

INTERLEUKIN-6, also named B-cell stimulatory factor, is a glycoprotein with a molecular weight of 26 kDa. Increased serum levels of interleukin-6 (IL-6) are found in several disease conditions. We investigated the importance of a deteriorated kidney function upon IL-6 serum concentrations. No relation was found between serum levels of IL-6 and s-creatinine, $r = 0.004$. On the other hand, the serum concentration of complement protein factor D and soluble IL-2 receptor showed a good correlation to s-creatinine, $r = 0.92$ and 0.79 , respectively. In conclusion, serum levels of IL-6 are not dependent upon a reduced kidney function.

Key words: Interleukin-6, Kidney, Low molecular weight protein

Serum levels of interleukin-6 are not dependent on the kidney function

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Introduction

Interleukins or cytokines are message molecules which regulate important immunological functions. All interleukins have low molecular weights. Increased levels of interleukin-6 (IL-6), also known as the B-cell stimulatory factor, or originally named interferon β_2 (IFN β_2) are found after a variety of tissue responses ranging from minor stress,¹ elective surgery,² severe sepsis,³ and in bacterial peritonitis in patients undergoing continuous ambulatory peritoneal dialysis, CAPD.⁴ IL-6 seems to be produced in most nuclear containing cells. It appears to be one of the major mediators of the reaction to viral and bacterial infections, inflammation and shock.⁵ It has also been experienced that the level of IL-6 increases earlier than acute proteins such as C-reactive protein and α_1 -antitrypsin.

IL-6 is a protein with 184 amino acids and a molecular weight of about 26 kDa, so it can be classified as a low molecular weight protein.⁶ Therefore, it can be filtered through the glomeruli. A reduced kidney function could be assumed to increase serum IL-6 levels. Increased serum levels of small molecular weight proteins i.e. β -microglobulin and factor D are seen in patients with reduced glomerular filtration rate.^{7,8} The aim of this study was to investigate the influence of kidney function upon serum levels of IL-6.

Materials and Methods

Patients: Twenty-one patients with adult polycystic kidney disease were included in the study. Except for their kidney disease they were all well and there were no signs of an ongoing infection, or other diseases. Serum creatinine values ranged from 80 to 1390 $\mu\text{mol/l}$.

Normal sera: Sera from 25 normal blood donors served as control for establishing the cut-off level in the IL-6 assay.

Methods: Serum creatinine were measured by a conventional technique at our department of clinical chemistry. Factor D was measured by a haemolysis technique.⁹ The concentration is given as a percentage of normal sera. Soluble interleukin-2 receptor (S-IL-2R) was measured using an ELISA method (T-cell Science, Boston). Briefly, wells were coated with a murine monoclonal antibody directed against an epitope on the S-IL-2R molecule. A horseradish peroxidase conjugated monoclonal antibody directed against another epitope on S-IL-2 is used as the detecting (secondary) antibody.

IL-6 assay: Serum interleukin-6 (IL-6) was assayed using an ELISA method (Innogenetics A.S., Antwerp, Belgium). Briefly, polystyrene microplates were coated with sheep polyclonal anti-IL-6 antibodies. One hundred microlitres of the respective samples were incubated for 2 h at 37°C. A murine monoclonal anti-IL-6 biotin-labelled antibody was then added, followed by peroxidase conjugated streptavidin. The substrate used was tetramethylbenzidine and the developed colour was read at 450 nm.

Results

Normal sera: The mean absorbance, when subtracting the background values, was 0.058. This value corresponds to a concentration of 10 pg/ml, when reading from the standard curve. The cut-off level was set at 10 pg/ml, which corresponds to the recommendation given by the manufacturer.

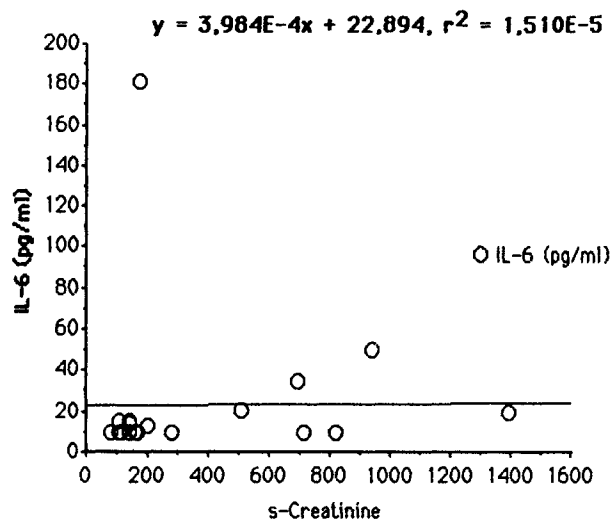


FIG. 1. Shows the relation between serum concentration of IL-6 and s-creatinine. The correlation coefficient was $r = 0.0004$.

Patients: Twelve of the patients had serum concentrations of IL-6 below 10 pg/ml. One patient had a very high serum concentration, 180 pg/ml. As seen in Figure 1 no correlation was seen between S-IL-6 and s-creatinine ($r = 0.004$). There was no correlation between serum levels of IL-6 and levels of factor D and S-IL-2R. The correlation coefficients were $r = 0.028$ and $r = 0.018$, respectively. On the other hand, a very high correlation was found between Factor D and s-creatinine as seen in Figure 2a ($r = 0.92$; $p = 0.0001$). A somewhat lower relation was observed between S-IL-2R and s-creatinine ($r = 0.79$; $p = 0.001$) (Figure 2b).

Discussion

IL-6 is a low molecular weight protein synthesized in monocytes, though it can be produced and secreted from all nuclear cells. It was first identified by its ability to induce antibody secretion by preactivated normal and Epstein—Barr virus transformed human B-cells¹⁰ without first inducing cellular proliferation. Apart from this effect, it has been found that IL-6 induces differentiation of cytotoxic T-cells from both mature and immature T-cells.¹¹

Several lines of evidence suggest that IL-6 is involved in the pathogenesis of certain autoimmune diseases.¹² High levels have been found in synovial fluid in rheumatoid patients, but not in those with active osteoarthritis. Furthermore, increased serum levels of IL-6 have been found during allograft rejection.¹³ A close relation is also seen between body temperature and IL-6 levels.¹⁴ Since serum levels of IL-6 are increased in several different diseases, it could be of great importance to investigate how a reduced kidney function

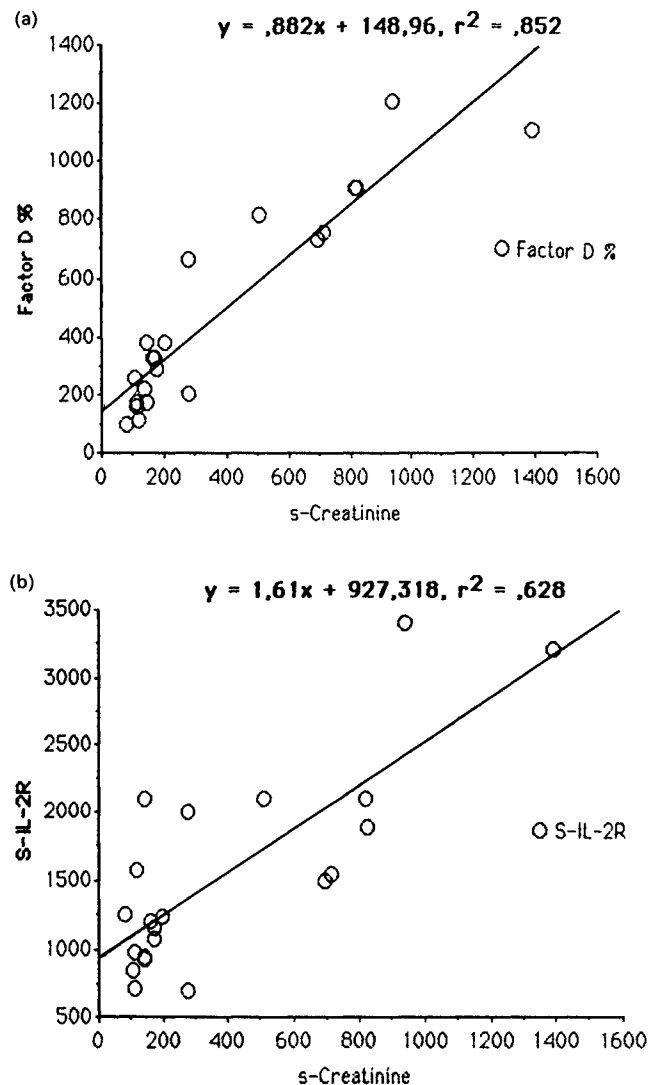


FIG. 2. (a) High correlation was found between serum levels of factor D and s-creatinine ($r = 0.92$, $p = 0.0001$). (b) Relation between serum concentrations of soluble IL-2R and s-creatinine ($r = 0.79$, $p = 0.001$).

influenced the measured concentrations. Under normal circumstances interleukins, including IL-6, are eliminated rapidly from plasma. There are many possible ways for inactivation and elimination other than the kidneys, for instance inactivation by proteases,¹⁵ binding to circulating soluble receptors such as soluble TNF receptor,¹⁶ to carriers such as α_2 -macroglobulin,¹⁷ or to autoantibodies.¹⁸ This study shows clearly that a reduced kidney function does not have a major effect on the serum levels of IL-6. Therefore, measured levels are not falsely high values recorded due to a deteriorated kidney function, but reflect an inflammatory response *per se*. These findings indicate that kidneys do not contribute to any great extent to the clearance of IL-6. The same condition seems to be valid for interleukin-1. Radiolabelled IL-1 was injected to nephrectomized rats, and there was only a small

increase of serum IL-1 levels in these rats compared to non-nephrectomized rats. The authors concluded that less than 10% of IL-1 was cleared by the kidney.¹⁹ Though renal catabolism may play a role, and tubular cells have been found to reabsorb interleukins, (cf. Ref. 20) other elimination pathways may account for an optimal clearance. In patients with renal dysfunction it could be assumed that some or several of the above mentioned pathways are stimulated, leading to an enhancement of inactivation of cytokines.

Factor D is a complement protein and the S-IL-2R is a T-lymphocyte derived product with a molecular weight of 45 kDa compared to 23.5 kDa for Factor D. Serum levels for both these proteins depend on kidney function and high correlation coefficients to s-creatinine were found. Factor D was seen to correlate even better for s-creatinine than S-IL-2R. Recently it has also been shown that circulating soluble tumour necrosis factor binding protein is highly correlated to s-creatinine²¹ in a manner similar to S-IL-2R.

The high level of IL-6 in one of the patients was puzzling. At the time of sampling there was no sign of infection or inflammatory state. It is known that IL-6 can be produced locally in the kidney due to hypercellularity, for instance in renal cell carcinoma²² or in mesangial proliferative glomerulonephritis.²³ It has also recently been shown that other tumours may cause IL-6 production, for instance myeloma and cardiac myxoma cells. It was not completely ruled out that our patient might have had a tumour, but at the time of sampling no indication of neoplasm or other disorder was observed. Follow-up has, however, not been possible. It has been demonstrated that *in vivo* administration of recombinant human tumour necrosis factor to patients with metastatic cancer leads to the induction of circulating levels of IL-6.²⁴ From an experimental point of view it has also been shown that tumour necrosis factor influences IL-6 kinetics.²⁵

In conclusion, when evaluating serum levels of IL-6 in different disease states there is no need for correction due to a reduced glomerular filtration rate.

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