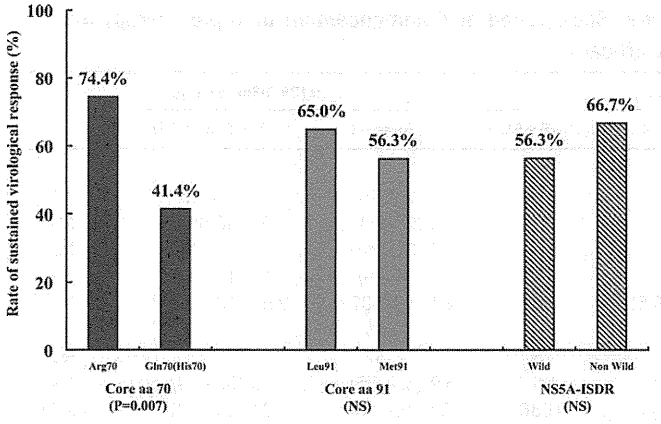

Fig. 1. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions showed sustained virological response than that of patients who showed Gln70(His70) ($P = 0.007$). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 and Met91. Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype and nonwildtype.

(56.3%) and nonwildtype (66.7%) (Fig. 1). Thus, sustained virological response was influenced by the substitution of core aa 70.

Sustained Virological Response According to Genetic Variation Near the IL28B Gene. According to the genetic variation in rs8099917, sustained virological response was achieved by 83.8% (31 of 37 patients), 29.6% (8 of 27 patients), and 0% (0 of 2 patients) in patients with genotype TT, TG, and GG, respectively. Thus, a significantly higher proportion of patients with genotype TT (83.8%) showed sustained virological response than that of patients who showed genotype non-TT (27.6%) (Fig. 2, $P < 0.001$) (Table 2).

According to the genetic variation in rs12979860, sustained virological response was achieved by 83.8% (31 of 37 patients), 34.5% (10 of 29 patients), and 0% (0 of 2 patients), in patients with genotype CC, CT, and TT, respectively. Thus, a significantly higher proportion of patients with genotype CC (83.8%) showed sustained virological response than that of patients who showed genotype non-CC (32.3%) (Fig. 2, $P < 0.001$) (Table 2).

Predictive Factors Associated with Sustained Virological Response. Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 4.12, $P = 0.007$), genetic variation in rs8099917 (genotype TT; OR 13.6, $P < 0.001$), and rs12979860 (genotype CC; OR 10.8, $P < 0.001$). Two factors were identified by multivariate analysis as independent parameters that significantly influenced sustained virological response (rs8099917 genotype TT; OR 10.6, $P < 0.001$; and Arg70; OR 3.69, $P = 0.040$) (Table 3).

Assessment of Amino Acid Substitutions in Core Region and Genetic Variation Near the IL28B Gene as Predictors of Sustained Virological Response. The ability to predict sustained virological response by substitution of core aa 70 and rs8099917 genotype near the IL28B gene was evaluated. The sustained virological response rates of patients with a combination of Arg70 or rs8099917 genotype TT were defined as PPV (prediction of sustained virological response). The nonsustained virological response rates of patients with a combination of Gln70(His70) or rs8099917 genotype non-TT were defined as NPV (prediction of nonsustained virological response).

In patients with rs8099917 genotype TT, the sensitivity, specificity, PPV, and NPV for sustained virological response were 79.5, 77.8, 83.8, and 72.4%, respectively. Thus, genotype TT has high sensitivity, specificity, and PPV for prediction of sustained virological response. In patients with Arg70 the sensitivity, specificity, PPV, and NPV were 76.9, 63.0, 75.0, and 65.4%, respectively. Thus, Arg70 has high sensitivity and PPV in predicting sustained virological response. Furthermore, when both predictors were used the sensitivity, specificity, PPV, and NPV were 61.5, 85.2, 85.7, and 60.5%, respectively. When one or more of the two predictors were used the sensitivity, specificity, PPV, and NPV were 94.9, 55.6, 75.5, and 88.2%, respectively. These results indicate that the use of the combination of the above two predictors has high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response (Table 4).

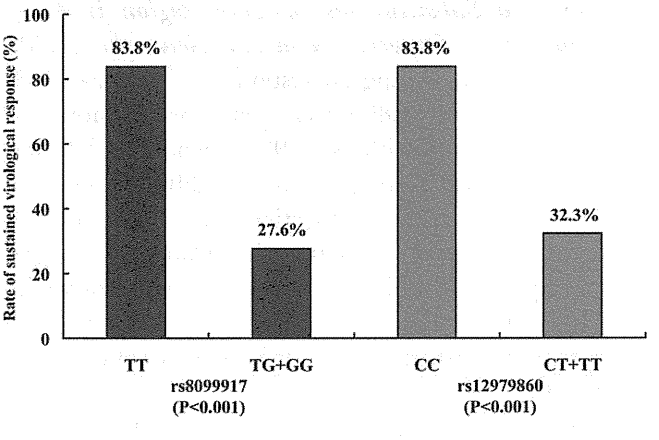

Fig. 2. According to the genetic variation in rs8099917 or rs12979860 near the IL28B gene, a significantly higher proportion of patients with genotype TT or CC showed sustained virological response than that of patients who showed genotype non-TT or non-CC, respectively ($P < 0.001$ or $P < 0.001$, respectively).

Table 2. According to Genetic Variation Near the IL28B Gene, Background at Commencement of Triple Therapy and Treatment Efficacy

	rs8099917 genotype			rs12979860 genotype		
	TT (n = 42)	TG+GG (n = 32)	TT vs. TG+GG *P*	CC (n = 42)	CT+TT (n = 34)	CC vs. CT+TT *P*
Demographic data						
Sex (M/F)	22 / 20	18 / 14	NS	22 / 20	19 / 15	NS
Age (years)*	54 (23-65)	56 (36-65)		54 (23-65)	55 (36-65)	NS
History of blood transfusion	15 (35.7%)	9 (28.3%)	NS	15 (35.7%)	9 (26.5%)	NS
Family history of liver disease	6 (14.3%)	6 (18.8%)	NS	6 (14.3%)	6 (17.6%)	NS
Body mass index (kg/m^2)*	22.1 (13.2-32.4)	22.4 (18.7-26.5)	NS	22.1 (13.2-32.4)	22.3 (18,7-26.5)	NS
Laboratory data*						
HCV genotype (1a / 1b)	0 / 42	1 / 31	NS	0 / 42	1 / 33	NS
Level of viremia (log IU/mL)	6.9 (5.4-7.5)	6.6 (5.1-7.4)	NS	6.9 (5.4-7.5)	6.5 (5.1-7.4)	NS
Serum aspartate aminotransferase (IU/L)	38 (15-118)	31 (20-137)	0.036	38 (15-118)	31 (20-137)	0.031
Serum alanine aminotransferase (IU/L)	50 (12-175)	36 (17-136)	0.029	50 (12-175)	35 (17-136)	0.014
Serum albumin (g/dL)	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS
Gamma-glutamyl transpeptidase (IU/L)	29 (9-194)	53 (9-154)	0.008	29 (9-194)	53 (9-229)	0.004
Leukocyte count (/mm^3)	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS
Hemoglobin (g/dL)	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS
Platelet count ($\times$ 10^4/mm^3)	16.8 (9.9-33.8)	17.1 (9.1-24.8)	NS	16.8 (9.9-33.8)	17.8 (9.1-28.8)	NS
Alpha-fetoprotein (μ g/L)	4 (2-39)	5 (2-38)	NS	4 (2-39)	5 (2-38)	NS
Total cholesterol (mg/dL)	184 (112-276)	178 (110-263)	NS	184 (112-276)	178 (110-263)	NS
Fasting plasma glucose (mg/dL)	97 (80-125)	90 (66-111)	0.038	97 (80-125)	91 (66-111)	0.030
Treatment regimen						
T12PR12 group / T12PR24 group	12 / 30	7 / 25	NS	12 / 30	7 / 27	NS
Ammo acid substitutions in the HCV genotype 1b						
Core aa 70 (arginine / glutamine [histidine])	30 / 12	13 / 18	0.016	30 / 12	13 / 20	0.009
Core aa 91 (leucine / methionine)	25 / 17	13 / 18	NS	25 / 17	14 / 19	NS
ISDR of NS5A (wild-type / non wild-type)	39 / 3	30 / 1	NS	39 / 3	32 / 1	NS
Past history of IFN therapy						
Treatment-naive / Relapsers to previous treatment / Nonresponders to previous treatment	16 / 24 / 2	7 / 6 / 19	<0.001	16 / 24 / 2	8 / 7 / 19	<0,001
Treatment efficacy**						
End-of-treatment response (%)	35 (94.6%)	23 (79.3%)	NS	35 (94.6%)	25 (80.6%)	NS
Sustained virological response (%)	31 (83.8%)	8 (27.6%)	<0.001	31 (83.8%)	10 (32.3%)	<0.001

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.
**Treatment efficacy according to rs8099917 genotype was evaluated in 66 patients, and that according to rs12979860 genotype was evaluated in 68 patients.

Predicting Sustained Virological Response by Amino Acid Substitutions in Core Region in Combination with Genetic Variation Near the IL28B Gene. Sustained virological response by core aa 70 in combination with rs8099917 genotype is shown in Fig. 3. In patients with rs8099917 genotype TT, sustained virological response was not different between Arg70 (85.7%) and Gln70(His70) (77.8%). In contrast, in patients with rs8099917 genotype TG and GG, a significantly higher proportion of patients with Arg70 (50.0%) showed sustained virological response than that of patients with Gln70(His70) (11.8%) (P = 0.038).

Based on a strong power of substitution of core aa 70 and rs8099917 genotype in predicting sustained virological response (Table 3), how they increase the predictive value when they were combined was evaluated. The results are schematically depicted in Fig. 3. Together they demonstrate three points: (1) the efficacy of triple therapy was high in patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70; (2) in patients having genotype TG and GG, those of Arg70 gained high sustained virological response (50.0%); and (3) sustained virological response (11.8%) was the worst in patients who possessed both of genotype TG and GG, and Gln70(His70).

Discussion

Two previous studies (PROVE1 in the US, and PROVE2 in Europe) showed that the T12PR12 and T12PR24 group of telaprevir, PEG-IFN, and ribavirin could achieve sustained virological response rates of 35%-60% and 61%-69%, respectively.[10,11] In the

Table 3. Multivariate Analysis of Factors Associated with Sustained Virological Response of Telaprevir, Peginterferon and Ribavirin Triple Therapy in Japanese Patients Infected with HCV Genotype 1

Factor	Category	Odds Ratio (95% CI)	P
rs8099917 genotype	1: TG+GG	1	
	2: TT	10.6 (3.07-36.5)	<0.001
Substitution of aa 70	1: Gln70 (His70)	1	
	2: Arg70	3.69 (1.06-12.8)	0.040

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression analysis are shown. 95% CI: 95% confidence interval.

present Japanese study, the sustained virological response rates were 45% and 67% in the T12PR12 and T12PR24 group, respectively, as in the two previous studies. There were differences at three points between the present study and two previous studies: (1) PEG-IFN in two previous studies was used at a fixed dose of PEG-IFNα-2a, but that of the present study was a body weight-adjusted dose of PEG-IFNα-2b; (2) The body mass index of our patients (median; 23 kg/m^2) was much lower than that of the participants of the previous study by McHutchison et al.[10] (median; >25 kg/m^2); and (3) The present study was performed based on Japanese patients infected with HCV-1b, except for only one patient with HCV-1a. Especially in PROVE-1, the viral breakthrough rate was higher in HCV-1a subjects compared to HCV-1b, and one of the reasons might be due to the low genetic barrier to the emergence of the R155K variant in HCV-1a.[10,27] Further studies of a larger number of patients matched for background, including genotype, race, body mass index, treatment regimen, and past history of IFN therapy are required to investigate the rate of the sustained virological response by triple therapy.

IL28A, IL28B, and IL29 (IFN-λ-2, IFN-λ-3, and IFN-λ-1, respectively) are novel IFNs identified recently.[28,29] They are similar to type 1 IFNs in terms of biological activities and mechanism of action, in contrast to their differences in structure and genetics.[30] The antiviral effects of IFN-λ against hepatitis B virus and HCV have been reported.[31] Furthermore, α and λ IFNs act synergistically against HCV.[32-34] Recent reports showed that genetic variation near the IL28B gene (rs8099917, rs12979860) are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,[18-21] and also affect clinical outcome, including spontaneous clearance of HCV.[22] At the 2009 meeting of the American Association for the Study of Liver Diseases, Thompson et al.[35] reported that genetic variation near the IL28B gene also affected the viral suppression in the first 2 to 4 weeks of PEG-IFN plus ribavirin, and this phenomenon probably explains much of the difference in treatment response rate. The present study is the first to report that genetic variation near the IL28B gene significantly also affect sustained virological response by triple therapy. These results should be interpreted with caution because races other than Japanese populations were not included. Any generalization of the results should await confirmation by studies of patients of other races to explore the relationship between genetic variation near the IL28B gene and the response to triple therapy.

The present study indicated that the use of the combination of aa substitution of the core region and genetic variation near the IL28B gene had high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response. The efficacy of triple therapy was high in the patients with TT, irrespective of substitution of core aa 70. In the patients having non-TT, those of Arg70 gained high sustained virological response, and sustained virological response was the worst in patients who possessed both non-TT, and Gln70(His70). Along with a high sustained virological response, combined PEG-IFN and ribavirin are accompanied by severe side effects and entail high

Table 4. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Sustained Virological Response, According to Substitution of Core aa 70 and Genetic Variation Near IL28B Gene

	% (Number)			
	Sensitivity	Specificity	PPV*	NPV**
(A) rs8099917 genotype TT	79.5 (31/39)	77.8 (21/27)	83.8 (31/37)	72.4 (21/29)
(B) Substitution at aa 70 of arginine (Arg70)	76.9 (30/39)	63.0 (17/27)	75.0 (30/40)	65.4 (17/26)
(A) and (B)	61.5 (24/39)	85.2 (23/27)	85.7 (24/28)	60.5 (23/38)
(A) and/or (B)	94.9 (37/39)	55.6 (15/27)	75.5 (37/49)	88.2 (15/17)

*PPV; Sustained virological response rates for patients with a combination of Arg70 or rs8099917 genotype TT (prediction of sustained virological response).

**NPV; nonsustained virological response rates for patients with a combination of Gln70(His70) or rs8099917 genotype non-TT (prediction of nonsustained virological response).

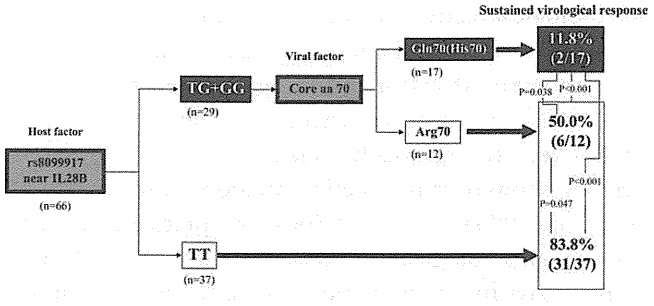

Fig. 3. Predicting sustained virological response by aa substitution in core region in combination with genetic variation near the IL28B gene. Efficacy of triple therapy was high in the patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70. In the patients having genotype TG and GG, those of Arg70 gained a high sustained virological response (50.0%), and sustained virological response (11.8%) were the worst in patients who possessed both genotypes TG and GG, and Gln70(His70).

costs. Hence, the patients who do not achieve sustained virological response need to be identified as early as possible, in order to free them of unnecessary side effects and high costs. The present study is the first to report that the combination of aa substitution of the core region and genetic variation near the IL28B gene are very useful as pretreatment predictors of sustained virological response by triple therapy, and further studies based on a larger number of patients are necessary to investigate the present results.

Other limitations of the present study were that aa substitutions in areas other than the core region and NS5A-ISDR of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR),[36] were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation,[37] were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both *in vivo* and *in vitro* studies.[38,39] Thus, viral factors before and during triple therapy should be investigated in future studies and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, triple therapy with telaprevir, PEG-IFN, and ribavirin in Japanese patients infected with HCV-1 and high viral load achieved high sustained virological response rates. Furthermore, the aa substitution pattern of the core region and genetic variation near the IL28B gene seem to affect treatment efficacy. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of triple therapy and further understanding of the complex interaction between virus- and host-related factors should facilitate the development of more effective therapeutic regimens.

Acknowledgment: This study was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

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Journal of Medical Virology 81:1032–1039 (2009)

Amino Acid Substitutions in the Hepatitis C Virus Core Region of Genotype 1b Are the Important Predictor of Severe Insulin Resistance in Patients Without Cirrhosis and Diabetes Mellitus

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Previous studies provided a direct experimental evidence for the contribution of HCV core protein in the development of insulin resistance (IR), but the clinical impact of HCV core region on IR is still not clear. The present study evaluated the impact of Amino acid (aa) substitutions of HCV-1b core region on IR in 123 Japanese patients infected with HCV-1b without cirrhosis and diabetes mellitus, and investigated the treatment efficacy of 48-week pegylated interferon (PEG-IFN) plus ribavirin (RBV) according to HOMA-IR values. Patients with IR (HOMA-IR $\geq$2.5) and severe IR (HOMA-IR $\geq$3.5) were present in 51.2% and 27.6%, respectively. Multivariate analysis identified body mass index ($\geq$25 kg/m^2) and hepatocyte steatosis ($\geq$5%) as significant determinants of IR. Furthermore, multivariate analysis identified hepatocyte steatosis ($\geq$5%), aa substitutions of the core region (Gln70 (His70) and/or Met91), and age ($\geq$55 years) as significant determinants of severe IR. Especially, significantly lower proportions of patients with Gln70 (His70) and/or Met91 were noted among those without severe IR (59.6%) than those with severe IR (82.4%). The rates of sustained virological response in patients with IR (50.0%) were not significantly different from those without IR (52.9%). Furthermore, the rates of non-virological response in patients with IR (28.9%) were not significantly also different from those without IR (20.6%). In conclusion, the present study indicated that substitutions of HCV-1b core region were the important predictor of severe IR in patients without cirrhosis and diabetes mellitus, but HOMA-IR values might be not useful as predictors of 48-week PEG-IFN plus RBV therapy. ***J. Med. Virol. 81:1032–1039, 2009.*** © 2009 Wiley-Liss, Inc.

KEY WORDS: HCV; core region; genotype; HOMA-IR; hepatocyte steatosis; cirrhosis; diabetes mellitus

INTRODUCTION

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [Dusheiko, 1998; Ikeda et al., 1998; Niederau et al., 1998; Kenny-Walsh, 1999; Akuta et al., 2001]. Furthermore, HCV infection also affects an increased risk of diabetes mellitus [Allison et al., 1994; Caronia et al., 1999; Mason et al., 1999; Mehta et al., 2000, 2003; Zein et al., 2000, 2005; Antonelli et al., 2005] or insulin resistance (IR) [Hickman et al., 2003; Hui et al., 2003; Lecube et al., 2004, 2006]. IR and glucose metabolism impairment are associated with liver necroinflammation [Hui et al., 2003], hepatocyte steatosis [Fartoux et al., 2005; Cammà et al., 2006; Conjeevaram et al., 2007], cirrhosis [Petrides et al., 1994], and HCC [El-Serag et al., 2001; Lai et al., 2006; Veldt et al., 2008]. Especially, in patients infected with HCV genotype 1 (HCV-1), significant fibrosis is associated with IR independent from hepatocyte steatosis [Moucari et al., 2008; Petta et al., 2008].

Previous studies reported that HCV core protein induced HCC and IR in transgenic mice, and provided

Grant sponsor: Ministry of Health, Labor and Welfare, Japan (partly supported).

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Accepted 5 February 2009

DOI 10.1002/jmv.21473

Published online in Wiley InterScience (www.interscience.wiley.com)

a direct experimental evidence for the contribution of HCV core protein in the development of HCC and IR in human HCV infection [Moriya et al., 1998; Shintani et al., 2004]. Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of genotype 1b (HCV-1b core region) were predictors of poor virological response to 48-week pegylated interferon (PEG-IFN) plus ribavirin (RBV) combination therapy [Akuta et al., 2005, 2006, 2007a,b,c; Donlin et al., 2007; Okanoue et al., 2008], and also risk factors for hepatocarcinogenesis [Akuta et al., 2007d, 2008]. Thus, previous reports supported the oncogenic potential of the HCV core region and clinically linked substitutions of aa 70 and/or 91 in HCV-1b core region to HCC [Akuta et al., 2007d, 2008], but the clinical impact of HCV-1b core region on IR is still not clear. IR develops type 2 diabetes mellitus as its major late feature, and is also associated with advanced fibrosis [Petrides et al., 1994; Petta et al., 2008]. Hence, the biological mechanisms underlying the association between HCV core region and IR are probably multifactorial, and study based on patients without diabetes mellitus and cirrhosis, that might affect IR, should be performed to investigate whether HCV core region might affect IR clinically.

Previous reports showed that IR might be predictors of poor virological response to PEG-IFN plus RBV combination therapy [D'Souza et al., 2005; Romero-Gómez et al., 2005]. Chu et al. [2008] reported that IR was a major determinant of sustained virological response (SVR) in HCV-1 patients receiving 24-week PEG-IFN plus RBV. However, to our knowledge, there is little evidence that IR affects treatment efficacy of HCV-1b patients receiving 48-week PEG-IFN plus RBV combination therapy.

The aims of the present study conducted in Japanese patients infected with HCV-1b without cirrhosis and diabetes mellitus, were the following. (1) To evaluate the HOMA-IR values of patients infected with HCV-1b. (2) To identify the impact of aa substitutions in the core region on IR in such patients, and determine the factors associated with IR, and (3) to investigate the treatment efficacy of 48-week PEG-IFN plus RBV combination therapy according to HOMA-IR values.

PATIENTS AND METHODS

Study Population

At Toranomon Hospital, Tokyo, Japan, 221 HCV-infected Japanese patients were consecutively recruited into the study protocol of the combination therapy with PEG-IFNα-2b plus RBV between December of 2001 and June of 2005. Among these, 123 patients were selected in the present retrospective study based on the following criteria. (1) Negativity for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positivity for anti-HCV (third-generation enzyme immunoassay, Chiron Corp, Emerville, CA), and positivity for HCV RNA qualitative analysis with PCR (Amplicor, Roche Diagnostics, Mannheim, Germany). (2) They were infected with HCV-1b alone. (3) HOMA-IR values and substitutions of aa 70 and 91 in the HCV core region were determined at the commencement of treatment. (4) They were free of cirrhosis and hepatocellular carcinoma, based on biopsy examination, laboratory tests, and imaging studies at baseline. (5) None had diabetes mellitus. (6) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (7) All were free of coinfection with human immunodeficiency virus. (8) None had other forms of hepatitis, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) Each signed a consent form of the study protocol that had been approved by the human ethics review committee. Table I summarizes the profiles and laboratory data of the 123 patients at the commencement of treatment. They included 71 males and 52 females, aged 20–70 years (median, 55 years). The treatment efficacy was evaluated by HCV-RNA positive based on qualitative PCR analysis at the end of treatment (non-virological response; NVR), and by HCV-RNA negative based on qualitative PCR analysis at 24 weeks after the completion of therapy (SVR).

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for alanine aminotransferase (ALT) and HCV-RNA levels. The serum samples were frozen at −80°C within 4 hr of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of NS5 region [Chayama et al., 1993]. HCV-RNA levels were measured by quantitative PCR (AMPLICOR GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems, Inc.) at least once every month before, during, and after therapy. The dynamic range of the assay was 5.0×10^3 to 5.0×10^6 IU/ml. Samples collected during and after therapy that showed undetectable levels of HCV-RNA ($<5.0 \times 10^3$ IU/ml) were also checked by qualitative PCR (AMPLICOR HCV v2.0, Roche Molecular Systems, Inc.), which has a higher sensitivity than quantitative analysis, and the results were expressed as positive or negative. The lower limit of the assay was 50 IU/ml.

Histopathological Examination of Liver Biopsies

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan). The biopsy material was fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens for examination contained six or more portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H.K.) who was blinded to the clinical data. Chronic hepatitis and liver cirrhosis were diagnosed

TABLE I. Profile and Laboratory Data of 123 Patients Infected With HCV Genotype 1b

Demographic data	
Number of patients	123
Sex (M/F)	71/52
Age (years)*	55 (20–70)
History of blood transfusion	41 (33.3%)
Family history of liver disease	37 (30.1%)
Body mass index (kg/m^2)*	23.6 (17.6–32.0)
Laboratory data*	
Serum aspartate aminotransferase (IU/L)	59 (17–266)
Serum alanine aminotransferase (IU/L)	81 (25–504)
Serum albumin (g/dl)	3.8 (3.1–4.5)
Gamma-glutamyl transpeptidase (IU/L)	50 (15–393)
Leukocytes ($/mm^3$)	4,800 (2,300–8,800)
Hemoglobin (g/dl)	14.4 (10.6–17.6)
Platelet count ($\times 10^4/mm^3$)	16.8 (7.5–27.7)
Indocyanine green retention rate at 15 min (%)	15 (4–41)
Serum iron (μg/dl)	138 (18–290)
Serum ferritin (μg/L)	130 (<10–711)
Creatinine clearance (ml/min)	99 (46–146)
Level of viremia (KIU/ml)	1,900 (23–>5,000)
Alpha-fetoprotein (μg/L)	6 (2–161)
Total cholesterol (mg/dl)	166 (96–294)
High-density lipoprotein cholesterol (mg/dl)	46 (10–83)
Low-density lipoprotein cholesterol (mg/dl)	101 (53–207)
Triglycerides (mg/dl)	95 (33–362)
Uric acid (mg/dl)	5.5 (2.3–9.4)
Fasting plasma glucose (mg/dl)	94 (62–120)
Fasting insulin (μU/ml)	10.5 (0.4–55.5)
HOMA-IR	2.6 (0.1–12.5)
Treatment*	
PEG-IFNα-2b dose (μg/kg)	1.4 (0.7–1.9)
Ribavirin dose (mg/kg)	11.0 (3.7–14.2)
Histological findings	
Stage of fibrosis (F1/F2/F3/ND)	53/32/20/18
Hepatocyte steatosis (<5% (Absent)/≥5% (Present)/ND)	38/64/21
Amino acid substitutions in the HCV	
Core aa 70 (arginine/glutamine (histidine))	69/54
Core aa 91 (leucine/methionine)	71/52
ISDR of NS5A (wild-type/mutant-type/ND)	94/22/7

HOMA-IR, homeostasis model for assessment of insulin resistance; ND, not determined.
Data are number and percentages of patients, except those denoted by *, which represent the median (range) values.

based on histological assessment according to the scoring system of Desmet et al. [1994]. Hepatocyte steatosis was assessed as the percentage of hepatocytes containing fat droplet, and subjects were considered to have steatosis in the presence of fat droplets in ≥5% of hepatocytes.

Diagnosis of Liver Cirrhosis, Insulin Resistance, and Diabetes Mellitus

Liver cirrhosis was diagnosed based on the presence of markedly irregular surface with nodular formation in the liver, evident on peritoneoscopy, histological assessment according to the scoring system of Desmet et al. [1994], or on computed tomography or ultrasonography. Ascites, edema, and esophageal varicosities, facilitated the diagnosis when present.

The diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association using a value of fasting plasma glucose of ≥126 mg/dl on at least two occasions [American Diabetes Association, 2000]. IR was assessed by the Homeostasis Model for Assessment of Insulin Resistance (HOMA-IR) method [Matthews et al., 1985], using the following equation: HOMA-IR = fasting plasma glucose (mg/dl) × fasting insulin (μU/ml)/405. HOMA-IR values of ≥2.5, and ≥3.5 were evaluated as IR, and severe IR, respectively.

Detection of Amino Acid Substitutions in Core Region and NS5A Region

With the use of HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of genotype 1b was determined and then compared with the consensus sequence constructed on 50 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. The sequence of 2209–2248 aa in the NS5A of genotype 1b (IFN-sensitivity determining region [ISDR]) reported by Enomoto et al. [1995, 1996] was also determined, and the numbers of aa substitutions in ISDR were defined as wild-type (≤1) or mutant-type (≥2).

In the present study, aa substitutions of the core region and NS5A-ISDR were analyzed by direct

sequencing [Enomoto et al., 1995, 1996; Akuta et al., 2005]. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids were amplified by PCR using the following primers: (a) Nucleotide sequences of the core region: The first-round PCR was performed with CC11 (sense, 5′-GCC ATA GTG GTC TGC GGA AC-3′) and e14 (antisense, 5′-GGA GCA GTC CTT CGT GAC ATG-3′) primers, and the second-round PCR with CC9 (sense, 5′-GCT AGC CGA GTA GTG TT-3′) and e14 (antisense) primers. (b) Nucleotide sequences of NS5A-ISDR: The first-round PCR was performed with ISDR1 (sense, 5′-ATG CCC ATG CCA GGT TCC AG-3′) and ISDR2 (antisense, 5′-AGC TCC GCC AAG GCA GAA GA-3′) primers, and the second-round PCR with ISDR3 (sense, 5′-ACC GGA TGT GGC AGT GCT CA-3′) and ISDR4 (antisense, 5′-GTA ATC CGG GCG TGC CCA TA-3′) primers. ([a]; hemi-nested PCR. [b]; nested PCR). All samples were initially denatured at 95°C for 15 min. The 35 cycles of amplification were set as follows: denaturation for 1 min at 94°C, annealing of primers for 2 min at 55°C, and extension for 3 min at 72°C with an additional 7 min for extension. Then 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

Statistical Analysis

Non-parametric tests were used to compare variables between groups, including the Mann–Whitney U test, chi-squared test, and Fisher's exact probability test. Univariate and multivariate logistic regression analyses were used to determine the independent predictive factors of IR. The odds ratios and 95% confidence intervals (95%CI) were also calculated. All P-values <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P<0.05$) or marginal significance ($P<0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors of IR included the following pretreatment variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), ALT, albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet, count, indocyanine green retention rate at 15 min (ICG R15), serum iron, serum ferritin, creatinine clearance, level of viremia, alfa-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, fasting plasma glucose, fasting insulin, HOMA-IR values, stage of fibrosis, hepatocyte steatosis, PEG-IFN dose/body weight, RBV dose/body weight, and aa substitution in the core and ISDR of NS5A. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL).

RESULTS

HOMA-IR Values of Patients Infected With HCV-1b Without Cirrhosis and Diabetes Mellitus

As a whole, 16.3% (20 of 123 patients), 32.5% (40 of 123), 23.6% (29 of 123), and 27.6% (34 of 123) indicated HOMA-IR values of ≤1.4, 1.5–2.4, 2.5–3.4, and ≥3.5, respectively (Fig. 1). Thus, patients with IR (HOMA-IR ≥2.5) were present in 51.2% (63 of 123), and exceeded 50%. Furthermore, patients with severe IR (HOMA-IR ≥3.5) were present in 27.6%. These results show that patients, infected with HCV-1b without cirrhosis and diabetes mellitus, might indicate IR frequently.

Factors Associated With Insulin Resistance in Univariate and Multivariate Analyses

The whole population sample of 123 patients were analyzed to determine factors that could be associated with IR. IR (HOMA-IR ≥2.5) was detected in 63 of 123 (51.2%) patients. Univariate analysis identified six parameters that tended to or significantly influenced IR. These included age (≥55 years, $P=0.072$), body mass index (≥25 kg/m^2, $P<0.001$), serum ferritin (≥200 μg/L, $P=0.071$), family history of liver disease (Absent, $P=0.077$), hepatocyte steatosis (Present (≥5%), $P=0.002$), and aa substitutions of the core region (Gln70 (His70) and/or Met91, $P=0.092$). Multivariate analysis identified two parameters that independently influenced IR, including body mass index (≥25 kg/m^2, $P=0.001$) and hepatocyte steatosis (Present (≥5%), $P=0.028$).

Severe IR (HOMA-IR ≥3.5) was detected in 34 of 123 (27.6%) patients. Univariate analysis identified five

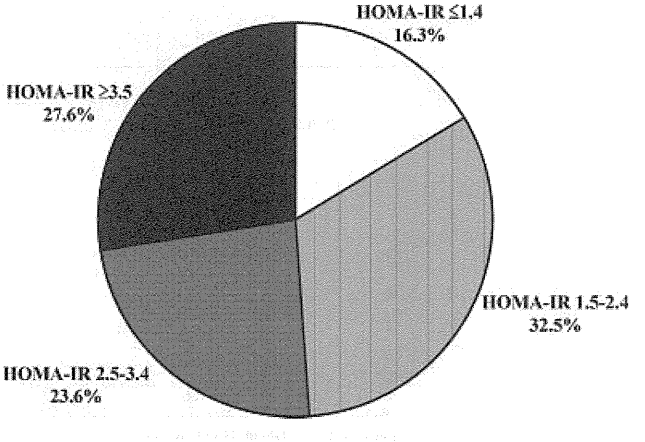

Fig. 1. HOMA-IR values of patients infected with HCV genotype 1b without cirrhosis and diabetes mellitus. As a whole, 16.3%, 32.5%, 23.6%, and 27.6% indicated HOMA-IR values of ≤1.4, 1.5–2.4, 2.5–3.4, and ≥3.5, respectively. These results show that patients, infected with HCV genotype 1b without cirrhosis and diabetes mellitus, might indicate IR frequently.

TABLE II. Factors Associated With Severe IR (HOMA-IR ≥3.5) in Patients Infected With HCV Genotype 1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	*P*
Hepatocyte steatosis	1: Absent (<5%)	1	
	2: Present (≥5%)	4.170 (1.235–14.08)	0.021
Substitution of aa 70 and 91	1: Arg70 and Leu91	1	
	2: Gln70 (His70) and/or Met91	3.654 (1.215–10.99)	0.021
Age (years)	1: <55	1	
	2: ≥55	3.015 (1.071–8.488)	0.037

parameters that tended to or significantly influenced severe IR. These included age (≥55 years, $P=0.015$), body mass index (≥25 kg/m^2, $P=0.025$), hepatocyte steatosis (Present (≥5%), $P=0.003$), triglycerides (≥100 mg/dl, $P=0.060$), and aa substitutions of the core region (Gln70 (His70) and/or Met91, $P=0.020$). Multivariate analysis identified three parameters that independently influenced severe IR, including hepatocyte steatosis (Present (≥5%), $P=0.021$), aa substitutions of the core region (Gln70 (His70) and/or Met91, $P=0.021$), and age (≥55 years, $P=0.037$) (Table II).

aa Substitutions of Core Region and HOMA-IR Values

The entire population sample was also analyzed to determine the relationship between aa substitutions of the core region and HOMA-IR values. HOMA-IR values of 81 patients with Gln70 (His70) and/or Met91 (median; 2.9) indicated the higher levels than those of 42 patients with Arg70 and Leu91 (median; 2.3), significantly ($P=0.022$) (Fig. 2).

Furthermore, the proportions of patients with Gln70 (His70) and/or Met91 among those with HOMA-IR values of ≤1.4, 1.5–2.4, 2.5–3.4, 3.5–3.9, and ≥4.0 were 60.0% (12 of 20 patients), 57.5% (23 of 40), 62.1% (18 of 29), 83.3% (5 of 6), and 82.1% (23 of 28), respectively (Fig. 3). Thus, the higher the proportion of patients with Gln70 (His70) and/or Met91, the higher HOMA-IR values, and significantly lower proportions of patients with Gln70 (His70) and/or Met91 were noted among those without severe IR (59.6% (53 of 89)) than those with severe IR (82.4% (28 of 34)) ($P=0.020$).

Treatment Efficacy of PEG-IFN Plus RBV Combination Therapy According to HOMA-IR Values

Of the 123 patients, 72 could be evaluated as 48-week regimen of PEG-IFN plus RBV combination therapy. Seventy-two patients received PEG-IFNα-2b combination therapy at a median dose of 1.5 μg/kg (range, 0.8–1.8 μg/kg) subcutaneously each week plus oral RBV at a median dose of 11.3 mg/kg (range, 9.7–14.2 mg/kg) daily for 48 weeks. 51.4% (37 of 72 patients) could achieve SVR, and 25.0% (18 of 72) had NVR.

In each groups with IR or without IR, SVR was achieved by 19 of 38 patients (50.0%) and 18 of 34 (52.9%), respectively. The proportions of SVR in group with IR was not significantly different from those in group without IR. Furthermore, in each groups with IR or without IR, NVR was identified in 11 of 38 patients (28.9%) and 7 of 34 (20.6%), respectively. The proportions of NVR in group with IR was not significantly different from those in group without IR.

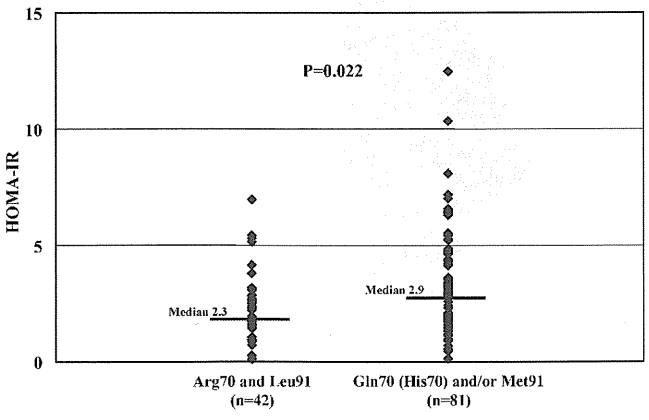

Fig. 2. aa substitutions of HCV core region and HOMA-IR values. HOMA-IR values of 81 patients with Gln70 (His70) and/or Met91 indicated the higher levels than those of 42 patients with Arg70 and Leu91, significantly ($P=0.022$).

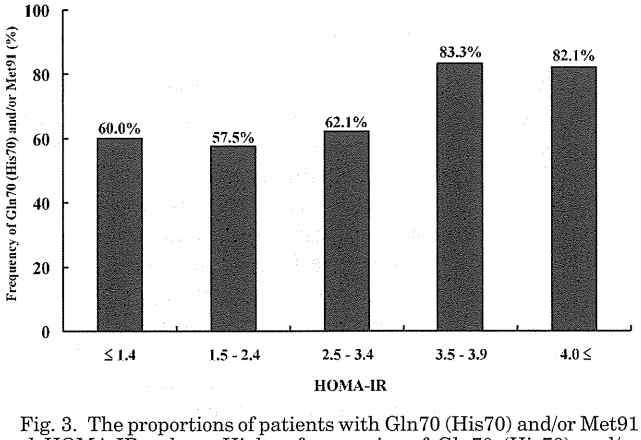

Fig. 3. The proportions of patients with Gln70 (His70) and/or Met91 and HOMA-IR values. Higher frequencies of Gln70 (His70) and/or Met91 correlated with higher HOMA-IR values. Significantly lower proportions of patients with Gln70 (His70) and/or Met91 were noted among those without severe IR (HOMA-IR <3.5) (59.6%) than those with severe IR (HOMA-IR ≥3.5) (82.4%) ($P=0.020$).

In each groups with severe IR or without severe IR, SVR was achieved by 11 of 20 patients (55.0%) and 26 of 52 (50.0%), respectively. The proportions of SVR in group with severe IR was not significantly different from those in group without severe IR. Furthermore, in each groups with severe IR or without severe IR, NVR was identified in 6 of 20 patients (30.0%) and 12 of 52 (23.1%), respectively. The proportions of NVR in group with severe IR was not significantly different from those in group without severe IR.

In this study, HOMA-IR values were not useful as pretreatment predictors of 48-week PEG-IFN plus RBV combination therapy in HCV-1b patients without cirrhosis and diabetes mellitus.

DISCUSSION

Shintani et al. [2004] reported that HCV core protein induced IR in transgenic mice, and provided a direct experimental evidence for the contribution of HCV core protein in the development of IR in human HCV infection. The results of the present study showed that higher frequencies of Gln70 (His70) and/or Met91 in HCV-1b core region might correlated with higher HOMA-IR values. Thus, the present results supported the potential of core region in the development of IR, and clinically linked substitutions of aa 70 and/or 91 in HCV-1b core region to IR. Especially, these findings without diabetes mellitus and cirrhosis suggest that the real connection between IR and HCV-1b infection is initiated at early stages of liver disease. The limitations of the present study were that it could not investigate an improvement of IR in patients who developed the viral eradication after antiviral treatment [Kawaguchi et al., 2007; Arase et al., 2008], as a direct evidence for the contribution of aa substitutions in HCV-1b core region. Further studies that examine the structural and functional impact of aa substitutions should be conducted to confirm the above finding.

To our knowledge, the present study is first report to identify the factors associated with IR of patients without diabetes mellitus and cirrhosis infected with HCV-1b. Especially, multivariate analysis identified age ($\geq$55 years), body mass index ($\geq$25 kg/m^2), hepatocyte steatosis (Present ($\geq$5%)), and aa substitutions of the core region (Gln70 (His70) and/or Met91) as significant determinants of IR (HOMA-IR $\geq$2.5) and/or severe IR (HOMA-IR $\geq$3.5). However, this study identified aa substitutions of the core region as significant determinants of severe IR, and did not identify as determinants of IR. The discrepant results may be due to one or more factors. The first reason for this is probably the small number of patients in the present study (e.g., possible type II error). Univariate analysis really identified aa substitutions of the core region that tended to influence IR. Furthermore, even if HOMA-IR values were also divided into two groups of $\geq$3.0 and $\leq$2.9, multivariate analysis identified aa substitutions of the core region as significant determinants of $\geq$3.0 (data not shown). Hence, further studies based on the large number of patients should be performed in the future. The second reason is probably the difference of objects, based on HCV-1b patients without diabetes mellitus and cirrhosis. Previous report indicated that HCV-related diabetes mellitus might occur in association with IR, hepatocyte steatosis, and high levels of both tumor-necrosis factor and CXCL10 [Antonelli et al., 2009], so patients with severe IR do not always have diabetes mellitus. However, IR develops type 2 diabetes mellitus as its major late feature, and is also associated with advanced fibrosis [Petrides et al., 1994; Petta et al., 2008]. Hence, the biological mechanisms underlying the association between HCV core region and IR are probably multifactorial, and the present study based on patients without diabetes mellitus and cirrhosis as confounding factors, that might affect IR, are very important for estimating the true relationship between HCV core region and IR. The present study is first report to identified aa substitutions of the core region (Gln70 (His70) and/or Met91) as significant determinants of severe IR, in HCV-1b patients without cirrhosis and diabetes mellitus.

Moriya et al. [1998] reported that HCV core protein induced HCC in transgenic mice, and provided a direct experimental evidence for the contribution of HCV core protein in the development of HCC in human HCV infection. Previous reports supported the oncogenic potential of the HCV core region and clinically linked substitutions of aa 70 and/or 91 in HCV-1b core region to HCC [Akuta et al., 2007d, 2008]. IR and glucose metabolism impairment are associated with HCC [El-Serag et al., 2001; Lai et al., 2006; Veldt et al., 2008]. The present study suggested the presence of IR-dependent pathway as a mechanism of HCV-1b core region-associated hepatocarcinogenesis, and the importance of eradication of the virus with Gln70 (His70) and/or Met91 in reducing the development of HCC through this pathway.

Treatment efficacy of 48-week PEG-IFN plus RBV combination therapy according to HOMA-IR values is controversial. Chu et al. [2008] reported that IR was a major determinant of SVR in HCV-1 patients receiving PEG-IFN plus RBV, but treatment duration was 24 weeks. Georgescu et al. [2008] reported that high HOMA-IR values could not affect treatment efficacy of 48-week PEG-IFN plus RBV therapy in HCV-1 patients, after excluding the patients of metabolic syndrome criteria. The present study based on HCV-1b patients without cirrhosis and diabetes mellitus also showed that HOMA-IR values might be not useful as predictors of 48-week PEG-IFN plus RBV therapy. This reason is probably related to exclude patients of diabetes mellitus as one of metabolic syndrome criteria, and the results might support the previous report of Georgescu et al. [2008]. To our knowledge, the present study is first report to investigate the relation between HOMA-IR values and treatment efficacy of HCV-1b patients, especially without cirrhosis and diabetes mellitus, receiving 48-week PEG-IFN plus RBV combination

therapy. Further studies based on the large number of patients should be performed in the future.

In conclusion, the results of the present study indicated that substitutions of HCV-1b core region were the important predictor of severe IR in patients without cirrhosis and diabetes mellitus. This finding highlights the importance of eradication of the virus with Gln70 (His70) and/or Met91 in reducing the development of severe IR. The limitations of the present study were that it did not investigate other genotypes apart from HCV-1b, the geographic diversities of HCV-1b core region (distribution of Arg70 or Gln70 (His70), and Leu91 or Met91), and the study of other races apart from Asians in Japan. Further prospective studies, matched for HCV genotype, aa substitutions of the core region, and race, of a large group of patients are required to determine the meaning of higher HOMA-IR values in HCV infection.

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Clinica Chimica Acta 413 (2012) 577–581

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Serum levels of IgG4 and soluble interleukin-2 receptor in patients with coronary artery disease

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ARTICLE INFO

Article history:
Received 30 July 2011
Received in revised form 22 November 2011
Accepted 23 November 2011
Available online 29 November 2011

Keywords:
IgG4-related systemic sclerosing disease
Lymphocyte activation
Coronary artery disease
Biomarkers

ABSTRACT

Background: Immunoglobulin G4 (IgG4)-related immuno-inflammation has been suggested to play a role in the development of remodeling of arterial wall. We investigated the association between serum concentrations of IgG4 or soluble interleukin-2 receptor (sIL-2R) and coronary artery disease (CAD).
Methods: Serum concentrations of IgG4 and sIL-2R were measured in 286 patients who underwent coronary angiography.
Results: In patients with CAD, the medians of serum concentrations of IgG4 (39.3 mg/dl) and sIL-2R (388 U/ml) were significantly higher than corresponding values in patients without CAD (IgG4 27.0 mg/dl, sIL-2R 312 U/ml). In receiver-operating characteristic curve analysis, the area under the curve of sIL-2R and IgG4 for the presence of CAD was 0.634 and 0.632, respectively. Age- and gender-adjusted logistic regression analysis showed that both of the fourth quartile of sIL-2R concentrations (≥509 U/ml) and that of IgG4 concentrations (≥57.7 mg/dl) were found to be associated with CAD with an odds ratio of 2.82 and 4.08, respectively, compared with the corresponding lowest quartile.
Conclusions: Serum concentrations of IgG4 and sIL-2R were increased in patients with angiographically-proven CAD, suggesting that IgG4-related immuno-inflammation may also have a role in the development and/or progression of coronary artery atherosclerosis.

1. Introduction

Recent studies have shown that the activation of immune system plays a pivotal role in various stages of atherosclerosis [1]. The focal recruitment of circulating monocytes and T lymphocytes is considered to be one of the earliest cellular responses in atherosclerotic lesion formation [2], and B lymphocytes are known to comprise the majority of adventitial inflammatory infiltrates in close proximity to intimal atherosclerotic plaques [3]. Interleukin-2 may modulate T-cell activation and responses [4], and a truncated form of the interleukin-2 receptor, termed soluble interleukin-2 receptor (sIL-2R), is secreted from activated T cells. Serum sIL-2R is elevated in a wide variety of diseases, such as hematological disorders [5], inflammatory bowel disease [6], sarcoidosis [7], and neoplastic diseases [8].

Until now, 4 immunoglobulin G (IgG) subclasses (IgG1, IgG2, IgG3 and IgG4) are known in humans, of which IgG4 is the rarest. It has become evident that increase of serum IgG4 concentrations and/or increased infiltration of IgG4-positive cells in various organs can be observed in immuno-inflammation-related disorders such as autoimmune pancreatitis [9], Mikulicz's disease [10], and idiopathic retroperitoneal fibrosis [11], leading to the proposal of a new clinicopathological entity, IgG4-related sclerosing disease (IgG4-SD) [12,13]. Intriguingly, IgG4-related immune activation may also underlie the cardiovascular conditions, such as inflammatory abdominal aortic aneurysm, lymphoplasmacytic aortitis [14,15], and coronary periarteritis [16,17]. Although these findings suggest that IgG4-related periarteritis may involve arteries of various sizes, it has not been clarified whether or not patients with coronary artery disease (CAD) have higher serum concentrations of IgG4 compared with those without. To this end, we have investigated the serum concentrations of these biomarkers, IgG4 and sIL-2R, in patients who underwent coronary angiography.

2. Methods

2.1. Subjects

The study was approved by the Ethical Committee of University of Tokyo, Tokyo, Japan. Written informed consent was obtained from all subjects. We enrolled 286 patients whose consent could be obtained

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doi:10.1016/j.cca.2011.11.023

and who underwent coronary artery angiography because of unstable angina, stable angina, silent myocardial ischemia, heart failure, and preoperative coronary artery screening between October 2005 and July 2008. The exclusion criteria were previous coronary artery interventions such as percutaneous coronary interventions (PCI) and coronary artery bypass graft surgery, acute myocardial infarction, and lack of informed consent for study enrollment. Among the 286 patients enrolled, 80 (28.0%) underwent preoperative screening for CAD before an operation for abdominal aortic aneurysm (AAA), thoracic aortic aneurysm (TAA), arteriosclerosis obliterans (ASO), or valvular heart disease (Table 1). Such pre-operative CAD screening would be performed especially when other coronary artery estimation, such as exercise testing and computed tomography angiography (CTA), cannot be performed or will not provide appropriate information, considering the high rate of coexisting coronary artery lesions. In the current study, obesity was defined as a body mass index (BMI) of $\geq 25\ kg/m^2$ [18]. Diagnosis of hypertension, hyperlipidemia, and diabetes, and assessment of smoking status were made by reference to those made at the time of admission, which were, in general, based on past history, laboratory data, and drugs taken.

2.2. Laboratory measurements

Blood samples were obtained by arterio- or venipuncture at the time of coronary artery angiography. The samples were then centrifuged within 15 min, and serum was stored at −80 °C. Serum concentrations of IgG4 were determined by turbidimetry. Serum concentrations of sIL-2R were measured by enzyme-linked immunosorbent assay (SRL, Tokyo, Japan). The measurements of serum IgG4 and sIL-2R concentrations were performed in duplicate (SRL, Tokyo, Japan) with coefficient variables of 5.2–5.7% and 1.7–3.9%, respectively. High-sensitivity C-reactive protein (hsCRP) was measured by an immunoturbidimetric assay.

Table 1
Baseline characteristics.

Variables	No CAD (n=119)	CAD (n=167)	P value
Male gender, n (%)	69 (58.0)	128 (76.6)	0.001
Age, years	63.6±11.8	68.2±8.5	0.001
BMI, kg/m^2	23.7±4.1	23.6±3.6	0.775
Old myocardial infarction, n (%)	0 (0.0)	27 (16.2)	<0.001
Congestive heart failure, n (%)	16 (13.4)	10 (6.0)	0.031
Renal failure on hemodialysis, n (%)	4 (3.4)	6(3.6)	0.916
Coronary risk factors			
Age≥70 y, n (%)	39 (32.8)	84 (50.3)	0.003
Obesity, n (%)	37 (31.1)	48 (28.7)	0.668
Hypertension, n (%)	87 (73.1)	140 (83.8)	0.027
Hyperlipidemia, n (%)	44 (37.0)	104 (62.3)	<0.001
Diabetes, n (%)	27 (22.7)	92 (55.1)	0.000
Preoperative screening for			
AAA, n (%)	11 (9.2)	14 (8.4)	0.800
TAA, n (%)	15 (12.6)	9 (5.4)	0.030
ASO, n (%)	5 (4.2)	16 (9.6)	0.086
Valvular heart disease, n (%)	18 (15.1)	8 (4.8)	0.003
Smoking status			
Never, n (%)	56 (47.1)	58 (34.7)	
Former, n (%)	37 (31.1)	76 (45.5)	
Current, n (%)	26 (21.8)	33 (19.8)	
Angiographic findings			
0 vessel disease, n (%)	119 (100)	0	
1 vessel disease, n (%)	0	61 (36.5)	
2 vessels disease, n (%)	0	40 (24.0)	
3 vessels disease, n (%)	0	66 (39.5)	

Values are presented as the mean±standard deviation unless described otherwise. CAD indicates coronary artery disease. BMI, AAA, TAA, and ASO indicate body mass index, abdominal aortic aneurysm, thoracic aortic aneurysm, and arteriosclerosis obliterans, respectively. Obesity was defined as a BMI of 25 kg/m^2 or more.

2.3. Coronary angiography

Angiographic images were analyzed by experienced observers who were unaware of the serum biomarkers tested: sIL-2R, IgG4, and hsCRP. CAD was defined to be present when narrowing of the normal contrast-enhanced lumen to <50% was identified within any of 13 segments (1–4, 5–9, 11–14) in accord with the American Heart Association coronary classification.

2.4. Statistical analysis

Data analysis was performed using Dr. SPSS II for Windows (SPSS Inc., Chicago, IL). Differences between groups were calculated by Mann–Whitney, and χ^2 tests. Correlations between variables tested were assessed by using Spearman's rank correlation coefficient. Logistic regression analysis was performed for binominal variables. A $P<0.05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics

The mean age of the 286 patients enrolled was 66.3±10.3 y (range 34–86 y). CAD cases were significantly older than non-CAD cases, and the male gender was more prevalent in CAD cases than non-CAD cases (Table 1). Serum concentrations of sIL-2R were greater in patients with diabetes (median 406 U/ml, interquartile range [IR] 300–562) than in those without (median 331 U/ml, IR 264–450, $P=0.001$ by Mann–Whitney test). In addition, sIL-2R concentrations were also greater in patients with hypertension (median 373 U/ml, IR 279–535) than in those without (median 312 U/ml, IR 247–412, $P=0.003$ by Mann–Whitney test). The relationship between age, estimated glomerular filtration rate (eGFR), and the three biomarkers tested was assessed by Spearman's rank correlation. There was a weak, but significant correlation between sIL-2R and eGFR with a correlation coefficient of −0.393 (Table 2). Although statistically significant, correlation between IgG4 and sIL-2R, between sIL-2R and hsCRP, and between IgG4 and hsCRP was found to be trivial.

3.2. Serum concentrations of sIL-2R, IgG4, and hsCRP and coronary artery disease

The serum concentrations of sIL-2R (median 388 U/ml, IR 301–552) and IgG4 (median 39.3 mg/dl, IR 21.5–66.5) were statistically significantly higher in CAD cases than corresponding values in non-CAD cases (sIL-2R, 312 U/ml, IR 256–450, $P<0.001$; IgG4, 27.0 mg/dl, IR 14.9–45.1, $P<0.001$) (Fig. 1). On the other hand, in this study population, hsCRP did not significantly differ between CAD and non-CAD

Table 2
Spearman's rank correlation coefficient of sIL-2R, IgG4, and hsCRP.

	Age	eGFR	sIL2R	IgG4	hsCRP
Age					
r	–				
P value	–				
eGFR					
r	−0.185	–			
P value	0.002	–			
sIL2R					
r	0.183	−0.393	–		
P value	0.002	<0.001	–		
IgG4					
r	0.005	−0.048	0.205	–	
P value	0.937	0.423	0.001	–	
hsCRP					
r	0.028	−0.046	0.222	0.136	–
P value	0.633	0.440	<0.001	0.023	–

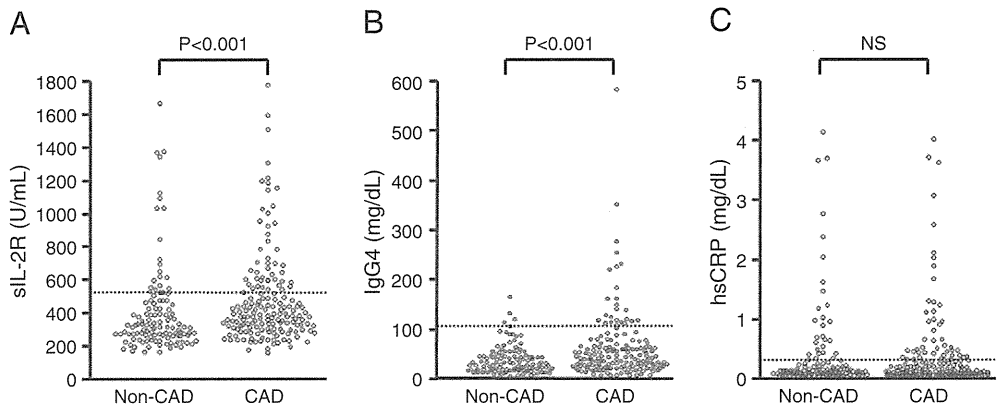

Fig. 1. Scattered plot of soluble interleukin-2 receptor (sIL-2R) (A), immunoglobulin G4 (IgG4) (B), and hsCRP (C) in patients with and without coronary artery disease (CAD). The values of the upper normal limit of each biomarker were shown by dotted lines. P values were calculated by Mann–Whitney *U* test.

cases. The prevalence of an sIL-2R concentration greater than the upper normal limit, which is 519 U/ml, was higher in CAD than in non-CAD cases (46 [27.5%] vs. 20 [16.8%], P = 0.037, by χ^2 test). In addition, the prevalence of an IgG4 concentration greater than the upper normal limit, which is 105 mg/dl, was higher in CAD than in non-CAD cases (25 [15.0%] vs. 4 [3.4%], P = 0.001, by χ^2 test). The sensitivity, specificity, positive predictive value and negative predictive value of sIL-2R of >519 U/ml were 27.5%, 83.1%, 69.7%, and 44.7%, respectively, and those of IgG4 of >105 mg/dl were 15.3%, 96.6%, 86.2%, and 45.2%, respectively. Receiver operating characteristic (ROC) curve analysis of the serum concentrations of sIL-2R, IgG4, and hsCRP for CAD is shown in Fig. 2. The area under the curve (AUC) of sIL-2R, IgG4, and hsCRP for the presence of CAD was 0.634 (SE: 0.034, P<0.001), 0.632 (SE: 0.033, P<0.001), and 0.509 (SE: 0.035, P = 0.801), respectively. In the current study population, the AUC of age, hypertension, dyslipidemia, and diabetes for CAD was 0.612, 0.554, 0.627, and 0.662, respectively. When each gender was analyzed separately, for the presence of CAD, the AUC of sIL-2R was 0.719 in women and 0.571 in men, and the AUC of IgG4 was 0.672 in women and 0.594 in men.

3.3. Multivariate analysis

Age- and gender-adjusted logistic regression analysis showed that the fourth quartile of sIL-2R concentrations (≥509 U/ml) and that of IgG4 concentrations (≥57.7 mg/dl) were both found to be associated with CAD with an odds ratio of 2.82 and 4.08, respectively, compared with the corresponding lowest quartile (Table 3). As compared with the lowest quartile, after adjusting for age and gender, the combined highest three quartiles of sIL-2R (≥275 U/ml) and those of IgG4 (≥18.1 mg/dl) were associated with CAD with an odds ratio of 2.60 (95% CI 1.45–4.68, P = 0.001) and 2.25 (95% CI 1.26–4.03, P = 0.006), respectively. When both IgG4 and sIL-2R were entered into the statistical model (model 2), these biomarkers remained to be significantly associated with CAD, indicating that the association between sIL-2R (IgG4) and CAD was, at least in part, independent of IgG4 (sIL-2R) (Table 3). Furthermore, when eGFR and coronary risk factors were added into the statistical model as covariates (model 3), a graded association of sIL-2R concentrations remained significant and the highest quartile of IgG4 concentrations also remained to be positively associated with CAD. When the usage of statins and angiotensin II receptor blockers was added as an additional covariate, the fourth quartile of sIL-2R concentrations (≥509 U/ml) and that of IgG4 concentrations (≥57.7 mg/dl) remained to be associated with CAD with an odds ratio of 2.68 and 4.29, respectively, compared with the corresponding lowest quartile.

4. Discussion

We found that, among patients who underwent coronary angiography, serum concentrations of IgG4 and sIL-2R were higher in patient with CAD than in those without. The combined highest three quartiles of sIL-2R (≥275 U/ml) and those of IgG4 (≥18.1 mg/dl) were both found to be associated with the presence of CAD with an odds ratio of 2.60 (95% CI 1.45–4.68, P = 0.001) and 2.25 (95% CI 1.26–4.03, P = 0.006), respectively, as compared with the lowest quartile. These findings suggest that IgG4-related immuno-inflammatory process may play a role in the pathogenesis of coronary artery lesions.

Several previous studies have also reported the relationship between serum sIL-2R concentrations and coronary artery atherosclerosis. Neri et al. have reported that patients with unstable angina (n = 29) had significantly higher serum sIL-2R concentrations than healthy subjects (n = 30) [19]. Similarly, Olsson et al. reported that sIL-2R concentrations were found to be increased in patients with stable angina than healthy controls [20]. Satoh et al. also found that

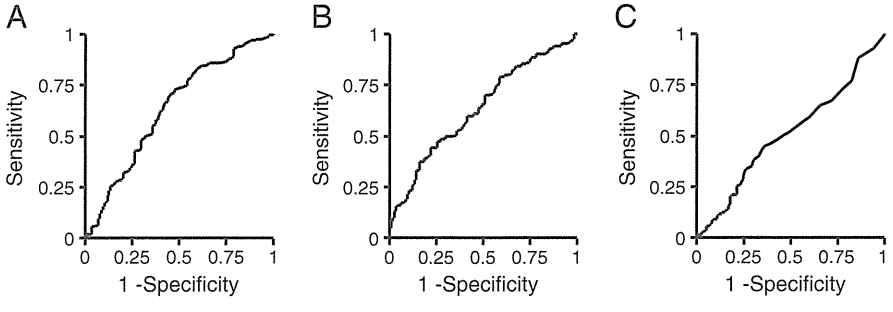

Fig. 2. Receiver-operating characteristic (ROC) curve analysis showing the prognostic value of biomarkers for coronary artery disease. ROC curves of sIL-2R (A), IgG4 (B), and hsCRP (C) for the presence of coronary artery disease are shown.

Table 3
Logistic regression analysis using the presence of CAD as a dependent variable.

	Model 1			Model 2			Model 3		
Independent variables	Odds ratio	(95% CI)	P value	Odds ratio	(95% CI)	P value	Odds ratio	(95% CI)	P value
sIL2R quartiles									
First (range 151–274)	1.00	Reference	–	1.00	Reference	–	1.00	Reference	–
Second (range 275–354)	2.33	(1.15–4.73)	0.019	2.54	(1.21–5.31)	0.014	2.41	(1.08–5.38)	0.031
Third (range 355–496)	2.71	(1.32–5.58)	0.007	2.90	(1.35–6.22)	0.006	2.54	(1.11–5.81)	0.027
Fourth (range 509–1770)	2.82	(1.37–5.80)	0.005	2.84	(1.32–6.13)	0.008	2.72	(1.08–6.86)	0.035
IgG4 quartiles									
First (range 3.0-17.8)	1.00	Reference	–	1.00	Reference	–	1.00	Reference	–
Second (range 18.1–32.7)	1.92	(0.95–3.88)	0.070	2.04	(1.00–4.17)	0.051	1.59	(0.73–3.48)	0.243
Third (range 33.4–57.6)	1.64	(0.82–3.30)	0.163	1.67	(0.82–3.38)	0.158	1.60	(0.74–3.46)	0.231
Fourth (range 57.7–580.0)	4.08	(1.90–8.76)	<0.001	3.91	(1.80–8.52)	0.001	3.75	(1.64–8.57)	0.002
hsCRP quartiles									
First (range 0.01–0.05)	1.00	Reference	–	1.00	Reference	–	1.00	Reference	–
Second (range 0.06–0.10)	0.53	(0.26–1.08)	0.081	0.44	(0.21–0.94)	0.033	0.46	(0.20–1.05)	0.065
Third (range 0.11–0.31)	0.80	(0.40–1.60)	0.525	0.67	(0.32–1.40)	0.290	0.65	(0.29–1.45)	0.289
Fourth (range 0.32–4.12)	0.89	(0.44–1.82)	0.752	0.59	(0.27–1.30)	0.191	0.87	(0.36–2.08)	0.752

Model 1: Independent variables include gender, age, and either of IgG4, sIL-2R, or hsCRP.
Model 2: Independent variables include gender, age, IgG4, sIL-2R, and hsCRP.
Model 3: Independent variables include those in Model 2 + eGFR, hypertension, hyperlipidemia, and diabetes.

sIL-2R concentrations were higher in CAD patients (n = 85) than normal controls (n = 50) [21]. Together with our results, these observations suggest the presence of continuous immune system activation and antigenic stimulation although the nature of such antigen remains to be investigated. In the above-mentioned Satoh et al.'s report, it was also reported that sIL-2R concentrations showed further elevation 30 days after successful PCI in patients who experienced intraluminal restenosis, but not in those who did not experience restenosis [21], suggesting that the activation of T-lymphocyte activation may also play a role in the development of coronary artery restenosis after successful PCI. On the other hand, Wadwa et al. have reported that elevated plasma sIL-2R is associated with progression of coronary artery calcification assessed by electron beam tomography that was independent of traditional coronary artery disease risk factors [22], suggesting the role of T-lymphocyte activation in coronary arteriosclerosis.

Possible involvement of IgG4-related autoimmunity in the development of fibrosclerotic lesion formation has been first discovered in autoimmune pancreatitis [9]. Growing body of evidence is accumulating that IgG4-SD may involve a wide variety of organs including cardiovascular system, including abdominal [14,15,23] and thoracic aorta, and pericardium [24]. Although it seems that only a few studies have analyzed the association between IgG4 and the presence or extent of coronary artery lesions thus far, several recent studies suggested the involvement of IgG4-related immuno-inflammation in coronary periarteritis. Matsumoto et al. presented a case with IgG4-related inflammatory pseudo-tumor in the peri-coronary artery region [16]. In addition, Maturen et al. have recently reported a patient with idiopathic retroperitoneal fibrosis, which is considered to belong, in part, to IgG4-SD, who had perivascular low-attenuation soft tissue surrounding the coronary arteries, illustrated by computed tomography [17]. In Matsumoto et al.'s and Maturen et al.'s papers, coronary periarterial fibrosclerosis was not seemed to be associated with flow-limiting coronary artery disease. In this sense, the current study is the first one that shows the possible relationship between CAD and serum IgG4 concentrations.

It may be questioned whether IgG4-related peri-coronary arterial fibrosclerosis would be associated with CAD. We recently reported a case who was admitted to the hospital owing to the chest symptoms suggestive of acute coronary syndrome [25]. In this patient, CT coronary angiography revealed a fibrous thickening of the peri-coronary arterial regions, and the luminal stenosis at the site of coronary periarteritis. Histologic and immunohistochemical analysis showed enhanced infiltration of IgG4-positive plasma cells in the observed periarterial lesion, and together with increased serum IgG4 concentrations (564 mg/dl), IgG4-related coronary periarteritis has been diagnosed in this patient. Although the causal role of IgG4-related immuno-inflammation in the development in luminal stenosis could not be concluded from that patient, it is possible that the presence of coronary periarteritis had been underdiagnosed until now.

In this study, hsCRP was not found to be higher in patients with CAD, which contrasted to the previous findings demonstrating that CRP and other circulating markers of inflammation, such as erythrocyte sedimentation rate and von Willebrand factor in the prediction of coronary heart disease [26]. Veselka et al. reported a similar observation, however, that CRP concentrations were not related to the extent or the presence of coronary atherosclerosis assessed by coronary angiography in patients referred for coronary angiography [27]. In addition, by prospectively analyzing the data from patients with stable angina or an abnormal stress test who were referred for diagnostic coronary angiography, Azar et al. reported that CRP concentrations were not correlated with the extent or severity of coronary narrowing; no significant difference in plasma CRP concentrations between subjects with normal coronary artery, mild CAD, single vessel disease, and multi-vessel disease [28]. Furthermore, Abdelmouttaleb et al. have found that CRP concentrations were higher in patients with unstable angina, but not in asymptomatic subjects or those with stable angina in which CAD was established by coronary angiography [29]. In the current study population, unstable angina was present in 21 (7.3%). It is possible that several factors, including the prevalence of acute coronary syndrome, may affect the relationship between CRP concentrations and CAD. In addition, statins and angiotensin II receptor blockers, which had already been taken in some patients, may have affected hsCRP concentrations [30–32].

In this study, subjects with aortic aneurysm but without CAD were also included in non-CAD cases, which may affect the inflammatory status in non-CAD subjects. In another set of on-going study, we are measuring serum IgG4 concentrations in the 127 subjects who have neither CAD nor aortic aneurysm. In that preliminary study, the median of serum IgG4 concentrations in subjects who had neither CAD nor aortic aneurysm was 28.5 mg/dl (IR 14.9–45.7) and that of sIL-2R concentrations was 317 U/ml (IR 260–450). Among them, 5 subjects (3.9%) were found to have serum IgG4 concentrations greater than 105 mg/dl, and 22 subjects (17.3%) were found to have serum sIL-2R

concentrations greater than 519 U/ml. These observations confirm that serum IgG4 and sIL-2R concentrations are indeed elevated in subjects who have neither CAD nor aortic aneurysm. We may have to clarify whether non-CAD subjects with increased IgG4 or sIL-2R would have higher incidence of future CAD or CAD-associated events in the near future.

There are several limitations of the current study. First, we investigated the relationship between these biomarkers and CAD in relatively high risk patients. Whether or not they represent a marker of coronary or peripheral atherosclerosis should be investigated in the general, i.e., relatively low risk, population. Second, due to the cross-sectional nature of the study, we cannot draw conclusions about the causal or resultant relationship between the elevation of sIL-2R and IgG4 concentrations and CAD. A longitudinal follow-up of the subjects should be performed to elucidate whether those subjects with higher concentrations of these markers would be more likely to experience the progression of CAD. Third, to assess the role of IgG4-related immuno-inflammation in the atherogenesis, we have to assess the IgG4-positivity in perivascular cells of coronary artery in CAD patients by histological and immunohistochemical approach as the degree of IgG4-positive staining does not necessarily correlate with elevated serum concentrations [33]. Finally, to elucidate the usefulness of the measurement of IgG4 and sIL-2R, whether the elevation of these biomarkers predicts the risk for future atherosclerotic episodes or restenosis following successful coronary intervention should be investigated.

In conclusion, we have shown that higher serum concentrations of IgG4 and sIL-2R, markers for lymphocytic activation, were associated with CAD in patients who underwent coronary artery angiography. Further studies are warranted to clarify whether these biomarkers are useful in the prediction of coronary artery lesions in patients who have a relatively low-risk profile.

Acknowledgments

The work was supported in part by a grant from Chiyoda Mutual Life Foundation, the St Luke's Grant for Epidemiological Research, Daiwa Securities Health Foundation, Gerontology Research Grant of Kowa Life Science Foundation, Foundation for Total Health Promotion, Gout Research Foundation of Japan, a Grant-in-Aid from the Ministry of Health, Labour, and Welfare, Japan, and the Japan Society for the Promotion of Science (JSPS) through its "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)". We are highly appreciative of Kyoko Furuta for her technical assistance and Dr. Hiroshi Noto for his comments on statistical analysis.

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Original Article

Impact of Changes in Obesity Parameters on Glucose Metabolism and Insulin Resistance Over a One-Year Period

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Aim: Changes in indexes of obesity, such as waist circumference (WC) and body mass index (BMI), may influence some glucose metabolism-related parameters in both obese and non-obese subjects. We have investigated the impact of changes in WC and in BMI on data related to glucose metabolism over a one-year period.
Methods: Data from 3213 individuals (2014 men, 1199 women) who underwent a general health screening two years running and were not taking antidiabetic medication were analyzed.
Results: In men, percent changes in WC (%dWC) and BMI (%dBMI) were both significantly correlated with percent changes in fasting glucose (%dFG), in hemoglobin A_{1c} (%dHbA_{1c}), and in HOMA-IR (%dHOMA-IR). In women, these relationships were not significant except for the relationship between %dBMI and %dHOMA-IR. In a multivariate linear regression analysis using age, %dBMI, and %dWC as independent variables, %dBMI, but not %dWC, was found to be an independent predictor of %dHOMA-IR in both genders. Furthermore, in men, %dBMI was also an independent factor predicting %dFG and %dHbA_{1c}.
Conclusion: During the one-year period, a reduction in BMI, and thus weight loss, was found to be associated with the improvement of insulin sensitivity, especially in men. A reduction in WC was also associated with an improvement in insulin sensitivity in men; however, this relationship did not remain significant after controlling for changes in BMI.

J Atheroscler Thromb, 2010; 17:1246-1255.

Key words; Waist circumference, Body mass index, Glucose metabolism, Insulin resistance, Health screening

Introduction

Elevated fasting glucose (FG) and hemoglobin A_{1c} (HbA_{1c}) concentarions, and enhanced insulin resistance are associated with an increased incidence of cardiovascular diseases[1]. Obesity, which may be reflected as an increase in waist circumference (WC) and in body mass index (BMI), is known to be associated with these glucose metabolism-related parameters[2-6]. In addition, the relative risk of developing type 2 diabetes increases with a gain in weight and BMI[7]. The relationship observed between insulin resistance and obesity may be explained by a disproportionate accumulation of visceral fat, leading to a change in levels of adipocytokines, which may underlie various metabolic disorders[8-10]. On the other hand, it has not been fully established whether changes in BMI or those in WC have the greater impact on glucose metabolism-related data. To this end, here we have analyzed the relationship between changes in obesity parameters and changes in diabetic parameters over a one-year period in Japanese individuals.

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Received: May 24, 2010
Accepted for publication: July 13, 2010

Table 1a. Baseline Characteristics at the First Visit According to %dWC

variables	%dWC-Q1 (range: −21.3−−3.4)	%dWC-Q2 (range: −3.4−−0.1)	%dWC-Q3 (range: 0.0-3.3)	%dWC-Q4 (range: 3.3-33.4)	*p* value
Women					
n	348	202	223	426	
Age, years	53 (52-54)	53 (51-54)	51 (50-53)	51 (50-52)	0.066
Height, cm	156 (156-157)	157 (156-158)	157 (156-158)	157 (157-158)	0.037
Weight, kg	50 (51-52)	52 (52-54)	52 (52-55)	51 (51-53)	0.009
WC, cm	79 (78-80)	77 (77-80)	76 (76-78)	72 (73-74)	<0.001
BMI, kg/m^2	20.7 (20.7-21.3)	21.1 (21.2-22.2)	21.1 (21.2-22.1)	20.8 (20.8-21.3)	0.028
Systolic blood pressure, mmHg	115 (116-119)	118 (118-123)	114 (115-120)	113 (115-118)	0.129
Diastolic blood pressure, mmHg	72 (72-75)	73 (73-76)	72 (72-75)	71 (71-74)	0.198
Pulse rate, bpm	63 (63-64)	63 (62-65)	63 (63-65)	63 (63-64)	0.937
LDL-cholesterol, mg/dL	131 (127-134)	130 (125-134)	127 (124-133)	122 (121-127)	0.021
HDL-cholesterol, mg/dL	68 (68-71)	66 (66-70)	68 (67-70)	68 (68-70)	0.329
Trigryceride, mg/dL	77 (81-91)	77 (84-99)	77 (79-93)	69 (76-83)	0.026
Uric acid, mg/dL	4.5 (4.5-4.7)	4.5 (4.4-4.7)	4.6 (4.5-4.7)	4.4 (4.4-4.5)	0.076
Fasting glucose, mg/dL	87 (87-90)	89 (89-93)	88 (88-91)	88 (88-91)	0.149
Hemoglobin A$_{1C}$, %	5.1 (5.1-5.2)	5.2 (5.1-5.2)	5.1 (5.1-5.2)	5.1 (5.1-5.2)	0.284
Blood urea nitrogen, mg/dL	13.0 (13.0-13.8)	13.0 (13.2-14.2)	13.0 (12.9-13.7)	13.0 (13.2-13.8)	0.705
Serum creatinine, mg/dL	0.60 (0.61-0.70)	0.60 (0.62-0.65)	0.60 (0.61-0.63)	0.60 (0.62-0.64)	0.408
Anti-dyslipidemic medication, *n* (%)	13 (3.7)	11 (5.4)	6 (2.7)	16 (3.8)	0.526
Anti-hypertensive medication, *n* (%)	27 (7.8)	18 (8.9)	9 (4.0)	17 (4.0)	0.022
Current smoker, *n* (%)	36 (10.3)	15 (7.4)	12 (5.4)	44 (10.3)	0.117
Men					
n	462	589	600	363	
Age, years	54 (53-55)	54 (53-54)	54 (53-54)	53 (51-53)	0.040
Height, cm	169 (169-170)	170 (169-170)	169 (169-170)	169 (169-170)	0.975
Weight, kg	68 (68-70)	68 (68-69)	67 (68-69)	67 (67-68)	0.328
WC, cm	88 (87-89)	87 (86-87)	85 (85-86)	82 (82-84)	<0.001
BMI, kg/m^2	23.8 (23.6-24.2)	23.7 (23.6-24.0)	23.6 (23.6-24.0)	23.3 (23.2-23.8)	0.150
Systolic blood pressure, mmHg	128 (127-131)	125 (127-130)	124 (125-127)	121 (121-124)	<0.001
Diastolic blood pressure, mmHg	81 (81-83)	80 (80-82)	79 (79-81)	77 (77-79)	<0.001
Pulse rate, bpm	62 (62-64)	62 (62-64)	62 (62-64)	61 (61-63)	0.347
LDL-cholesterol, mg/dL	132 (129-134)	130 (128-133)	129 (127-132)	125 (124-131)	0.225
HDL-cholesterol, mg/dL	54 (55-58)	54 (54-57)	53 (54-56)	55 (55-58)	0.328
Trigryceride, mg/dL	111 (122-136)	111 (123-134)	111 (126-140)	100 (115-133)	0.037
Uric acid, mg/dL	6.1 (6.0-6.2)	6.1 (6.1-6.3)	6.0 (6.0-6.2)	6.2 (6.0-6.2)	0.290
Fasting glucose, mg/dL	95 (97-100)	95 (97-99)	94 (95-97)	93 (94-97)	0.008
Hemoglobin A$_{1C}$, %	5.3 (5.3-5.4)	5.3 (5.3-5.4)	5.2 (5.2-5.3)	5.2 (5.2-5.3)	0.005
Blood urea nitrogen, mg/dL	14.0 (14.3-15.0)	14.0 (14.4-14.9)	14.0 (14.0-14.6)	14.0 (14.1-14.7)	0.405
Serum creatinine, mg/dL	0.80 (0.83-0.92)	0.80 (0.85-0.87)	0.85 (0.85-0.87)	0.80 (0.84-0.86)	0.647
Anti-dyslipidemic medication, *n* (%)	18 (3.9)	25 (4.2)	28 (4.7)	16 (4.4)	0.942
Anti-hypertensive medication, *n* (%)	58 (12.6)	77 (13.1)	84 (14.0)	42 (11.6)	0.736
Current smoker, *n* (%)	137 (29.7)	194 (32.9)	175 (29.2)	121 (33.3)	0.352

Methods

Study Population

The study was approved by The Ethics Committee of Mitsui Memorial Hospital. Between October 2005 and October 2006, 11558 individuals underwent a general health screening at our institute. Of these, 3325 (2113 men, 1212 women) individuals