of NAG, an enzyme found in the lysosomes of proximal renal tubular cells, has been demonstrated to be more specific for renal tubular pathology (15). The molecular weight of NAG is large enough to preclude passage through the normal glomerular basement membrane. Thus an increased excretion of NAG reflects active tubular damage and has also been reported in patients with glomerulonephritis or under nephrotoxic drug treatment (16-18). The increase in NAG excretion found in diverse renal pathologies is consistent with the underlying disease process of injury to the proximal tubular cells. Increased glomerular filtration of protein by itself can seemingly cause tubular pathology, since patients with nephrotic syndrome due to minimal change glomerulonephritis were shown to have large lipid and protein-laden vacuoles in their proximal tubular cells (19, 20). The correlation between albumin excretion and NAG loss might be consistent with proteinuria causing tubular injury, but also could result from the primary disease process in another way. Although our patients received aggressive treatment in a burns unit, the 24-hr urinary excretion of NAG increased to a maximal level by 1 week after the burn and was still elevated and remained above five times of the level of day 0 on day 21 after the burn injury.

Burn injury initiates an appreciable oxidative stress and

inflammation-induced hyper-metabolic state that can lead to severe multiple organ failure (21). Oxidants are major products of inflammation and lipids peroxides have been shown to increase in the plasma of burn animals and patients (6, 13-21). Free radical production is associated with inflammation, and circulating lipid peroxides have been shown to increase in burn patients during the first week post injury (6, 21, 22, 24). A close relationship between the intensity of lipid peroxidation and complications after burns has been reported (25, 26). Urinary MDA excretion was greatly increased in burn patients which was approximately 20 times higher than normal (27) during the first week post-burn, confirming that lipid peroxidation is strongly activated after burns (9). Therefore, urinary MDA appears to be a very sensitive biochemical parameter and may well be useful in assessing renal oxidation status (6, 9). The 24-hr urinary excretion of MDA was slightly decreased on day 1 post-burn and thereafter progressively elevated to reach a maximal level on day 21 post-burn in this study, which was a reverse to the findings of other reports (9). It is suggested that renal inflammation and tubular injury in burned patients persist during the 3 weeks after the burn injury in spite of early and aggressive management.

In conclusion, our results show a persistent renal tubular damage and inflammation in spite of recovery of general renal function as demonstrated by BUN, serum creatinine, and creatinine clearance after a transient acute renal dysfunction. We suggest that an early intensive care of burn-induced renal damage be necessary in order to prevent renal complications as well as to lower the mortality in patients with major burns.

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