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A Study of Urinary Protein Pattern Using Polyacrylamide Gel Disc Electrophoresis in Nephrotic Syndrome

Neeta Biyani*, Nandkumar Dravid**

*Assistant Professor **Professor and Head, Department of Pathology; ACPM Medical College, Dhule.

Abstract

Introduction: The Nephrotic syndrome aptly termed "the syndrome of Proteinuria" by the lord platt has stimulated provoked and puzzled doctors for years or more. The characteristic toad face, be if "Caucasian Negriod" or " Mongoloid" is so pathognomic that together with bloated belly, legs and genitalia it has been recognized as a spectacular and specific since the time interest in thedisease of children began. *Aims and Objectives*:To Study Urinary Protein Pattern Using Polyacrylamide Gel Disc Electrophoresis in Nephrotic Syndrome. *Meterials and Methods*: This is a retrospective study. The study was carried out in department of ACPM Medical College, Dhule. Diagnosed cases of Nephrotic syndrome of different age and sex admitted to hospitals during one year were included in study. Clinical diagnosis of Nephrotic syndrome was recommended by international study of kidney disease for diagnosis used were as follows (more than 3 gm in 24 hrs/1.73 m^2/more than 40 mg/hr/m^2) (serum cholesterol more than 200 mg/dl) (hypoalbuminaemia-serum albumin less than 2.5 g/dl. *Result:* Maximum number of patient 54(64.28%) were-observed in 0-10 years age group. Of this 54 patients, 28(51.85%) were male and 26 (48.14%) were female. So male female ratio is 1.07:1 The prevalence of nephrotic syndrome decreases as age advance. Male female ratio is 1.5:1. Oedema was the most common presenting symptom and it was observed in all patients (100%). The next common presenting symptom was fever which was observed in 47(55.95%) patients. Associated diseases were found in 23 cases of nephrotic syndrome the most common disorder associated with nephrotic syndrome was glomerulonephritis which was observed in 15(65.22%) patients. The next common associated systemic disorder was tuberculosis which was observed in 5 cases (21.74%) Commonest pattern observed was presence of albumin and transferrin (II) among 36 patient (42.85%) followed by presence of albumin, transferrin, post albumin and post transferrin (IV) in 15 patients (17.85%). Albumin, transferrin and post albumin (III) was observed in 9 patients (10.71%) and only albumin (I) in seven patient (8.33%). Whereas presence of all proteins (VI) was noted in 3 patients (3.57%) and presence of albumin, transferrin, post albumin and post transferrin and 7S (V) in 14 patients (16.66%). Pattern 'I' protein in range of 2 -2.9 g/24hours, in thepattern II protein in range of 2-2 g/24 hours . Type 'III' protein levels of 2-2.9gm/24 hours- pattern 'IV' -3-3.9gm/24 hours. Pattern 'V'- 3-3.9 gm/24 hours . Pattern VI- in 3-3.9. *Conclusion*: Polyacrylamide Gel Disc Electrophoresis in Nephrotic Syndrome is useful of quantification and identification of the nephrotic syndrome.

Keywords: Nephrotic Syndrome; Polyacrylamide Gel Disc Electrophoresis.

Introduction

The Nephrotic syndrome aptly termed "the syndrome of Proteinuria" by the lord platt has stimulated provoked and puzzled doctors for years or more. The characteristic toad face, be if "Caucasian Negriod"or " Mongoloid" of is so pathognomic that together with bloated belly, legs and genitalia it has been recognized as a spectacular and specific since the time interest in the disease of children began.Prior to 1950 the therapeutic attempts other than antimicrobials were horrific example hot cages, purges

Corresponding Author: Neeta Sandeep Biyani, 1582/1, Opp Sonya Maruti Mandir, Lane no -5 Dhule- 424001 Maharashtra.
E-mail: biyani.dhule@gmail.com

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subcutaneous drainage and tubes, mercurial diuretics, repeated doses of TAB iatrogenic infection with measles and malaria were used. Treatment with IV gum acacia and thyroxin being used without reasonable indications, (Arneil G C et al, 1982).The patient and incidence of nephrotic syndrome in India is similar observation in western countries (Srivastava R.N. et al 1975).The present study was undertaken with a view to correlate various clinical,biochemical and immunogical parameters of nephrotic syndrome cases to assess the stage of disease in order to help the clinician for the better management.

Aims and Objectives

Retrospective Study Urinary Protein Pattern Using Polyacrylamide Gel Disc Electrophoresis in Nephrotic Syndrome.

Meterials and Methods

The retrospective study was carried out in department of Pathology ACPM Medical college, Dhule.Diagnosed cases of nephrotic syndrome of different age and sex admitted to the hospital during one year were included in study. Clinical diagnosis of Nephrotic syndrome was recommended by international study of kidney disease for diagnosis used were as follows(more than 3 gm in 24 hrs/1.73 m^2/more than 40 mg/hr/m^2) (serum cholesterol more than 200 mg/dl) (hypoalbuminaemia-serum albumin less than 2.5 g/dl)

Result

Age and sex wise distribution of 84 cases of nephrotic syndrome is tabulated in above table. Maximum number of patient 54(64.28%) were-observed in 0-10 years age group. Of this 54 patients, 28(51.85%) were male and 26 (48.14%) were female. So male female ratio is 1.07:1

The prevalence of nephrotic syndrome decreases as age advance. This is evident from above table number 1.13(15.4%) patients were seen in 11-20 years age group of which, 7 patient were made and 6 patients were female. So male female ratio is 1.16:1

In age group of 21-30 years, 10(11.90%) patients were observed, of which 6 patients were male 4 patients were female. So male female ratio is 1.5:1 minimum number of patients i.e. only 7(8.33%) were noted in age group 31-60 years with same male female ratio.

Out of overall 84 patients, 45 patient were male and 39 patients were female. So male female ratio is 1.15:1.

Oedema was the most common presenting symptom and it was observed in all patients (100%). The next common presenting symptom was fever which was obserbed in 47(55.95%) patients . In decreasing order the presenting symptom were oliguria, distention of abdomen and ascites,

hypertension, upper respiratory tract infection, diarrhea and vomiting.Oliguria and distention of abdomen and ascites was observed in 22 cases (26.19%), 19 patients (22.61%) had hypertension while 11(13.093%) patients had upper respiratory tract infection and diarrhea and vomiting.

Associated diseases were found in 23 cases of nephrotic syndrome. The most common disorder associated with nephrotic syndrome was glomerulonephritis which was observed in 15(65.22%) patients. The next common associated systemic disorder was tuberculosis which was observed in 5 cases (21.74%) other rarely observed associated diseases in cases of nephrotic syndrome were collagen disorder, renal vein thrombosis and leukemia seen in one case each (1.19%).

Table 1: Distribution of Nephrotic syndrome according to age and sex

Age (in yrs)	Male Patients	Female Patients	Total	M/F Ratio
0-10 yrs	28(48.14%)	26(48.14%)	54(64.28%)	1.07:1
11-20 yrs	7(53.84%)	6(46.15%)	13(15.47%)	1.16:1
21-30 yrs	6(60.00%)	4(40.00%)	10(11.90%)	1.5:1
31-60 yrs	4(57.14%)	3(42.85%)	7(8.33%)	1.3:1
total	45(53.57%)	39(46.42%)	84(100%)	1.15:1

Table 2: Distribution of principle presenting clinical features in cases of nephrotic syndrome

Clinical features	No. of cases	Percentage
Oedema	84	100%
Fever	47	55.95%
Oliguria	22	26.19%
Distention of abdomen, ascites	22	26.19%
Hypertension	19	22.61%
Upper respiratory tract infection	11	13.09%
Diarrhea and vomiting	11	13.09%

Table 3: Distribution of associated diseases in cases of nephrotic syndrome

Associated diseases	No. of cases	Percentage found in 84 cases
Glomerulonephritis	15	17.85%
Tuberculosis	5	5.95%
Collagen disorder	1	1.19%
Renal vein thrombosis	1	1.19%
Leukemia	1	1.19%
total	23	

Table 4: Distribution of total 24 hours urine protein

Total urine protein g/24 hrs	No. of cases (%)		Total
	Upto 15 yrs	Above 15 yrs	
2-2.9	24(40.67%)	9(36.00%)	33(39.28%)
3-3.9	25(42.37%)	8(32.00%)	33.(39.28%)
4-6	10(16.94%)	7(28.00%)	17(20.23%)
>6	----	1(4.00%)	1(1.19%)
Total	59(70.23%)	25(29.76%)	84

Table 5: Patterns of electrophoresis of urine on polyacrylamide gel disc electrophoresis in patients of nephrotic syndrome.

Group	PAGDE pattern	No. of cases	Percentage
I	Presence of only albumin	7	8.33%
II	Presence of albumin and transferrin	36	42.85%
III	Presence of albumin transferrin and post albumin	9	10.71%
IV	Presence of albumin, transferrin, post albumin, and post transferrin	15	17.85%
V	Presence of albumin, transferrin post albumin, post transferrin and 7S	14	16.66%
VI	Presence of all proteins	3	3.57%
Total		84	100%

Table 6: Correlation between urine polyacrylamide gel disc elcetrophoresis pattern and blochemical urinary protein values

Urinary PAGDE pattern	No. of cases	Biochemical urinary protein in g/24 hrs (%)			
		2-2.29	3-3.9	4-6	6
I	7	7(100%)	--------	------	----
II	36	20(55.55%)	15(14.66%)	1(2.77%)	-----
III	9	1(11.11%)	2(22.22%)	5(55.55%)	(11.11%)
IV	15	------	8(53.33%)	7(46.66%)	-----
V	14	------	4(28.57%)	10(71.42%)	------
VI	3	------	1(33.33%)	1(33.33%)	(33.33%)
Total	84	28(33.33%)	30(35.71%)	24(28.57%)	2(2.38%)

Pattern of polyacrylamide gel disc electrophoresis in urine proteins also could be grouped in 6 groups according to the pattern showing presence of one of more number or proteins fraction. Commonest pattern observed was presence of albumin and transferrin (II) among 36 patient (42.85%) followed by presence of albumin, transferring, post albumin and post transferrin (IV) in 15 patients (17.85%).Albumin, transferrin and post albumin (III) was observed in 9 patients (10.71%) and only albumin (I) in seven patient (8.33%). Whereas presence of all proteins (VI) was noted in 3 patients (3.57%) and presence of albumin, transferrin, post albumin and post transferrin and 7S (V) in 14 patients (16.66%).

The above Table 6 shows the correlation of urine polyacrylamide gel electrophoresis pattern with biochemical urine protein values in nephritic syndrome cases. The correlation was found between urinary polyacrylamide gel electrophoresis pattern and urinary protein level.

Pattern 'I' (presence of only albumin was seen in 7 cases and responded 100% with the presence of urinary protein in range of 2 -2.9 g/24 hrs in 7 cases.The urinary polyacrylamide gel disc electrophoresis pattern 'II' of albumin and transferrin) was seen in 36 cases with presence of urinary protein in the range of 2-2 g/24 hours in 20 cases and 3-3.9g/ 24hours in 15 cases and 4-6 gm/24 hours in single

case. The polyacrylamide gel disc. Electrophoresis type 'III' (presence of albumin. Transferrin and post albumin) was seen in patient with urine protein levels of 2-2.9gm/24 hours in single cases , 3-3.9gm/24hrs in 2 cases and 4-6 gm/24 hrs in 5 cases and single showed 7gm/24hrs. 15 cases showed polyacrylamide gel disc electrophoresis, pattern 'IV' (albumin, transferrin, post albumin, post transferrin)was associated with high urinary protein level of 3-3.9 gm/ 24 hours in 8 cases and 4-6 gm/24 hrs in 7 cases. In 14 cases polyacrylamide gel disc electrophoresis, pattern 'V' (presence of albumin, transferrin, post albumin, post transferring and 7s) was associated with the urinary protein level of be 3-3.9 gm/24 hours in 4 cases and 4-6 gm/24 hours in 10 cases. Pattern VI of urine showed presence of high urinary protein level of in 3-3.9, 4-6 and more than 6 gm/24 hours in one patient each only.

Discussion

Nephrotic syndrome is a common chronic disorder, characterized by alterations of permselectivity at the glomerular capillary wall, resulting in its inability to restrict the urinary loss of protein. Nephrotic range proteinuria is defined as proteinuria exceeding 1000 mg/m² per day or spot (random) urinary protein-tocreatinine ratio exceeding 2 mg/mg. The proteinuria in childhood nephrotic syndrome is relatively selective, constituted primarily by albumin. Estimates on the annual incidence of nephrotic syndrome range from [4-9] per 100,000 children, and prevalence from 12-16 per 100,0001. There is epidemiological evidence of a higher incidence of nephrotic syndrome in children from south Asia [4]. The condition is primary (idiopathic) in 95 per cent cases. An underlying disorder that might be identified in less than 5 per cent cases, includes systemic lupus erythematosus, Henoch Schonleinpurpura, amyloidosis and infection with HIV, parvovirus B19 and hepatitis B and C viruses [1,3,4]. More than 80 per cent patients with nephrotic syndrome show minimal change disease (MCD) characterized by normal renal histology on light microscopy. The remaining is contributed by focal segmental glomerulosclerosis (FSGS) and mesangioproliferative glomerulonephritis (MesPGN). MCD and FSGS are often considered to represent the same pathophysiological process. Membranoproliferative glomerulonephritis and membranous nephropathy are uncommon conditions in childhood [7-9].

The glomerular capillary wall consists of three structural elements that constitute the permselectivity barrier: endothelial cells separated by fenestrae, the glomerular basement membrane made up of a network of matrix proteins, and specialised epithelial cells (podocytes) connected to each other via an interdigitating network of slit diaphragms. Normally, proteins the size of albumin (69 kd) and larger are excluded from filtration, a restriction that depends substantially on the integrity of the slit diaphragms. In nephrotic syndrome, glomeruli appear greatly changed— adjacent podocytes appear fused together, assuming a flattened rather than foot-like morphology. Three observations provide important clues to the primary pathophysiology of idiopathic nephrotic syndrome. Mutations in several podocyte proteins have been identified in families with inherited nephrotic syndrome, highlighting the central importance of the podocyte. A plasma factor may alter glomerular permeability, especially among patients with steroid-resistant nephrotic syndrome. Altered T-lymphocyte responses seem to be important; a primary T-cell event could result in the production of a permeability factor that interferes with the expression, function, or both, of key podocyte proteins to cause proteinuria. The podocyte target of such a putative factor is, however, unclear. A higher rate of certain gene polymorphisms among nephrotic patients than among controls suggests the existence of disease susceptibility genes. Risk of progressive FSGS may also be determined by genotype. Nephrin was the first slit-diaphragm protein identified [10-20]. Mutations in this transmembrane protein cause congenital (Finnish-type) nephrotic syndrome that occurs with a frequency of one per 8200 livebirths in Finland. Among children with inherited nephrotic syndrome, investigators have identified mutations in other genes that encode podocyteproteins. Many laboratories are actively investigating how disruptions in the podocyte network (from the slit diaphragm to its contractile cytoskeleton) cause pathological proteinuria. The role of podocyte proteins in the pathogenesis of sporadic cases of so-called idiopathic nephrotic syndrome requires further investigation. Genetic mutations have been identified in some children with sporadic steroid-resistant nephrotic syndrome. In particular, mutations in the gene encoding podocin have been identified in patients with sporadic FSGS [21-23]; mutations in WT-1 have been reported in children with isolated diffuse mesangial sclerosis [24,25]. Steroid-responsive nephroticsyndrome is occasionally seen in more than one family member. At least one locus has been mapped to chromosome 1q25, close to but distinct from the podocin gene [26].

In our study we have studies that Maximum number of patient 54(64.28%) were-observed in 0-10 years age group. Of this 54 patients, 28(51.85%) were male and

26 (48.14%) were female. So male female ratio is 1.07:1 The prevalence of nephrotic syndrome decreases as age advance. Male female ratio is 1.5:1. Oedema was the most common presenting symptom and it was observed in all patients (100%). The next common presenting symptom was fever which was obserbed in 47(55.95%) patients. Associated diseases were found in 23 cases of nephrotic syndrome he most common disorder associated with nephrotic syndrome was glomerulonephritis which was observed in 15(65.22%) patients. The next common associated systemic disorder was tuberculosis which was observed in 5 cases (21.74%) Commonest pattern observed was presence of albumin and transferrin (II) among 36 patient (42.85%) followed by presence of albumin, transferrin, post albumin and post transferrin (IV) in 15 patients (17.85%). Albumin, transferrin and post albumin (III) was observed in 9 patients (10.71%) and only albumin (I) in seven patient (8.33%). Whereas presence of all proteins (VI) was noted in 3 patients (3.57%) and presence of albumin, transferrin, post albumin and post transferrin and 7S (V) in 14 patients (16.66%).

Conclusion

Polyacrylamide Gel Disc Electrophoresis in Nephrotic Syndrome is useful of quantification and identification of the nephrotic syndrome.

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