Title: Underestimation of impaired glucose tolerance and usefulness of a continuous glucose monitoring system in chronic liver disease.

Running Head: Glucose monitoring in liver cirrhosis

Authors: Yutaro Ogawa[1,2], Takashi Nakahara[1,2], Masafumi Ono[3], Takumi Kawaguchi[4], Hiroshi Isoda[5], Akira Hiramatsu[1,2], Shinsuke Uchikawa[1,2], Hatsue Fujino[1,2], Eisuke Murakami[1,2], Tomokazu Kawaoka[1,2], Masami Yamauchi[1,2], Masataka Tsuge[1,2,6], Kensuke Munekage[7], Tsunehiro Ochi[7], C Nelson Hayes[1,2], Michio Imamura[1,2], Hiroshi Aikata[1,2], Hirokazu Takahashi[5], Takuji Torimura[4], Kazuaki Chayama[2,8,9]

[1] Department of Gastroenterology and Metabolism, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan.

[2] Research Center for Hepatology and Gastroenterology, Hiroshima University, Hiroshima, Japan.

[3] Division of Innovative Medicine for Hepatobiliary & Pancreatology Faculty of Medicine, Kagawa University, Takamatsu, Japan.

[4] Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume, Japan.

[5] Liver Center, Saga University Hospital, Saga, Japan.

[6] Natural Science Center for Basic Research and Development, Hiroshima University, Hiroshima, Japan.

[7] Department of Gastroenterology and Hepatology, Kochi Medical School, Nankoku, Japan.

[8] Collaborative Research Laboratory of Medical Innovation, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan.

[9] RIKEN Center for Integrative Medical Sciences, Yokohama, Japan.

Corresponding author

Takashi Nakahara, M.D., Ph.D., Japan.

Department of Gastroenterology and Metabolism, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan.

Phone: +81-082-257-5191, Fax: +81-082-257-5194

E-mail: nakahara@hiroshima-u.ac.jp

Electronic word count

2636 words

Number of figures and tables

This paper contains 5 figures and 2 tables.

<Abstract>

Background & Aims

The prevalence of glucose intolerance in chronic liver disease patients is high, but glucose intolerance may be overlooked in a single blood test. The purpose of this study is to evaluate blood glucose variability in patients with chronic liver disease by a continuous glucose monitoring system (CGMS) and to examine the discrepancy between HbA1c levels estimated from average blood glucose levels and HbA1c.

Methods

This study included 335 patients with chronic liver disease associated with glucose intolerance. A fasting blood test and 72-hour CGMS were performed. The estimated HbA1c was calculated from the average blood glucose level, and the correlation between hepatic functional reserve and blood glucose-related parameters was analyzed. From the obtained data, we created a new formula to calculate HbA1c without using CGMS.

Results

As hepatic functional reserve decreased, average blood glucose and insulin resistance increased while HbA1c decreased ($p<0.0001$). The discrepancy between the estimated HbA1c calculated from the mean blood glucose level and the serum HbA1c (ΔHbA1c) increased as the liver reserve decreased. Using multiple regression analysis, a formula based on fasting blood glucose, HbA1c, body mass index, albumin, and liver function was constructed, and its validity was demonstrated in a study using a different control group.

Conclusions

HbA1c may be underestimated due to decreased hepatic functional reserve. CGMS was useful in assessing accurate glycemic control of blood glucose and in detecting

postprandial hyperglycemia and nocturnal hypoglycemia. Patients with chronic hepatic impairment should be corrected for hepatic functional reserve before glycemic control.

Keywords

liver cirrhosis (LC), continuous glucose monitoring system (CGMS), HbA1c

Abbreviations:

ADAG study: A1c-derived average glucose study

AG: average blood glucose

BMI: body mass index

CGMS: continuous glucose monitoring system

CH: chronic hepatitis

CLD: chronic liver disease

CP: Child-Pugh

FBG: fasting blood glucose

HbA1c: hemoglobin A1c

HOMA-IR: homeostasis model assessment of insulin resistance

LC: liver cirrhosis

NAFLD: nonalcoholic fatty liver disease

NASH: nonalcoholic steatohepatitis

Introduction

It has long been known that glucose intolerance and insulin resistance occur in patients with chronic liver disease [1,2]. The liver is responsible for maintaining blood glucose levels by storing glucose as glycogen and breaking down glycogen to produce glucose [3]. Skeletal muscle also responds to insulin and lowers blood glucose levels by taking up glucose. In patients with liver cirrhosis, hepatocytes and skeletal muscle mass are reduced, and glucose intolerance is thought to occur [4].

Diabetes mellitus is diagnosed by measuring fasting and casual blood glucose levels, the oral glucose tolerance test, and hemoglobin A1c (HbA1c) levels. The goal of diabetes treatment is to improve metabolic abnormalities caused by hyperglycemia and to prevent the onset and progression of diabetic complications. In daily practice, fasting blood glucose and HbA1c levels are commonly used as indicators for glycemic control [5]. HbA1c is the percentage of glycosylated hemoglobin in total hemoglobin and reflects blood glucose levels over a 1–2 month period. However, in some conditions, blood glucose levels are not correctly reflected in HbA1c. HbA1c is low in conditions such as abnormal hemoglobin molecules, anemia due to bleeding, renal failure, pregnancy, cirrhosis, and splenomegaly that shorten the lifespan of red blood cells [6,7]. On the other hand, iron deficiency anemia, B12 deficiency, and folate deficiency anemia, which prolong the lifespan of red blood cells, result in higher HbA1c levels. Lead poisoning and chronic intake of alcohol, salicylates, and opioids have also been reported to falsely elevate HbA1c levels [8]. Glycoalbumin is used as an alternative, but in cirrhosis it is apparently higher due to the prolonged albumin lifespan associated with impaired albumin synthesis [9,10]. In addition, cirrhotic patients are characterized by postprandial hyperglycemia despite normal fasting glucose, and postprandial hyperglycemia has been

reported to increase cardiovascular mortality [4,11]. Thus, underestimation of blood glucose related biomarkers is a problem in the management of diabetes in patients with cirrhosis. A Continuous glucose monitoring system (CGMS) is a method of measuring glucose concentration in interstitial fluid by percutaneously implanting a sensor under the skin. The glucose concentration in the interstitial fluid correlates with blood glucose levels and can be used to measure average blood glucose levels [12]. In the ADAG study, it was reported that average blood glucose (AG) correlated with HbA1c level, and estimated HbA1c (eHbA1c) = (AG + 46.7) / 28.7 can be used to calculate [13]. Using this method, we thought it would be possible to calculate the true HbA1c level reflecting the average blood glucose level of patients with chronic liver disease.

This study examined the discrepancy between serum HbA1c levels and eHbA1c levels in patients with liver disorders. We also constructed and validated a new formula to estimate HbA1c in patients with chronic liver disease without using CGMS.

Patients and Methods

Patients

We studied 335 patients with liver disease who underwent CGMS for glucose intolerance from December 2013 to October 2017 at our hospital. CGMS was performed during hospitalization for liver dysfunction or liver cancer. Physical measurements were taken on the day of admission, and blood tests were performed during an early morning fast the day after admission. During the CGMS, no new therapeutic interventions affecting blood glucose were performed (the currently administered therapeutic drugs were continued), and the results of the CGMS were used to determine the therapeutic strategy for blood glucose control. Patients were diagnosed as diabetic according to the diagnostic criteria

of the Japanese Clinical Practice Guideline for Diabetes 2019 [5]. Fasting blood glucose level of 126 mg/dl or higher, oral glucose tolerance test (OGTT) 2-hour value of 200 mg/dL or higher, blood glucose at any time of 200 mg/dL or higher, and HbA1c of 6.5% or higher are defined as diabetic type. Diabetes mellitus was diagnosed when (1) diabetic type is observed two or more times (one of which must be confirmed by blood glucose level), (2) blood glucose level shows diabetic type, and typical symptoms of diabetes such as thirst, polydipsia, polyuria, weight loss, or obvious diabetic retinopathy are observed, and (3) diabetes mellitus had been diagnosed in the past. The diagnosis of chronic hepatitis or cirrhosis was made comprehensively by biochemical liver function tests, histological tests in percutaneous liver biopsy and hepatectomy, and imaging tests such as abdominal computed tomography scan and ultrasonography [14,15]. Cirrhosis was further subdivided according to the Child-Pugh classification [16,17].

CGMS and estimated HbA1c

We used iPro2 (Medtronic, Dublin, Ireland) for CGMS. A sensor was implanted under the skin of the abdomen for 72 hours to continuously measure glucose concentration, and the maximum, minimum, and average blood glucose levels were recorded. A maximum blood glucose level of 200 mg/dl or higher was considered postprandial hyperglycemia, and a nocturnal blood glucose level of less than 70 mg/dl was considered nocturnal hypoglycemia. Blood glucose was measured in peripheral blood four times per day (before breakfast, lunch, dinner, and bedtime), and it was confirmed that there was no deviation from the CGMS readings. The obtained average blood glucose levels were used to calculate the estimated HbA1c values using the ADAG study conversion formula [13]. The estimated HbA1c was subtracted from the serum HbA1c to obtain ΔHbA1c.

Construction of a new calculation formula and validation study

A new HbA1c calculation formula was developed using linear single and multiple regression analysis with eHbA1c level as the objective variable. The validity of the formula was examined in a validation study using a different control group. A total of 231 patients with chronic liver disease from multiple institutions who underwent CGMS complicated by glucose intolerance between November 2017 and March 2019 were included in the study. A total of 136 cases were from our institution and 95 cases were from collaborating institutions (Kochi University Hospital, Saga University Hospital, and Kurume University Hospital). iPro2 or Freestyle Libre Pro (Abbott, Chicago, USA) were used for CGMS. We examined the correlation between eHbA1c and HbA1c calculated from the conversion formula in this study (cHbA1c).

Statistical analysis

The correlation between average glucose and HbA1c was analyzed using Spearman's rank correlation coefficient test. Differences in each parameter with respect to hepatic functional reserve were tested using the Jonckheere-Terpstra test, and one-way ANOVA was performed when there was no significant difference. The estimated HbA1c and ΔHbA1c between the two groups were compared using the Mann-Whitney U test. Statistical analysis was performed using JMP® pro 15 (SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Ethics

This study was approved by the Ethics Review Committee of Hiroshima University (project identification code number E-624-4). This was a retrospective analysis of records stored in a database and official approval was received based on the Guidelines for Clinical Research issued by the Ministry of Health and Welfare in Japan. All procedures complied with the Declaration of Helsinki.

Result

Patient characteristics

Patient characteristics are shown in Table 1. The median age was 69 years (range 24-84). The median FBG was 122 mg/dl and the median HbA1c was 6.9 % (51.9 mmol/mol). Diabetes mellitus was diagnosed in 254 patients (76%) before the CGM, and 193 of them were treated with hypoglycemic drugs. Hepatic functional reserve was classified as follows: CH, 171 (51%); CP-A, 81 (24%); CP-B, 63 (19%); CP-C, 20 (6%). The causes of liver injury were HCV in 119 cases (36%), HBV in 37 cases (11%), HCV-HBV coinfection in 2 cases (1%), nonalcoholic fatty liver disease (NASH) in 126 cases (38%), and alcoholic and autoimmune hepatitis, primary cholestatic cholangitis, and others in 51 cases (15%). The number of patients with hepatocellular carcinoma (HCC) was 133 (40%).

Markers for the stage of chronic liver disease

Fig. 1 shows a comparison of the parameters for each stage of liver damage. Hb were lower as liver damage progressed ($p<0.0001$). Fasting blood glucose did not differ between stages. Insulin, HOMA-IR, and average blood glucose level levels were higher as liver damage progressed ($p=0.0278$, $p=0.0296$, and $p<0.0001$, respectively), and

HbA1c was lower as liver damage progressed ($p<0.0001$). The percentage of postprandial hyperglycemia was more than 40% in all stages of cirrhosis. The highest rate of nocturnal hypoglycemia was 20% in CP-C.

Correlation between average blood glucose level and HbA1c

Average median blood glucose levels were 146.5 mg/dl for chronic hepatitis and 167 mg/dl for cirrhosis. Median HbA1c values were 7.0% (53.0 mmol/mol) for chronic hepatitis and 6.8% (50.8 mmol/mol) for cirrhosis. The correlation between average blood glucose level and HbA1c level in patients with chronic hepatitis and cirrhosis is shown in Fig. 2. Both chronic hepatitis ($r=0.706$, $p<0.0001$) and cirrhosis ($r=0.496$, $p<0.0001$) showed significant correlation, while the correlation was lower in cirrhosis.

Deviation between HbA1c and eHbA1c

Mean eHbA1c values were 7.0% (53.0 mmol/mol) for CH, 7.7% (60.6 mmol/mol) for CP-A, 7.7% (60.6 mmol/mol) for CPB, and 8.0% (63.9 mmol/mol) for CP-C. Mean ΔHbA1c values were 0.3% (3.3 mmol/mol) for CH, -0.5% (-5.5 mmol/mol) for CP-A, -1.0% (-10.9 mmol/mol) for CP-B, and -2.2% (-24.0 mmol/mol) for CP-C. The difference in ΔHbA1c for CP-C was significantly greater than that for the other stages (Fig. 3). When eHbA1c levels were used, 18 of the 81 patients (22%) who did not meet the diagnostic criteria for diabetes before CGM met the new diagnostic criteria.

Multiple regression analysis with estimated HbA1c as the objective variable

Univariate analysis of each item with estimated HbA1c as the objective variable showed significant differences in BMI, HbA1c, Alb, FBG, HbA1c, ChE, and liver function.

Further multiple regression analysis revealed that FBG, HbA1c, BMI, ALB, and liver function were explanatory variables. Decreased liver function was positively correlated with HbA1c level. From the obtained coefficients, a new conversion formula was constructed (Fig. 4). We referred to the HbA1c obtained from this equation as calculated HbA1c (cHbA1c).

Validation study

The characteristics of the control group of the validation study are shown in table 2. The median age was 69 years (range 16-87). The median FBG was 112 mg/dl and the median HbA1c was 6.9 % (51.9 mmol/mol). Hepatic functional reserve was classified as follows: CH, 163 (71%); CP-A, 31 (13%); CP-B, 31 (13%); CP-C, 6 (3%). There was insufficient data collection on LDL cholesterol levels and the presence of a history of diabetes treatment. The correlation between the eHbA1c and cHbA1c was analyzed using Spearman's rank correlation coefficient test, and a high correlation was observed ($r=0.749$, $p=<0.0001$) (Fig. 5).

Discussion

In a large cohort study, diabetes mellitus was shown to be an independent risk factor for chronic liver disease and hepatocellular carcinoma [18]. The liver also plays a central role in glucose metabolism, and almost all patients with cirrhosis show insulin resistance, 60% to 80% are glucose intolerant, and about 20% will develop diabetes [19]. In nonalcoholic fatty liver disease (NALFD), diabetes mellitus was reported to be an important risk factor for liver fibrosis progression [20]. It was also reported that increased HbA1c was involved in the progression of histological fibrosis in NASH [21]. Thus, there is a close relationship

between diabetes and chronic liver disease. It is also important to maintain good glycemic control status because patients with inadequate glycemic control have a poor prognosis [22].

In this study, we first compared blood glucose trends measured by CGMS at each liver reserve with blood glucose parameters measured during fasting. When compared by hepatic reserve, fasting blood glucose showed no difference, while average blood glucose levels were higher and serum HbA1c was lower as hepatic reserve decreased. In addition, both insulin secretion and insulin resistance increased as liver reserve decreased. These results were consistent with previous reports [23]. In patients with chronic hepatic disease, the rate of insulin metabolism in hepatocytes is decreased, resulting in hyperinsulinemia. In the presence of a portal-systemic shunt, insulin levels in the periphery increase, while insulin levels in the portal vein decrease, resulting in decreased glucose uptake into hepatocytes [24,25]. As a result, as liver damage progresses, diurnal blood glucose fluctuations increase. A previous study using CGMS at our institution reported that decreased liver reserve was associated with worse glycemic variability [26]. In this study, cirrhotic patients also had higher rates of postprandial hyperglycemia and nocturnal hypoglycemia than chronic hepatitis patients. It was thought that the use of CGMS would enable us to identify such trends in blood glucose variability and to select appropriate therapeutic agents such as insulin preparations and oral medications.

Next, we examined the correlation between average blood glucose levels and serum HbA1c levels. The correlation was reduced in patients with cirrhosis. Comparing eHbA1c and serum HbA1c levels, the difference increased as liver reserve decreased, and CP-C showed a discrepancy of -2.2% (-24.0 mmol/mol) in ΔHbA1c. These results suggest that a decrease in liver reserve may lead to a low serum HbA1c despite worsening glucose

intolerance and that diabetes may be underestimated. It was reported that HbA1c levels were lower than the actual glycemic control state in patients with chronic liver disease, which was consistent with the results of the previous study [6]. One of the factors contributing to the decrease in HbA1c in patients with cirrhosis might be an increase in splenic blood flow due to the progression of liver fibrosis and the shortening of the half-life of red blood cells due to increased splenic function [2,7,27].

From these results, we concluded that in the treatment of diabetes in patients with cirrhosis, CGMS should be used for evaluation, rather than fasting glucose and HbA1c as the only indicators for management. In addition, patients who have not been diagnosed with diabetes and whose glucose intolerance has worsened due to the progression of cirrhosis may not be screened and may be overlooked. CGMS is also useful in clarifying the possibility of postprandial hyperglycemia and nocturnal hypoglycemia in cirrhotic patients.

One disadvantage of CGMS is that the device must be worn at all times during the measurement, which takes several days. We attempted to construct a new formula for cHbA1c. Multivariate analysis identified HbA1c, FBG, ALB, BMI, and liver reserve as independent factors contributing to eHbA1c. These factors were used to construct a formula for determining cHbA1c without CGMS. This formula may also be useful in facilities where CGMS cannot be worn due to skin diseases or that are not equipped for CGMS. By including Child-Pugh classification, ALB, and BMI, the formula reflects decreased liver reserve. In the case of the same HbA1c value, the worse the liver reserve, the higher the true HbA1c. BMI was also included in the prediction equation. This indicates that the higher the BMI, the greater the discrepancy between serum HbA1c and the true HbA1c. Although the detailed mechanism is unknown, iron deficiency may occur

in obese patients [28], and it is thought that prolongation of red blood cell lifespan may lead to higher apparent HbA1c. This prediction formula enables correction of the discrepancy between liver reserve and obesity-induced HbA1c. A validation experiment using another group of subjects showed a correlation between eHbA1c and cHbA1c. We propose that the handling of serum HbA1c levels in the daily treatment of patients with liver cirrhosis should be corrected in this way before controlling blood glucose.

There are some limitations to our study. Since this study included only patients with type 2 diabetes, we did not evaluate patients with chronic liver disease without type 2 diabetes. We were also unable to evaluate the glycemic parameters of diabetic patients without hepatic impairment as a control group. In addition, the diet and diabetes medications during CGMS measurement were not standardized, which may have affected the incidence of postprandial hyperglycemia and nocturnal hypoglycemia.

Conclusion

In this study, serum HbA1c levels were found to be lower than that predicted from average blood glucose levels due to decreased hepatic functional reserve. CGMS enables us to understand the correct blood glucose dynamics and to make appropriate treatment choices. Patients with chronic hepatic disease should be managed with correction for hepatic functional reserve and not just daily blood glucose control based on serum HbA1c.

<Acknowledgements>

Financial support statement

This research is supported by Japan Agency for Medical Research and Development (AMED) [grant number JP21fk0210090]. We thank all study participants for their cooperation. We also thank our staff for recruiting subjects and for their technical assistance.

Authors contributions

Y.O., T.N. contributed to this paper with conception, literature review and writing the manuscript. M.O, T.K., H.I., A.H., S.U., H.F., E.M., T.K., M.Y., M.T., K.M., T.O., C.N.H, M.I., H.A., H.T, T.T., K.C. were involved in study design and data interpretation. All authors critically revised the report, commented on drafts of the manuscript, and approved the final report.

Conflict of interest statement

T. Kawaguchi has received honoraria from Mitsubishi Tanabe Pharma, Otsuka Pharma. M. Imamura has received research funding from AbbVie. H. Takahashi has received research funding Astellas Pharma, Mitsubishi Tanabe Pharma, EA Pharma. K. Chayama has received honoraria from MSD K.K., Bristol- Myers Squibb, Gilead Sciences, and AbbVie, and research funding from Dainippon Sumitomo Pharma, TORAY, Eisai, Otsuka Pharma, Mitsubishi Tanabe Pharma, Daiichi Sankyo, Janssen, and Bristol-Myers Squibb.

References

[1] Muting D, Wohlgemuth D, Dorsett R. Liver cirrhosis and diabetes mellitus. *Geriatrics*. 1969; **24**: 91-9.

[2] Cacciatore L, Cozzolino G, Giardina MG*, et al.* Abnormalities of glucose metabolism induced by liver cirrhosis and glycosylated hemoglobin levels in chronic liver disease. *Diabetes Res*. 1988; **7**: 185-8.

[3] Roden M, Bernroider E. Hepatic glucose metabolism in humans--its role in health and disease. *Best Pract Res Clin Endocrinol Metab*. 2003; **17**: 365-83.

[4] Imano E, Kanda T, Nakatani Y*, et al.* Impaired splanchnic and peripheral glucose uptake in liver cirrhosis. *J Hepatol*. 1999; **31**: 469-73.

[5] Araki E, Goto A, Kondo T*, et al.* Japanese Clinical Practice Guideline for Diabetes 2019. *J Diabetes Investig*. 2020; **11**: 1020-76.

[6] Subhiyah BW, al-Hindawi AY. Red cell survival and splenic accumulation of radiochromium in liver cirrhosis with splenomegaly. *Br J Haematol*. 1967; **13**: 773-8.

[7] Trenti T, Cristani A, Cioni G, Pentore R, Mussini C, Ventura E. Fructosamine and glycated hemoglobin as indices of glycemic control in patients with liver cirrhosis. *Ric Clin Lab*. 1990; **20**: 261-7.

[8] Radin MS. Pitfalls in hemoglobin A1c measurement: when results may be misleading. *J Gen Intern Med*. 2014; **29**: 388-94.

[9] Bianchi R, Mariani G, Pilo A, Toni MG. Serum albumin turnover in liver cirrhosis. *J Nucl Biol Med*. 1974; **18**: 20-9.

[10] Stefanovíc S, Bosnjakovic V, Stajnfl S, Vujnić V, Petrić J, Ristić M. Characteristics of albumin turnover in different stages of cirrhosis of liver evaluated by means of radioisotope technique and analog computer analysis. *Radiobiol Radiother (Berl)*. 1972; **13**: 105-11.

[11] Nakagami T. Hyperglycaemia and mortality from all causes and from cardiovascular disease in five populations of Asian origin. *Diabetologia*. 2004; **47**: 385-94.

[12] Rebrin K, Steil GM. Can interstitial glucose assessment replace blood glucose measurements? *Diabetes Technol Ther*. 2000; **2**: 461-72.

[13] Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C assay into estimated average glucose values. *Diabetes Care*. 2008; **31**: 1473-8.

[14] Tokushige K, Ikejima K, Ono M*, et al.* Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. *J Gastroenterol*. 2021; **56**: 951-63.

[15] Yoshiji H, Nagoshi S, Akahane T*, et al.* Evidence-based clinical practice guidelines for liver cirrhosis 2020. *Hepatol Res*. 2021; **51**: 725-49.
[16] Child CG, Turcotte JG. Surgery and portal hypertension. *Major Probl Clin Surg*. 1964; **1**: 1-85.
[17] D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol*. 2006; **44**: 217-31.
[18] El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology*. 2004; **126**: 460-8.
[19] Petrides AS. [Hepatogenic diabetes: pathophysiology, therapeutic options and prognosis]. *Z Gastroenterol*. 1999; **Suppl 1**: 15-21.
[20] Nakahara T, Hyogo H, Yoneda M*, et al.* Type 2 diabetes mellitus is associated with the fibrosis severity in patients with nonalcoholic fatty liver disease in a large retrospective cohort of Japanese patients. *J Gastroenterol*. 2014; **49**: 1477-84.
[21] Daijo K, Nakahara T, Inagaki Y*, et al.* Risk factors for histological progression of non-alcoholic steatohepatitis analyzed from repeated biopsy cases. *J Gastroenterol Hepatol*. 2020; **35**: 1412-9.
[22] Bianchi G, Marchesini G, Zoli M, Bugianesi E, Fabbri A, Pisi E. Prognostic significance of diabetes in patients with cirrhosis. *Hepatology*. 1994; **20**: 119-25.
[23] Iwasaki Y, Ohkubo A, Kajinuma H, Akanuma Y, Kosaka K. Degradation and secretion of insulin in hepatic cirrhosis. *J Clin Endocrinol Metab*. 1978; **47**: 774-9.
[24] Bosch J, Gomis R, Kravetz D*, et al.* Role of spontaneous portal-systemic shunting in hyperinsulinism of cirrhosis. *Am J Physiol*. 1984; **247**: G206-12.
[25] Johnson DG, Alberti KG, Faber OK, Binder C. Hyperinsulinism of hepatic cirrhosis: Diminished degradation or hypersecretion? *Lancet*. 1977; **1**: 10-3.
[26] Honda F, Hiramatsu A, Hyogo H*, et al.* Evaluation of glycemic variability in chronic liver disease patients with type 2 diabetes mellitus using continuous glucose monitoring. *PLoS One*. 2018; **13**: e0195028.
[27] Nomura Y, Nanjo K, Miyano M*, et al.* Hemoglobin A1 in cirrhosis of the liver. *Diabetes Res*. 1989; **11**: 177-80.
[28] Aigner E, Feldman A, Datz C. Obesity as an emerging risk factor for iron deficiency. *Nutrients*. 2014; **6**: 3587-600.

Table 1. Patient characteristics

	median		min		max	
Age, years	69	(	32	-	91	)
BMI, kg/m^2	24.7	(	15.4	-	67	)
Hemoglobin, g/dl	13.3	(	4.2	-	19.1	)
Platelet count, ×10^4/μl	15.9	(	3.3	-	71.3	)
Total bilirubin, mg/dL	0.8	(	0.3	-	7.3	)
Albumin, g/dL	3.9	(	1.7	-	5.3	)
AST, U/L	32	(	10	-	324	)
ALT, U/L	28	(	6	-	409	)
Cholinesterase, U/L	277	(	46	-	605	)
Total cholesterol, mg/dL	178	(	82	-	300	)
LDL cholesterol, mg/dL	112	(	38	-	202	)
Triglyceride, mg/dL	111	(	31	-	461	)
Uric acid, mg/dL	5.4	(	1.6	-	11.3	)
FBG, mg/dL	122	(	68	-	388	)
HbA1c, %	6.9	(	3.8	-	14.3	)
HbA1c, mmol/mol	51.9	(	18.0	-	132.8	)
IRI, μU/mL	11.8	(	0.6	-	94	)
HOMA-IR	3.6	(	0.1	-	41.5	)
FIB-4 index	2.78	(	0.47	-	21.4	)
Diabetes mellitus, (+ / -)		(	254 / 81			)
Hepatic functional reserve, (CH / CP-A / CP-B / CP-C)		(	171 / 81 / 63 / 20			)
Etiology, (HCV / HBV / B+C / NAFLD / Other)		(	119 / 37 / 2 / 126 / 51			)
HCC , (+ / -)		(	133 / 202			)

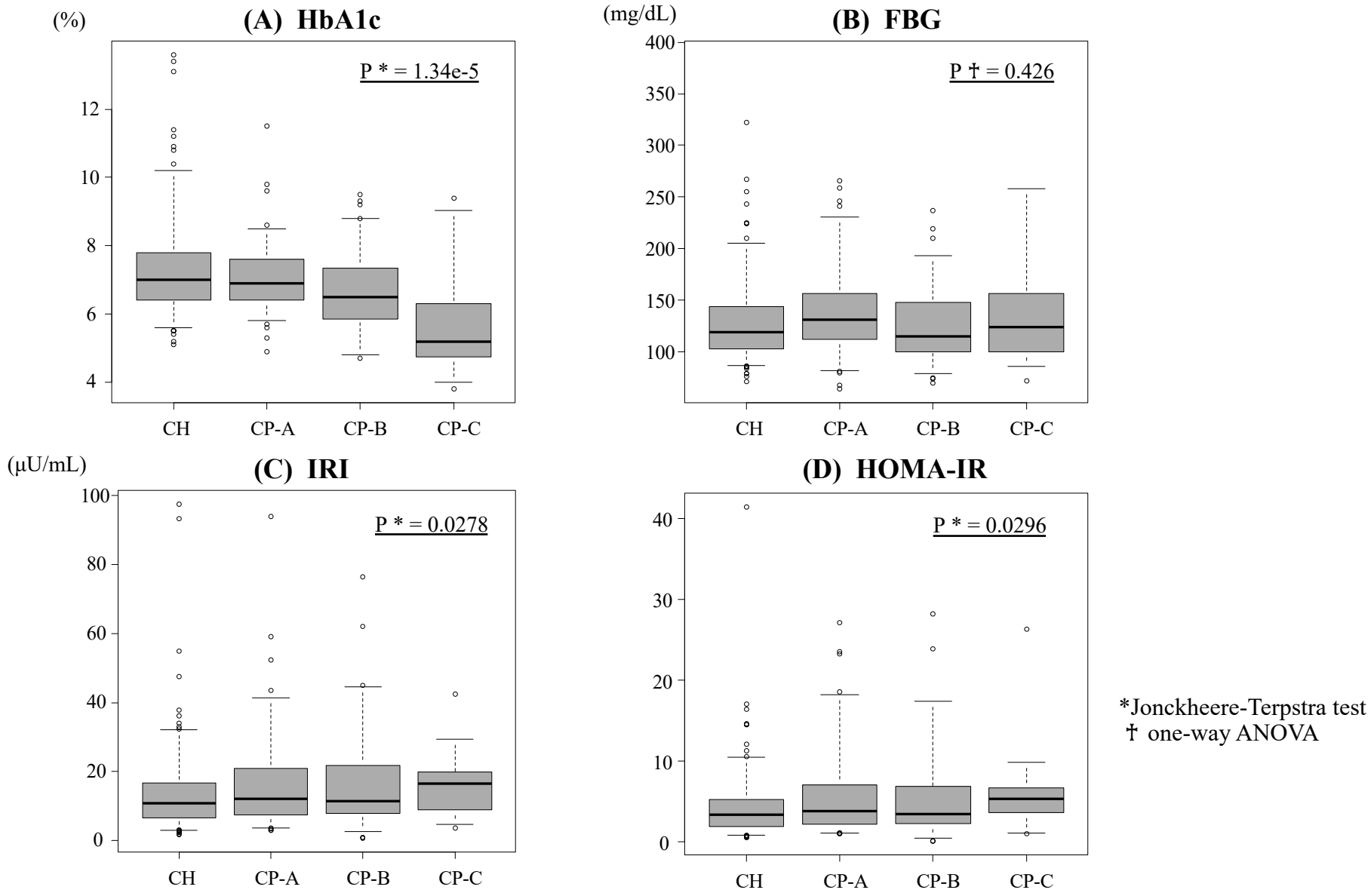
Data are presented as median (maximum and minimum) or number of subjects.
BMI, body mass index; AST, aspartate amino transferase; ALT, alanine amino transferase; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; FIB-4 index, fibrosis-4 index; CH, chronic hepatitis; CP, Child-Pugh; NAFLD, nonalcoholic fatty liver disease.

Table 2. Patient characteristics of validation study

	median		min		max	
Age, years	69	(	16	-	87	)
BMI, kg/m^2	24.7	(	16.6	-	48.7	)
Hemoglobin, g/dl	13.6	(	7.8	-	17.8	)
Platelet count, ×10^4/μl	13.6	(	2.0	-	67.8	)
Total bilirubin, mg/dL	0.9	(	0.3	-	7.2	)
Albumin, g/dL	3.9	(	2.0	-	5.3	)
AST, U/L	40	(	14	-	174	)
ALT, U/L	36	(	7	-	312	)
Cholinesterase, U/L	244	(	69	-	583	)
Total cholesterol, mg/dL	174	(	65	-	296	)
Triglyceride, mg/dL	100	(	25	-	451	)
Uric acid, mg/dL	5.1	(	1.6	-	9.7	)
FBG, mg/dL	112	(	62	-	408	)
HbA1c, %	6.6	(	3.8	-	13.2	)
HbA1c, mmol/mol	48.6	(	18.0	-	120.8	)
IRI, μU/mL	12.3	(	1.4	-	123.5	)
HOMA-IR	3.4	(	0.3	-	32.6	)
FIB-4 index	3.47	(	0.27	-	31.4	)
Hepatic functional reserve, (CH / CP-A / CP-B / CP-C)		(	163 / 31 / 31 / 6			)
Etiology, (HCV / HBV / B+C / NAFLD / Other)		(	95 / 16 / 0 / 93 / 27			)
HCC, (+ /-)		(	62 / 169			)

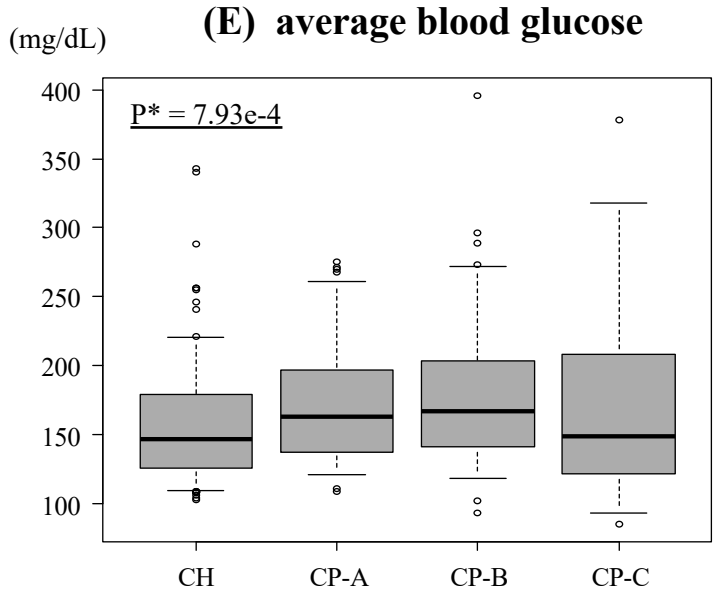
Data are presented as median (maximum and minimum) or number of subjects.
BMI, body mass index; AST, aspartate amino transferase; ALT, alanine amino transferase; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; FIB-4 index, fibrosis-4 index; CH, chronic hepatitis; CP, Child-Pugh; NAFLD, nonalcoholic fatty liver disease.

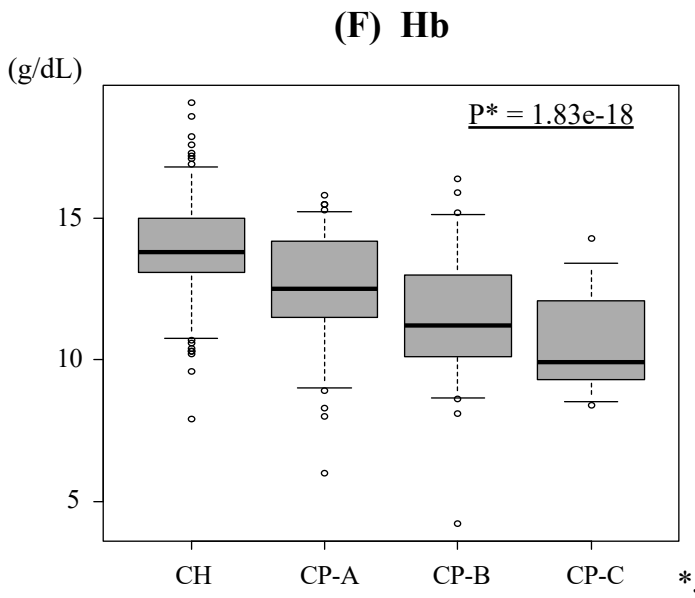
Fig. 1. Various markers in the stages of chronic liver disease



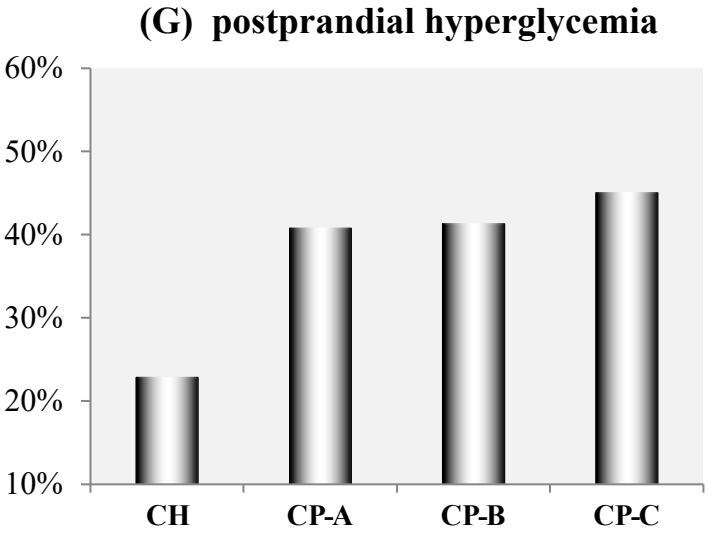
*Jonckheere-Terpstra test
† one-way ANOVA

Fig. 1. Various markers in the stages of chronic liver disease.





*Jonckheere-Terpstra test



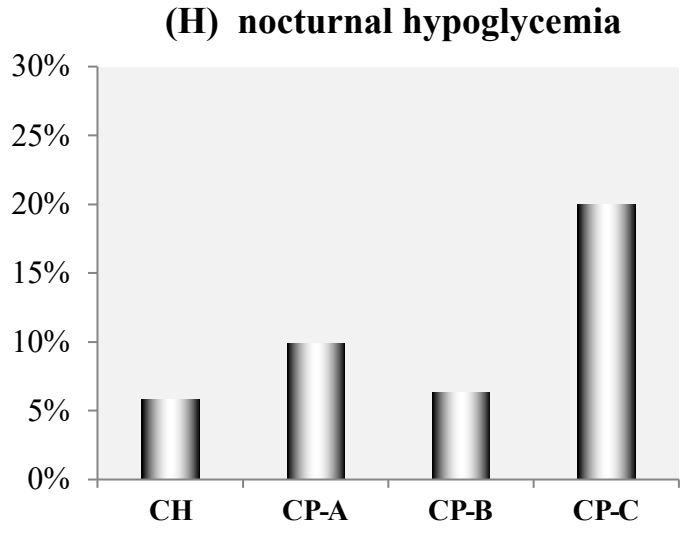
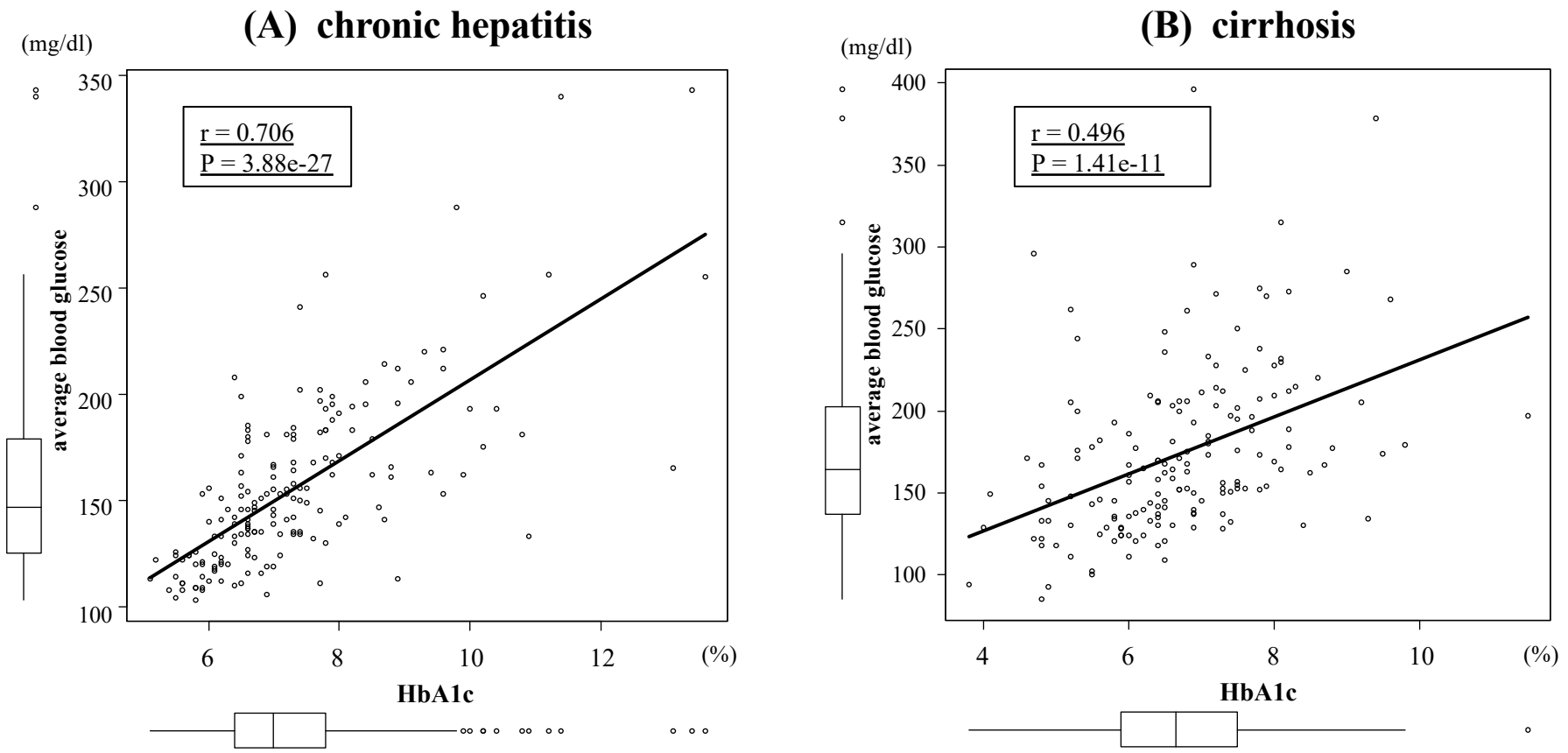


Fig. 1. Various markers in the stages of chronic liver disease

(A) HbA1c, (B) fasting blood sugar (FBS), (C) immunoreactive insulin (IRI), (D) homeostasis model assessment of insulin resistance (HOMA-IR), (E) average blood glucose, (F) hemoglobin (Hb), (G) postprandial hyperglycemia, and (H) nocturnal hypoglycemia according to the severity of background liver disease in patients with chronic hepatitis (CH), Child-Pugh grade A (CP-A), B (CP-B), and C (CP-C). In these box-and-whisker plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 25th and 75th percentiles, respectively; and the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively.

Fig. 2. Correlation between average blood glucose and HbA1c in chronic hepatitis and cirrhosis

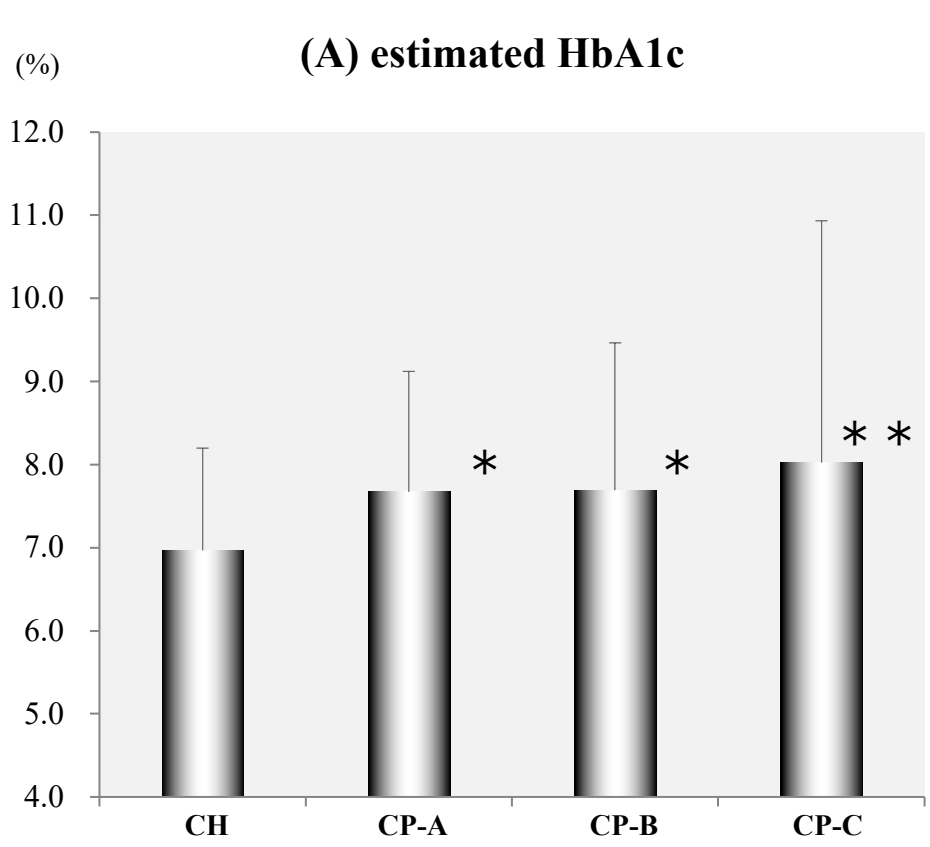


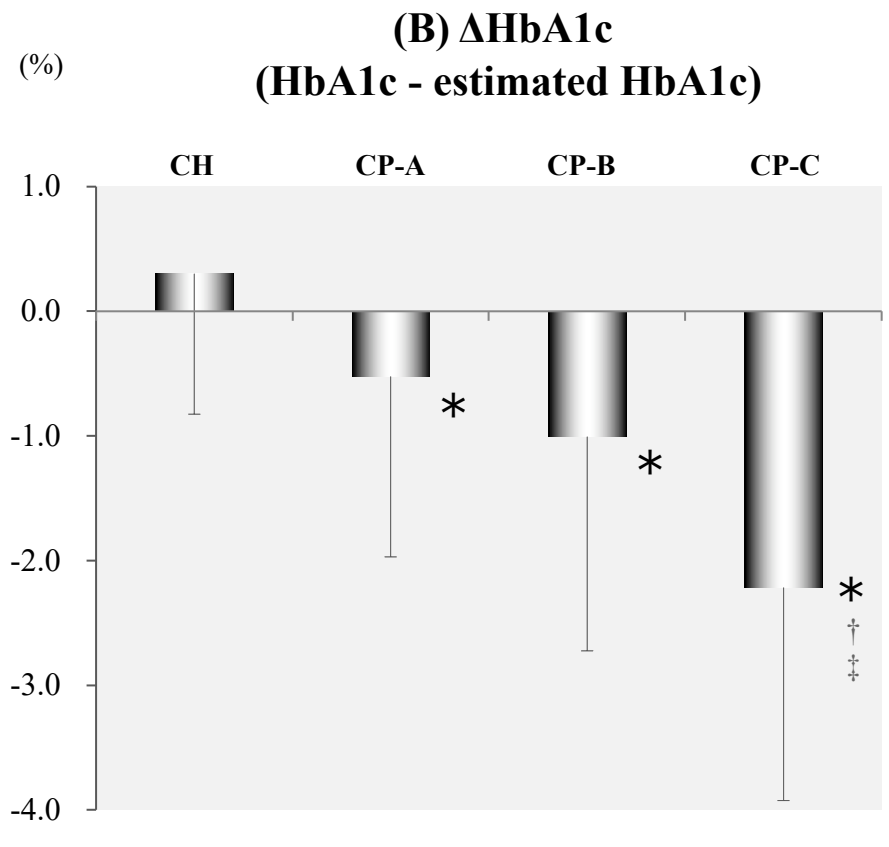
Spearman's rank correlation coefficient

Fig. 2. Correlation between average blood glucose and HbA1c in chronic hepatitis and cirrhosis

(A) chronic hepatitis (B) cirrhosis. In these box-and-whisker plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 25th and 75th percentiles.

Fig. 3. Deviation between estimated and measured value of HbA1c





estimated HbA1c =
(average glucose level + 46.7) / 28.7

＊ P < 0.01 vs CH
＊＊ P < 0.05 vs CH
† P < 0.01 vs CP-A
‡ P < 0.05 vs CP-B
(Mann–Whitney U test)

Fig. 3. Deviation between estimated and measured value of HbA1c

(A) estimated HbA1c and (B) ΔHbA1c according to the severity of background liver disease in patients with chronic hepatitis (CH), Child-Pugh grade A (CP-A), B (CP-B), and C (CP-C). The bar graph shows the mean value, and the error bars show the standard deviation.

Fig. 4. Multiple regression analysis with estimated HbA1c as the objective variable

(A)

	Estimate	S.E	tStat	P value
(Intercept)	5.376	0.896	6.0	<.0001
FBG	0.016	0.002	8.7	<.0001
HbA1c	0.353	0.057	6.19	<.0001
BMI	-0.046	0.012	-3.9	0.0001
ALB	-0.424	0.168	-2.52	0.0124
Child-Pugh	0.262	0.120	2.18	0.0303

(B)

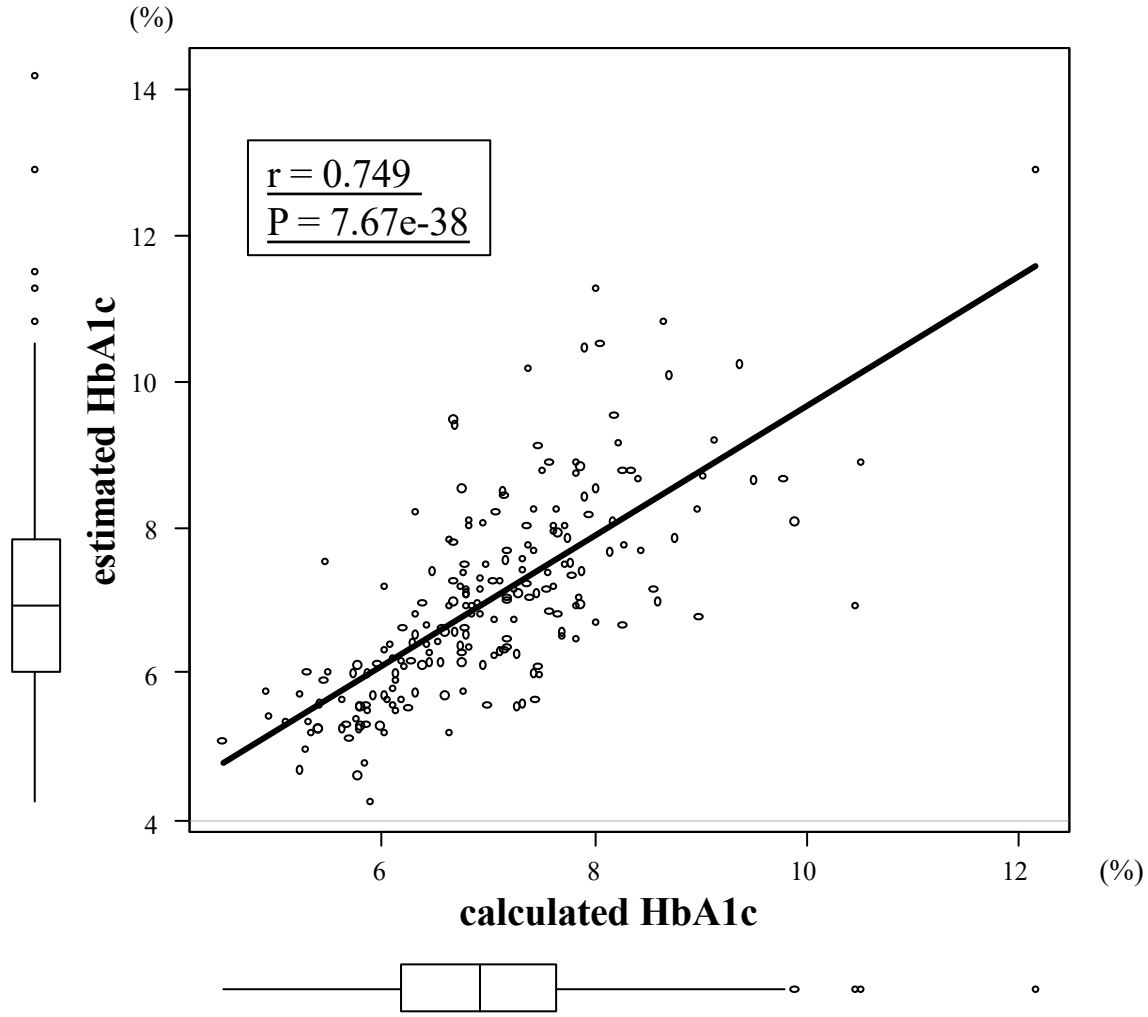
calculated HbA1c (%)
≒ 5.38 + (FBG×0.016) + (HbA1c×0.353) - (BMI×0.046) - (Alb×0.424)
+ 0 ← **CH**
+ 0.262 ← **CP-A**
+ 0.524 ← **CP-B**
+ 0.786 ← **CP-C**

Fig. 4. Multiple regression analysis with estimated HbA1c as the objective variable

(A) Results of multiple regression analysis with HbA1c as the objective variable.

(B) HbA1c prediction formula constructed by multiple regression analysis.

Fig. 5. Plot of estimated HbA1c from CGMS and calculated HbA1c from the prediction formula (Validation study)



Spearman's rank correlation coefficient

Fig. 5. Plot of estimated HbA1c from CGMS and calculated HbA1c from the prediction formula (Validation study)

The vertical axis shows the estimated HbA1c value calculated from the average glucose level, and the horizontal axis shows the HbA1c calculated from the prediction formula constructed in this study.