Malaysian J Pathol 2001; 23(1) : 27 – 30

Expression of proliferating cell associated protein, Ki-67, supports cellular proliferation in WHO Class IV lupus nephritis

PL CHEAH, *MBBS, FRCPath*, **R KUNASEEGARAN,** *MBBS, MPath,* **and** *LM* LOOI, *FRCPA, FRCPath*

Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur

Abstract

Ki-67 expression in diffuse proliferative lupus nephritis, WHO Class IV, was compared against normal controls to establish that cellular proliferation is involved in the production of glomerular hypercellularity. Twenty-three histologically confirmed WHO Class IV lupus nephritis and 23 normal control renal tissue were immunohistochemically stained with a polyclonal antibody to Ki-67 (Dako) using the peroxidase labelled streptavidin bioitin kit (Dako). There were 20 females and 3 males, with 17 Chinese and 6 Malays in the WHO Class IV lupus nephritis group. Ages of patients ranged between 10-56 years with a mean of 31.9 years. The normal controls, 20 males and 3 females, and ethnically 9 Indians, 7 Malays, 2 Chinese, and 5 foreign nationals (4 Indonesians and 1 Bangladeshi), had an age range between 15-33 years (mean = 23.3 years). Sixteen (69.6%) WHO Class IV lupus nephritis and 8 (34.8%) normal controls demonstrated Ki-67 immunoreactivity in at least 1 glomerulus ($p<0.05$). Of the 256 WHO Class IV lupus nephritis non-sclerosed, glomeruli studied, 37 (14.5%) were Ki-67 immunopositive compared with normal controls where 16 (0.7%) of 2159 glomeruli demonstrated Ki-67 ($p< 0.01$). Cellular proliferative activity, as evidenced by Ki-67 expression, was significantly increased in WHO Class IV lupus nephritis confirming that cell proliferation contributes to glomerular hypercellularity.

Key words: Ki-67, lupus nephritis, WHO Class IV

INTRODUCTION

Histological diagnosis of most "proliferative" glomerulonephritis is based on visual detection of increased number of cells in the glomeruli. A natural assumption would be that the increase is due to proliferation of cells within the glomeruli. Nevertheless, a loss of cellular obsolescence could produce a similar morphological picture. Although the physical implications to the glomerulus may remain the same, whatever the underlying mechanism of the increased cellular numbers, a clear distinction of what causes the increase is important if there is to be detailed understanding of the pathogenesis of the "proliferative" glomerulonephritides. In the Malaysian context, glomerulonephritis resulting from underlying systemic lupus erythematosus (SLE), is probably the most common glomerular disease leading to biopsy of the renal tissue for diagnostic, management and prognostic purposes. And, of all the histological types of glomerulonephritis arising from SLE, the diffuse proliferative type, or Class IV according to the World Health Organisation (WHO) classification system,[1] is the most commonly encountered entity.[2] WHO Class IV, diffuse proliferative lupus nephritis, is therefore one of the most important proliferative glomerulonephritis occurring in Malaysians and it was opted that it be studied.

The Ki-67 antibody, originally raised against a crude nuclear fraction of the Hodgkin's disease derived cell line L428, reacts with a 395 kilodalton non-histone protein in the nucleolus which is encoded by a single gene on chromosome 10.[3-6] The function of the Ki-67 antigenic protein has not been conclusively established but appears to be related to the enzyme α-polymerase, involved in DNA synthesis.[1] Expression occurs throughout the cell cycle except in G_0, rising from G_1 through S, reaches a peak in G_2 and M,[4,6,8-10] hence making it a good marker of proliferating cells. A study was undertaken at the Department of Pathology, University Hospital Kuala Lumpur to ascertain cellular proliferation in WHO Class IV diffuse "proliferative" lupus nephritis by determining expression of Ki-67.

Address for correspondence and reprint requests: Professor PL CHEAH, Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

MATERIALS AND METHODS

All cases of clinically diagnosed SLE which satisfied the American Rheumatology Association criteria for diagnosis,'' with renal biopsies performed for assessment of renal involvement between January 1994 to December 1996 were retrieved from the files of the Department of Pathology, University Hospital Kuala Lumpur. All histological sections and results of immunofluorescence studies were reviewed. The cases were classified according to the WHO classification system and only cases which fulfilled the following were admitted into the study. Hence, all cases (1) should be unequivocally classified as WHO Class IV, (2) showed at least 5 non-sclerosed glomeruli in the biopsy tissue and (3) had sufficient residual paraffin-embedded tissue for immunohistochemical staining. Renal tissue obtained during necropsy performed, within 24 hours from time of death, on victims who succumbed to various traumatic injuries formed the normal controls. Victims' kin were interviewed and only cases with no known past medical history of significance were considered. Both kidneys were also macroscopically examined during the necropsy and ensured to demonstrate no gross pathology before a cortical wedge biopsy was performed from either kidney. The tissue was immediately immersed in 10% buffered formalin and left to fix overnight before being routinely processed for histopathological examination. All cases were histologically studied for any incidental microscopic lesions before being accepted as normal.

2-pm thick sections of the formalin-fixed, paraffin-embedded tissues of the tests and controls were cut on to aminopropyltriehtoxysilane (TESPA) coated slides. Antigen retrieval was carried out via microwave treatment ((Energy Beam Sciences, Inc., 600 watts, 100% power) at 100°C for 20 min before immunohistochemical staining with a polyclonal antibody to a synthetic peptide deduced from a 62 base pair region of the human Ki-67 gene (Dako: 1:100), sharing reactivity with the monoclonal anti Ki-67, clone MIB 1,[12] using the peroxidase labelled streptavidin bioitin kit (Dako). Visualisation was via 3'3' diaminobenzidine hydrochloride. Positive controls consisting of tissue sections from a reactive lymph node and negative controls made up substituting phosphate buffered saline for primary antibody in the staining of the positive controls were included in each batch stained.

Only unequivocal nuclear staining for Ki-67 was considered immunopositive. For a glomerulus to be considered as exhibiting Ki-67 immunoreactivity, at least one mesangial, endothelial, visceral or parietal epithelia1 cell of the glomerulus must exhibit Ki-67 staining. The number of positive glomeruli was charted together with the total number of non-sclerosed glomeruli of the tests and normal controls as seen in the histological sections. Statistical analysis was carried out using the chi square test.

RESULTS

Twenty-three cases of lupus nephritis which satisfied the criteria of the study were admitted. Of these, 20 were females and 3 males. There were 17 Chinese and 6 Malays. Ages of patients ranged between 10-56 years with a mean of 31.9 years. Of the 23 normal controls, 18 kidney tissue were from cases succumbing to injuries arising from motor vehicular accidents, 3 hanging, 1 chest stab wounds and 1 lightning strike. Of these, there were 20 males and 3 females. Ethnically, there were 9 Indians, 7 Malays, 2 Chinese, and 5 foreign nationals (4 Indonesians and l Bangladeshi). Mean age of the cases was 23.3 years while their ages ranged from 15-33 years.

Table 1 shows the number of Ki-67 positive glomeruli versus the total number of glomeruli assessed in diffuse proliferative lupus nephritis (WHO Class IV) and normal controls. Sixteen (69.6%) WHO Class IV lupus nephritis and 8 (34.8%) normal controls demonstrated Ki-67 immunoreactivity in at least 1 glomerulus ($p<0.05$). A total of 256 glomeruli were studied in the WHO Class IV lupus nephritis cases (mean = 11.1 glomeruli/case) compared with 2159 in the normal controls (mean = 93.9 glomeruli/case). Of the 256 WHO Class IV lupus nephritis affected glomeruli, 37 (14.5%) were Ki-67 immunopositive compared with normal controls where 16 (0.7%) of 2159 glomeruli demonstrated Ki-67 ($p< 0.01$). Fig. 1 shows a case of WHO Class IV lupus nephritis with nuclear Ki-67 staining.

DISCUSSION

As expected, there was a marked female predominance (M:F = 0.2:1) in the lupus nephritis group. The inverse (M:F = 6.7:1) was seen in the control group whereby a male predominance was evident. 78% of the control kidney tissue

TABLE 1: Ki-67 glomerular immunopositivity in diffuse proliferative lupus nephritis (WHO Class IV) and normal controls

	Diffuse proliferative lupus nephritis, WHO Class IV, (n=23)				Normal controls (n=23)		
	Ki-67 positive glomeruli				Ki-67 positive glomeruli		
	No.	%	Total assessed		No.	%	Total assessed
1	0	0	6	1	3	3.8	79
2	1	10.0	10	2	3	3.1	98
3	2	25.0	8	3	2	2.5	80
4	3	23.1	13	4	1	1.3	80
5	1	7.7	13	5	0	0	96
6	2	9.5	21	6	0	0	78
7	1	16.7	6	7	0	0	108
8	0	0	9	8	0	0	88
9	0	0	6	9	3	3.9	76
10	2	22.2	9	10	1	1.0	102
11	0	0	6	11	0	0	79
12	4	16	25	12	0	0	120
13	0	0	8	13	2	2.4	82
14	2	15.4	13	14	0	0	96
15	1	8.3	12	15	0	0	70
16	2	16.7	12	16	1	1.1	88
17	5	50.0	10	17	0	0	108
18	0	0	9	18	0	0	114
19	1	11.1	9	19	0	0	118
20	2	22.2	9	20	0	0	96
21	6	66.7	9	21	0	0	89
22	2	8.7	23	22	0	0	100
23	0	0	10	23	0	0	114
	37	14.4	256		16	0.7	2159

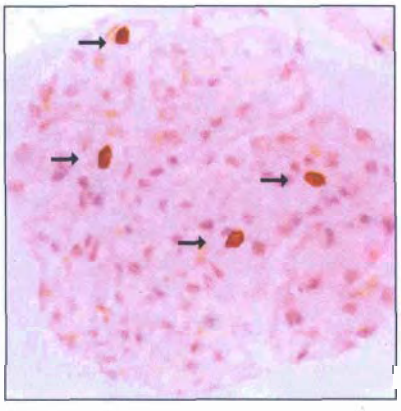

FIG. 1: A case of diffuse proliferative lupus nephritis, WHO Class IV, showing glomerular cells with Ki-67 nuclear immunopositivity (arrows).

were from victims succumbing from injuries sustained in motor vehicular accidents, a mode of death more common among males, and probably explains for the male bias. The ethnic predilection of lupus nephritis for Chinese[2] was also noted in this study. The racial breakdown of the controls reflected the ethnic distribution of the cases succumbing to traumatic injuries at the University Hospital Kuala Lumpur. The lupus nephritis cases were about a decade older than the controls. It would be expected that the proportion of sclerosed glomeruli would be increased in this group compared with the controls for two reasons i.e. natural aging obsolescence and disease destruction. However, the study was designed to discount sclerosed glomeruli and should not have created any unaddressed bias. It has nevertheless to be reiterated that in this

study, it was assumed that proliferative capacity of glomerular cells was similar for both sexes, for all ethnic groups of tests and controls and for differing age groups.

There was a significant increase in the number of cases of diffuse "proliferative", WHO Class IV, lupus nephritis which expressed Ki-67 (69.6%) compared with normal controls (34.8%). This difference was further enhanced when comparing the ratio of glomeruli exhibiting Ki-67 in WHO Class IV lupus nephritis (14.5%) with normal controls (0.7%). Thus, an increased cellular proliferation appears to contribute to the morphologically increased number of glomerular cells in diffuse proliferative lupus nephritis. Although there has been a general paucity of studies on proliferative index using cell cycle markers such as Ki-67 in glomerular diseases, the capability of glomerular cells, including mesangial, endothelial, visceral and parietal epithelia1 cells to proliferate under certain conditions has been documented.[13-18] Nevertheless, it still has not been excluded that decreased obsolescence or apoptosis does not contribute to the morphological hypercellularity. In fact, Uda et al showed that besides increased proliferation, a loss of apoptosis was noted in WHO Class IV diffuse proliferative lupus nephritis and IgA nephropathy as evidenced by the significant increase in the expression of Bcl-2, an antiapoptotic marker.[15] This aspect would prove interesting for further study as would detailed delineation of proliferating cell type in different proliferative glomerulonephritides.

ACKNOWLEDGEMENT

This study was funded by the Malaysian Ministry of Science, Technology and Environment IRPA research grant: University of Malaya 06-02-03-0719.

REFERENCES

1. Churg J, Bemstein J, Glassock RJ. Renal disease; classification and atlas of glomerular diseases. 2nd ed. New York: Igaku-Shoin 1995:151-6
2. Looi LM. The pattern of renal disease in Malaysia. Malays J Pathol 1994; 16:19-21
3. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983; 31:13-20
4. Gerdes J, Lemke H, Baisch H, Wacker H-H, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984; 133:1710-5
5. Verheijen R, Kuijpers HJH, Schlingmann RO et al. Ki-67 detects a nuclear matrix-associated proliferation-related antigen. I. Intracellular localization during interphase. J Cell Sci 1989; 92:123-30
6. Verheijen R, Kuijpers HJH, van Driel R et al. Ki-67 detects a nuclear matrix-associated proliferation-related antigen. II. Localization in mitotic cells and association with chromosomes. J Cell Sci 1989; 92:531-40
7. Fontana D, Bellina M, Gubetta G et al. Monoclonal antibody Ki-67 in the study of the proliferative activity of bladder carcinoma. J Urol 1992; 148:1149-51
8. Sasaki K, Murukami T, Kawasaki M, Takahashi M. The cell cycle associated change of the Ki-67 reactive nuclear antigen expression. J Cell Physiol 1987; 133:579-84
9. Guillard P, du Manoir S, Seigneurin D. Quantification and topographical description of Ki-67 antibody labelling during the cell cycle of normal fibroblastic (MRC-5) and mammary tumour cell lines (MCF-7). Anat Cell Pathol 1989; 1:25-39
10. Wersto RP, Herz F, Gallagher RE, Koss LG. Cell-cycle-dependent reactivity with the monoclonal antibody Ki-67 during myeloid cell differentiation. Experimental Cell Res 1988; 179:79-88
11. Tan EM, Cohen AS, Fries JF et al. The 1982 revised criteria for the classification of SLE. Arthritis Rheum 1982; 25:1271-7
12. Cattoretti G, Becker MHG, Key G, Duchrow M, Schluter C, Galle J et al. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. J Pathol 1992; 168:357-63
13. Matsuda M, Shikata K, Makino H, Sugimoto H, Ota Z. Glomerular expression of macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in patients with various forms of glomerulonephritis. Lab Invest 1996; 75;403-12
14. Groma V, Marcussen N, Olsen S. A quantitative immunohistochemical study of the expression of mesangial alpha-smooth muscle actin and the proliferation marker Ki-67 in glomerulonephritis in man. Virchows Arch 1997; 431:345-50
15. Uda S, Yoshimura A, Sugenoya Y, Inui K, Taira T, Ideura T. Mesangial proliferative nephritis in man is associated with increased expression of the cell survival factor, Bcl-2. Am J Nephrol 1998; 18:291-5
16. Shankland SJ, Eitner F, Hudkins KL, Goodpaster T, D'Agati V, Alpers CE. Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease: role in podocyte proliferation and maturation. Kidney Int 2000; 58;674-83
17. Barisoni L, Mokrzycki M, Sablay L, Nagata M, Yamase H, Mundel P. Podocyte cell cycle regulation and proliferation in collapsing glomerulopathies. Kidney Int 2000; 58:137-43
18. Chung WY, Kim YJ. Expression of Ki-67 antigen using monoclonal antibody MIB-l in children with post-streptococcal glomerulonephritis. Pediatr Nephrol 2000; 14:389-92