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Glucocorticoid Receptors and the Pattern of Steroid Response in Idiopathic Nephrotic Syndrome

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Abstract

Introduction: Little is known about the relationships between the T lymphocytes (CD3+) expression of glucocorticoid receptors (GCR) and the response to glucocorticoid treatment in children with idiopathic nephrotic syndrome (NS). The aim of the current study is to determine whether steroid responsiveness is dependent on the amount of T lymphocytes GCR expression

Methods: We studied 60 children with idiopathic NS in the age group from 2-10 years. According to the response to steroids we classified our patients into early responders (ER; n=46) and late responders (LR; n=14). Sixty age and gender matched healthy children represented the control group. The clinical and laboratory findings at baseline and GCR expression by T lymphocytes (CD3+) as determined by flow cytometry were compared between the three groups.

Results: The T lymphocytes (CD3+) expression of GCR was significantly lower in the LR than that in the control group (P<0.01), whereas it was similar in the ER and control groups. GCR expression was also decreased in the LR group compared to the ER group (P<0.01). Furthermore, the T lymphocytes (CD3+) expression of GCR correlated inversely with the time to complete remission (CR) (r = -0.54, P<0.05), but not with urinary protein excretion at baseline.

Conclusion: The levels of T lymphocytes (CD3+) expression of GCR may be a useful predictor of steroid responsiveness in children presenting with idiopathic NS.

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Introduction

It is known that glucocorticoids (GC) therapy is the treatment of choice for young patients with idiopathic nephrotic syndrome (NS); however some patients fail to respond to the treatment even when given high-dose GC. For those patients, the treatment should be bolstered by synergizing GC with other immunosuppressant [1].

Although biochemical alterations and clinical manifestations in most nephrotic patients seem to be quite similar, substantial differences are encountered regarding the course of disease. Relapse of proteinuria is experienced in approximately 60% to 80% of steroid-sensitive NS patients, and despite initial complete remission some remain steroid-dependent or become steroid-resistant [2].

The lack of response to corticosteroids has been explained by several mechanisms. It may be ascribed to overwhelming disease severity, poor compliance, abnormalities in glucocorticoid metabolism or poor absorption, especially in patients with the NS, who often develop heavy proteinuria and hypoalbuminemia, and, finally, by GC resistance due to a glucocorticoid receptor (GCR) or post-receptor abnormality. GCR was incriminated in worsening the response to steroids earlier [3,4], but inadequate response to steroids, either due to inherited target tissue defective response or acquired impaired responsiveness was reported in a number of patients [5].

Table 1: Demographic and baseline laboratory parameters of the study groups

	Control (n = 60)	ER (n = 46, 76.7%)	LR (n = 14, 33.3%)
Time to complete remission (days)		13 ± 3.2	34 ± 4.1*
Age	5 ± 2.3	5 ± 2.9	4 ± 3.8
Male/Female	38/22	32/14	8/6
Haemoglobin (g/dl)	13.8 ±1.8	14.5 ± 0.9	13.4 ± 0.2
BUN (g/dl)	13.7 ± 1.1	13.5 ± 1.6	12..9 ± 1.9
Creatinine (mg/dl)	0.4 ± 0.3	0.5 ± 0.4	0.4 ± 0.6
Total cholesterol (mg/dl)	165.4 ± 31.9	387.7 ± 22.3†	358.4 ± 41.5†
Serum albumin (g/dl)	4.3 ± 0.5	1.8 ± 0.9†	2.1 ± 0.7†
Urinary albumin/creatinine	-	7 ± 1.5	8.9 ± 2.3
24 h urinary protein excretion (g/24 h)	-	10 ± 0.5	8.9 ± 2.3
Selective protein index(SPI)	-	0.12 ± 0.01	0.13 ± 0.07

Values are expressed as mean ± SEM; ER: early responders, complete remission achieved within 4 weeks of steroid therapy; LR: late responders, complete remission achieved after 4 weeks of steroid therapy.

* P < 0.05 compared to ER
† P < 0.01 compared to controls

Prediction of clinical response before starting steroid therapy may lead to administration of synergized therapy at the beginning of the treatment and this will help avoid the side effects of chronic high-dose hormone therapy, improve the individual response to GC therapy and benefit more patients [6].

To our knowledge, there have been no published studies of the relationship between the expression of lymphocytes GCR and the time required to achieve complete remission in pediatric patients with steroid sensitive NS.

We hypothesized that evaluation of GCR expression in children by a suitable method before treatment might be beneficial in predicting response to steroids. To investigate whether steroid responsiveness is dependent on the amount of T lymphocytes (CD3+) expression of GCR, we compared the expression of GCR by lymphocytes obtained from idiopathic NS patients who were categorized according to their response to glucocorticoids. Furthermore, we analyzed the correlations between the time interval from the start of GC therapy to complete remission and the amount of T lymphocytes (CD3+) expression of GCR.

Methods

This case control study was conducted in the nephrology and dialysis unit in Madinah Maternity and Children Hospital (MMCH), Saudi Arabia, between February 2009 and January 2011. During this period 60 children in the age group 2-10 years with new-onset nephrotic syndrome

(NS) were prospectively studied. NS was characterized by the presence of edema, proteinuria of at least 2.0 g/24 hours (or urine protein/creatinine ratio of at least 2.0) and hypoalbuminemia (serum albumin <2.0 g/dl). Steroid responsiveness was defined as patients who went into complete remission within 2 months of oral prednisolone. Based on the response to glucocorticoid treatment, the patients were divided into two groups: early responders (ER) in whom complete remission was achieved within four weeks of steroid treatment and late responders (LR) in whom complete remission was achieved after four weeks of steroid treatment. Sixty, age and sex matched, children admitted for elective surgeries were recruited as a control group.

We excluded patients with atypical NS as indicated by hypertension, elevated creatinine, or macroscopic hematuria. We also excluded patients with abnormal urinary sediments (abnormal casts or crystaluria) and patients with history of steroid intake for any cause during the previous 6 months.

All groups were assessed for basic demographic data and laboratory parameters (urinalysis, hemoglobin, blood urea nitrogen, serum creatinine, serum albumin, serum cholesterol, 24 hour urinary protein excretion, urinary protein to creatinine ratio, selective proteinuria index). The expression of intracellular glucocorticoid receptors was measured as the percentage of lymphocytes containing the receptors (CD3/GCR) and was determined in Taibah University Research Laboratory by flow cytometry

Figure 1: Percentage of intracellular glucocorticoid receptor expression by lymphocytes (CD3/GCR) in controls, early responders and late responders
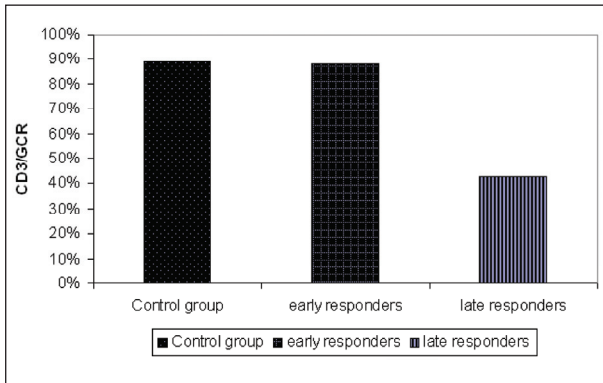
* P < 0.01 when compared to control group and early responders.

Figure 2: The mean percentage of expression of intracellular glucocorticoid receptors by lymphocytes (CD3/GCR) in nephrotic syndrome patients according to the number of weeks required to achieve complete remission.
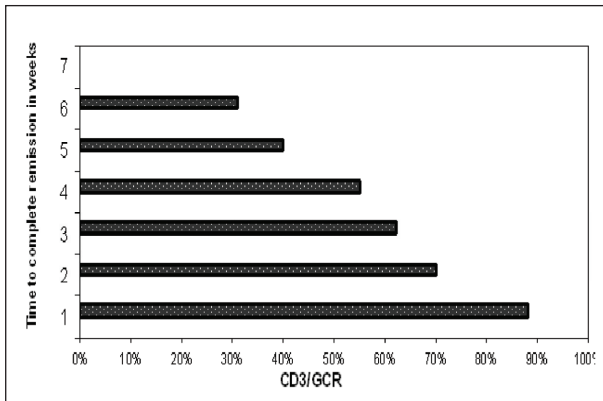
(FCM) using monoclonal antibody and FITC-Dex probes as described by Berki et al [7].

All Patients were treated according to the best clinical practice and evidence based guidelines [8]. They received oral prednisolone in single doses of 2.0 mg/kg/day followed by gradual tapering of the dosage if complete remission was achieved. The time interval from the start of glucocorticoid treatment to complete remission was calculated. Complete remission was defined as negative or trace proteinuria on urine dipstick tests and urinary protein to creatinine ratio <0.3 without oedema. Informed consent was obtained from parents and the protocol of the study was approved by the MMCH ethics committee.

Statistical analysis was performed using the statistical package SPSS for Windows Ver. 13.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were summarized as the mean ± SEM. Results were analyzed using the chi-square test or Mann-Whitney U non-parametric test for multiple comparisons. Correlation between the expression of intracellular glucocorticoid receptors shown as the percentage of lymphocytes containing the receptors (CD3/GCR) and the time from the start of steroid treatment to complete remission was determined by Spearman correlation analysis. Statistical significance was determined when P values were less than 0.05.

Results

A total of 60 children with typical features of minimal change NS were recruited. Based on the response to glucocorticoid treatment, the patients were divided into two groups: 46 early responders (ER: 76.7%) and 14 late responders (LR: 23.3%). There were no differences between the two groups regarding age, gender, or baseline laboratory parameters. The time from the start of steroid therapy to achievement of complete remission was significantly longer in the LR group (34 ± 4.1 days) than in the ER group (13± 3.2 days), P<0.05. Demographic characteristics and laboratory findings of the study population are shown (Table-1).

The shortest time interval between the onset of steroid treatment and complete remission was 5 days while the longest time interval was 42 days.

The mean percentage of lymphocyte expression of glucocorticoid receptor (CD3+/GCR) was significantly lower in the LR group than that in the control group (42.8 ± 1.5 versus 89.1 ± 2.3; P < 0.01), whereas it was similar in the ER (88.2 ± 3.1) and control groups. GCR expression was also decreased in the LR compared with the ER group (P < 0.01) (Figure-1). The CD3/GCR% was significantly higher in the patients who achieved complete remission within the first week of steroid treatment (88.2 ± 0.3) compared to patients who did not achieve complete remission till the 6th week after initiation of steroid therapy (31.0 ± 2.3; P < 0.01) (Figure-2).

The expression of intracellular GCR shown as the percentage of lymphocytes containing the receptors was negatively correlated to the time interval from the start of steroid treatment to complete remission (r = -0.54 and P < 0.01) (Figure-3)

The expression of intracellular GCR shown as the percentage of lymphocytes containing the receptors was not correlated to the urinary albumin/creatinine ratio (r = -0.01 and P > 0.01) (Figure-4).

Figure 3: Scatterplot demonstrating the negative correlation between the expression of intracellular glucocorticoid receptors by lymphocytes (CD3/GCR) and the time interval from starting steroid therapy to complete remission (r=0.54)
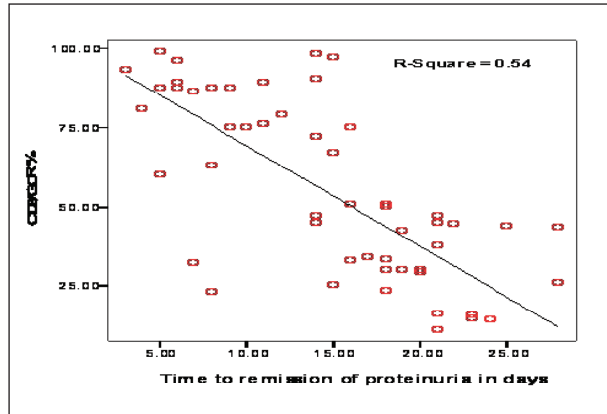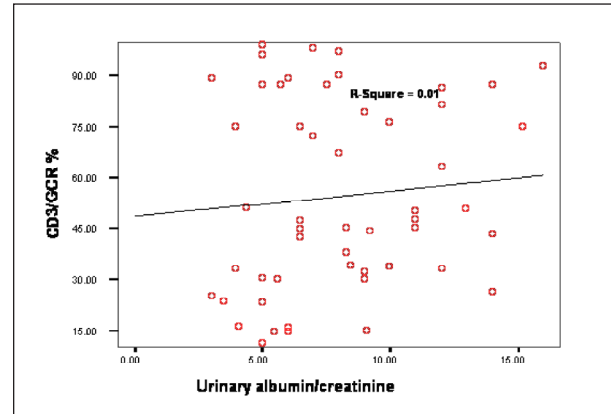

Figure 4: Scatterplot demonstrating lack of correlation between the expression of intracellular glucocorticoid receptors by lymphocytes (CD3/GCR) and the urinary albumin/creatinine ratio (r=0.01)

Discussion

One of the most important indicators for the outcome of children with idiopathic NS is their initial response to GC treatment. Eventual cure is mostly a consequence of a good initial response of proteinuria to GC, even in the case of frequent relapses. Meanwhile, progression to renal failure may be the final destination for children who show an incomplete or absent response initially [9].

No conclusive explanation has been provided regarding the discrepancy in NS patients' response to GC. Variations in the clearance rates of GC between patients are expected, and variations in the density of specific GC receptors may also exist [1-3]. Mononuclear leukocytes (MNL) expression of GC receptors was found to reflect the in-vivo effects of GC in healthy men as well as in patients with various autoimmune diseases such as rheumatoid arthritis, lupus nephritis and asthma [10]. Age, renal function at onset and selectivity of proteinuria were found to be associated with the response to steroid therapy in adults [4].

A pervious study explored the factors affecting the pattern of response to steroid therapy by dividing patients according to their response to steroids into early and late responders with a cut-off period of four weeks [11]. That study compared the frequency of relapses, but did not evaluate the differences in clinical or laboratory findings between the two groups. It reported that the age of onset in minimal change disease NS was not significantly correlated with steroid-responsive rate or with the time interval to remission [11]. The number or density of cellular GC receptors was found to determine cell response and the efficacy of GC on tumour cells in lymphoid leukaemia and lymphoma [12].

The results of the current study are in accordance with a previously cited study which showed that glomerular glucocorticoid receptor expression was reduced in late responders to steroids in adult-onset minimal change disease [10]. Moreover, current results showed a significant inverse correlation between the expression of intracellular glucocorticoid receptors shown as the percentage of lymphocytes containing the receptors (CD3/GCR) and time interval from the start of steroid therapy to complete remission. In a retrospective study, Vivarelli *et al* [13] investigated whether, in children with idiopathic NS, the period of time between the onset of steroid therapy and complete remission can serve as a prognostic indicator, and concluded that the length of time between steroid treatment onset and remission is an early prognostic indicator for patients with idiopathic NS. During follow-up, the median time to achieve remission was less than seven days in non-relapsing and infrequent relapsing NS patients, while it was more than seven days in frequent relapsing and steroid-dependent NS patients [13].

Conclusion

We conclude that evaluation of the expression of intracellular glucocorticoid receptors shown as the percentage of lymphocytes containing the receptors (CD3/GCR) can directly predict early and late responders to steroid therapy, and as a result the outcome of nephrotic syndrome patients regarding future relapses.

Acknowledgment

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