β2-Glycoprotein I in Rheumatoid Arthritis

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KOSAKA, S. β2-Glycoprotein I in Rheumatoid Arthritis. Tohoku J. exp. Med., 1977, 122 (3), 223-228 — Serum β2-glycoprotein I of the patients with rheumatoid arthritis was studied by means of single radial immunodiffusion method. There was a significant lowering of β2-glycoprotein I concentration in patients with rheumatoid arthritis. An inverse proportional correlation was seen between the concentrations of β2-glycoprotein I and of α1-antitrypsin, and between the former and C-reactive protein (CRP) rates in individual specimens. Slightly positive relationship was observed between the concentrations of β2-glycoprotein I and of α2-HS glycoprotein. The β2-glycoprotein I concentrations in healthy adults were significantly higher than those of previous reports — serum β2-glycoprotein I; rheumatoid arthritis

In the past decade, several new proteins were isolated from the β-globulin fraction of human serum, e.g., β2-glycoprotein I (Schultze et al. 1961; Haupt and Heide 1966), β2-glycoprotein II (Haupt and Heide 1965) and β2-glycoprotein III (Schwick et al. 1968). They can be distinguished by certain physico-chemical and immunological properties.

In 1961 Schultze and associates demonstrated the presence of so far unknown β2-globulin which they called β2-glycoprotein I. This is a low molecular weight protein (molecular weight, 40,000) with a sedimentation constant (S20, W) of 2.9 (Schultze et al. 1961). This molecule consists of a protein moiety (82%) and carbohydrate chains composed of hexoses (6.7%), acetyl hexosamine (5.8%), sialic acid (4.4%) and fucose (0.2%) (Schultze et al. 1961). In contrast to its heterogeneity in starch gel electrophoresis, there is only one precipitation line in immunoelectrophoresis which proves the immunological uniformity of the individual components. Purified β2-glycoprotein I (Haupt and Heide 1966) has been used for the production of specific antisera and for the construction of calibration curves, thus making possible its quantitative immunological determination.

Although there have been several reports on the genetic analysis and the population frequency of serum β2-glycoprotein I concentration, little is known about the biological function as well as the pathological condition of this protein. In the present study, the concentrations of β2-glycoprotein I were determined and were compared with other glycoproteins belonging to α-globulin fraction and C-reactive protein (CRP) in the sera of patients with rheumatoid arthritis.

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Material and Methods

Determination of $\beta_2$-glycoprotein I in serum was carried out by the single radial immunodiffusion method as follows (Mancini et al. 1965): 10 ml of warm 1.25% agar gel were mixed with 0.25 ml of anti-$\beta_2$-glycoprotein I rabbit serum (Behringwerke/Marburg) and poured on a Partigen plate to give a 2 mm thick layer. After congelation, circular wells with a diameter of 2 mm were punched out. Exactly 2 $\mu$l of test serum diluted 1:3 were introduced into each of the wells by means of a micropipette. After the plate was incubated for 48 hr at room temperature, the formation of a visible ring of precipitate around the wells was examined. The $\beta_2$-glycoprotein I concentration was estimated from the calibration curve which was designed for a series of dilution of standard human serum (Behringwerke) in reference to variations of the ring diameter.

Serum concentrations of $\alpha_1$-antitrypsin, $\alpha_2$-HS-glycoprotein and $\alpha_1$-acid glycoprotein were simultaneously measured with Partigen plate kit (Behringwerke) according to the procedure of single radial immunodiffusion method. In order to quantify the CRP, commercially available anti-CRP prepared from sheep and goat (Hyland Lab./Los Angeles) was used.

Results

$\beta_2$-Glycoprotein I concentrations in sera from healthy persons

Serum samples of controls were taken from 43 males and 46 females at the age of 20 to 25.

The $\beta_2$-glycoprotein I levels of these cases were in the range between 21 and 52 mg/100 ml, and the values seemed to represent the normal range. The average values for males and females were 35 mg/100 ml and 30 mg/100 ml, respectively. Thus the average value for the males seems to be somewhat higher than that for the females. No case with a $\beta_2$-glycoprotein I concentration above 38 mg/100 ml was found in females (Fig. 1).

![Fig. 1. $\beta_2$-Glycoprotein I concentrations in healthy controls. Open column for male; shaded column for female.](image)

$\beta_2$-Glycoprotein I concentrations in sera from patients with rheumatoid arthritis

In 65 cases of classical or definite rheumatoid arthritis which were composed of 18 males and 47 females, the $\beta_2$-glycoprotein I concentrations were between 18 and
42 mg/100 ml with an average of 30 mg/100 ml in most of the patients (Fig. 2). This indicates that the values were significantly decreased in patients with rheumatoid arthritis as compared with the normal healthy persons (p<0.01).

**Relationship between \( \beta_2 \)-glycoprotein I and other glycoprotein in rheumatoid arthritis**

Kosaka and Kitabatake (1970, 1971) reported significant increases in \( \alpha_1 \)-antitrypsin and \( \alpha_1 \)-acid glycoprotein concentrations, and a decrease in \( \alpha_2 \)-HS glycoprotein concentration in the sera of patients with rheumatoid arthritis.

There was an inverse high correlation between \( \beta_2 \)-glycoprotein I and \( \alpha_1 \)-
antitrypsin but was a low correlation between $\beta_2$-glycoprotein I and $\alpha_2$-HS glycoprotein, showing -0.29 of coefficient of correlation for $\alpha_1$-antitrypsin and 0.20 for $\alpha_2$-HS glycoprotein (Figs. 3 and 4). On the other hand, there was no definite relationship between $\beta_2$-glycoprotein I and $\alpha_1$-acid glycoprotein (Fig. 5).

Relationship between $\beta_2$-glycoprotein I and CRP in rheumatoid arthritis

CRP tests were performed in 54 cases. An inverse correlation was found between the $\beta_2$-glycoprotein I levels and degrees of the CRP rates, since the $\beta_2$-glycoprotein I concentrations for the positive group of CRP were significantly lower than for the negative group of CRP ($p<0.001$, Fig. 6).

**DISCUSSION**

Cleve (1968) showed that, although the concentration of $\beta_2$-glycoprotein I in serum is influenced by factors like age, diseases (e.g., chronic liver diseases such
β₂-Glycoprotein I

as liver cirrhosis) and pregnancy, the individual levels of this protein are also controlled by genetic factors. The results of family studies suggested that these concentrations are controlled by a pair of autosomal co-dominant alleles: Bg¹ and Bg². Bg¹ and Bg² with individual homozygous for the common allele Bg¹ were found to have β₂-glycoprotein I levels between 16 and 30 mg/100 ml, and heterozygous ones between 6-4 mg/100 ml. Individuals homozygous for the allele Bg² showed deficiency of β₂-glycoprotein I (less than 5 mg/100 ml). Haupt et al. (1968) have also demonstrated such a case.

Apart from the original data of Cleve (1968) and Cleve and Rittner (1969), there were observed population frequencies for several Caucasoid, Mongoloid and Negroid populations by Koppe et al. (1970), who showed all samples of Caucasoid origin to have approximately similar mean values, whereas the Negroid and Mongoloid races had a much lower frequency, of the more common Bg¹ allele. Subsequently, Atkin and Rundle (1974) reported that the levels of serum β₂-glycoprotein I were estimated on 381 healthy adult subjects of English origin and the frequency of Bg¹ gene was found to be 0.941 (s.e. 0.09), which is in close agreement with pooled data of Caucasian subjects of 0.937 introduced by Koppe et al. (1970).

Cleve (1968) demonstrated that serum concentrations of β₂-glycoprotein I in 94% of healthy persons varied from 16-30 mg/100 ml with a mean value of 21.3 ± 3.6 mg/100 ml, whereas the lower concentrations were found in 6% of the sera: x = 10.0 ± 1.3 mg/100 ml. Similar observations were published by Cohnen (1970), who found 22.6 mg/100 ml for a mean value of the serum concentrations in healthy adults. However, the normal values of the healthy Japanese adults in the present study are remarkably higher than those in the previous reports. On the contrary, it is of interest that Koreans belonging to the same oriental as Japanese have considerably low β₂-glycoprotein I concentrations in their sera (Koppe et al. 1970).

Besides the genetic mechanism, the concentration of β₂-glycoprotein I is influenced by other factors such as age, sex and various pathological conditions. In newborn serum, the concentration is about a half of that of adults (Cleve 1968). Men are reported to have slightly higher β₂-glycoprotein I levels than women (Cleve 1968). This tendency corresponds to the present finding.

Serum concentrations of β₂-glycoprotein I are significantly decreased in the patients with rheumatoid arthritis as compared with the healthy control persons in the present study. This result did not agree with the earlier observations by Cleve (1968), who showed values corresponding to the normal range in 25 patients with active rheumatoid arthritis. It is the known fact that the α₁-antitrypsin and α₁-antichymotrypsin belong to the group of the acute phase proteins which respond to inflammatory stimuli with a substantial rise in concentration (Kueppers 1968; Aronsen et al. 1972; Kosaka and Tazawa 1976). In rheumatoid arthritis, CRP is present in practically all patients with clinical evidence of disease activity. It trends to parallel the sedimentation rate closely (Ziff and Baum 1966). Because β₂-glycoprotein I levels are inversely proportional to α₁-antitrypsin concentrations
and CRP rates in rheumatoid arthritis, the present findings suggest that this new glycoprotein has also some relation with the acute phase protein and the activity of the disease in the patients.

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References