

Serum Levels of Sialic Acid and Neuraminidase Activity in Cardiovascular, Diabetic and Diabetic Retinopathy Patients

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Abstract

Background: Sialic acid is a component of serum that is elevated in diseases such as diabetes and certain malignancies. The normal range of SSA concentration and serum neuraminidase activity in different populations are varied, probably due to racial differences.

Objective: The purpose of the present study was to obtain the average SSA concentration and serum neuraminidase activity, in an Iranian population, and to show whether these indices could indicate the severity, and serve as risk factors, for diabetes and CVD.

Methods: Serum sialic acid (SSA) concentration and neuraminidase activity were measured in 214 male and female patients and 150 normal individuals. The patient groups were composed of diabetics, diabetics with vascular disease and CVD patients. A mean±SEM value of 60.06±3.36 mg/100 ml for SSA and 50.82±2.93 mU/ml for serum neuraminidase activity were obtained in the randomly selected normal controls.

Results: SSA was significantly higher in the patient groups as compared to the values in the age and sex-matched controls. Increased SSA in the diabetics with vascular complications was significantly higher than that for diabetics without retinopathy. The serum neuraminidase activity was also increased in the patient groups. In contrast to the pattern for SSA levels, serum neuraminidase activity in the diabetic patients was not significantly lower than that for diabetics with retinopathy.

Conclusion: While serum neuraminidase activity may serve as a factor which tends to increase in CVD, diabetic and retinopathic patients, it may not be as reliable as the SSA level which correlates the severity or monitoring of these diseases. However, it can be a useful index to be used along with SSA measurements.

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Keywords • Sialic acid • neuraminidase • diabetes • retinopathy

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Introduction

Sialic acid is an important component of serum that is elevated in diseases such as diabetes and certain malignancies. In a prospective study in 1991, Linderberg et al found that serum sialic acid (SSA) concentration was a strong predictor of CVD mortality.¹ Other studies showed that SSA was elevated in diabetes mellitus.² The concentration of SSA was reported to further increase in diabetic patients with retinopathy.² This increase, however, was not present in the study on United Arab Emirate population.³ Also in diabetic patients with or without retinopathy or CVD complications, the elevation of serum neuraminidase activity has been shown in all populations studied.⁴ The enzyme neuraminidase releases sialic acid from sialic acid containing lipoproteins and glycoproteins. The normal ranges of SSA concentration and serum neuraminidase activity in different populations are varied, probably due to racial differences. The purpose of the present study was to obtain the average SSA concentration and serum neuraminidase activity in 150 randomly selected healthy control subjects from the university personnel and students in Shiraz and to compare these values with those for the patient groups which comprised 214 individuals. It was also attempted to show whether these indices could indicate the severity, and serve as risk factors, for diabetes and CVD.

Patients and Methods

A control group comprising 150 apparently healthy individuals (78 F and 72 M), aged 20 to 79 years with no history of any serious disease, together with a total of 214 patients in the following four groups were studied. Group 1 consisted of 45 diabetic (NIDDM) cases with CVD, (22F and 23 M) aged 36 to 80 years, with a history of 6 months to 20 years of diabetes. These patients had been referred to the angiography department of Faghihi Hospital for angiography. Group 2 included 45 CVD patients, (19 F and 26 M) 40 to 72 years of age with no history of diabetes. Group 3 contained 62 diabetic (NIDDM) patients, (30 F and 32 M), 34 to 73 years of age with a history of diabetes ranging between 1 to 25 years. Group 4 comprised 62 diabetics (29 F and 33 M) with retinopathy and 6 months to 21 years of diabetes.

Five ml of fasting venous blood were collected from each subject and serum was separated immediately and stored at -70 °C until used. Analysis of serum samples for a given component from all groups was carried out under the same conditions. SSA was measured using the colorimetric thiobarbituric acid procedure developed by Danny.⁵ Measurement of the serum neuraminidase activity was performed employing a coupled enzyme assay as described by Ziegler and Hutchinson,⁶ This procedure was based on the measurement of sialic acid release from bovine submaxillary mucin. The released sialic acid was subsequently quantitated

Table 1: Concentration of serum sialic acid (SSA) and serum neuraminidase activity in different male and female age groups in normal subjects.

| Age (yr) | Sex | SSA (mg/100ml) | Neuraminidase activity (mU/ml) |
|------------------------------|-----|--------------------|--------------------------------|
| 20-29 | F | 60.88±3.37 (n=32) | 50.68±2.55 (n=32) |
| | M | 58.13±3.41 (n=30) | 50.24±2.53 (n=30) |
| 30-39 | F | 60.25±3.1 (n=16) | 50.13±2.91 (n=16) |
| | M | 58.00±2.98 (n=13) | 49.46±2.27 (n=13) |
| 40-49 | F | 62.09±3.40 (n=23) | 52.26±2.86 (n=23) |
| | M | 57.47±3.14 (n=15) | 51.00±2.49 (n=15) |
| 50-59 | F | 68.82±3.59 (n=10) | 54.73±1.96 (n=10) |
| | M | 59.29±3.23 (n=6) | 53.29±2.61 (n=6) |
| 60-79 | F | 72.00 (n=1) | 60.00 (n=1) |
| | M | 56.00±2.91 (n=4) | 51.66±3.14 (n=4) |
| 20-79 (Overall for each sex) | F | 61.88±3.48 (n=82) | 51.17±2.67 (n=82) |
| | M | 57.95±3.24 (n=68) | 50.40±3.14 (n=68) |
| 20-79 (Overall) | | 60.06±3.36 (n=150) | 50.82±2.93 (n=150) |

Values are mean±SEM, n = number of subjects

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Table 2: Concentration of SSA and serum neuraminidase activities of 5 patient groups compared with normal cases adjusted for age and sex.

| Groups | No. of subjects | SSA (mg/100ml) | Neuramidase Activity (mU/ml) |
|-------------------|-----------------|--------------------------|------------------------------|
| Normal | 59 | 61.20±3.01 ^a | 52.25±2.70 ^a |
| Group I (D + CVD) | 45 | 104.35±4.81 ^b | 65.49±2.69 ^b |
| Group II (CVD) | 45 | 69.66±2.66 ^c | 64.60±2.96 ^{bc} |
| Group III (D) | 62 | 82.98±3.68 ^d | 64.71±2.69 ^{bd} |
| Group IV (D + R) | 62 | 91.80±4.90 ^b | 64.61±2.94 ^{be} |

Values are mean±SEM. $p < 0.05$ (according to the one-way ANOVA and Duncan's multiple range as *post hoc* test)
D = Diabetes, CVD = Cardiovascular disease

by a modified thiobarbituric acid method.⁷

Statistical Analysis

Descriptive statistics were reported as the mean and standard error of the mean (SEM) for all data. The mean for SSA and serum neuraminidase activity, were adjusted for age and sex and analyzed using Student's *t* test and one-way ANOVA with Duncan's multiple range as the *post hoc* test.

Results

The normal subjects were classified into five age subgroups for each sex. The mean values for the concentration of SSA and serum neuraminidase activity, in these subgroups, as well as an overall value for each gender and both sexes are given in Table 1. The SSA levels in various male age groups were not statistically significant (by one-way ANOVA and Duncan's test). For the females using one way ANOVA and Duncan test, SSA level, in comparison with other age subgroups showed a significant increase ($p < 0.05$) from 62.09 to 72.00 mg/100 ml in postmenopausal period (50-79 years). The mean values of SSA concentration and serum neuraminidase activity for males and females and an overall value for each sex and both sexes for four patient groups and normal controls are reported in Table 2. Only data for 59 out of 150 normal subjects could serve as age- and sex-matched control groups. SSA in all four patient groups was significantly higher than their normal controls. Serum neuraminidase activity was increased in all groups as compared with the normal controls.

Discussion

The mean value for SSA, first reported in Iranians, in a population of 150 healthy subjects was 60.06±3.36 mg/100 ml which is lower than those reported for south Asians^{5,3} Europeans⁸ and Swedish populations.⁹ The difference in SSA levels for various

populations has been shown to affect the risk of CVD, and attributed to race and to the serum levels of acute phase proteins.¹⁰ Results from our patient groups demonstrated that the SSA levels were significantly higher compared to those of normal controls, meaning that pathological conditions such as CVD, diabetes and retinopathy may lead to an increase in SSA level as shown by others.^{1,2,3} In patients with CVD, an increase in SSA level may be due to the release of free and bound sialic acid from damaged myocardial cells or vascular endothelium.¹¹ The anticipated higher SSA level in diabetic patients is explained by the high level of serum acute phase proteins, known to be present in both type 1 and type 2 diabetes mellitus.¹² Abnormalities of red blood cell membrane in diabetic patients, with subsequent release of sialic acid, were also found to be yet another contributing factor to increased SSA levels in diabetic patients.¹² In the diabetic patients when complications of CVD or retinopathy were present, the SSA levels were still higher than those found in diabetics alone. However, CVD was significantly more effective than retinopathy in increasing the SSA in diabetic patients. Groom et al³ found that diabetic patients with retinopathy had similar levels of SSA to the diabetic patients with no retinopathic complication while the results from other studies indicated a significantly higher SSA level in diabetic patient with retinopathy as compared with that of diabetics with no retinopathy.³ Our results are in keeping with the latter finding. Serum neuraminidase activity in our population of 150 healthy subjects, had a mean value of 50.82 ± 2.93 mU/ml. The only reported serum neuraminidase level we found in the literature is that obtained in Turkey as 55.56±5.1 mU/ml which is somewhat higher than that of our population.⁴ Serum neuraminidase activity, however, was increased in their patient groups although the rise was not present or statistically significant for some of the patient groups. Therefore, while serum neuraminidase activity may serve as a factor which tends to increase in CVD, diabetic and retinopathic patients, it may not be as reliable as the SSA level which affects the severity or monitoring of these

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