

Higher Serum Levels of Indirect Bilirubin and Polyunsaturated Fatty Acids in Japanese Centenarians with Better Performance and Nutrition Status

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Abstract

The involvement of oxidative stress in aging and age-related diseases is well known. It is of interest that indirect (unconjugated) bilirubin as an antioxidant and polyunsaturated fatty acids susceptible to peroxidation can be associated with longevity. Twenty-seven community-dwelling centenarians were studied in relation to performance and nutrition status. The centenarians with better performance status had higher serum levels of total protein, albumin, creatinine, indirect bilirubin, total cholesterol, low-density lipoprotein cholesterol, triglyceride and dihomo- γ -linolenic acid, in addition to higher blood pressure. Serum albumin significantly correlated positively with total protein, iron, total cholesterol, low-density lipoprotein cholesterol, dihomo- γ -linolenic acid and eicosapentaenoic acid, and negatively with high-sensitivity C-reactive protein. Serum indirect bilirubin significantly correlated with serum albumin, dihomo- γ -linolenic acid, arachidonic acid and docosahexaenoic acid. Serum indirect bilirubin level was associated with the degree of physical activity in Japanese centenarians. Considering antioxidant effects of indirect bilirubin and health benefits of polyunsaturated fatty acids as well as the relevant literature, the present study suggests that centenarians with better performance status have a higher content of polyunsaturated fatty acids protected from peroxidation.

Keywords: Centenarians; Performance Status; Bilirubin; Polyunsaturated Fatty Acids; Oxidative Stress

Introduction

In our previous report [1] on leukocyte telomere length in community-dwelling centenarians, it was noted that centenarians with better performance status had higher serum albumin levels probably reflecting better nutrition status. In the present report, serum biochemical data were reassessed in relation to performance and nutrition status in the same centenarians. It was found that serum levels of indirect bilirubin and polyunsaturated fatty acids (PUFAs) as well as albumin were higher in centenarians with better performance status. Considering the involvement of oxidative stress in aging and age-related diseases [2,3], it is of interest that indirect (unconjugated) bilirubin works as an antioxidant in multiple ways [4,5].

Subjects and Methods

As described in the previous report [1], people aged 100 years and over (centenarians) during the fiscal year 2010 in Matsumoto city (135 people out of 242 thousand citizens) and Shiojiri city (35 people out of 68 thousand citizens) were asked by post whether they would agree to participate in the study, which was approved by the Ethical Review Board of National Hospital Organization Matsumoto Medical Center. The written consent was obtained from 39 centenarians by return of post, but we were able to visit only 28 centenarians in their own homes or nursing homes due to their inconvenience to our schedule. In the present report, one

centenarian with the gastrostomy feeding was excluded in the present study. Subjects of 27 centenarians were classified according to the Eastern Cooperative Oncology Group scale of performance status (PS) [6]: PS 0 means normal activity ($n = 0$); PS 1 means some symptoms, but still nearly fully ambulatory ($n = 4$); PS 2 means less than 50% of daytime in bed ($n = 11$); PS 3 means greater than 50% of daytime in bed ($n = 8$); and PS 4 means completely bedridden ($n = 4$).

Blood samples were taken between 11:00 and 18:00 after meals, and stored in serum at -20°C until used. Serum PUFAs were analyzed by gas chromatography. Reference ranges are 22.6 - 72.5 $\mu\text{g/ml}$ for dihomo- γ -linolenic acid, 135.7 - 335.3 $\mu\text{g/ml}$ for arachidonic acid, 10.2 - 142.3 $\mu\text{g/ml}$ for eicosapentaenoic acid and 54.8 - 240.3 $\mu\text{g/ml}$ for docosahexaenoic acid. Serum levels of total protein, albumin, urea nitrogen, creatinine, uric acid, direct bilirubin, indirect bilirubin, glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride were measured by an automated analyzer. High-sensitivity C-reactive protein was measured by a latex-enhanced nephelometric assay. All these measurements were performed by a referee laboratory (SRL, Inc., Tokyo, Japan).

Results are expressed as mean \pm SD. Differences between two groups were analyzed by two-sided unpaired t test. Linear associations among continuous variables were assessed by Pearson's correlation coefficient (r). A statistical significance was set at $p < 0.05$.

Results

Table 1 shows clinical characteristics in centenarians classified by performance status, PS 1&2 (better performance status) or PS 3&4 (poorer performance status). The mean body mass index was low in both groups: 20.0 kg/m² in PS 1&2 and 18.6 kg/m² in PS 3&4 without a significant difference. Blood pressure was significantly higher in PS 1&2 than in PS 3&4 ($p < 0.05$ for systolic blood pressure, $p < 0.001$ for diastolic blood pressure). Ten out of 15 centenarians with PS 1&2 and 6 out of 12 centenarians with PS 3&4 were treated with antihypertensive medications. As in Table 2, the centenarians with PS 1&2 had significantly higher serum levels of total protein, albumin, creatinine, indirect bilirubin, total cholesterol, LDL cholesterol, triglyceride and dihomo- γ -linolenic acid. Among them, serum albumin showed the biggest difference ($p < 0.001$).

Performance status (PS)	1&2	3&4
<i>n</i>	15	12
Male/Female	3/12	3/9
Age (years)	101.3 ± 1.4	101.8 ± 1.8
Body height (cm)	144.9 ± 9.2	143.4 ± 8.2
Body weight (kg)	42.2 ± 8.0	38.4 ± 7.2
Body mass index (kg/m ²)	20.0 ± 2.0	18.6 ± 2.3
Systolic blood pressure (mmHg)	134.9 ± 13.2	122.0 ± 17.5*
Diastolic blood pressure (mmHg)	80.8 ± 12.3	62.7 ± 10.2***
Hypertension therapy (<i>n</i>)	10	6
Diabetes therapy (<i>n</i>)	0	1
Hypercholesterolemia therapy (<i>n</i>)	0	1

Table 1: Clinical characteristics in centenarians classified by performance status (PS) 1&2 or 3&4.

Mean ± SD; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by unpaired *t* test.

Performance status (PS)	1&2	3&4
Total protein (g/dl)	6.94 ± 0.60	6.11 ± 0.75**
Albumin (g/dl)	3.91 ± 0.48	3.03 ± 0.52***
Urea nitrogen (mg/dl)	21.6 ± 4.9	19.8 ± 8.3
Creatinine (mg/dl)	0.89 ± 0.29	0.62 ± 0.30*
Uric acid (mg/dl)	5.38 ± 2.13	4.50 ± 1.08
Direct bilirubin (mg/dl)	0.16 ± 0.06	0.15 ± 0.07
Indirect bilirubin (mg/dl)	0.34 ± 0.17	0.20 ± 0.10*
Iron (μg/dl)	61.1 ± 28.9	46.7 ± 23.2
Glucose (mg/dl)	86.5 ± 19.5	80.6 ± 26.0
Total cholesterol (mg/dl)	189.4 ± 34.1	147.5 ± 28.1**
Low-density lipoprotein cholesterol (mg/dl)	107.8 ± 27.3	81.6 ± 22.3*
High-density lipoprotein cholesterol (mg/dl)	47.1 ± 15.2	42.7 ± 11.7
Triglyceride (mg/dl)	133.9 ± 64.4	81.1 ± 29.8*
Dihomo- γ -linolenic acid (μg/ml)	32.2 ± 11.2	22.9 ± 6.7*
Arachidonic acid (μg/ml)	169.1 ± 41.5	136.8 ± 41.8
Eicosapentaenoic acid (μg/ml)	58.8 ± 27.5	40.9 ± 21.2
Docosahexaenoic acid (μg/ml)	154.9 ± 42.5	130.7 ± 61.2
High-sensitivity C-reactive protein (μg/l)	4606 ± 6985	9922 ± 18557

Table 2: Laboratory data in centenarians classified by performance status (PS) 1&2 or 3&4.

Mean ± SD; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by unpaired *t* test.

Tables 3 and 4 show correlation coefficients between serum albumin or indirect bilirubin level and other laboratory parameters in all centenarians. Serum albumin level significantly correlated with serum levels of total protein, indirect bilirubin, iron, total cholesterol, LDL cholesterol, dihomo- γ -linolenic acid and eicosapentaenoic acid. The correlation coefficient was the highest for serum total protein ($r = 0.794$, $p < 0.001$) and the second highest for serum indirect bilirubin ($r = 0.621$, $p < 0.001$). Serum albumin negatively correlated with serum high-sensitivity C-reactive protein ($r = -0.553$, $p = 0.003$). Serum indirect bilirubin level significantly correlated with serum levels of total protein, albumin, direct bilirubin, total cholesterol, LDL cholesterol, HDL cholesterol, dihomo- γ -linolenic acid, arachidonic acid and docosahexaenoic acid. Especially, it strongly correlated with serum arachidonic acid ($r = 0.638$, $p < 0.001$), similar to serum albumin.

	Correlation coefficient (<i>r</i>)	<i>p</i> value
Total protein	0.794	<0.001
Urea nitrogen	0.367	0.060
Creatinine	0.297	0.133
Uric acid	0.275	0.164
Direct bilirubin	0.052	0.799
Indirect bilirubin	0.621	<0.001
Iron	0.462	0.015
Glucose	0.072	0.721
Total cholesterol	0.499	0.008
Low-density lipoprotein cholesterol	0.484	0.011
High-density lipoprotein cholesterol	0.177	0.378
Triglyceride	0.360	0.065
Dihomo- γ -linolenic acid	0.500	0.008
Arachidonic acid	0.376	0.053
Eicosapentaenoic acid	0.385	0.047
Docosahexaenoic acid	0.361	0.064
High-sensitivity C-reactive protein	-0.553	0.003

Table 3: Correlation coefficients between serum albumin and other laboratory parameters in total centenarians.

Discussion

It was demonstrated that the centenarians with better performance status (PS 1&2) had higher serum levels of total protein, albumin, creatinine, indirect bilirubin, total cholesterol, LDL cholesterol, triglyceride and dihomo- γ -linolenic acid, in addition to higher blood pressure. As the role of serum albumin as a useful and convenient biomarker of nutrition status is well known [7-9], it is conceivable that the centenarians with better performance status (PS 1&2) had better nutrition status. In view of serum creatinine level reflecting muscle mass [10-12], it is also indicated that their muscle mass was bigger than that of the centenarians with poorer performance status (PS 3&4). Elderly people with high cholesterol levels seem to live longer [13,14]. The present study showed that

	Correlation coefficient (r)	p value
Total protein	0.381	0.049
Albumin	0.621	<0.001
Urea nitrogen	0.108	0.591
Creatinine	0.050	0.804
Uric acid	0.262	0.186
Direct bilirubin	0.393	0.042
Iron	0.262	0.187
Glucose	0.089	0.658
Total cholesterol	0.543	0.003
Low-density lipoprotein cholesterol	0.384	0.048
High-density lipoprotein cholesterol	0.437	0.023
Triglyceride	0.203	0.311
Dihomo-γ-linolenic acid	0.432	0.024
Arachidonic acid	0.638	<0.001
Eicosapentaenoic acid	0.369	0.058
Docosahexaenoic acid	0.418	0.030
High-sensitivity C-reactive protein	-0.285	0.150

Table 4: Correlation coefficients between serum indirect bilirubin and other laboratory parameters in total centenarians.

centenarians with higher serum cholesterol levels were physically more active, probably in association with better nutrition status. As inferred from the report of a systematic review and meta-analysis on blood biomarkers of malnutrition in older adults [9], it was found that serum albumin positively correlated with total protein, iron, total cholesterol and LDL cholesterol, and negatively correlated with high-sensitivity C-reactive protein in the present study.

Indirect bilirubin, serum levels of which are mildly elevated in Gilbert syndrome (a common genetic variant), is thought to have antioxidant effects by acting as a direct oxidant scavenger and by inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [4,5,15,16]. Epidemiological studies have demonstrated that Gilbert syndrome has some health benefits to reduce risk of age-related diseases [17,18]. Interestingly, a recent study reported that low serum bilirubin level is a strong predictive biomarker for disability in activities in daily living in Japanese elderly patients with diabetes [19]. Similarly, the present study indicated that serum indirect bilirubin level was associated with the degree of physical activity in Japanese centenarians.

Bilirubin, a final decomposition product of heme metabolim, is bound to albumin in order to be transported in the blood circulation. Albumin-bound bilirubin was demonstrated to protect albumin-bound linoleic acid from peroxy radical-induced oxidation [20]. Indirect bilirubin and free fatty acids are bound to albumin in the blood. In the present study, serum indirect bilirubin was found to significantly correlate with serum dihomo-γ-linolenic acid, arachidonic acid and docosahexaenoic acid, in addition to serum

albumin. This may suggest that albumin-bound bilirubin protects PUFAs from peroxidation while not competing for albumin binding, although dietary factors, not studied, should be taken into account. Arachidonic acid, despite the precursor of pro-inflammatory eicosanoids, also has potential health benefits [21,22]. On the contrary, a higher content of PUFAs susceptible to peroxidation in the blood or membrane bilayers is postulated to be unfavorable for longevity [23,24]. From the present results, it is inferred that centenarians with better performance status have a higher content of PUFAs protected from peroxidation.

Conclusions

It was found that serum levels of indirect bilirubin and PUFAs as well as albumin were higher in the centenarians with better performance status. Considering also the possible involvement of indirect bilirubin and PUFAs in longevity, the present study suggests that centenarians, the longest-lived people, with better performance status have a higher content of PUFAs protected from peroxidation.

Conflict of Interest

The author has indicated no potential conflict of interest.

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