LEVELS OF PLASMA SOLUBLE COMPLEMENT RECEPTOR 1 (sCR1) IN NORMAL INDIAN ADULT POPULATION

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ABSTRACT

A decrease in the membrane anchored erythrocyte complement receptor 1 (CR1) is reported as an acquired phenomenon in a number of inflammatory and autoimmune diseases with concomitant rise in soluble CR1 (sCR1) levels in plasma. There is a need to establish the normal range of sCR1 in Indian adults to assess the function and disease association of this protein. The plasma sCR1 levels of 50 healthy individuals have been estimated by an indigenously developed sandwich ELISA. sCR1 levels from 26 patients suffering from nephropathies had also been assayed which was much higher than the normal controls. This observation suggests sCR1 as a potential marker for the assessment of disease activity in nephropathies.

KEY WORDS : Soluble complement receptor 1, Renal diseases, Prognostic marker.

INTRODUCTION

Complement Receptor 1 (CR1, CD35, C3b / C4b receptor) is an intrinsic membrane glycoprotein found on a variety of cells like erythrocytes, polymorphonuclear leukocytes, monocytes, B lymphocytes, T lymphocytes, mast cells and renal podocytes (1). This protein is endowed with the dual function of inactivating the complement cascade as a complement regulatory protein and clearance of immune complexes from circulation (2). CR1 is also present in the plasma in a soluble form (soluble CR1 or sCR1) which is indistinguishable in size and antigenicity from erythrocyte CR1 (3). Though its function is not established, sCR1 is

shown to be secreted by leukocytes (3). A recombinant soluble form of CR1(rCR1) (4) has been shown to be a potent inhibitor of the complement cascade (5) and to reduce inflammation in animal models of glomerulonephritis (6) and myocardial infarction (7). These findings suggest an association of soluble CR1 with disease activities. To explore this issue further, we needed to establish normal levels of sCR1 in healthy individuals. So far we do not have data from our own population. This paper reports our preliminary data on plasma sCR1 levels in normal Indian adults estimated by an indigenous sandwich enzyme linked immunosorbant assay (ELISA). We have also collected few data on samples from patients with nephropathies to

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assess the potential use of plasma sCR1 levels as a marker of disease activity in nephropathies.

MATERIALS AND METHODS

Patients

Heparinized blood was collected from 50 normal healthy individuals and 26 clinically diagnosed adult nephropathy patients. Proteinuria was the single criteria for random sampling. Plasma was separated from the whole blood by centrifuging at 800g for 15 minutes at 4°C.

Storage

Plasma separated from the blood was stored at -70°C till use.

Ingredients of ELISA

Two monoclonal antiCR1 antibodies, recognising two different epitopes on CR1 were used. Monoclonal mouse antibody to human CR1 (J3D3) was a kind gift from Prof. M.D. Kazatchkine (France). Monoclonal rabbit antibody to human CR1 was a kind gift from Prof. M.R. Daha (The Netherlands). HRP conjugated antimouse IgG was obtained from Sigma Chemical Co., U.S.A. Recombinant CR1 was a gift from Prof. J.P. Atkinson (USA). High binding ELISA plates were from Costar.

Methods

An indigenous ELISA was developed by a modification of the method described by Schifferli et. al. (1). Fig.1 displays the standard curve for ELISA. Steps were as follows: washing was carried out with PBS-T (PBS containing 0.05%

Fig. 1. Standard Curve for soluble CR1 ELISA

Tween) for four times in between every step. (a) wells were first coated with 100µl of mouse monoclonal antibody to human CR1 at a concentration of 0.3µg per well in carbonate bicarbonate buffer pH 9.6, left overnight at 40°C and washed. (b) unbound sites were blocked with 1% BSA-PBS-T for 2 hrs at 37°C. (c) plates were washed and 100µl of plasma samples at various dilutions were added to each well and incubated overnight at 40°C. (d) after washing, plates were incubated with 100µl of monoclonal rabbit antibody to human CR1 diluted 1:3000 (Stock 0.56 Ig/ml) for 3 hrs at 37°C. (e) after washing, plates were incubated with 100µl of HRP conjugated antirabbit IgG diluted at 1:3000 (Stock 0.5mg/ml) for 1 hr. at 37°C and washed finally. Color was developed with 250 µl of freshly prepared substrate solution containing 4mg/ml of OPD in 0.1M citrate phosphate buffer pH 5.0 in each well. The enzymatic reaction was allowed to proceed for 30 minutes and then stopped with 50µl of 0.2N H₂SO₄. The absorbance was read in an ELISA reader (Anthos, Austria) at 450nm. A standard

curve was run using recombinant CR1 in every plate.

Statistical methods

Statistical evaluation was done by student's t test.

RESULTS

Soluble CR1 levels in normal controls and in patients.

The soluble CR1 concentration was determined in normal adult individuals, and the

Fig. 2. Soluble CR1 Levels in normal healthy individuals and in nephropathy patients.

mean concentration was found to be 44.68 ± 12.5 (range 24-74) ng/ml (Fig.2). Patients with nephropathies had a significantly higher mean concentration of sCR1 (106.40 ± 24.34 ng/ml), compared to adult controls ($p < 0.01$). There was

no difference in the levels of CR1 between the serum and plasma samples.

Sample storage conditions

Freeze - thawing of samples stored at -70°C did not give any difference in their sCR1 levels as compared to the fresh sample. Hence samples were stored at -70°C till assayed.

DISCUSSION

In many of the inflammatory or autoimmune diseases there had been an alteration in the levels of CR1 on erythrocytes and podocytes (8,9). Plasma sCR1 levels reported earlier by Schifferli et.al. (2) was 31 ± 7.8 ng/ml. Mean levels of sCR1 from our studies were found to be higher. Plasma samples from nephropathy patients had a marked rise in their sCR1 levels as compared to the normals. The reason for increased sCR1 levels in patients suffering from nephropathies is not clear at present. However sources like shedding off from the erythrocytes, increased levels due to increased leukocytosis or induced synthesis under infectious, autoimmune or inflammatory conditions (3) may not be ruled out.

To conclude, although higher levels of sCR1 in patients with nephropathies indicate the possibility of using plasma sCR1 as a marker for disease activity in these patients, further extension of this study is needed, to suggest sCR1 as a prognostic marker for nephropathies.

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