Serum Glycoproteins in Coronary Artery Disease

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SUMMARY Serum glycoprotein levels were compared in two groups of age- and sex-matched patients, 15 with coronary artery disease and 14 normal controls. While total glycoprotein levels were increased in the coronary group, significantly higher levels were found in only five of 16 glycoproteins — $C_4$, haptoglobin, GC-globulin, $\alpha_1$-acid glycoprotein, and $C_4$ activator — with no change in 10 other glycoproteins and significant decrease in transferrin.

This study demonstrates what appears to be a glycoprotein profile in coronary artery disease and reviews possible interactions of glycoproteins with known risk factors in atherogenesis.

ELEVATION OF SERUM PROTEIN-BOUND CARBOHYDRATE has been reported in a number of unrelated disease entities including diabetes,1 infections and inflammations,2,3 malignancy,4 and hyperlipidemic subjects of all types.5 To account for this elevation in hyperlipidemic subjects, a specific profile of glycoprotein response has been described. This is characterized by elevations of $\alpha_1$-acid glycoprotein, hemopexin, haptoglobin, ceruloplasmin, and the complement proteins $C_4$, $C_2$, and $C_4$ activator.5,6

In addition, there have been reports describing elevation of total serum glycoproteins in chronic atherosclerotic cardiovascular disease.7 However, because there is no information as to the precise glycoprotein changes in this latter condition and also because of a possible association between glycoprotein abnormalities and increased atherogenesis, the following study was undertaken.

Methods

Fifteen patients who had documented chronic symptomatic coronary arterial disease but were free of any other disease entities were selected for this study. These patients all had myocardial infarctions at least one year in the past, as defined by the usual clinical and laboratory criteria or else had significant symptomatic angina pectoris with coronary visualization documenting significant atherosclerosis. None were hypertensive or diabetic nor were any hyperlipidemic subjects included (based on their having normal lipid profiles). In addition, no patient who had an acute infarction was included if it had occurred within a year of the study. None of the patients showed evidence of diabetes even with the occurrence of myocardial infarction.

Also excluded were patients with a history of any recent infectious process or acute inflammatory process within the year preceding the study.

Fourteen normal control subjects were used for comparison. These were free of any apparent disease and were age and sex-matched. The mean age of the normal controls was 48.9 ± 3.8 and this group contained six females and eight males. The mean age for the coronary patients was 50.1 ± 5.5 and there were also six females in this group. They were also matched for race.

Both groups of patients and controls were recruited from volunteers. In the case of controls they were hospital employees. The patients were ambulatory outpatients who have informed consent for venipuncture. No patient was on any drug (including estrogens, androgens or steroids) which has ever been shown to affect blood protein concentrations. Random glucose values in the study patients were at all times under 105 mg%, fasting cholesterol under 240 mg%, and fasting triglyceride under 130 mg%. In the control group, these studies were also in the same limits.

Single samples from both patients and controls were collected over a three week period. Red blood cells were removed from plasma by centrifugation and the serum stored at $-10^\circ$ C for studies.

Protein-bound carbohydrate — a measure of the total serum glycoprotein level — was determined by the Phenol-Sulfuric method using a standard composed of galactose, mannose, and fucose in the ratio of 5:5:1. This is similar to the carbohydrate composition in serum glycoproteins.

Quantitation of individual serum glycoproteins was performed by standard radial immunodiffusion using plates containing monospecific antiserum obtained from Behring Lab.

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Diagnostics. All samples were run against three provided standards for each glycoprotein.

Samples and standards were done in duplicate and in a blind fashion with no knowledge of the source of any specimen. Three standards were run on each immunodiffusion plate along with the specimens. Diameters of precipitin rings were measured with an instrument provided by Behring Diagnostic Company using a dissecting microscope. Diameters of standard rings were plotted on semilog paper against concentrations. All samples of each single protein were determined on a single day and all proteins were studied within a two week period.

The precision of these determinations when compared to serum of known concentrations was found to be within ± 4%. The results of glycoprotein determinations when repeated on successive days and successive weeks were found to show no statistically significant variation using Student's t-test.

When blood was redrawn three weeks later on selected individuals to determine stability of glycoprotein profile, no statistically significant variation was seen.

With regard to statistical analyses, independent random samples were drawn within the two groups of patients who were later age- and sex-matched between groups. Mean values and standard errors for each glycoprotein measured were calculated and compared between the two groups by the difference of means test. The significance was determined from Student's t distribution. Since the sample size was relatively small, the correct degree of freedom was calculated using the following formula:

\[
\text{df} = \frac{[s_1^2/(N_1 - 1) + s_2^2/(N_2 - 1)]^2}{[s_1^2/(N_1 - 1)]^2/(N_1 + 1) + [s_2^2/(N_2 - 1)]^2/(N_2 + 1)} - 2
\]

Rejection of the null hypothesis was set at the 5% level or below.

Our studies measured all of the serum glycoproteins which could be quantitated by immunodiffusion.

Results

Table 1 indicates that there is a significant elevation of the total serum protein-bound carbohydrate in the patients with vascular disease. This is a good measure of total serum glycoprotein levels.

When individual glycoproteins were measured it can be seen (table 2) that significant increases occurred in five of these — C\(_4\)-C, haptoglobin, GC-globulin, \(\alpha_1\)-acid glycoprotein, and C\(_3\) activator (B\(_2\) glycoprotein II). The greatest changes occurred in haptoglobin, \(\alpha_1\)-acid glycoprotein, and C\(_3\) activator. In addition, it can also be seen that transferrin is significantly decreased when compared to the normal controls. Ten other specific serum glycoproteins show no significant changes in coronary artery disease.

Table 3 gives the individual values of protein-bound carbohydrate for all patients plus the individual values for each serum protein which was altered statistically by coronary artery disease.

Discussion

This study demonstrates that patients with documented coronary arterial disease have elevations of total serum glycoproteins as measured by an elevated serum protein-bound carbohydrate level. This test measures total levels of hexasose and fucose bound to serum glycoprotein. Thus serum protein-bound carbohydrate might be increased in conditions producing uniformly increased levels of all glycoproteins, increased levels of only certain glycoproteins, or even in conditions producing increased amounts of carbohydrate bound to the same amount of total glycoprotein. The latter event would be very unusual and it is to be expected that an increment in serum protein-bound carbohydrate would correlate with actual increases in the levels of certain or all of the serum glycoproteins. There is, however, no specificity in this test as to which of the glycoproteins are affected.

In addition to the hexasose and fucose bound to the serum glycoproteins there are also other protein-bound carbohydrates including sialic acid and amino sugars but these are not measured by the Phenol-Sulfuric Assay. There is no evidence to suggest that they would affect the assay. One should therefore expect a reasonable parallel of data from this assay with total protein-bound hexasose and fucose levels and therefore with total glycoprotein levels.

However, this Phenol-Sulfuric method is nonspecific. Certain glycoproteins may have increased which were not assayed immunologically by our studies. In addition, it is possible that there were increments in the carbohydrate composition of certain glycoproteins as a result of coronary disease. The results obtained from the Phenol-Sulfuric method may not correspond precisely with the results obtained by immunodiffusion.

In order to determine specificity, then, the individual serum glycoproteins are analyzed separately. The data indicate that not all of them are significantly elevated. Rather there is a significant profile of response characterized by elevations of several, a depression of one, and no significant change in ten others.

This pattern of glycoprotein response is not identical to that previously reported in hyperlipidemic subjects without apparent vascular disease where similar elevations in the complement components C\(_4\)C and C\(_4\) activator as well as haptoglobin and \(\alpha_1\)-acid glycoprotein were noted but also those patients had elevation of C\(_6\), ceruloplasmin and hemopexin and did not have any change in GC-globulin or transferrin unlike the patients with coronary disease in this study.

In a recent study of glycoprotein changes immediately after acute myocardial infarction there are a number of similarities to the data presented here. However, in that study many additional proteins were observed to increase and in the case of GC-globulin that study found decreases while an increase is seen in chronic coronary disease. Conversely, transferrin has been found to decrease in chronic coronary disease but increase after infarction.

The present study thus identifies a pattern of abnormalities in serum glycoproteins in patients with documented...
coronary artery disease. This pattern of response is not quite identical to any glycoprotein profile previously described nor has a similar study been performed on a group of patients with coronary artery disease. Whether this difference is real or a result of the small numbers of patients in the varying studies is not yet certain. However, statistical significance at $P < 0.05$ was found for this pattern of glycoprotein response. In any case it appears that in chronic coronary artery disease there are abnormalities in serum glycoproteins and that this process does not affect all of them.

The etiology of these changes is uncertain for they do not appear to be related to the so-called acute phase reaction of infection or inflammation. This reaction consists of simultaneous rises in haptoglobin, ceruloplasmmin, $\alpha_1$-acid glycoprotein, and $\alpha_1$-antitrypsin with a fall in transferrin. Moreover, none of these patients had any clinically apparent acute infection or acute vascular event within the year preceding the study.

Regardless of the etiology of these glycoprotein changes abnormalities in serum glycoproteins have been linked with atherogenesis. One interaction may be between serum glycoproteins and serum lipoproteins. Some studies suggest an

### Table 2. Glycoprotein Levels in Coronary Artery Disease

<table>
<thead>
<tr>
<th></th>
<th>Increased (mg%)</th>
<th>Normal (mg%)</th>
<th>Coronary disease (mg%)</th>
<th>% Change</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-acid GP</td>
<td>86.1 &lt; 3.0</td>
<td>135 &lt; 14</td>
<td>57.1% &lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$_s$ activator</td>
<td>22.3 &lt; 1.5</td>
<td>33.6 &lt; 3.2</td>
<td>50.7% &lt; 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased</td>
<td>20.3 &lt; 4.0</td>
<td>22.5 &lt; 1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transferrin</td>
<td>308 &lt; 8</td>
<td>279 &lt; 10</td>
<td>9.3% &lt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
actual physical interaction of the two proteins in the arterial wall initiating atherosclerotic plaque formation. There is other additional evidence even linking serum glycoproteins with the induction of hyperlipidemia. In certain cases hyperlipidemic subjects may have inhibition of the enzyme lipoprotein lipase. This may be due to a circulating enzyme inhibitor which appears to be an alpha globulin — a fraction rich in the serum glycoproteins. Thus lipoprotein lipase may be inhibited by serum glycoproteins as well as by the already described apolipoprotein inhibitors. This could occur locally in the arterial wall even in the absence of hyperlipidemia.

There is also evidence associating abnormal platelet aggregability with the presence of atherosclerotic disease in the form of both angina pectoris and myocardial infarction. Even without definite vascular disease, hyperlipidemic states likewise are associated with platelet aggregation abnormalities. The exact interaction on atherogenesis of such abnormalities is not yet clear; however, studies are available to show that abnormal platelet aggregability may result from a disordered ratio of fractions of serum, each containing different glycoproteins. It would appear from those studies that an abnormality of serum glycoproteins from whatever cause could result in increased platelet aggregation and thus possibly in an increased rate of atherogenesis. There is no evidence suggesting, in contrast, that the increased rate of platelet aggregation might affect serum glycoprotein levels. This effect of serum glycoprotein changes on platelet function might be exemplified by the recently described inhibitory effects of alpha-acid glycoprotein on platelet aggregation. This inhibitory effect might be more than compensated for if it can later be shown that other glycoproteins which we have found to increase in coronary disease facilitate platelet aggregation. Another example of a possible interaction of glycoproteins and platelet function might be in hyperlipoproteinemia in which both levels of certain serum glycoproteins as well as platelet aggregation have been found to be distinctly abnormal.

Similarly, abnormalities of the complement system have been implicated in atherogenesis and it is known that the components of this system are glycoproteins.

This study, however, in no way shows how these abnormalities could be involved in the atherosclerotic process. Still, data are clearly evident from the literature linking abnormalities of serum glycoproteins to known factors correlated with atherogenesis such as platelet aggregation or hyperlipidemia. Most of these studies have at best identified only fractions of serum, each containing multiple glycoproteins rather than individual glycoproteins. Therefore, it can only be said at this time that the demonstrated abnormalities in serum glycoproteins in this study could conceivably be involved in the atherosclerotic process. This effect could be as a primary disturbance of glycoproteins resulting in secondary effects on lipids and platelets with their known atherogenic effect. It is also possible that the abnormalities in glycoproteins may result from the atherosclerotic process itself and potentiate already present contributory factors such as hyperlipidemia or abnormal platelet aggregation. This is all speculative and current studies are under way in an attempt to define the role of these glycoprotein abnormalities.

References

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