SERUM N-ACETYL-β-D-GLUCOSAMINIDASE ACTIVITY
IN A LARGE POPULATION
— A Useful Index of Cardiovascular Impairment —

Gakuji Nomura, M.D., Shoji Sakai, M.D., Michimasa Sumie, M.D.
Hiromi Tashiro, M.D., and Hironori Toshima, M.D.

Serum N-acetyl-β-D-glucosaminidase (NAG) levels were measured in 1080 residents in Tanushimaruko, Fukuoka, aged 20 to 84 years old, during a periodical epidemiological survey performed in 1982. Thirteen pregnant women were excluded from this study.

Serum NAG levels showed an increase with age, but were not different between sexes. We found high serum NAG values in those with high blood pressure, high serum total cholesterol, low serum HDL-cholesterol, or reduced creatinine clearance rate, and women with high serum uric acid, increased skinfold thickness, or high hematocrit.

Multiple regression equation was as follows: \[ NAG = 3.53 + 0.07 \text{(age)} + 0.14 \text{(hematocrit)} + 0.03 \text{(total skinfold thickness)} + 0.04 \text{(systolic blood pressure)} - 0.03 \text{(HDL-cholesterol)} - 0.04 \text{(mean blood pressure)} - 0.01 \text{(creatinine clearance)}. \] The multiple correlation coefficient was 0.37 (F = 24.4).

We suggest that NAG may be a useful index in screening cardiovascular impairment and for cardiovascular risk factors.

Increased serum N-acetyl-β-D-glucosaminidase (NAG) levels have been reported in diabetes mellitus,1-4 myocardial infarction,1-5 some types of liver diseases,1 malignancies,1,2 atherosclerosis,6 essential hypertension,7 and renovascular hypertension.8 Therefore, NAG may be not a specific but a useful index of the cardioangiopathy, when the existence of non-cardiovascular diseases such as malignancies, liver diseases and so forth have been precluded.

The present study investigates the levels of serum NAG in a large population to discover the relationship between NAG and the risk factors for cardiovascular diseases.

MATERIAL AND METHODS
During periodical epidemiological survey performed in 1982 in Tanushimaruko, Fukuoka, Japan, we examined 77.3% of the population who were 20 years old or older (Table I). The age range was found to be from 20 to 84 years old involving 460 males and 620 females; a total of 1080 people. Thirteen of these were pregnant women and were excluded from our study.

The group examinations were performed from 8:00 AM to 5:00 PM between Sept. 20, 1982

Key Words:
N-acetyl-β-D-glucosaminidase (NAG)
Age
Sex
Cardiovascular risk factor

(Received April 23, 1984; accepted October 23, 1984)
Third Department of Internal Medicine, Kurume University School of Medicine, Fukuoka, Japan
This study was supported in part by the Research Grant of the Kimura Memorial Heart Foundation.
Mailing address: Gakuji Nomura, M.D., Third Department of Internal Medicine, Kurume University School of Medicine, Kurume, Fukuoka 830, Japan

Japanese Circulation Journal Vol. 49, January 1985
TABLE I  SUBJECTS IN EACH AGE GROUP AND SEX

<table>
<thead>
<tr>
<th>Sex</th>
<th>20–29</th>
<th>30–39</th>
<th>40–49</th>
<th>50–59</th>
<th>60–69</th>
<th>70–</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>42</td>
<td>76</td>
<td>100</td>
<td>119</td>
<td>73</td>
<td>50</td>
<td>460</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td>103</td>
<td>120</td>
<td>135</td>
<td>125</td>
<td>79</td>
<td>607</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>179</td>
<td>220</td>
<td>254</td>
<td>198</td>
<td>129</td>
<td>1067</td>
</tr>
</tbody>
</table>

Age (mean ± SD): Male 50.2 ± 15.1, Female 51.6 ± 15.0.
13 pregnant women were not included in this study.

Fig. 1. Serum NAG in each age group and sex.

and Nov. 12, 1982. There were no dietary restrictions. Some careful detailed questioning and measurements of body weight and height were undertaken. Electrocardiograms were taken, and blood pressures were measured, with subjects in the supine position. Then, blood was withdrawn for the estimation of serum total protein, total cholesterol, HDL-cholesterol, uric acid, creatinine, NAG, blood urea nitrogen and hematocrit, in the sitting position.

Skinfold thickness was measured in the area of the triceps muscle of the arm and in the subscapular area using a subcutaneous fat meter (Eiken method: Meiko Co.), and the sum of these is presented as total skinfold thickness.

The blood pressure was measured using a sphygmomanometer. The mean arterial pressure was calculated as the diastolic blood pressure plus one-third of the pulse pressure.

Serum total protein, total cholesterol, HDL-cholesterol, creatinine, uric acid, and blood urea nitrogen were measured by an autoanalyzer. NAG was measured using a Shionogi-Kit® which was a colorimetric method using artificial substrate (m-cresolsulfophthaly1-N-acetyl-β-D-glucosaminide). The creatinine clearance rate was evaluated from the serum creatinine value using the formula of Duarte®.

Results are presented as mean ± standard deviation (mean ± SD). The statistical analysis of
Fig. 2. Serum NAG of healthy subjects in each age group.

Fig. 3. Serum NAG in normotensives, borderline hypertensives and hypertensives by WHO classification.
the data was made using Student's t-test, and was interpreted as significant when p-values were < 0.05. Partial correlation coefficients and a multiple regression equation were also calculated. The statistical analysis was performed using a FACOM MI30F computer.

RESULTS

Results showed that serum NAG levels increased with age as shown in Fig. 1. However, the difference between the groups of 60–69 years old and of 70 years or older was not statistically significant. Serum NAG levels were not different between the sexes except in the group of those 30–39 years old. In this age group, the NAG of males was significantly higher than that of females. When subjects were restricted to apparently healthy 375 persons (i.e. blood pressure less than 140/90 mmHg, total cholesterol less than 220 mg/dl, creatinine clearance rate more than 70 ml/min, uric acid less than 6.7 mg/dl (male) or 5.5 mg/dl (female), and normal urinalysis), then serum NAG levels tended to an increase with age (Fig. 2).

When the full population was classified as normotensive, borderline hypertensive, or hypertensive, according to systolic and diastolic blood pressures (shown in Table II), then the serum NAG level of the hypertensive group was significantly higher than that of the normotensive group. This trend was more clear when the population was subdivided into the 3 groups according to the classification of hypertension by the World Health Organization\(^1\) (Fig. 3).

Groups with high total cholesterol, or low HDL-cholesterol, presented high serum NAG, and those with low total cholesterol or high HDL-cholesterol presented low serum NAG, respectively (Table II and Fig. 4).

When all subjects were divided by renal function into 2 groups, then serum NAG level of the group with normal creatinine clearance rate was lower than that of the group with reduced creatinine clearance rate (less than 69 ml/min), (Table II).

In females, the higher the uric acid, the thicker the total skinfold thickness and the higher the hematocrit, the more increased NAG was (Table III). These trends were not evident in males.

The partial correlation coefficients between the age adjusted serum NAG levels and other various parameters are shown in Table IV.
Fig.4. Serum NAG versus HDL-cholesterol.

### TABLE III  SERUM NAG IN EACH GROUP SUBDIVIDED BY URIC ACID, TOTAL SKINFOLD THICKNESS, HEMATOCRIT AND SEX

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Uric acid (mg/dl))</td>
<td>( -4.4 )</td>
<td>( 4.5–6.4 )</td>
</tr>
<tr>
<td>NAG (U/L)</td>
<td>12.8 ± 4.7</td>
<td>12.7 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>** ns</td>
<td>** ns</td>
</tr>
<tr>
<td>(Total skinfold thickness (mm))</td>
<td>( -19 )</td>
<td>( 20–29 )</td>
</tr>
<tr>
<td>NAG (U/L)</td>
<td>12.6 ± 4.3</td>
<td>12.7 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>(Hematocrit (%))</td>
<td>( -42.9 )</td>
<td>(43.0–48.9)</td>
</tr>
<tr>
<td>NAG (U/L)</td>
<td>12.7 ± 4.7</td>
<td>12.7 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

$\times = p < 0.05; \times\times = p < 0.01; \times\times\times = p < 0.001$

A multiple regression equation of serum NAG was calculated as follows:

\[
NAG = 3.53 + 0.07 \text{(age)} + 0.14 \text{(hematocrit)} + 0.03 \text{(total skinfold thickness)} + 0.04 \text{(systolic blood pressure)} - 0.03 \text{(HDL-cholesterol)} - 0.04 \text{(mean blood pressure)} - 0.01 \text{(creatinine clearance)}.
\]

The multiple correlation coefficient was 0.37
TABLE IV  PARTIAL CORRELATION COEFFICIENTS BETWEEN AGE ADJUSTED SERUM NAG AND PARAMETERS DESCRIBED BELOW

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>0.14</td>
<td>***</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.12</td>
<td>***</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>-0.11</td>
<td>***</td>
</tr>
<tr>
<td>Total skinfold thickness</td>
<td>0.11</td>
<td>***</td>
</tr>
<tr>
<td>Mean blood pressure</td>
<td>0.10</td>
<td>***</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.09</td>
<td>**</td>
</tr>
<tr>
<td>Total protein</td>
<td>0.08</td>
<td>**</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>-0.07</td>
<td>*</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.07</td>
<td>*</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.07</td>
<td>*</td>
</tr>
</tbody>
</table>

\( x = p < 0.05; \ xx = p < 0.01; \ xxx = p < 0.001 \)

\( F = 24.4 \).

DISCUSSION

Woollen and Turner\(^1\) reported that serum NAG increased with age in 148 normal subjects. On the other hand, Welman et al.\(^3\), Belfiore et al.\(^3\), and Simon and Altfan\(^7\) reported no increase in serum NAG with age. The latter studies were carried out on small numbers of subjects; i.e. 18–76 persons. Our study, done on a large number of subjects, not only confirmed the results of Woollen and Turner\(^1\) in normal subjects (Fig. 2), but also revealed the increase in serum NAG levels with age in a general population of rural district in Japan (Fig. 1). Notably, we report that the serum NAG level of the population older than 70 years was no more increased than that of the group of 60 to 90 years old, suggesting that serum NAG reaches the plateau at sometime during the seventh decade.

Since Woollen and Turner\(^1\) reported a marked increase in serum NAG level during pregnancy, 13 pregnant women were excluded in our study. Then we found no difference in NAG with sex, confirming previous results of other investigators\(^1,3,5,7\).

As a group, hypertensive patients showed a significantly higher serum NAG value as compared with normotensive. This is consistent with the results of Simon and Altfan\(^7\). Furthermore, the populations with high total cholesterol, low HDL-cholesterol, or reduced creatinine clearance rate (Table II), and women with high serum uric acid, increased skinfold thickness, or high hematocrit (Table III), showed high serum NAG value. These data suggest that increased in serum NAG are parallel with cardiovascular risk factors.

The partial correlation coefficients between serum NAG levels and several parameters which are generally accepted to be risk factors for cardiovascular diseases were calculated. The coefficients accurately reflect the wide general population involved in our survey (Table IV). The results of the multiple regression analysis indicated that serum NAG level was dependent on age, hematocrit, total skinfold thickness, systolic blood pressure, HDL-cholesterol, mean blood pressure and creatinine clearance rate. In regards to the positive correlation between NAG and hematocrit, it is interesting to note that Letcher and coworkers\(^11\) reported elevated blood viscosity in patients with borderline essential hypertension. They suggested that the increased blood viscosity was a consequence of increased hematocrit, plasma viscosity and red cell aggregation.

It has been reported that there were increased serum NAG levels in those patients with diabetes mellitus, and especially with diabetic angiopathy\(^3,4\). Since dietary intake was not restricted prior to the blood collection in our study, we did not attempt to correlate serum NAG levels with blood sugar levels or the diabetic state in this survey.

The results of our study combined with those of others\(^1,3,5,7\) suggest that serum NAG is a useful index for cardiovascular impairment, when malignancies, liver diseases and inflammation were excluded. Since NAG is richly contained in the heart, kidney, liver, aortic smooth muscle, skeletal muscle, lung and leukocytes\(^8\) and is released into serum from the cells by an exocytosis and/or a breakdown of cells, it is logical that increased serum NAG level accompanies many various cardiovascular diseases such as ischemic heart diseases, atherosclerosis, hypertension, diabetic angiopathy and others. Therefore, increased NAG indicates rather nonspecific abnormalities. Nevertheless, the estimation of serum NAG may be useful in screening cardiovascular impairment, and serum NAG level may also be a useful index for cardiovascular risk factors, although a long-term follow-up study

*Japanese Circulation Journal Vol. 49, January 1985*
is needed before we will reach a final conclusion.

Acknowledgement

The authors thank Kohei Harada, Ph.D. for his statistical analysis and Ms. Mihoko Nakamura for her secretarial assistance.

REFERENCES

8. SHIONOGI-SEIYAKU: NAG test Shionogi.

*Japanese Circulation Journal* *Vol. 49, January 1985*