The role of Ectonucleotidases Pathway (CD39/CD73) in Childhood Renal Failure

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Abstract: Background and Aim: Chronic renal failure (CRF) is defined as an irreversible reduction in glomerular filtration rate (GFR). In children, CRF may be the result of congenital, acquired, inherited or metabolic renal disease. In addition to progressive injury with ongoing structural/ metabolic genetic diseases, renal injury may progress despite removal of the original insult. The clinical presentation of CRF is quite varied and dependent on the underlying renal disease. Hypoxia is considered as the master factor in the pathogenesis of renal failure. In the last few years, studies have demonstrated the protective anti-inflammatory role of CD39/CD73 molecules pathway during hypoxia. CD39 and CD73 molecules have been described on circulating T lymphocytes, endothelial cells and minimally, expressed on granulocytes. CD39, (ectonucleotidase triphosphate diphosphohydrolase 1, ENTPD1) is responsible for the conversion of proinflammatory ATP to ADP and AMP whereas CD73 (ecto-5'-nucleotidase) converts AMP into adenosine. Adenosine plays a central role in tissue protection via anti-inflammatory and immune modulatory effect. We aimed to assess the role of CD39/CD73 axis in immuno-inflammatory pathogenesis of renal failure. Also, we aimed to define the expression pattern of both phenotypes on circulating T lymphocytes. Patients and method: This cross sectional study was conducted at Al Zahraa University Hospital from December 2011 to May 2012. An informed consent was obtained from parents of all children. The study included twenty children with chronic renal failure, (on regular hemodialysis) and twenty apparently healthy children as control group. The routine laboratory investigations were performed for patients (blood urea, serum createnin,serum alkaline phosphatase and complete blood count).Using flowcytometry, the patients and control groups were investigated for the frequency of CD39 and CD73 T lymphocytes. In addition, C-reactive protein (CRP) was performed for patients as inflammatory marker. Results: We found that both CD39% and CD73% of patient group were significantly lower than those of control Group. In patient group, we observed that both CD39% and CD73% were negatively correlated to blood urea.Also,CD73% was negatively correlated to serum createnine. In addition, patient group showed negative correlation between CRP as an inflammatory marker and the percentage of both CD39/CD73.Furthermore, there was negative correlation between CD73% and serum alkaline phosphates. Conclusion: The significant decline in both CD39 and CD73 molecules on patients T lymphocytes confirmed the relation between down modulation of ectonucleotidases and deregulation of renal immunological/inflammatory cascade. Also, the negative correlation between both molecules and blood urea proved that CD39/CD73 deficiency can impact renal function. Moreover, the negative relation between CRP and the mean percentages of both phenotypes revealed the protective anti-inflammatory role of the pathway.

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1. Introduction

Extra cellular nucleotides and nucleosides act as signaling molecules involved in a wide spectrum of biological effects. Their levels are controlled by a complex cell surface-located group of enzymes called ectonucleotidases. There are four major families of ectonucleotidases, nucleoside triphosphate diphosphohydrolase (ENTPD/CD39), ectonucleo-hydrophosphatase, alkaline phosphatase and ecto-5'-nucleotidase (5'NT/CD73) (Rosa et al., 2008).). It is generally accepted that ectonucleotidase, CD39 (ENTPD1) hydrolyse pro-inflammatory adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and adenosine monophosphate (AMP) whereas CD73 (5'NT) catalyses the dephosphorylation of AMP into adenosine (Hilaire et al., 2011).

Ectonucleotidases pathway participates in numerous important immunological functions, It has been appreciated that adenosine attenuates potentially harmful aspects of inflammation via controlling interaction between lymphocytes and vascular endothelium (Shirley et al., 2009).

The vascular endothelium is the primary interface between tissue inflammatory signals and circulating leukocytes (Colgan et al.,2006). As such, the endothelium is central to orchestration of lymphocytes activation in response to chemotactic stimuli (Hasko et al., 2011).

The ectonucleotidases (CD39/CD73) pathway has been widely implicated as an adaptive response to hypoxia (Robson, 2011).

It has been suggested that hypoxia up-regulates the expression of CD39 /CD73 molecules on T lymphocytes (Hernandez et al., 2011). CD39 and CD73 deficient T lymphocytes seem to occupy the central role in the pathogenesis of hypoxia-induced inflammatory disorders. They act as mediator of ischemic injury and modulator of immune response. The mechanism by which T lymphocytes exert their modulatory effect remain poorly understood (Thompson et al., 2004, Friedman et al., 2009).

Many experimental studies demonstrated the protective and modulatory role of ectonucleotidases (CD39 and CD73) in different inflammatory models. In human, controversial results have been published about their function and their pattern of expression on circulating leukocytes (Nitschk et al., 2011).

Previous studies reported that CD39 and CD73 deficient T lymphocytes is considered as proinflammatory phenotype of circulating lymphocytes, enhanced expression of adhesion molecules and predisposed to autoimmune inflammation (Zernecke et al.,2005)

In murine model, it was demonstrated that chronic lack of CD73 leads to interstitial nephritis with subsequent renal impairment. The underlying mechanisms depend upon activation of lymphocytes to secrete excessive inflammatory cytokines (Blume et al., 2012).

Research on adenosinergic mechanisms of inflammation, revealed that CD39 deficiency impacts nephropathy in murine models and CD39 gene mutation; also appear to influence renal outcomes (Shirley et al., 2009).

Chronic renal failure is considered as inflammatory disorder. It may results from various forms of glomerulonephritis The prevalence of chronic renal failure in the pediatric population is approximately 18 per 1 million (Vogt and Avner, 2004). Therefore, we aimed to assess the contribution of CD39/CD73 pathway to the immuno- inflammatory pathogenesis of renal failure.

2. Patients and Methods

The study group consisted of 20 patients with chronic renal failure (on regular hemodialysis), they were 11 males and 9 females with ages ranged from 7 to 16 years. They were recruited from the pediatric nephrology and dialysis unit at Al zahraa Hospital. Inclusion criteria were patients on regular dialysis, with CRF caused by various forms of glomerulonephritis. Exclusion criteria were CRF caused by congenital abnormalities such as renal hypoplasia, dysplasia and/or obstructive uropathy, CRF related to metabolic disorders (cystinosis, hyperoxaluria) and CRF resulted from inherited disorders (Alport. syndrome, polycystic kidney disease). Twenty age- and sex- matched apparently healthy children were included as control group. All children included in the study were subjected to complete history taking, thorough clinical examination and routine laboratory investigations as blood urea, serum createnine, serum alkaline phosphatase, CBC and CRP.

The patients and control groups were investigated for the frequency of CD39 and CD73 T lymphocytes using flowcytometry. C-Reactive protein (CRP) was performed for patients group by using Teco diagnostics (latex slide test)

Methodology:

Venous samples were taken in the plain vacutainers for C-reactive protein and sterile EDTA vacutainers for immunophenotyping.

Immunophenotyping:

We used one test tube for each sample (20 patients tubes and 20 control tubes). Each tube contain 20 microliteres of conjugated flurescine isothiocynate (FITIC) labeled CD39 monoclonal antibodies and 20 microliteres conjugated phycoerythrin (PE) labeled CD73 monoclonal antibodies 100 microliteres of the test samples was add to each tube, vortex the tubes gently.

The tubes were incubated 20 minutes at room temperature in the dark, then add 1 mL of fix and lyse mixture, vortex the tubes immediately for one second and incubated again for 10 minutes in the dark at room temperature centrifugation of tubes at low speed for 5 minutes followed by aspiration of supernatant and resuspension of pellet in residual fluid. 2 mL of phosphate buffer saline was add to each tube, the suspension was centrifuged at low speed. The supernatant was discarded, then the residual suspension were passed through the flow-cytometer.

For analysis, gats were set around lymphocytes on the bases of the forward and side scatter profile.

3. Results

Twenty children with chronic renal failure, on regular hemo-dialysis and twenty apparently healthy children were included in our study. To assess the expression pattern of CD39/CD73 on T lymphocytes during renal failure, we compared the expression of both phenotypes in patients group and control group. Table and figure (1) show highly statistically significant decrease in patients compared to control group regarding CD39 and

CD73 T lymphocytes (P<0.001, P <0.002, respectively).

In order to detect the influence of CD39 and CD73 T lymphocytes on renal function, correlation study between the two parameters was performed.

As shown in Table (2) and figures (2, 3), both phenotypes (CD39 andCD73) were negatively correlated to blood urea (P <0.01, P P <0.001, respectively).

Also, figure (4) show statistically significant negative correlation between CD73 T lymphocytes and serum createnine (P <0.012).

To evaluate the role CD39/CD73 in inflammatory pathogenesis of renal failure, correlation study between both phenotypes and C-reactive protein (CRP) as inflammatory marker was performed.

Table (3) and figure (5) show statistically significant negative correlation between the percentages of both CD39, CD73 T lymphocytes and CRP values (P <0.001, P <0.05, respectively).

The relation between CD39, CD73 T lymphocytes and serum alkaline phsophatase was studied. Table (3) shows statistically significant negative correlation between CD73 T lymphocytes and serum alkaline phosphatase, whereas no correlation between CD39 T lymphocytes and serum alkaline phosphate (P <0.05, P = 0.630, respectively).

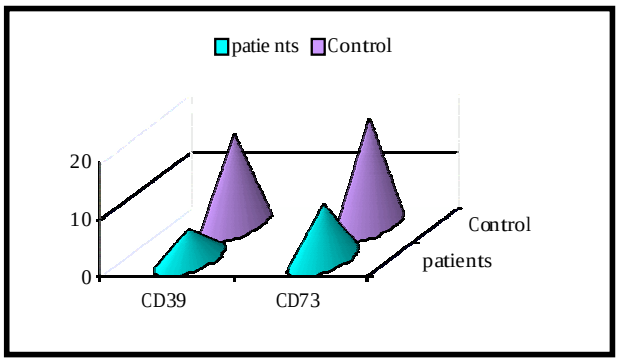
In summary, the comparison between the two studied groups showed a highly statistically significant decrease regarding to CD39 and CD73 in patients compared to controls.

There was a statistically significant negative correlation between CD39 and urea. Also there was a statistically significant negative correlation between CD73 and S. createnine and a highly statistically significant negative correlation between CD73 and urea.

There was a highly statistically significant negative correlation between both CD39 and CD73 T lymphocytes and CRP.

Table (1) and figure (1): Comparison between the studied groups regarding CD39% and CD73%.

Parameters	Groups				Independent t-test	
	Patients		Control		T	p-value
	Mean	±SD	Mean	± SD		
CD39%	5.44	±1.90	16.20	±1.62	-15.283	<0.001
CD73%	9.74	±2.95	18.66	±1.13	-9.160	<0.002



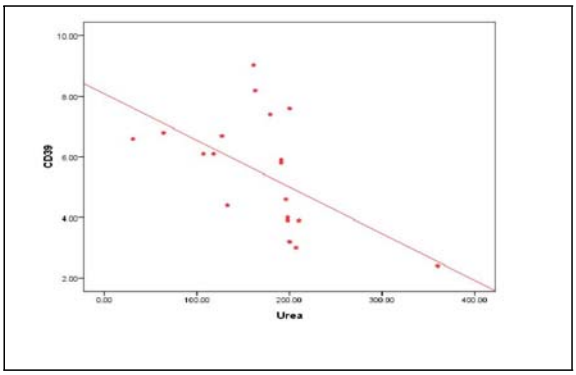


Figure 2: Correlation between CD39 and urea

Table 2: Correlation between CD39% and CD73% with blood urea and Serum creatinine

Parameters	CD39%		CD73%	
	R	P	R	P
Urea(mg/dL)	-0.543*	0.01	-0.708**	<0.001
Serum creatinine(mg/dL)	0.579	20	-0.552*	<0.012

*. Correlation is significant at the 0.05 level (2- tailed).

**. Correlation is significant at the 0.001 level (2- tailed).

Table 3: Correlation between CD39% and CD73% with alkaline Phosphatase and C-reactive protein (CRP)

	CD39%		CD73%	
	R	P	R	P
Alkaline phosphatase (u/l)	-0.115	0.630	-0.441*	< 0.05
CRP (mg/dl)	-0.801**	<0.001	-0.429*	< 0.05

*. Correlation is significant at the 0.05 level (2-tailed).

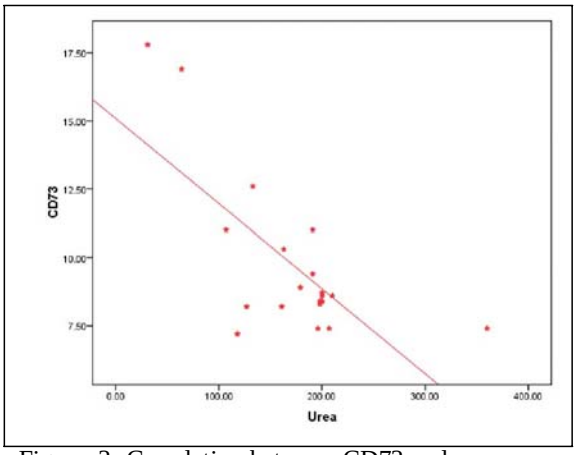


Figure 3: Correlation between CD73 and urea

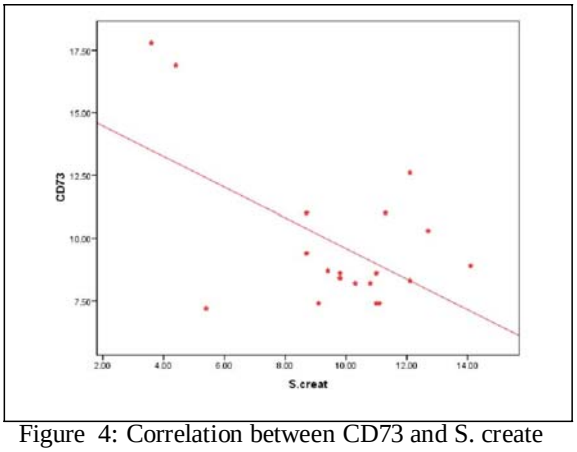


Figure 4: Correlation between CD73 and S. create

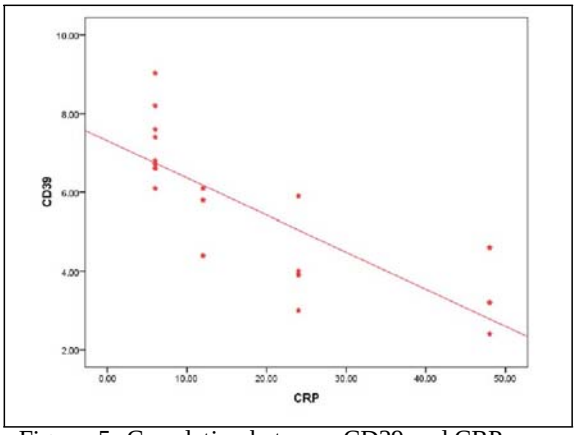


Figure 5: Correlation between CD39 and CRP

4. Discussion

Cells with anti-inflammatory and immuno-suppressive functions raise particular interest in renal impairment because of their potential role in determination of disease course and their prospective use in therapy. CD39 and CD73 T lymphocytes are believed to be one of the immuno-suppressive cells, contradictory reports are, however, available describing their frequency and function during ischemic diseases (Allam et al., 2009).

CD39 and CD73 molecules are members of ectonucleotidases family, it has been reported that both phenotypes are expressed on human and murine T lymphocytes. In particular, these molecules suppress T lymphocytes functions and control inappropriate or exaggerated immune response (Nicolova et al., 2011).

Also, CD39 molecule in concert with CD73 molecule generate adenosine which is important to balance between activation and regulation of immuno-inflammatory cascade (Kasper et al., 2007 and Cekic., 2012).

Many experimental studies have demonstrated the anti-inflammatory role of CD39/CD73 pathway in murine model (Blume et al., 2012).

In vitro tissue culture studies revealed that the lack of CD39/CD73 pathway is likely to predispose to increased inflammatory activity via disruption of cytokines expression and dysregulation of cell adhesion molecules cascade (Bell et al., 2010,Libra et al., 2011 and Zhang et al., 2011).

The in vivo studies on human CD39/CD73 axis are scarce and contradictory (Bonner et al., 2010). Therefore, our study aimed to define the expressive pattern of both phenotypes on circulating lymphocytes. Also, to assess the role of CD39/CD73 pathway in the immuno- inflammatory pathogenesis of renal impairment.

In the present study, we noted the down-modulation of CD39 in tandem with CD73.These findings may be due to deficiency of CD39 and subsequent accumulation of ATP which exhibits feed forward inhibition of CD73 (Robson,(2012).

On comparing the frequency of CD39 and CD73 T lymphocytes in patient group and control group, we observed that T lymphocytes of patients group displayed lower expression of both CD39 and CD73 molecules as matched to control group. These observations were in agreement with Blume et al. (2012), who demonstrated that CD39/CD73 deficiency exhibit glommerular injury and renal impairment.

Interestingly, Romio et al. (2011) have suggested the trafficking of murine circulating T lymphocytes into lymphoid tissues during inflammation.

Moreover, it was demonstrated that CD73 deficiency is associated with pro-inflammatory phenotype of the vasculature; increased attachment of lymphocytes to the endothelium and enhanced

expression of cell adhesion molecules (Zernecke et al.,2005 and Lecka et al., 2010).

Our results showed significant negative correlation between the percentages of both phenotypes and blood urea; also, CD73 was negatively correlated to serum createnine. These finding confirmed that CD39/CD73 deficiency can impact renal function.

In consistent with our findings, Grenz et al. (2008) showed deterioration of renal hemodynamics including serum createnine and blood urea in CD73 deficient mice. Also, Shirley et al. (2009) suggested that deficiency of ectonucleotidase, CD39 impacts nephropathy and renal impairment in murine model.

As regard, the role of CD39/CD73 pathway in inflammatory pathogenesis of renal failure, our finding proved significant negative relation between both phenotypes and CRP as inflammatory marker. Melbourne et al. (2012) suggested the anti-inflammatory role of AMP derived ectonucleotidases in ischemic induced renal impairment.

Also, the nephropathology of CD73 null mutant mice revealed marked tubulonephritis and glomerulonephritis (Grenzi et al., 2008).

In addition, in vitro studies found that the lack of adenosine derived ectonucleotidases pathway enhance release of inflammatory cytokines and chemokines by T lymphocytes (Romio et al., 2011and Bonner et al., 2012).

In another inflammatory disorder model, Ham and Rees (2008), supported the hypothesis that CD39/CD73 which metabolize pro-inflammatory and pro-coagulant nucleotides into anti-inflammatory adenosine might have fundamental role in the pathophysiology of inflammatory disorders.

The association between deficiency of CD73 and activation of inflammatory process was reported by Bell et al. (2010).

Similar to the previous experimental models, we demonstrated significant negative correlation between the proportion of patients CD73 T lymphocytes and the mean value of serum alkaline phosphotase. Hilarie et al. (2011) proposed the link between down modulation of CD73 and increased alkaline phosphatase level in mutant mice.

Our data were similar to the previous findings by Bell et al.(2010) and Robson et al.(2011),who clarified the association between CD39 and CD73 deficiency and developing renal impairment. Also,they confirmed the protective immuno-modulatory role of ectonucleotidases during ischemic renal injury.

We concluded that the down modulation of CD39 and CD73 molecules by patients T lymphocytes may predispose to ischemic nephritis and subsequent renal impairment via dysregulation of trafficking inflammatory mechanism. Also, the significant relation between the deficiency of both phenotypes and CRP may support the notion that the pathway is able to modulate inflammatory process via degradation of proinflammatory ATP as well as production of AMP and adenosine, Our results confirmed the association between the deficiency of the two molecules and deterioration of renal function.

In fact, the ectonucleotidases therapy might be promising in the future for nephroprotection during kidney inflammation.

Of note, our study was limited to circulating lymphocytes while, the CD39 and CD73 molecules were also expressed by secondary lymphoid organs. Thus, further future studies on tissue ectonucleotidases are recommended.

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