URINARY IL-10 IN RENAL TRANSPLANT CASES, DOES IT PREDICT A STATE OF TOLERANCE OR REJECTION?

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Introduction:

Both increased knowledge of the importance of cytokines in the activation of the immune system and the development of specific and accurate assays for their quantitation now offer the possibility of their study as markers to predict or diagnose renal allograft rejection. **Interleukin-10** (IL-10) is one of the most important cytokines that have been implicated in the development of renal allograft rejection.

Patients and methods:

We aim at studying the effect of IL-10 on renal allograft outcome. We measured serum and urinary levels of IL-10 in renal transplant patients more than one year with and without renal impairment, and compared them with chronic renal failure patients on conservative treatment and normal healthy control.

Results:

We found that serum IL-10 levels were significantly higher in the impaired transplant patients than in the normal transplants. Also, the urinary level of IL-10 was significantly higher in the impaired transplant patients more than normal transplants and healthy controls.

Conclusion:

Measuring IL-10 in the serum and urine of renal allograft recipients can help to differentiate between the effect of immunosuppressive drugs and the occurrence of true rejection.

Keywords: Cytokines, Renal transplantation, IL-10

Introduction:

Acute cellular rejection of renal allografts remains a principal cause of graft loss in human kidney transplantation. Approximately 10-15% of allograft kidneys are lost due to rejection in the first year post transplant, making it one of the leading causes of end stage renal disease in developed countries[(2)]. The acute cellular rejection is a consequence of a complex series of events that ultimately leads to graft destruction [(1)]. This complexity suggests the possibility of multiple signals which initiate, modulate and affect this process; these signals do exist and are termed cytokines [(1-3)].

Cytokines are hormone like polypeptides or glycoproteins of low molecular weight (6-60 Kd MW), which are secreted by certain cells and affect other cells, in the course of immunological and inflammatory responses. Cytokines produced by lymphocytes are called lymphokines whereas those produced by macrophages are called monokines. They regulate all the important biological processes:

cell growth, cell activation, inflammation, immunity, tissue repair, fibrosis and morphogenesis.

IL-10 is a growth cofactor for thymocytes, spleen and lymph node cells. It was originally called cytokine synthesis inhibitory factor because of its ability to inhibit cytokine production by activated T lymphocytes. IL-10 produced late in the activation process by TH2 cells, IL-10 can completely prevent antigen specific T cell proliferation, IL-10 has a direct stimulatory effect on B cell activation, proliferation and differentiation into antibody secreting cells, IL-10 can inhibit TH1 cytokine synthesis [4-10].

IL-10 is one of the most important cytokines that have been implicated in the development of renal allograft rejection. It may be of value in treatment of inflammatory diseases, IL-10 is attractive candidate in treating a variety of T cell mediated autoimmune diseases such as type I diabetes and multiple sclerosis, IL-10 has been found in the supernatent of several human cancer cell lines as ovarian cancer and other intra-peritoneal cancers.IL-10 plays a critical role in the emergence of EBV related lymphomas in AIDS patients [11-15].

Patients and methods:

We aim at studying the effect of IL-10 on renal allograft outcome. This is a prospective study conducted on 40 individuals. Were classified to the following 4 groups:

Group A: Ten patients with transplanted renal allograft for more than one year and serum creatinine less than 1.4 mg/dl aged from.

Group B: Ten patients with transplanted renal allograft for more than one year and serum creatinine more than 1.4 mg/dl.

Group C: Ten patients with end stage renal disease under conservative treatment.

Group D: Ten normal individuals as control group.

The evaluation of patients included:

1. Complete history taking and full clinical examination.
2. Measurement of serum urea, creatinine and creatinine clearance.
3. Measurement of serum and urinary interleukin 10.
4. Measurement of trough cyclosporine level for the renal transplant groups.
5. Complete urine analysis. Liver function tests: serum bilirubin (Total and direct), ALT, AST, Alkaline phosphatase, and serum albumin level.

We measured serum and urinary levels of IL-10 in renal transplant patients more than one year with and without renal impairment, and compared them with chronic renal failure patients on conservative treatment and normal healthy control. We measured serum and urinary levels of TGF-B1 and PDGF in the four groups.

The kit used to measure IL-10 in urine and serum. Isolation and culture of peripheral blood mononuclear cells may be realized by usual methods. Serum was removed as soon as possible from the clot of red cells after clotting and centrifugation, and kept at 4 ^{0}C. Plasma collected on sterile EDTA or heparin tubes and rapidly separated after centrifugation. Serum plasma samples must be kept at -20 ^{0}C for maximum 2 months, and for long storage (maximum one year) at -70 ^{0}C [3,13].

Data were collected coded and analyzed using SPSS software version 11 under Windows XP.

Results

A total of 40 patients were identified and recruited into our study, of whom 31 were male and 9 were female.

Fig (1) Comparison of Interleukin-10 in Urine in the four groups

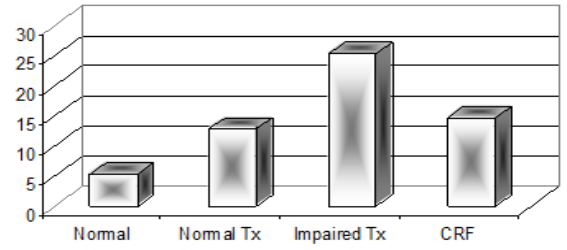


Fig (2) Comparison of Interleukin-10 in Serum in the four groups

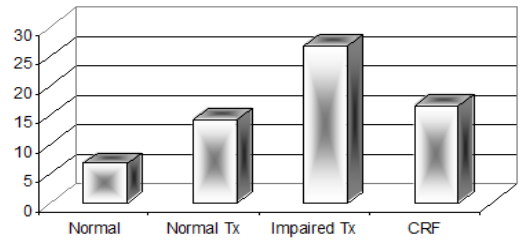


Fig (3) Correlation between Creatinine & Interleukin - 10 in Serum in the whole sample

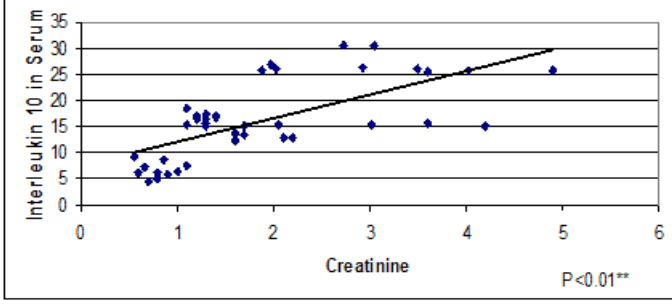


Fig (4) Correlation between Creatinine & Interleukin-10 in Urine in the whole Sample

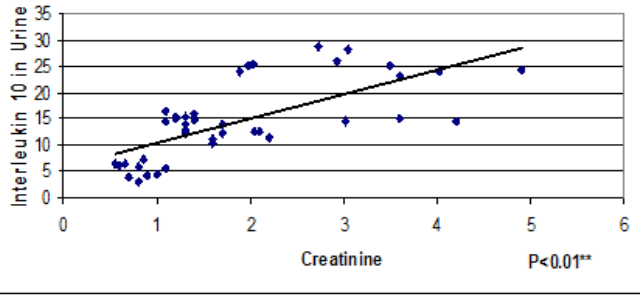


Fig (5) Correlation between Creatinine & Interleukin -10 in serum in (Controls & CRF groups)

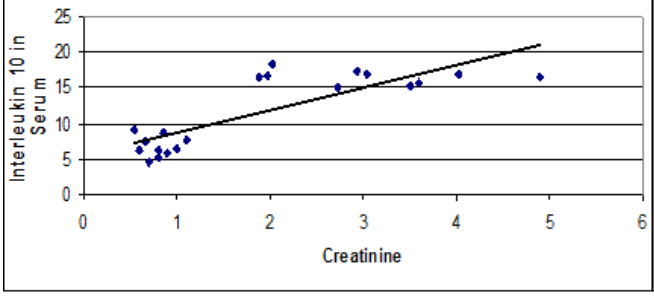


Fig (6) Correlation between Creatinine & Interleukin-10 in Urine in (Controls & CRF groups)

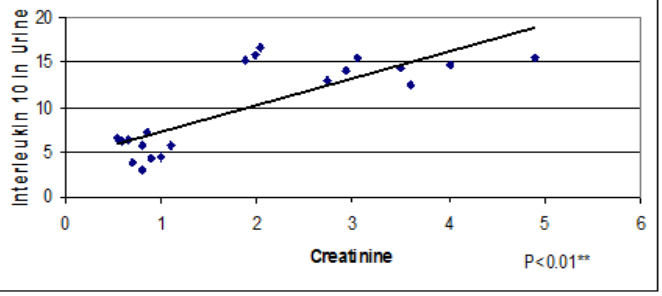


Fig (7) Correlation between Creatinine & Interleukin-10 in serum in (Transplantation Groups)

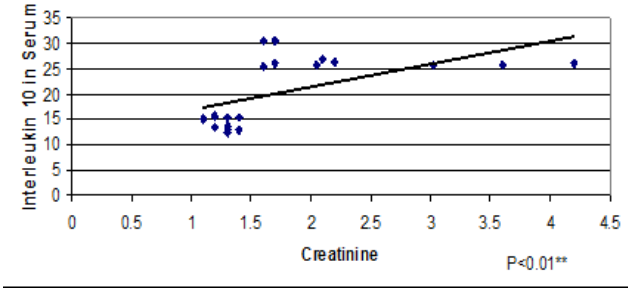
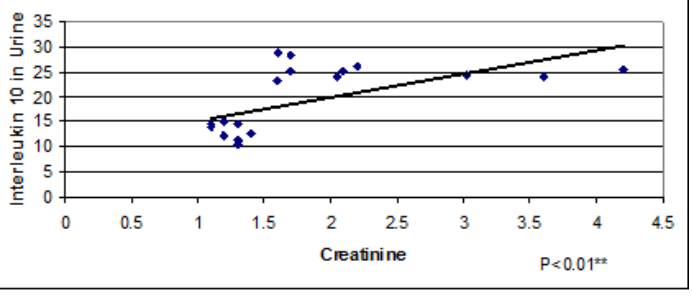
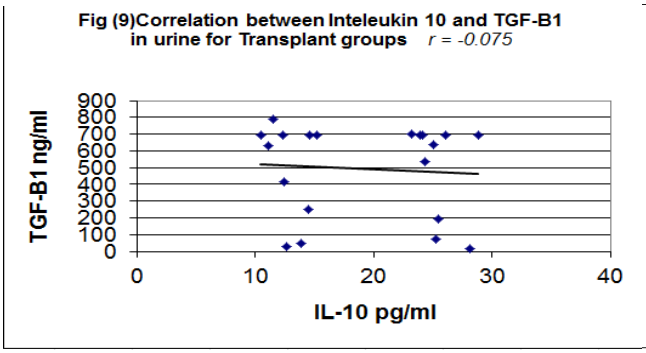
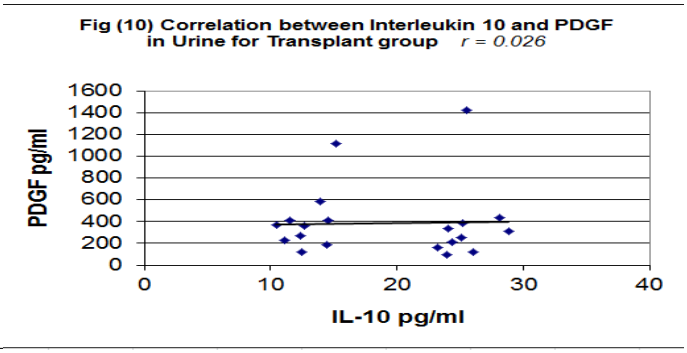
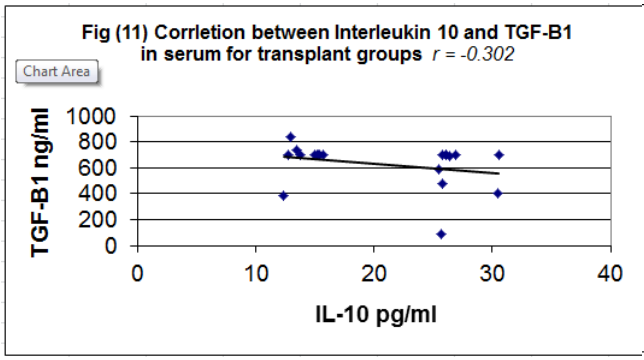


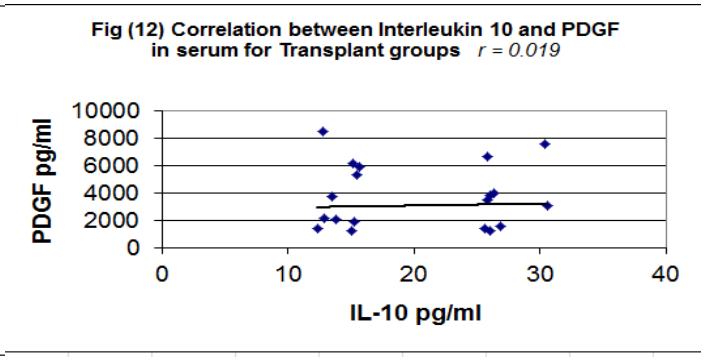
Fig (8) Correlation between Creatinine & Interleukin-10 in Urine in (Transplantation Groups)











Discussion

The serum and urinary levels of IL-10 (26.930 ± 1.931 pg/ml) and (25.48 ± 1.8 pg/ml) respectively and serum and urinary levels of TGF-B1 (712.53 ± 45.53 ng/ml) and (653 ± 101 ng/ml) respectively in the transplanted group of patients with impaired renal function were significantly higher than the transplanted group with normal kidney function (14.218 ± 1.269 pg/ml), (12.884 ± 1.602 pg/ml) and (317.77 ± 94.6 ng/ml), (415.459 ± 101 ng/ml) respectively, This would suggest an active rejection which needs further immunosuppression.

The presence of relatively higher serum and urinary IL-10 as compared to controls together with mild elevation of TGF-B1 in the normal transplant group indicates underlying immune stimulation with subclinical impending rejection which needs monitoring of other cytokines, kidney functions and cyclosporine A level together with further immunosuppression.

The results of serum and urinary PDGF carried the same correlative value to IL-10 as the TGF-B1. And as the levels of serum and urinary IL-10 in the transplanted group of patients with impaired renal function (26.930 ± 1.931 pg/ml) and (25.48 ± 1.18 pg/ml) respectively were significantly higher than that of the CRF group (16.560 ± 0.987 pg/ml) and (14.37 ± 1.3 pg/ml) respectively [$p<0.05$], and who had comparable serum creatinine [CRF group (3.061 ± 0.974 pg/dl) and impaired Tx group (2.377 ± 0.917 pg/dl)] [$p>0.05$ i.e non-significant], suggests a greater impact of the rejection process than mere retention of the cytokine due to reduced clearance (Fig 9-12).

Serum creatinine was found to be significantly directly correlated with serum and urinary IL-10 in the four groups ($p< 0.01$) (Fig 3-8).

Serum creatinine was also significantly directly correlated with serum and urinary IL-10 in groups not receiving cyclosporin A (control and CRF group) ($p<0.01$), and in groups receiving cyclosporin A (transplantation groups) [$p< 0.01$] respectively (Fig 3-8).

The significantly higher serum IL-10 levels in CRF group (16.56 ± 0.987 pg/ml) more than the control group (6.707 ± 1.481 pg/ml)

[p< 0.01] suggests an ongoing inflammatory reaction rather than retention of the cytokines as the level of urinary IL-10 was significantly higher in the CRF group than control group (14.37 ± 1.3 pg/ml) and (5.362 ± 1.38 pg/ml) respectively (Fig 1-2).

Urinary IL-10 level in the transplanted group of patients with impaired renal function (25.478 ± 1.812 pg/ml) and in the transplanted group with normal kidney function (12.88 ±1.602 pg/ml) were also significantly higher than the control group (5.362 ± 1.380 pg/ml) [p< 0.01 and < 0.01 respectively] reflecting the state of incompletely suppressed immune reaction in the graft. And being significantly higher in the transplanted group of patients with impaired renal function (25.478 ±1.812 pg/ml) more than the transplanted group with normal kidney function group (12.885±1.602 pg/ml) [p< 0.01] signifies an important active rejection process more than a process of retention related to renal function, inducing elevation of the serum level of the cytokines due to significant higher urinary IL-10 in the Tx group with impaired kidney function (Fig 1-2).

Increased IL-10 in normal transplants would suggest increasing CsA dose and subsequent TGF-B1 to abolish IL-10 activity completely, yet higher CsA dose was not reflecting by higher TGF-B1 levels suggesting that CsA toxicity and even effect may be mediated by other effect of mediators than TGF-B1, and IL-10 level was negatively correlated with CsA dose.

Lower IL-10 in serum and urine of normal transplants than impaired transplants indicates lesser rejection activity which might be still requiring further immunosuppression.

In impaired transplant patients the concomitant increase in both IL-10 and TGF-B1 suggests that the higher TGF-B1 is more relevant to the rejection process rather than CsA effect, and that the higher the IL-10 the greater is the rejection activity.

Conclusion

Human renal allograft rejection is associated with high serum and urinary levels of IL-10 and TGF-B1 among other cytokine changes. IL-10 can be induced to a greater extent by an active rejection process and to a lesser extent by an incompletely suppressed immune reaction. A rise of serum and urinary levels of IL-10 more than four folds the normal value suggests active rejection. Higher serum IL-10 levels in CRF patients than normal individuals are due to ongoing inflammatory reaction.

RECOMMENDATIONS:

The cytokine IL-10 is an indicator rather than a mediator of rejection which can be induced to a greater extent by an active rejection process and a lesser extent by an incompletely suppressed immune reaction. Measurement of this cytokine would help to segregate both conditions and direct the adjustment of the dose of the immunosuppressive agent particularly in patients with normal graft function having elevations of the serum and urinary levels of these cytokines.

Measurement of IL-10 can be used in conjunction with other cytokines to assess the immune state of transplant recipients. Whether IL-10 can be used therapeutically to suppress an ongoing rejection process requires further investigations.

Conflicts of interest

There are no conflicts of interest

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