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Diagnostic Value of Urinary CD80 in Typical and Atypical Nephrotic Syndrome

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Abstract

Background: *Cluster of differentiation 80 is one of the important biomarker which is present on podocytes. Urinary CD80 concentration increases only in patients with typical nephrotic syndrome in active state and level remains normal in patients with atypical nephrotic syndrome.*

Objective: *To evaluate urinary CD80 concentration as a diagnostic marker of typical and atypical nephrotic syndrome.*

Methods: *This was a cross sectional study, performed in the Department of Paediatrics & Paediatric Nephrology, Chittagong Medical College Hospital, Chattogram. Thirty nine with typical and 36 with atypical nephrotic syndrome were enrolled from January 2020 to July 2021.Urinary CD80 concentration was measured and compared between typical and atypical nephrotic syndrome.*

Results: *Mean urinary CD80 concentration in typical nephrotic syndrome (671.92 ±328.45) was found significantly higher (p<0.001) than atypical nephrotic syndrome patients (122.30 ±158.47). The area under the curve for typical nephrotic syndrome was 0.946 for urinary CD80 concentration (95% confidence interval: 0.899–0.992) (p<0.001). Urinary CD80 concentration 114.9 ng/g of creatinine was found most appropriate cut-off value with the sensitivity 92.3% and the specificity 27.8% for the differentiation of typical (active state) and atypical nephrotic syndrome.*

Conclusion: *Urinary CD80 level found significantly higher in patients with typical nephrotic syndrome than atypical nephrotic syndrome. Along with the other diagnostic criteria, urinary CD80 may be used as a non-invasive biomarker to differentiate typical and atypical nephrotic syndrome.*

Keywords: *Typical Nephrotic Syndrome, Atypical Nephrotic Syndrome, Urinary CD80.*

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Introduction:

Nephrotic Syndrome (NS) is typically presented with nephrotic range proteinuria, hypoalbuminaemia, oedema and hyperlipidaemia.[1] Atypical nephrotic syndrome associated with any one of gross hematuria, hypertension, sustained serum creatinine elevation, low serum C3 Level and low C4 level or in combination.[2,3] These clinical changes are correlated with specific structural changes in the foot processes of glomerular visceral epithelial cells, podocytes which form the glomerular filtration barrier.[4] The incidence of Nephrotic Syndrome is ranging between 2 to 7 newly diagnosed cases per 100000 children per year and the prevalence is about 16 cases per 100000 children per year.[5] Minimal Change Disease (MCD) is the most common cause of idiopathic childhood-onset nephrotic syndrome which accounts for about 85% of cases followed by Focal Segmental Glomerulosclerosis (FSGS) in almost 10% of cases.[6]

Cluster of differentiation 80 (CD80) is a transmembrane protein found on the surface of activated B lymphocytes and monocytes that helps T-cell activation.[7] It works as a co-stimulatory signal and activates primitive T cells by binding to the receptor CD28 which is a pre-requisite in the activation of adaptive immune responses.[8] Abnormal T-cell functions cause the release of cytokines and lymphokines lead to altered glomerular basement permeability.[9] Over-expression of CD80 in podocytes leads to slit diaphragm protein rearrangement in vitro and proteinuria in animal model.[10]

Renal biopsy is the gold standard for diagnosis of the underlying pathology of nephrotic syndrome especially in atypical types. But it is an invasive procedure, not available in every settings, need expert hand and having risk of many complications. Sometimes biopsy is needed in later part of the disease which make the diagnosis delay and delay the therapeutic decision.[5]

Heterogeneity of nephrotic syndrome and a lack of validated biomarkers limits intervention and reduces the ability to examine outcome.[11] Many urinary and serum markers like urine interleukin-13, CD80, CD28, matrix metalloproteinase-2 and granzyme-B are already used in some study to evaluate the activity and pathogenesis of nephrotic syndrome as an alternative way of renal biopsy.[12] Among them, urinary CD80 is one of the important biomarker. Measurement of urinary CD80 is a non-invasive procedure; sample collection and preservation are easier; no related complication occurs.[12]

Urinary CD80 excretion increases in MCD than in those in Focal Segmental Glomerulo Sclerosis (FSGS), MCD in remission and other glomerulopathies.[13] Liao et al.[14] found the urinary CD80 concentration change as reliable predictor of SSNS recurrence.

Other studies reported that elevation of urinary CD80 only in patients with MCD in active stage of disease with level remains normal in control, patients with MCD in remission, FSGS or other renal diseases.[15,16] The aim of this study was to observe the possibility of using urinary CD80 as a biomarker to diagnose and differentiate typical and atypical nephrotic syndrome which may help to early predict in prognosis, take proper decision and avoid unnecessary treatment burden.

Materials and Methods:

This was a hospital based cross sectional study and was carried out in The Department of Paediatrics and Paediatric Nephrology of Chittagong Medical College Hospital, Chattogram, Bangladesh during the period of January 2020 to June 2021. Thirty-nine cases of typical and thirty-six cases of atypical presentation of Nephrotic Syndrome were included by consecutive sampling technique having age between 1-12 years with initial episode of nephrotic syndrome in active state, typical nephrotic syndrome in relapse state and atypical nephrotic syndrome in relapse or remission state. Secondary causes like SLE nephritis, HSP nephritis, other vasculitis, drug induced, infection associated and those who refused to give consent were excluded from the study. In this study, nephrotic syndrome patients, age 1-12 years with normal blood pressure, normal complement level and normal renal function was considered as typical nephrotic syndrome and nephrotic patients age of onset more than 10 years with gross hematuria, hypertension, sustained serum creatinine elevation, low complement (C3,C4) level was considered as atypical nephrotic syndrome.[1]

Patients with initial episode were considered to have active disease if they had edema, a serum albumin level < 3.0 g/dl and a urinary protein/creatinine(mg/mg) ratio of > 2.0.[17]

Patients were considered to be in relapse if they had oedema and their uPCR ≥200 mg/mmol or >2(mg/mg) or ≥3+ protein on urine dipstick for 3 consecutive days and to be in remission if they had no oedema

and their uPCR <20 mg/mmol or <0.2(mg/mg) or <1+ protein on urine dipstick for 3 consecutive days.[4,18] Hematuria was considered in presence of both microscopic and macroscopic hematuria.[19] Hypertension was considered as systolic blood pressure and/or diastolic blood pressure >95thcentrile for age, gender and height.[20] Poor renal function was determined on the basis of more than upper limit of age matched serum creatinine level.[21]

After taking informed written consent from the parents, a detailed history was obtained and a complete clinical examination was performed. Then relevant investigations were done and recorded. Patients were grouped into typical and atypical nephrotic syndrome. About 10 ml of freshly voided morning urine sample was collected into a sterile tube from all patients. After centrifugation at 2000-3000 rpm for 20 minutes at 4^{o}C and supernatants were stored at -20^{o}C until further analysis. All urine samples were stored for maximum of 12 months and all samples were brought to room temperature before analysis. Urinary creatinine was measured by Beckman Coulter, U.S.A, Model: Beckman Coulter AV-480 works in Enzymatic principle. Urinary CD80 concentrations were measured afterward by Convergent Technologies, Germany, Model: Convergys EL-Reader 96X. Human T lymphocyte activation antigen CD80 (Human sCD80 Instant ELISA kit),(Catalogue number: BMS291INST, LOT no: 231022-00S) by Thermo-Fisher Scientific, U.S.A, was used to measure urinary CD80 level using an Enzyme Linked Immunosorbent Assay (ELISA) method.

Data were processed and analyzed by using computer-based software SPSS-25. Optimal cut off value of uCD80 concentration for predicting typical NS was defined at which the sum of sensitivity and specificity is maximum. P value was considered as statistically significant when it was less than 0.05 and confidence interval was set at 95% level.

Results:

Thirty-nine patients (52%) were in typical nephrotic syndrome group and 36 patients (48%) were in atypical nephrotic syndrome group. Mean age of typical NS was found 6.04±2.23 years (±SD) vs atypical NS 8.26±2.90 years (±SD). So mean age was found higher in atypical NS group. In both group majority of the children were male Table I.

Table I

Age and gender distribution of the study patients (n=75)

Characteristics (unit)		Nephrotic Syndrome Typical (n=39)	Atypical (n=36)	p value
Age (years)	Mean±SD	6.04±2.23	8.26±2.90	<0.001^{S}
Gender	Female	15 (38.5%)	11 (30.6%)	0.472^{NS}
	Male	24(61.5%)	25 (69.4%)	

Student-t test

Among the 39 children of typical nephrotic syndrome, most of them (51.3%) were initial episode of nephrotic syndrome followed by 30.8% were relapse (infrequent and frequent). Similarly, among 36 children of atypical nephrotic syndrome, most of them (52.8%) were initial episode of nephrotic syndrome followed by 22.2% and 8.3% children were Steroid-dependent nephrotic syndrome (SDNS) and steroid resistant nephrotic syndrome (SRNS) respectively (Fig.-1).

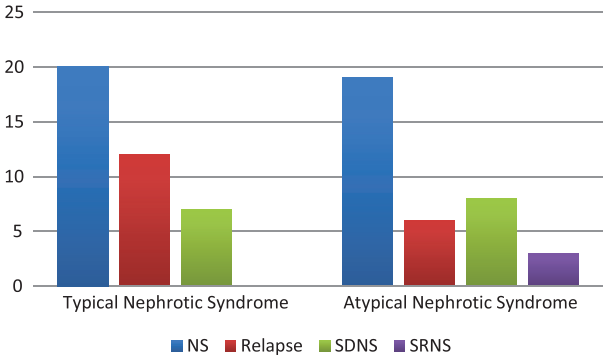

Fig.-1: *Pattern of nephrotic syndrome among the patients*

Histogram showed distribution of urinary CD80 concentration among the study patients. Mean urinary CD80 concentration was 408.11 ± 379.077 ng/g of creatinine (Fig.-2).

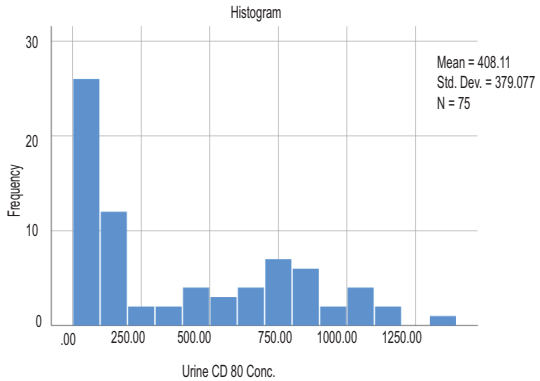

Fig.-2: *Distribution of Urine CD 80 concentration among the study patients, (n=75)*

Table II

S. Creatinine and Urinary CD80 concentration among the study patients (n=75)

Variables (unit)	Nephrotic Syndrome		p value
	Typical (n=39)	Atypical (n=36)	
Urinary CD80 concentration (ng/g of creatinine)			
Mean ±SD	671.92±328.45	122.30±158.47	<0.001[S]

Mean urinary CD80 concentration (ng/g of creatinine) in typical NS group was 671.92 ±328.45 (±SD) and in atypical NS group was 122.30 ±158.47(±SD). So urinary CD80 concentrations (ng/g of creatinine) were significantly higher in typical NS group than atypical NS group (P<0.001) [Table II]

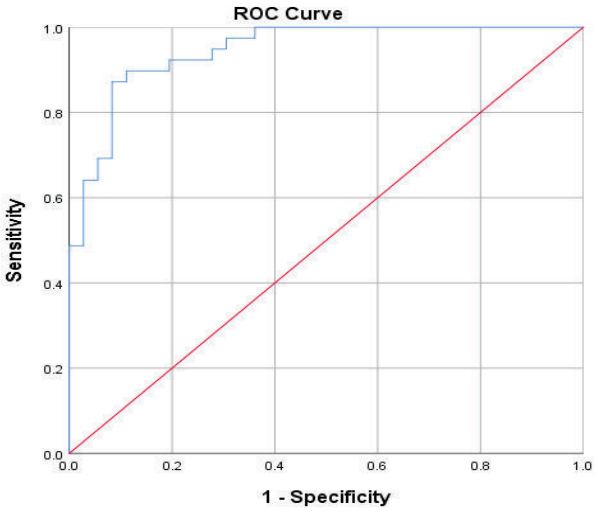

Fig.-3: *Receiver operator characteristic (ROC) curve for urinary CD80 concentration in typical nephrotic syndrome*

AUC for typical nephrotic syndrome was 0.946 for urinary CD80 concentration, (95% confidence interval: 0.899–0.992). Urinary CD80 concentration of 114.9 ng/g of creatinine was found most appropriate cut-off value with the sensitivity 92.3% and the specificity 27.8% to differentiate typical and atypical nephrotic syndrome (Fig.-3). In typical NS uCD80 was >114.9 ng/ml.

Discussion:

Clinical changes of Nephrotic Syndrome are correlated with specific structural changes in the foot processes of glomerular visceral epithelial cells, podocytes which form the glomerular filtration barrier.[4] It is increased in MCD patients in initial attack & relapse compared with MCD patients in remission or those with FSGS.[15] Total 75 patients ranging 1-12 years of age were enrolled for this purpose. Because upper limit of age for admission in paediatrics ward was upto 12 years as no adolescent ward was in paediatrics.

This study showed that mean age was higher in atypical NS group (8.26 ±2.90) than typical NS group (6.04 ±2.23). This difference found significant which correlates with a study done by Ling et al.[16] where mean age was 5.9 ±3.9 years in MCD group. But dissimilar with Ahmed et al.[5] where mean age was 8.4 ±3.2 years in MCD group.

Current study showed that male children were predominant in typical and atypical NS group (61.5% and 69.4% respectively). This is similar to other studies.[5,14,16,17,22]

Mean urinary CD80 concentration was 408.11 ± 379.077 ng/g of creatinine which is higher than Ling et al. (334.58 ± 208.92 ng/g of creatinine) may be due to higher number of atypical nephrotic syndrome patients.[16]

Mean urinary CD80 concentration in typical nephrotic syndrome (671.92 ±328.45 ng/g of creatinine) was higher than atypical nephrotic syndrome (122.30 ±158.47 ng/g of creatinine) found statistically significant (p<0.001). This result consistent with Ahmed et al.[5]

Urinary CD80 concentration found elevated in MCD group than FSGS group by Ling et al.[16] (689.66± 378.21 vs 123.49±167.88 ng/g creatinine) and by Cara-Fuentes et al.[23] (448±564 vs 60.2±73.4 ng/g creatinine). But Minamikawa et al.[22] had reported low urinary CD80 concentration in MCD group & high CD80 concentration in other than MCD group.

In the present study, ROC curve analysis showed, the AUC for typical nephrotic syndrome was 0.946 for urinary CD80 concentration (95% confidence interval: 0.899–0.992) (p<0.001), which is similar to AUC 0.977 (95% confidence interval, 0.899 to 0.999) by Ahmed et al.[5] and 0.925 (95% confidence interval: 0.873–0.978) by Ling et al.[16] for diagnosis of MCD.

A urinary CD80 concentration of 114.9 ng/g of creatinine was found most appropriate cut-off value

with the sensitivity 92.3% and the specificity 27.8% to differentiate typical and atypical nephrotic syndrome. The analysis rendered an optimal cut-off value of 1.5 ng/mg creatinine corresponding to 100% sensitivity and 86% specificity by Ahmed et al.[5] and a cut off value of 328.98 (ng/g creatinine) with a sensitivity of 81.1 % and specificity of 94.4 % by Ling et al.[16] Another cut off value of 108.9 ng/g of creatinine with a sensitivity of 65 % and specificity of 83 %. by Guerrico et al.[11] for diagnosis of MCD.

In this study, urine was collected before initiation of corticosteroid therapy in all initial attack cases and in other cases irrespective of corticosteroid therapy. Ahmed et al. and Ling et al. found no impact of immunosuppressive therapy on urinary CD80 concentration & the effect of taking medicine on the result was ignored.[5,16] Limitations of the study were-study duration was short and for covid pandemic renal biopsy was not done in all cases .

It has been found that urinary CD80 concentration is elevated in MCD.[5,15,16,17] The present study is consistent with those study results. So, urinary CD80 can be used as a non- invasive biomarker to differentiate typical and atypical nephrotic syndrome.

Conclusion:

Urinary CD80 levels found significantly higher in patients with typical nephrotic syndrome than in patients with atypical nephrotic syndrome. Urinary CD80 may be used as a non-invasive diagnostic biomarker in association with other investigations to differentiate typical and atypical nephrotic syndrome. Further studies needed to correlate urinary CD80 level, clinical and histo-pathological pattern of nephrotic syndrome

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