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Renal bcl-2/bax expression in childhood Henoch–Schönlein nephritis: prognostic importance?

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Aim: To investigate the renal expression of apoptosis regulatory proteins b-cell lymphoma 2 (bcl-2) and bcl-2 associated X (bax) in children with Henoch–Schönlein nephritis (HSN)

Materials and methods: Renal expression of antiapoptotic bcl-2 and proapoptotic bax proteins were studied immunohistochemically in 22 children with HSN. The kidneys of 5 children who died due to nonrenal reasons were used as the control. The patients were compared according to their renal histopathology and prognosis.

Results: Nephrotic or nephrotic-nephritic renal involvement was the most common presentation. Histopathological findings were Class I in 1, Class II in 5, Class III in 10, Class IV in 4, and Class V in 2 patients. IgA staining was shown in all biopsies. Bcl-2 and bax proteins mainly showed tubular and crescentic expression. This was significantly different for bcl-2 in tubules and bax in distal tubules compared to the controls ($P < 0.05$). No significant difference between bax and bcl-2 expression was observed among patients. However the mean values of the bcl-2/bax ratio, that is, the balance between survival and apoptosis, were significantly higher in crescents of the severe histopathologic group and in distal tubules of the poor prognosis group ($P < 0.05$).

Conclusion: A higher bcl-2/bax ratio, that is relative to the predominance of cell survival in distal tubules and crescents in HSN, seems to be a poor prognostic factor in children.

Key words: Apoptosis, bax, bcl-2, children, Henoch–Schönlein nephritis, prognosis

1. Introduction

Henoch–Schönlein purpura (HSP) is the most common small vessel vasculitis of children. Renal involvement determines the prognosis. In the pathogenesis of Henoch–Schönlein nephritis (HSN), glomerular deposition of abnormally glycosylated immunoglobulin A (IgA), intraglomerular nitric oxide elevation, peroxidative damage, apoptosis, and sclerosis are involved (1). The b-cell lymphoma 2 (Bcl-2) gene family is an important group of genes encoding a group of proapoptotic and antiapoptotic regulatory proteins. The best known and most extensively studied ones are antiapoptotic *bcl-2* and proapoptotic *bcl-2 associated X* (*bax*), which block each other by heterodimerisation. Their relative expressions at cellular level determine the fate of involved cells, either in the direction of apoptosis or survival (2–5).

In this study we investigated renal expression of these 2 proteins with opposing actions by immunohistochemical methods in children with HSN and evaluated their prognostic significance.

2. Materials and methods

2.1. Subjects

Twenty-two children with HSN who followed at the Dr Sami Ulus Obstetrics and Children's Hospital, Paediatric Nephrology Department, in Ankara, Turkey, were enrolled in the study. The American College of Rheumatology criteria were used for the diagnosis of HSP (6). Patients with another systemic disease, no adequate urinalysis data, or no follow-up were excluded from the study. In 2 patients a rebiopsy was performed because of the progression of renal findings. Histopathologic findings

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were graded from Class I to Class VI according to severity, as previously suggested (1). The clinical and laboratory parameters were defined as follows: 1) haematuria: 5 or more red blood cells per high power field in a centrifuged specimen, or more than 10 red blood cells per microlitre on microscopy without proteinuria; 2) mild proteinuria: urinary protein excretion of 4–40 mg m^{-2} h^{-1}; 3) nephrotic range proteinuria: urinary protein > 40 mg m^{-2} h^{-1}; 4) acute nephritic syndrome: haematuria associated with at least 2 of the following: raised serum urea or creatinine, hypertension, and oliguria; and 5) nephrotic syndrome: proteinuria > 40 mg m^{-2} h^{-1}, with serum albumin level below 2.5 g/dL and oedema. Hypertension was defined as average systolic or diastolic blood pressure values greater than or equal to the 95th percentile for age, sex, and height. The Schwartz formula was used to estimate creatinine clearance.

As the control group, the autopsy kidney materials of 5 age-matched children who died due to extrarenal pathologies were used. Patients were divided into 2 groups according to histopathologic findings: those having crescents of less than 50% (first 3 classes) and those having crescents of >50% (Class >III). They were also grouped according to their prognosis as being in complete remission if all clinical and laboratory findings were normal; partial remission if minimal renal involvement with normal albumin, urea, and creatinine was present; and unresponsive or progression if renal findings persisted or deteriorated.

The study was approved by the hospital's ethics committee.

2.2. Immunohistochemical examination

Bcl-2 and *bax* specific staining was applied to 3–5 μm sections of paraffin blocks of kidney tissue using the human specific antigen kits suitable for the tissues held in formol or paraffin (*bax*: *Ab-1*, *clon 2D2*, 1 kDa antigen; *bcl-2α*: *Ab-1*, *clon100/D5*, 25–26 kDa antigen, NeoMarkers, USA).

Expression of *bcl-2* and *bax* proteins were scored semiquantitatively between "0" and "+++" according to the percentage of the stained cells by a pathologist blind to the clinical features of the patients, as suggested by Charalambous et al. (7). The *bcl-2/bax* ratio was calculated by using the percentages of stained cells.

2.3. Statistical analysis

The chi-square test was used to compare the renal expression of bax and bcl-2 in patient and control groups. Kruskal–Wallis and Mann–Whitney U tests were used to compare the means of the *bcl-2/bax* ratio between groups. $P < 0.05$ was accepted as significant. SPSS 10.0 was used for the statistical analyses.

3. Results

Kidney biopsies of 22 children (17 males, 5 females) with HSN, aged between 6 and 15 years (mean: 11.04 ± 2.5), were evaluated immunohistochemically. The mean follow-up time was 35.5 (6–140) months. Initial clinical findings were palpable purpura in 22 patient (100%), gastrointestinal involvement in 17 (78%), arthritis/arthralgia in 15 (68%), oedema in 8 (36%), hypertension in 6 (27%), and central nervous system involvement in 1 (4%). Laboratory tests revealed an elevated erythrocyte sedimentation rate in 14 patients (63%), low C_3 level in 2, and low C_4 level in 3. Serum IgA levels were high in 4 patients (18%), low in 1 (4%), and normal in 17 (78%). Group A beta haemolytic streptococcus was isolated in 1 patient's throat culture, and in 8 patients, antistreptolysin-O antibody titres were elevated. The number of glomeruli in specimens ranged from 10 to 125 (mean: 33.6). IgA deposition was detected in all biopsies, ranging from + to +++ (+: 8.4%, ++:54.1%, and +++:37.5%). Nephrotic (9 patients; 40%) or nephrotic-nephritic (9 patients; 40%) presentations were the most common types of renal involvement. Persistent nonnephrotic proteinuria was shown by 3 patients (13%), and 1 patient (4%) was nephritic. Histopathological findings of the patients were Class I in 1, Class II in 5, Class III in 10, Class IV in 4, and Class V in 2 patients. There were no Class VI patients. Rebiopsy was performed in 2 patients with Class V HSN with deteriorating kidney functions who were unresponsive to treatment. These second biopsies were also of Class V and were included in the study. In 4 Class IV patients, the percentage and types of crescents were as follows: 27 crescents in 54 glomeruli (50%, all cellular), 6 crescents in 11 glomeruli (55%, all fibrous), 6 crescents in 10 glomeruli (60%, 4 fibrous, 2 cellular), and 7 crescents in 10 glomeruli (70%, 6 cellular, 1 fibrocellular). In 2 Class V patients and their 2 rebiopsies, crescent distribution was as follows: 25 crescents in 32 glomeruli (78%, 4 fibrous, 3 cellular, and 18 fibrocellular), 4 crescents in 35 glomeruli (11%, 2 fibrous and 2 fibrocellular crescents, and 19 obsolescent glomeruli; diagnosed as Class V due to chronicity findings), 36 crescents in 44 glomeruli (81%, 6 fibrous, 11 cellular, and 19 fibrocellular), and 49 crescents in 52 glomeruli (94%, 30 fibrous, 3 cellular, and 16 fibrocellular).

There were 9 patients with rapidly progressive glomerulonephritis who were given triple therapy, as suggested by Öner et al. (8). The patients with gastrointestinal system involvement were given oral prednisolone. The outcomes of treatment according to histopathologic classes are given in the Table.

Bcl-2 and *bax* proteins mainly showed tubular and crescentic expression (Figure 1). Other than crescents, glomerular expression of *bcl-2* and *bax* was detected only in 1 patient. Therefore glomerular involvement was not included in the statistical analysis. In the control group there were no crescents, as expected, and minimal tubular *bcl-2* and *bax* expression was detected. In the patients

Table. Treatment response rates according to histopathologic class.

Prognosis	Histopathologic classification				
	Class I	Class II	Class III	Class IV	Class V
Remission		4	4		
Partial remission	1	1	6	3	
Progression				1	2

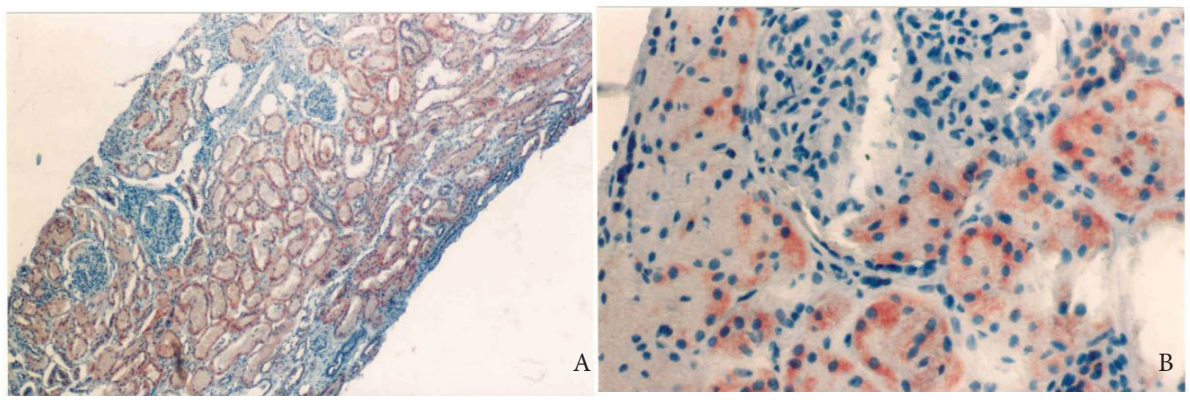

Figure 1. Tubular (+++) and crescentic (+) immunostaining with *bax* (A: 100×) and *bcl-2* (B: 400×).

significantly higher *bcl-2* expression in proximal and distal tubules, and *bax* expression in distal tubules, were detected, both in the histopathologic and prognostic subgroups, when compared to the controls ($P < 0.05$ for all). When patients were compared with each other, no significant difference was observed between the histopathologic or prognostic subgroups. Therefore, the means of the *bcl-2/bax* ratio, showing the balance between proapoptotic and antiapoptotic proteins, were used for comparison. For all sites (proximal tubules, distal tubules, crescents), the means of the *bcl-2/bax* ratio were the highest in higher histopathologic classes (Classes IV and V) and the progression group. These differences were statistically significant for crescentic expression in histopathologic classes ($P = 0.049$) and for distal tubular expression in prognostic groups ($P = 0.045$) (Figures 2 and 3).

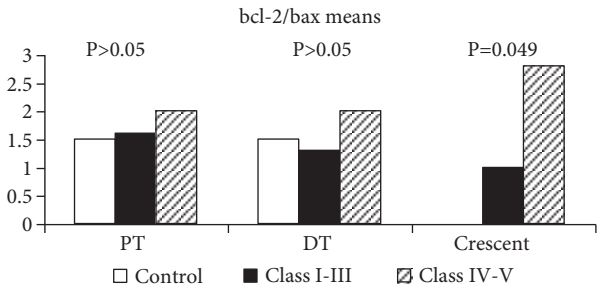

Figure 2. Means of *bcl-2/bax* ratio in patients according to their histopathology (PT: proximal tubule, DT: distal tubule).

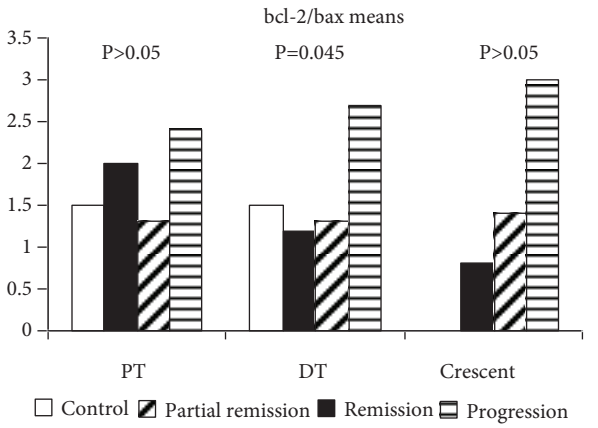

Figure 3. Means of *bcl-2/bax* ratio in subjects according to their prognosis (PT: proximal tubule, DT: distal tubule).

4. Discussion

The degree of renal involvement in HSP determines the prognosis (9–11). In our study, 3 patients (13.6%) progressed to end-stage renal disease (ESRD). Multiple factors, including apoptosis, are important in progression to ESRD, but there are many unexplained or controversial points about the role of apoptosis in renal diseases (12–16). For example, in resolving postinfectious glomerulonephritis, proliferation and cell death coincide. However, in lupus nephritis, proliferation predominates. Proliferative glomerulonephritis shows increased mitotic activity in glomeruli or infiltrating inflammatory cells. Thus, recovery of the lesions probably depends on increased apoptotic removal of these cells (4,17). Our results support this theory. This is because the significantly increased *bcl-2/bax* ratio in crescents and distal tubules of the patients with more severe histopathology, or with poorer prognosis, indicates cell survival predominance or insufficient apoptosis. In glomerular diseases *bcl-2* is responsible for hypercellularity while *bax* is important for glomerulosclerosis. Thus, the balance between them is critical for the progression of glomerular diseases. *Bcl-2* expression correlates with proliferation/activation of mesangial cells (4,18,19). However, *bax* expression correlates with mesangial matrix expansion and type-IV collagen deposition. Therefore, their relative expression is important in disease progression (4). Involvement of oxidative stress and reactive oxygen molecules (ROMs) in HSP is more indirect evidence for the role of apoptosis in HSN pathogenesis. Besides, ROM levels are under the control of the *Bcl-2* gene product (20,21).

The mainly tubular expression of *bax* and *bcl-2* in our patients is in agreement with the literature (18,22). However, the variable biopsy timing in our study is a drawback and thus immunohistochemical findings may represent different apoptotic stages. In order to alleviate this limitation, we used the means of the *bcl-2/bax* ratio instead of the individual expression of each protein, and we found significantly higher means of the *bcl-2/bax* ratio in crescents in the more severe histopathologic group. For the prognostic groups, a significant increase was detected in the means of the *bcl-2/bax* ratio in distal tubules, going from favourable to poor prognosis groups.

In proliferative glomerulonephritis models, apoptosis is important for the removal of hypercellularity in the repair process (23). In our study, higher crescentic and distal tubular *bcl-2/bax* ratios in severe histopathologic or poor prognostic groups may represent the relative predominance of cell survival, preventing repair at a critical phase of HSN. In the remnant kidney model, however, increased apoptosis is reported to be responsible for scarring, tubular atrophy, or fibrosis (24). These contradictory results might be due to the different roles of apoptosis in different pathogenetic processes. In proliferative inflammatory conditions of the kidney, apoptosis is essential for resolution, but for resident kidney cells, uncontrolled apoptosis is definitely harmful with the potential risk of renal failure by reducing functional renal mass (23,24). Our findings seem to contradict the results of the nephrotoxic nephritis rat model of Yang et al. (25), as well. They reported a strong increase in the *bax/bcl-2* ratio in favour of apoptosis through caspase-3 activation. However, they also reported differences between mRNA and tissue expression of these proteins, and they attributed this to further translational or posttranslational control mechanisms within the diseased kidney.

Apoptosis is a complex and dynamic process with many pathways and regulatory proteins involved. Expression of a single molecule might not necessarily represent the dominant pathway in that moment, since a kidney biopsy provides a static picture of the pathogenesis of a specific disease. Despite all these limitations, to our knowledge, this is the first study investigating the renal expression of these 2 proteins in the most frequent vasculitis of children.

In conclusion, our results indicate, but do not prove, that insufficient apoptosis or prolonged cell survival in crescents and distal tubules at a critical and probably proliferative stage of HSN in children is associated with an unfavourable histology and a poor prognosis.

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