50 Years of Nephrotic Syndrome in Children, and Hereafter

RN SRIVASTAVA
Consultant Pediatric Nephrologist, Apollo Indraprastha Hospitals, New Delhi 110076, India. drrnsri@gmail.com

Nephrotic syndrome in children has been well recognized and extensively reported for several decades. Earlier descriptions of this disorder mentioned the clinical picture of the massively bloated, young child with muscle wasting and passage of scanty urine. Heavy proteinuria, decreased levels of serum proteins and hyperlipidemia were observed. An underlying etiologic condition was absent in most cases. Few effective therapeutic measures were available. Most patients died of bacterial infections such as peritonitis, pneumonia and sepsis, although in some cases spontaneous recovery was seen [1].

Early efforts towards management were focused on control of edema. Various drugs were employed including xanthine derivatives and mercurial compounds (these were effective but nephrotoxic and could cause heavy proteinuria). Chlorothiazide and aldosterone antagonists were of little benefit in patients with massive edema. Infusion of salt poor human albumin and induction of measles were attempted [1]. Frusemide and other top diuretics have been used since early 1960s, which lead to better control of edema.

The advent of antibiotics and their extensive use impacted the mortality from infections and the mortality rates declined from 40% in the pre-antibiotic era to 16%. In early 1950s, ACTH was employed in the management and its efficacy observed in causing loss of edema, diminution of proteinuria and increase in serum proteins [2]. With availability of cortisone, ACTH therapy (requiring IM injections), was discontinued. Cortisone and later prednisolone were widely used resulting in complete remission of disorder in most cases, and the mortality rate further fell down to 3-7% [3]. The procedure of percutaneous renal biopsy was applied in 1962 in children with nephrotic syndrome and other forms of glomerular diseases [4].

The association of nephrotic syndrome with *Plasmodium malariae* disease in some African countries had been earlier recognized. Congenital/infantile nephrotic syndrome, collagen vascular diseases and other glomerulopathies, and more recently hepatitis B and HIV infection, were also observed to cause heavy proteinuria in some cases, and thus *secondary* nephrotic syndrome, which was differentiated from the *idiopathic* form that constituted about 90% of all cases of nephrotic syndrome [5].

INTERNATIONAL STUDY GROUP

Major contributions towards the understanding of nephrotic syndrome in children were made by a multinational investigative group called "International Study for Kidney Disease in Children" (ISKDC), established in 1965. The participating countries included USA, Canada, Mexico, UK, Europe, Japan and Hong Kong. The Group defined heavy proteinuria (>40 mg/m^2 /hour), dose regimen of prednisolone (60 mg/m^2/day for 4 weeks followed by 40 mg/m^2 on 3 days a week for next 4 weeks), response to such therapy and criteria for infrequent and frequent relapses, steroid dependence and steroid resistance. Clinicopathologic studies were carried out in children with the initial episode of nephrotic syndrome who had not received corticosteroids. Renal biopsies were examined by three experts without having knowledge of the clinical and laboratory details. Light microscopy, immunofluorescence evaluation and electronmicroscopy were performed. 521 patients aged between 16 weeks-16 years from 24 clinics were investigated during 1967-74 [6]. Controlled trials on the use of azathioprine and cyclophosphamide were conducted. The presence of underlying minimal change (minimal change nephrotic syndrome: MCNS) in the majority of patients (76.6%) and their satisfactory response to prednisolone were observed. The morphology of "non-minimal" glomerular lesions, chiefly focal segmental glomerulosclerosis (6.9%), mesangial proliferative and membranoproliferative, were identified. Patients with such abnormalities had a poor response to prednisolone [7]. A number of reports from different countries [8,9], including one from Delhi [10], confirmed a similar pattern of nephrotic syndrome in children. The ISKDC prednisolone regimen (although not evidence based) was widely employed so that the observations in different studies could be compared.

MANAGEMENT

Corticosteroid responsive patients

Corticosteroids have remained in use for the initial attack of nephrotic syndrome as well as subsequent relapses. The ISKDC regimen for the initial episode was modified in 1980s to "6 weeks daily + 6 weeks alternate day", which was found superior to the former and employed at most centres. For patients having frequent relapses, small doses of prednisolone were used on alternate days for prolonged periods provided remissions were maintained. However, a large proportion of children required high doses and about 20% were prednisolone dependent. Cyclophosphamide has been employed in such cases since late 1960s, with excellent results [11]. With the currently used 12-week regimen the side effects are uncommon and the gonadal toxicity not a serious concern. Levamisole, an immunomodulatory agent, has been used since 1980s and is beneficial in 50-60% of patients with milder forms of MCNS and has a steroid sparing effect [12]. Investigations are being carried out to determine its optimal regimen. Cyclosporine, a calcineurin inhibitor, initially used in renal transplantation, has been employed since 1985 to treat patients who fail to benefit from cyclophosphamide and found to be very effective in maintaining remissions for prolonged periods [13]. More recently tacrolimus, also a calcineurin inhibitor, has been used with similar results [14], but often preferred over cyclosporine because of the latter's cosmetic side effects. Judicious administration using small doses minimizes their nephrotoxicity. Since early 2000s, there were several reports of using mycophenolate mofetil in steroid dependent patients with impressive benefit, which has been confirmed in two controlled trials [15,16].The present recommendation is to use mycophenolate before exposing the child to a calcineurin inhibitor. A novel approach has been the introduction of Rituximab in 2007. It is a CD20 monoclonal antibody that causes B cell depletion. Rituximab has been used in steroid dependent nephrotic syndrome, mostly as "rescue therapy" when other medications have failed. The short term results have been very impressive [17]. Besides the use of specific agents, supportive care, avoidance of iatrogenic side effects and prompt management of complications have been emphasized [18].

Corticosteroid resistant patients

The small proportion of children with idiopathic nephrotic syndrome who fail to respond to corticosteroids (about 10%, called "corticosteroid resistant") present very difficult therapeutic problems. A majority of such patients show underlying "non-minimal" glomerular lesions, but about 20% have minimal change. Cyclophosphamide, calcineurin inhibitors, mycophenolate and, more recently, retuximab have been employed in an effort to abolish or reduce proteinuria. Such therapies have met with variable, but mostly discouraging response [19]. Genetic abnormalities have been detected in a small proportion of such cases, particularly those with "familial" FSGS. These patients do not respond to immunosuppressive agents and usually have a rapidly worsening course. The underlying abnormality; however, does not recur in the renal allograft, which frequently occurs in non-genetic FSGS. Unnecessary exposure to toxic medications is avoided and the patients managed with non-specific agents such as ACE inhibitors and angiotensin receptor blockers to reduce proteinuria [19].

Long term care and outcome

With rationalization of management, most children with MCNS can be kept in remission and normal growth and development, schooling and social adjustments ensured. However, the unpredictable, long term course of the disorder and the variable response to specific drugs in an individual patient make the management challenging, and often frustrating for the family. Although complete recovery eventually occurs, the question most often asked *"when will the child be cured?"* cannot be answered. The small proportion of patients with steroid resistant disorder forms a heterogeneous group with an overall unsatisfactory prognosis [19].

MECHANISMS OF PROTEINURIA

The morphological and molecular structure of glomerular capillary wall were defined in early 1970s. The thin fenestrated endothelial layer, basement membrane and the epithelial cell layer made up of interdigitating foot processes that abut the basement membrane and extend from the podocytes, which are interposed between slit diaphragms of 200-300 A in width and are separated form the GBM by slit diaphragms. Slit pore diaphragm was identified as a major barrier to filtration. The passage of protein molecules across glomerular capillary wall depends upon its size, stereotaxic arrangement and its electrical charge. Elegant experiments using dextran tracers of varying charges demonstrated increased clearance of anionic dextran and decreased clearance of cationic dextran. Several negatively charged molecules were found in the capillary GBM and epithelial cell surface [20].

Recent investigations have emphasized the pivotal role of podocytes and their foot processes in controlling the passage of proteins through the capillary wall. Several proteins have been identified that encode structural elements of the slit diaphragm and the podocyte

cytoskeleton, and are responsible for podocyte development and function. Mutations in these genes have been found in familial FSGS and congenital nephrotic syndrome. The extensive effacement of foot processes, characteristically observed in MCNS, is regarded as suggestive of a primary injury to podocyte [21,22].

Proteinuria in nephrotic syndrome

Varying degrees of proteinuria in "non minimal" lesion nephrotic syndrome, and in various other forms of glomerulopathies can be explained by the underlying glomerular capillary damage. In these conditions, urinary proteins consist of albumin as well as globulins, indicating a more profound injury to capillary filter. In MCNS urinary protein mostly consisted of albumin, which observation (termed *proteinuria selectivity*) was earlier suggested to differentiate MCNS from others from those with significant lesions.

Mechansims of heavy proteinuria in MCNS

The mechanism of heavy loss of protein in MCNS remains obscure. Absence of glomerular inflammation and any evidence of immunological involvement in MCNS were confirmed early. Loss of anionic charge from the glomerular capiilary wall was suggested as a possible contributor factor [20]. In experimental animals with a puromycin aminonucleoside-induced heavy proteinuria that closely resembles human MCNS, toxic oxygen radicals were considered to have a role in causing injury to glomerular filter [22]. The clinical significance of these findings in the pathogenesis of MCNS is not clear.

T- cell dysfunction and circulatory factors, other immune abnormalities, podocyte injury

In 1974, Shalhoub hypothesized that in MCNS there may be an underlying abnormality of T lymphocytes resulting in a "circulating chemical mediator" that had a deleterious effect on glomerular capillaries leading to heavy proteinuria. During 1970-1980, a large number of studies were carried out reporting a variety of abnormalities (such as presence of a circulating lymphocytotoxin/ vascular permeability factor, impaired lymphocyte response, T cell subset abnormalities) [20]. The significance of these observations remains unclear. The efficacy of rituximab suggests a role for B cells in the pathogenesis [21].

Search for a putative proteinuria-inducing circulatory factor has recently been intensified and cytokine as well as non-cytokine substances have been postulated to play a role. Altered signal transduction in T cells and deficiency of T cell regulatory function, and a likely involvement of B cells have also been proposed [21]. All these abnormalities are considered to affect podocyte function. It has been suggested that various glomerular diseases with varying degrees of protienuria may be regarded as *podocytopathies* and podocyte injury presumably responsible for the development as well as progression of the underlying disorder [23].

Drug therapy and mechanisms

A very limited number of drugs are available for treatment of MCNS. For about 60 years, prednisolone has remained the initial agent to induce remission as well as manage infrequent relapses. The ideal prednisolone regimen is still being investigated. Cyclophosphamide has been used for more than 40 years for frequently relapsing and steroid dependent cases. Levamisole, mycophenolate , cyclopsporine, tacrolimus and rituximab complete the therapeutic armamentarium. These various agents act through different mechanisms, although it is now hypothesized that they may target the podocyte directly. Calcineurin inhibitors, known to act via inhibition of nuclear factor-activated T cell signaling, may also stabilize actin cytoskeleton in podocyte and preserve its architecture. ACTH and corticosteroids, hitherto believed to act through immunosuppressive properties, are now considered to directly affect podocyte structure and function. Angiotensin converting enzyme inhibitors and angiotensin receptor blockers, which attenuate proteinuria by causing a reduction in intraglomerular vascular resistance and thus decreasing filtration of proteins, may also have a direct beneficial effect on the podocytes [24]. Studies over the past decade have focused on the cytoskeleton of the podocyte, the foot processes and the slit diaphragm, various signaling mechanisms, and mediators of apoptosis and fibrosis, and identification of the circulatory factor/s, which should eventually lead to defining the etiology of MCNS [24].

PAST AND FUTURE

Although the pathophysiology of idiopathic nephrotic syndrome and the mechanisms of proteinuria have become more clear, very little progress has been made towards defining the etiology of MCNS, and what leads to its eventual cure. A myriad of abnormalities have been observed in MCNS but their role in the pathogenesis is undefined. The initiating mechanisms in MCNS remain enigmatic. Decades old drugs still form the sheet anchor of management. Most investigative efforts over the past 50 years have been towards finding effective treatment regimens (interestingly, the newer agents found beneficial in nephrotic syndrome were initially used in renal transplantation), while the pathogenesis of MCNS remained mostly unaddressed. Genetic abnormalities detected in familial cases and FSGS appear to have little

relevance in MCNS, which has a remarkably similar pattern in various parts of the world. Intensive efforts are under way to examine more efficacious regimens of the currently employed medications. However, only once the initiating mechanisms are identified, specific molecules could be developed to counteract them, used initially and perhaps lead to permanent cure [25].

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