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Research Article

Clinical Significance of Hilar Hyalinosis in Glomeruli of Children with Idiopathic Nephrotic Syndrome

Abstract

Introduction: Hilar hyalinosis (HH) of glomeruli has been thought for a long time to be a precursor or a variant of focal glomerulosclerosis (FGS). In view of its implications on treatment and prognosis of nephrotic syndrome, a retrospective study of hilar hyalinosis in children with the idiopathic nephrotic syndrome (INS), but without any other histologic evidence of FGS, was performed to determine the clinic-pathologic significance of this lesion.

Methods: A total of 92 Children with INS who had kidney biopsies for frequent relapses, steroid dependency or resistance were included. Eight of them had a biopsy-proven diagnosis of either minimal change disease or mild IgM nephropathy with HH in 6-25% of glomeruli were compared to control group consisting of 84 children with either minimal change disease (n=57) or IgM nephropathy (n=27) but without HH.

Results: Clinically HH patients presented with NS and followed a steroid-dependent relapsing course prior to biopsy. Around 75% of the HH patients received at least one course of cytotoxic therapy after biopsy. At last visit, 6 patients were in remission, 2 were protein-free but on medication. None had hypertension or renal insufficiency. Apart from having a shorter interval between presentation and biopsy ($P<0.001$) and a lower remission rate ($P<0.05$), control patients followed a similar course. Pathologically HH patients showed less mesangial change ($P<0.001$) than the controls but similar mean glomerular size and tubuloinerstitial damage.

Conclusion: The results do not support the concept of HH being a precursor lesion of FGS.

Abbreviations

HH: Hilar Hyalinosis; INS: Idiopathic Nephrotic Syndrome; FGS: Focal Glomerulosclersis

Introduction

Idiopathic Nephrotic syndrome (INS) is the most common glomerular kidney disease in pediatric age group. It is a triad of significant proteinuria, hypoalbuminaemia, and edema with an associated phenomenon of hyperlipidemia. The clinical pattern of IN and response to treatment mainly reflect the underlying histopathology of the disease with a good prognosis in Minimal change disease and IgM nephropathy and a n unfavorable outcome in Focal Glomerulosclerosis (FGS).

Hyalinosis is a lesion containing amorphous material composed of serum proteins with characteristic histologic appearance and staining properties [1]. Glomerular hyalinosis is frequently observed in focal glomerulosclerosis (FGS), both primary and secondary, and in diabetic glomerulosclerosis [2-4]. Its pathogenesis is uncertain although there is evidence that hyperfiltration and maladaptive responses to other injuries leading to loss of functioning nephron play an important role in its development [2,4-6]. The significance of glomerular hilar (and parahilar) hyalinosis (HH) in children is uncertain. Several investigators have suggested that the lesion is an early form of FGS or even considered as one of the five variants of FGS as per the Columbia classification [7-9]. However Spear and Kibara [10] have reported HH in the kidneys of children without clinical evidence of renal disease. In view of the possibility that Hilar and perihilar hyalinosis is a precursor or a variant of FGS with its implications for treatment and prognosis, a retrospective study of HH in children with the nephrotic syndrome (NS) but without any other histologic evidence of FGS was performed to determine the clinic-pathologic significance of this lesion.

Patients and Methods

For the purposes of this study HH was diagnosed in children with the NS when a renal biopsy hyaline was present in the hila or parahilar segments of glomeruli and there was no other evidence of FGS such as segmental sclerosis or visceral epithelial changes characteristic of early FGS [11]. In a retrospective review of all pediatric renal biopsies with a diagnosis of minimal change disease, idiopathic mesangioproliferative glomerulonephritis, IgM nephropathy and FGS done over 16 years period, 8 patients were found to have HH on kidney biopsy and were labeled as either minimal change disease (n=7) or IgM nephropathy (n=1). These 8 patients were compared to a group of children with either minimal change disease or IgM nephropathy biopsied during the same period (n=84) but had no HH in the glomeruli. Clinical and laboratory information for each patient was obtained from hospital charts. Laboratory tests were performed by standard hospital laboratory methods. Nephrotic syndrome was defined as the presence of protienuria , hypoalbuminemia and generalized edema. Nephrotic range proteinuria is defined the spilling

of ≥ 40 mg/hour/m^2 of protein in the urine or a urinary protein (mg): creatinine (mg) ratio on an early morning spot urine of >0.2 in a child >2 years old and ≥0.5 in children <2 years old. Urinary 24-hour protein excretion (mg/M^2/day) was calculated by multiplying the Protein: creatinine ratio by 0.63. Remission was defined as the disappearance of proteinuria i.e. < 4 mg/hour/m^2 urinary protein or negative or trace protein on dipstick examination) on at least 3 consecutive examinations. Relapse is defined as the reappearance of proteinuria on at least 3 consecutive days. Early response was defined as remission within 4 weeks of initiation of treatment with corticosteroids, while late response is that which is achieved within 4-8 weeks of start of steroid treatment. Steroid resistance was defined as failure to achieve remission in spite of a complete course of full dose prednisone 60 mg/M^2/day for 8 weeks while Steroid dependence was defined as a state in which relapse frequently occurs while receiving or within 14 days of discontinuing steroids. Frequent relapses is defined as 2 or more relapses within 6 months or 4 or more relapses within 12 months of the initial response.

Kidney biopsy

Kidney tissue obtained by biopsy was divided into 3 portions for histology, direct immunofluorescence (IF) and electron microscopy and processed as previously described [12]. Intensity of IF was graded on a scale of 0 to 3+ where 0 = no fluorescence, 1 + was mild, 2+ moderate and 3+ marked. Mesangial expansion was semi-quantitatively evaluated by the method of Bank et al. [13] using either PAS-stained 2.0 micron paraffin-embedded sections or periodic acid-silver methenamine (PASM)-stained 1.0 micron methacrylate-embedded sections. All glomeruli in a section were individually graded on a scale of 0 to 3 according to the degree of mesangial increase. Grade 0 is no mesangial increase. Grade 1 applies to glomeruli showing a mild increase in mesangial matrix that appeared to be twice that of a normal glomerulus. The increase could be global or localized to one lobule. In grade 2 change the increase is confined to 2 lobules or is diffuse and the amount of mesangium appears to be triple that of a normal glomerulus. The increase could be global or localized to one lobule. Grade 3 change is reserved for glomeruli exhibiting increased mesangium at least 4 times normal in either 3 or more lobules or diffusely. For each biopsy the sum of grades was divided by the number of glomeruli evaluated. This value was multiplied by 100 to obtain the score. Each section was evaluated twice to check reproducibility. The degree of tubular atrophy was estimated semi-quantitatively on a scale of 0 to 3. For no tubular atrophy the score was 0. A score of 0.5 was assigned for tubular atrophy involving up to 5% of the tubules. Tubular atrophy of 6 to 25%, 26 to 50% and greater than 50% of tubules were given scores of 1, 2 and 3 respectively.

For the measurement of glomerular cross-sectional areas a method similar to that of Fogo et al. [14] was employed. Only those glomeruli that had been sectioned through the hilum or equator (for maximum circumference) were assessed. One micron thick PASM-stained methacrylate sections or 2.0 micron thick PAS-stained paraffin sections were used. The area limited by Bowman's capsule was measured with the aid of a digitizer and a morphometric computer program (Bioquant II, R & M Biometrics, Nashville, TN, USA).

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 22. Student's test, the chi-square test and the Mann-Whitney U test were utilized where appropriate. $P<0.05$ was considered significant for all tests.

Results

Clinical data

The important clinical and laboratory findings of both the HH and control groups at presentation are depicted in Table 1. All of the HH patients presented with the NS. Seven of the HH group were treated with prednisone and all showed an early response.One patient with tetralogy of Fallot who was not treated with steroids remitted spontaneously. All but two of the control group received prednisone with 91% showing an early response and 2% a late response. The mean number of relapses per 6 months per patient was 1.29 ± 0.87 and 2.57 ± 2.1 for the HH and control groups respectively in the interval between presentation and diagnostic biopsy, the difference being insignificant. The mean dose of prednisone at the time of relapse was similar in both groups (HH=0.46 ± 0.40mg/kg/day, control = 0.63 ± 0.43 mg/kg/day). At the time of biopsy the mean age of the HH group (10.4 ± 3.4 years) was greater than that of the control group (7.8 ± 4.6 years) but the difference was not significant.

Indication for biopsy in both groups was steroid dependent frequently relapsing NS in all patients except in 1(14%) of the hilar hyalinosis patients and in 6 (8%) of the control group who had initial steroid resistance. The difference in being insignificant.In one HH patient the indication for biopsy was the development of NS in the setting of tetralogy of Fallot (TOF).

Following the biopsy, all patients in both groups (except the TOF patient with HH) underwent one or more courses of cytotoxic therapy (cyclophosphamide, chlorambucil and Cyclosporine A).

Follow-up clinical data is presented in Table 2. Eighteen (21%) control patients were followed for less than 3 months after biopsy and were considered lost to follow-up. Most treated HH (83%) and control (65%) patients followed an infrequently relapsing post-biopsy course. For those patients who continued to relapse, the mean number of relapses per 6 months per patient and the mean dose of prednisone at relapse were very similar in both groups (Table 2). At last visit none of the patients in both groups were hypertensive or had renal insufficiency.

Table 1: Clinical and laboratory findings of presentation in hilar hyalinosis (HH) and control (C) patients

Parameter	HH	C
No. of patients	8	84
Male:Female	03:05	53:31
Mean age (year)	3.7± 2.3	5.3 ± 4.1*
% of patients with hematuria	13	26
Mean (± SD) serum Cr (µmol/l)	30 ± 11	43 ± 40*
Mean (± SD) 24 hour urinary protein (g/day)	4.1 ± 2.3	5.5 ± 5.4*

* Difference is not significant.

Biopsies

In 4 of the HH patients only cortex was available for study while in the other 4 biopsies both cortex and medulla were present. The mean number of glomeruli present in each biopsy was 25 with a range of 12-28 glomeruli. Apart from HH, glomeruli were either normal or showed only mild variable mesangial expansion. HH was observed in 2 to 7 glomeruli per biopsy (6-25% of all glomeruli). The lesion was characterized as one or 2 nodules of hyaline in the glomerular hilum (or in a parahilar segment). The nodules varied in size from small to moderately large. The nodules were initial in some glomeruli but more deeply located in others. Continuation with intimal arteriolar hyaline was observed in one glomerulus. Separate foci of arteriolar intimal hyalinosis were noted in 3 biopsies. The arteriolar hyaline was very focal and inconspicuous. No segmental sclerosis or glomerular epithelial cell changes were present. In 2 biopsies there were rare small areas (grade 0.5) of tubular atrophy and interstitial fibrosis. No other vascular lesions were seen.

The results of quantitative analysis of the biopsies of both HH patients and controls are summarized in Table 3.

Direct IF showed mesangial deposition of IgM in all HH biopsies. In all but one case the amount was very small (<1+). In one case diagnosed as IgM nephropathy the IgM deposition was graded as 1+ to 2+ with diffuse mesangial pattern. Mesangial complement C3 and/or C1q was detected in 5 of the 8 HH cases. Trace amounts of IgG and IgA were observed in one biopsy. Electron microscopy of one or 2 glomeruli was done in all 8 cases. This revealed variable effacement of visceral epithelial cell foot processes and no significant abnormalities of glomerular basement membrane structure or mesangium. No electron dense deposits was observed either within the mesangium or along glomerular basement membranes.

Table 2: Follow-up data for hilar hyalinosis (HH) and control (C) patients

Parameter	HH	C
Mean (±SD) interval between presentation and last visit (months)	109±68	66±50[a]
Mean (±SD) interval between presentation and biopsy (months)	79.8±61.2	24.4±39[b]
Number of patients in remission (%)[c]	6(75)	13(20)[d]
Number of patients with no proteinuria but on medication (%)c	2(25)	33(50)[e]

[a]P < 0.05
[b]P < 0.001
[c]18 C patients were lost to follow-up
[d]P < 0.05
[e]Difference is not significant.

Table 3: Results of quantitative analysis of the kidney biopsies of (HH) and control (C) patients

Parameter	HH	C
Mean (±SD) mesangial score	118.6±34.3	346.2±2[a]
Mean (±SD) glomerular cross-sectional area (M^2)	22717±4626	18774±7047[b]
Mean (±SD) tubulointerstitial damage	0.13±23	0.16±0.27[b]

[a]P < 0.001(Mann-Whitney U test)
[b]Difference is not significant

Discussion

HH has been considered by some to be an early or precursor lesion of FGS [7-9]. This view is based on several facts. First, the finding of hilar sclerosis (FGS) on repeat biopsy in children whose initial biopsies showed hyalinosis but not sclerosis in the para-hilar region [9]. Secondly, a particularly high frequency of this lesion in patients with reflux nephropathy and secondary FGS [15]. Thirdly, experimental studies in which hyalinosis is an early glomerular lesion in animals which develop FGS secondary to marked reduction of renal mass [5,6]. Thus the presence of HH is worrisome in nephrotic children with otherwise normal or minimally altered glomeruli since the prognosis for FGS is considerably poorer than that for minimal change disease [16-18]. Contrary to expectations the results of the present study indicate that HH as an isolated finding does not predict a clinical course similar to FGS. Indeed its association with a low relapse rate and long interval between presentation and biopsy suggest that is associated with relatively mild disease, good response to therapy and a non-progressive course. This is underscored by the failure to demonstrate in the biopsies with isolated HH significant mesangial expansion or glomerular hypertrophy both of which are associated with progressive disease and relatively poor outcome [14,19-21]. The pathogenesis of HH is unknown. This lesion is frequently seen in circumstances characterized by glomerular hyperfiltration [2,5,6] and it has been postulated that visceral epithelial cell damage is critical for its development [6]. However it is unknown whether hyperfiltration occurs in primary FGS [3] in which glomerular hyalinosis is common [2-4]. In the hilar hyalinosis patients there was no morphological evidence of hyperfiltration such as glomerular hypertrophy, glomerular capillary microaneurysms, microthrombi or prominent mesangial expansion [6].

Olson et al. [5] demonstrated that endothelial cell injury precedes the development of glomerular hyalinosis in the remnant kidney model suggesting that the endothelial cell damage may play an important role in the pathogenesis of hyalinosis. Although none of the present cases showed any histological evidence of endothelial injury, we cannot exclude it as a factor in the pathogenesis of HH since these cells were not examined critically at the ultra-structural level.

Pathologically the only significant difference between HH patients and the control group was the mean mesangial score which was higher in the controls. This was most likely due to the higher proportion in the control group of IgM nephropathy cases which usually has more mesangial expansion than minimal change disease.

Conclusion

The presence of isolated HH in otherwise histologically normal or minimally altered glomeruli does not appear to be a precursor for FGS or to have a deleterious effect on the course of the renal disease in children with either minimal change disease or IgM nephropathy.

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