Clinical Chemistry

PD-01

Serum and plasma lysophosphatidic acid analysis using column chromatography and MALDI-TOF mass spectrometry

Makoto Yamaura, Sunao Morita, Atsushi Hori, Hiroya Hidaka

Department of Biomedical and Laboratory Sciences, Shinshu University School of Medicine, Japan.

【Background and Aims】

Lysophosphatidic acid (LPA) is a lipid mediator with potent bioactivity, and its binding with specific receptors is influenced by the identity of its fatty acid side chain. LPA has been found in serum, and its level in the blood has been found to correlate with the occurrence of various diseases. However, there is no appropriate analytical method available for quantifying LPA species in the clinical laboratory.

In this study, we examined LPA species in serum and plasma using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and column chromatography.

【Methods】

1. The plasma sample was collected from whole blood in a plastic tube containing EDTA-2K.
2. Samples were mixed with LPA(C17:0, the internal standard) and a citrate-phosphate buffer (pH4.0). The lipids were extracted with 1-butanol and water-saturated 1-butanol solution.
3. The LPA-rich fraction was partially purified by column chromatography, and then LPA species were analyzed with the negative ion mode method by MALDI-TOF MS.

【Results】

1. Mass spectrum of serum LPA

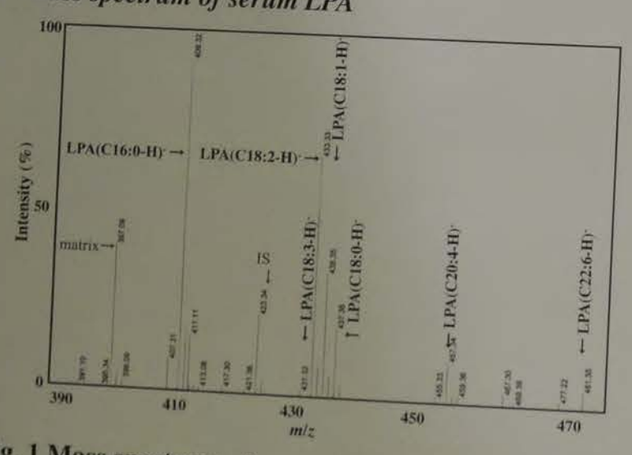


Fig. 1 Mass spectrum of serum LPA.
Seven peaks in the range of *m/z* 390–490 were detected, and these corresponded to LPA species as confirmed by MS/MS analysis.

2. Product ion analysis of LPA

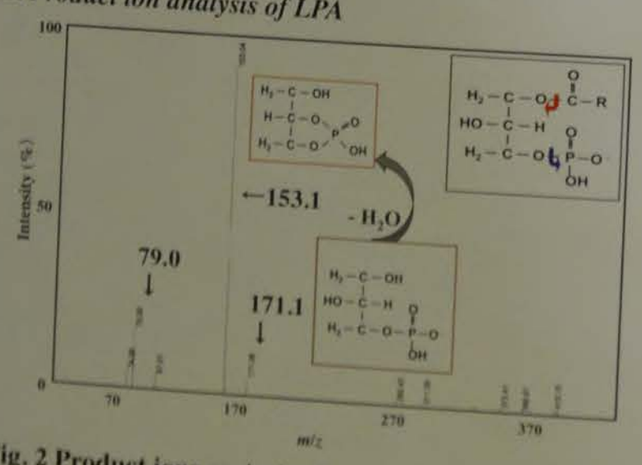


Fig. 2 Product ions analysis of serum LPA (*m/z* 435).
Serum LPA at *m/z* 435, LPA(C18:1-H)⁻, was analyzed with the negative ion mode method. The product ion peaks at *m/z* 79.0 and *m/z* 171.1 were phosphate and glycerophosphate respectively, and the peak at *m/z* 153 was the cyclic version of *m/z* 171.1, i.e., a dehydrated glycerophosphate.

3. Calibration

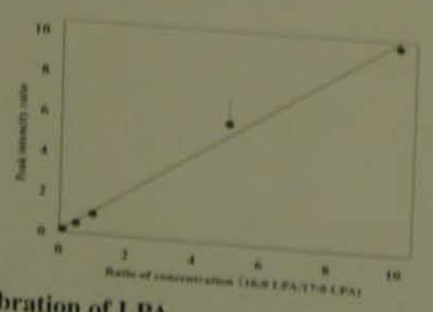


Fig. 3 Calibration of LPA .
The diluent preparations of the LPA C16:0 with the internal standard, LPA C17:0, were measured by MALDI-TOF mass spectrometry. (n=3).

4. Reproducibility of LPA species measurement

Table 1. Reproducibility of LPA species measurement

LPA	Within assay (Mean ± SD, CV%)	Between assay (Mean ± SE, CV%)
LPA C16:0	1.43±0.019 (1.35)	1.46±0.034 (2.35)
LPA C18:3	0.02±0.014 (28.6)	0.05±0.006 (14.3)
LPA C18:2	1.24±0.030 (2.39)	1.30±0.078 (6.00)
LPA C18:1	0.56±0.036 (6.38)	0.56±0.010 (1.72)
LPA C18:0	0.28±0.020 (7.29)	0.28±0.010 (3.64)
LPA C20:5	0.05±0.008 (16.6)	0.04±0.009 (21.6)
LPA C20:4	0.25±0.010 (3.80)	0.36±0.013 (3.56)
LPA C22:6	0.09±0.007 (8.71)	0.09±0.003 (3.45)

Within each well is the mean value (μmol / L), SD, and CV of triplicate measurements in the same well. Between wells is the mean value (μmol / L), SE, and CV of respective measurements in the three wells.

5. Linearity of serum LPA species

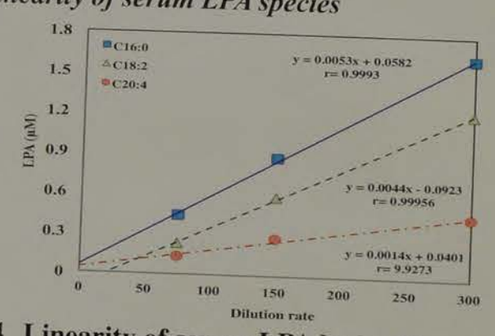


Fig. 4 Linearity of serum LPA by healthy young human subjects.
LPA in serum from the healthy young subjects was measured with MALDI-TOF MS. The internal standard material (LPA [C17:0]) was used in the analysis. Closed square is LPA C16:0, closed triangle is LPA C18:2, and closed diamond is LPA C20:4.

6. Concentrations of LPA species in serum and plasma.

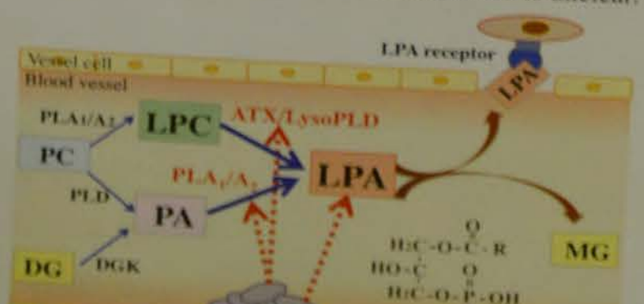
Table 2. Concentrations of LPA species in serum and plasma

Species / time*	Serum 0h	Serum 1h	Plasma 0h	Plasma 1h
LPA (C16:0)	N.D.	1.33±0.12	N.D.	N.D.
LPA (C18:3)	N.D.	N.Q.	N.D.	N.D.
LPA (C18:2)	0.14±0.05	1.13±0.23	N.D.	N.D.
LPA (C18:1)	N.D.	0.44±0.04	N.D.	N.D.
LPA (C18:0)	N.D.	0.32±0.04	N.D.	N.D.
LPA (C20:5)	N.D.	N.Q.	N.D.	N.D.
LPA (C20:4)	0.13±0.03	0.34±0.16	N.D.	N.D.
LPA (C22:6)	N.D.	0.07±0.01	N.D.	N.D.

Sample lipids were extracted as described above, and LPA species measured with the internal standard material. Time*: incubation room temperature. 0h; fresh serum and plasma, 1h; incubation a temperature.

【Discussions】

In this study, we present the analysis using column chromatography and MALDI-TOF MS method to analyze various LPA species from serum and plasma. However, fresh plasma levels were all lower than sensitivity of the measurement, i.e., <100 nmol/L. Serum LPA might be released from peripheral cells by blood clotting. Subsequently autotaxin (ATX), an LPA-producing enzyme, was activated in the serum such that LPA might be produced. The serum LPA level may reflect a state of LPA production in vitro, but the clinical association is unclear.



【Conclusion】

This study showed that LPA can be analyzed simply and sensitively by partial purification with column chromatography followed by measurement using MALDI-TOF MS. Serum LPA may show degrees of release from the peripheral blood cells, and production by ATX.

Clinical Chemistry

PD-02

Analysis of human serum sphingomyelin species by MALDI-TOF mass spectrometry in negative ion mode

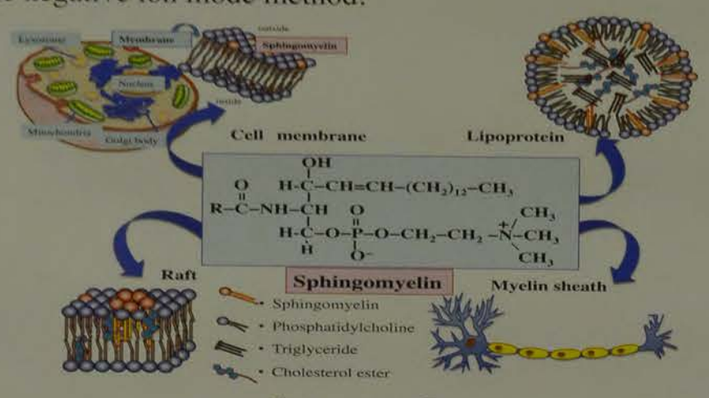
Sunao Morita, Makoto Yamaura, Atsushi Hori, Hiroya Hidaka

Department of Biomedical and Laboratory Sciences, Shinshu University School of Medicine, Japan

【Background and Aims】

Sphingomyelin (SM) plays important roles in biological membrane maintenance and cell signaling.

This study shows a simple method for profiling human serum SM by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the negative ion mode method.



【Methods】

1. Serum was treated with phospholipase A_2 (from *Crotalus adamanteus*) in Tris-HCl buffer (containing $CaCl_2$, pH 8.0).
2. Enzymatic treated serum lipid with an internal standard material (SM[d18:1C12:0]) was extracted with chloroform/ methanol (2/1 v/v).
3. The lipid was mixed with a matrix material.
4. The SM species and product ions were measured using MALDI-TOF MS (AB SCIEX TOF/TOF 5800 system).

【Results】

1. Mass Spectrum of serum sphingomyelin

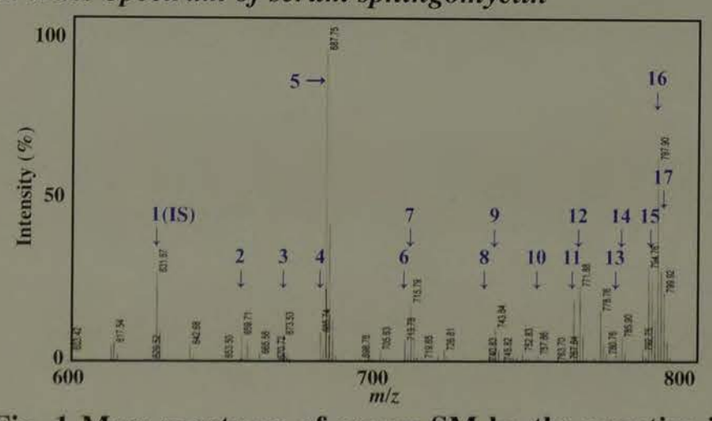


Fig. 1 Mass spectrum of serum SM by the negative ion mode method.

The lipid was measured in the range of *m/z* 600–800, and 16 peaks were detected. The peaks were identified as SM by product ion analysis.

2. Product ion analysis of serum sphingomyelin species

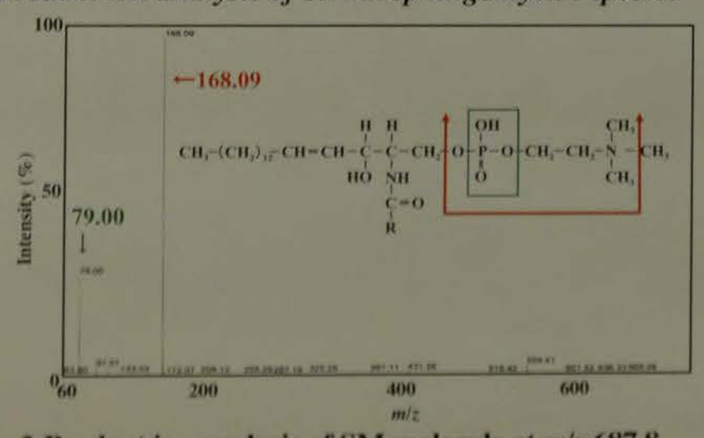


Fig. 2 Product ion analysis of SM molecule at *m/z* 687.8.

The product ion peaks at *m/z* 79.0 and 168.1 corresponded to phosphate residue and demethylated phosphorylcholine, respectively. The insert figure shows the cleavage positions of the SM molecule detected by the product ion analysis.

3. Assignment of SM species

Table1. Assignment of SM species

peak No	*m/z*	molecular ion	LCB	FA
1(IS)	631.7	$[M-CH_3]^-$	d 18:1	C12:0
2	659.7	$[M-CH_3]^-$	d 18:1	C14:0
3	673.7	$[M-CH_3]^-$	d 18:1	C15:0
4	685.7	$[M-CH_3]^-$	d 18:1	C16:1
5	687.8	$[M-CH_3]^-$	d 18:1	C16:0
6	713.8	$[M-CH_3]^-$	d 18:1	C18:1
7	715.8	$[M-CH_3]^-$	d 18:1	C18:0
8	741.8	$[M-CH_3]^-$	d 18:1	C20:1
9	743.8	$[M-CH_3]^-$	d 18:1	C20:0
10	757.9	$[M-CH_3]^-$	d 18:1	C21:0
11	769.9	$[M-CH_3]^-$	d 18:1	C22:1
12	771.9	$[M-CH_3]^-$	d 18:1	C22:0
13	783.9	$[M-CH_3]^-$	d 18:1	C23:1
14	785.9	$[M-CH_3]^-$	d 18:1	C23:0
15	795.9	$[M-CH_3]^-$	d 18:1	C24:2
16	797.9	$[M-CH_3]^-$	d 18:1	C24:1
17	799.9	$[M-CH_3]^-$	d 18:1	C24:0

4. Calibration

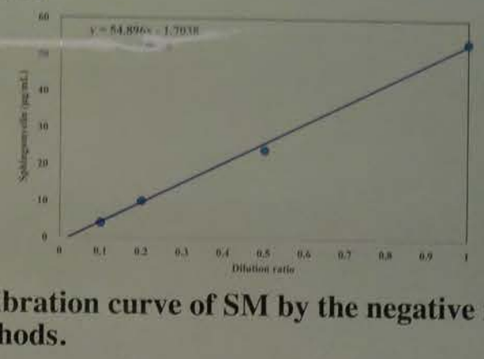


Fig. 3 Calibration curve of SM by the negative ion mode methods.

The hexadecanoyl sphingomyelin (SM[C16:0]) was diluted with C/M (2/1 v/v). The diluted solutions mixed with the internal standard material (SM [C12:0]) were measured by the negative ion mode method.

5. Reproducibility of serum SM by the negative mode and positive ion mode method

Table 2. Reproducibility of serum SM species

SM species	mean ±SD (μg/mL)(CV%) Positive ion mode	Negative ion mode
C14:0	6.6 ± 0.89 (13.4)	6.8 ± 0.35 (5.2)
C15:0	7.0 ± 0.98 (13.9)	2.6 ± 0.43 (16.6)
C16:1	8.6 ± 1.59 (18.4)	10.2 ± 0.57 (5.6)
C16:0	48.7 ± 4.35 (9.0)	96.9 ± 3.93 (4.1)
C18:1	6.1 ± 0.67 (10.8)	7.3 ± 0.53 (7.2)
C18:0	9.0 ± 0.98 (10.8)	16.4 ± 0.73 (4.5)
C20:1	3.7 ± 0.49 (13.3)	5.1 ± 0.48 (9.5)
C20:0	7.6 ± 1.14 (15.1)	11.2 ± 0.92 (8.2)
C21:0	3.8 ± 0.78 (20.7)	3.0 ± 0.20 (6.7)
C22:1	8.8 ± 1.05 (9.0)	17.7 ± 1.30 (7.3)
C22:0	11.1 ± 0.95 (8.6)	22.6 ± 1.77 (7.8)
C23:1	4.6 ± 0.86 (18.5)	7.4 ± 0.46 (6.1)
C23:0	5.6 ± 0.75 (13.4)	8.7 ± 0.79 (9.1)
C24:2	16.2 ± 1.04 (6.4)	27.7 ± 3.85 (13.9)
C24:1	26.2 ± 2.34 (8.9)	66.4 ± 5.18 (7.8)
C24:0	13.7 ± 1.31 (9.7)	21.2±2.71 (12.8)
total	187.1 ± 15.16 (8.1)	329.2 ± 17.88(5.4)

6. Serum SM by the negative mode and positive ion mode

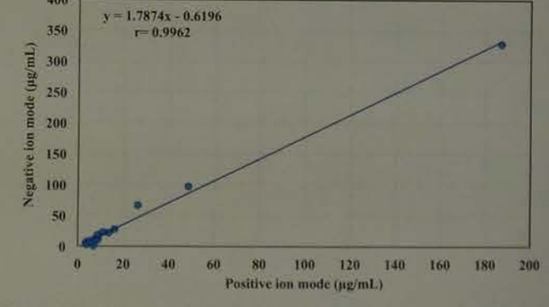


Fig. 4 Correlation of serum SM species with the negative and the positive ion mode.

Serum SM species was measured with the negative ion mode and the positive ion mode method.

7. Serum sphingomyelin species in healthy young subjects

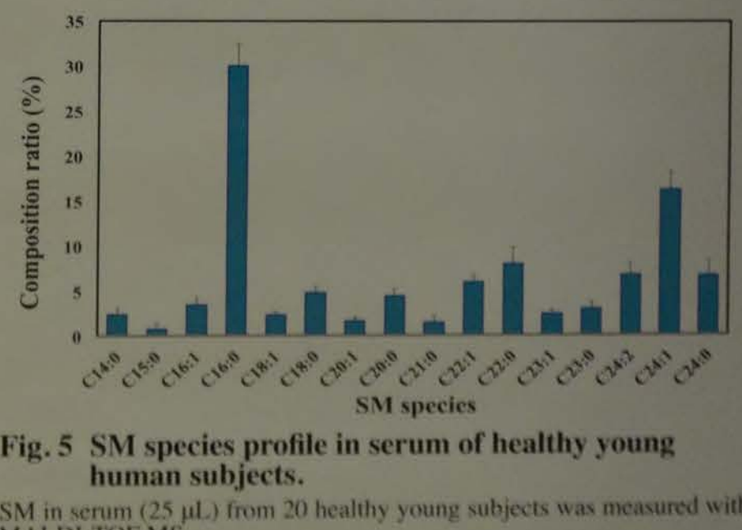


Fig. 5 SM species profile in serum of healthy young human subjects.

SM in serum (25 μL) from 20 healthy young subjects was measured with MALDI-TOF MS.

【Discussion】

MALDI-TOF MS in the negative ion mode for profiling serum SM was simple and highly accurate. In the negative ion mode, only the demethylated species were observed in the mass spectrum. Because of this, such measurements were simple for data processing and had better reproducibility than those in the positive ion mode. Measuring operation and daily maintenance of MS are simpler and easier than those of LCMS, and thus the method might be applicable in a clinical study. The SM profile of the healthy subject can be used to detect SM metabolic disorders.

【Conclusion】

The analysis of serum SM species with the negative ion mode method by MALDI-TOF MS was simple and highly accurate and might be applicable in a clinical study.

Clinical Chemistry

PD-03

To Integrate Point-of-Care and Laboratory Automation System of BNP testing

Mei-E Yang[1], Hsin-Hsin Chang[1], Ching-Yi Lin[1], Chi-Kuan Chen[12]

Department of Laboratory Medicine[1] and Department of Pathology[2] ,

Mackay Memorial Hospital ,Taipei ,Taiwan

Abstract

Congestive heart failure (CHF) is a complex syndrome occurs when the heart is unable to pump sufficiently to maintain blood flow to meet the body's need, and may cause dysfunction of heart and lead the fluid retention in peripheral vascular and lung. When under the stress, the heart secret a 108-amino acid pro form of natriuretic peptide (proBNP), then proBNP will be cleaved into two molecular: a 32-amino acid B-type natriuretic peptide (BNP) and a 76-amino acid N-terminal pro B-type natriuretic peptide (NT-proBNP). According to 2013 ACCF/AHA Heart Failure Guideline, both BNP and NT-proBNP have good correlation with heart failure severity, and both of them can be used to heart failure diagnosis and prognosis.

There are multiple commercial BNP and NT-proBNP testing in the market, include automation system and point-of-care testing (POCT) system, each of them has its own characteristic. POCT is a global trend to obtain patient data in 15-20 minutes at the bed site, it is a suitable tool for physicians to diagnose emergency cases immediately. However, for central laboratory, how to control the data quality and consistency is the most important objective. Therefore, how to integrate POCT and automation system in the hospital is imperative. Triage® BNP perform good correlation between Triage® MeterPro (POCT system) and Beckman® DxI 600 (Beckman BNP = 1.095 x Triage BNP - 20.85, R=09938). It means that we can diagnose emergency patients by Triage® BNP in a short TAT, then monitoring CHF patients by Beckman system (true TAT: 24.7 mins v.s. 38.3 mins). In addition, we also investigate the clinical performance of BNP and NT-proBNP on Triage® system, to find out which biomarker is more suitable for the group difference in our hospital.

Methods

Alere Triage® MeterPro is a Point-of-Care testing system which can perform both BNP and NT-proBNP tests by EDTA whole blood specimens. The Alere Triage® BNP Test for Beckman Coulter® Immunoassay Systems (BCI BNP) empowers higher throughput BNP and automation. Provides clinically interchangable BNP results between the Alere Triage® MeterPro and Beckman Coulter UniCel® DxI 800 or Beckman Coulter Access® 2 Immunoassay System.

Precision Study*:
The study used three different levels liquid quality control (Liquichek™ Cardiac Markers Plus, Bio-Rad) to perform the precision test: (1) within-run precision: testing each level controls for 10 times (2) between-day precision: this study was conducted over 5 days, testing each level controls 5 times per day.

Correlation Study*:
We collected 68 random ED patients and used the EDTA whole blood samples to performed BNP testing on Triage® MeterPro, then centrifuged the specimens by 3000 rpm, 10 minutes to obtain the plasma to perform NT-proBNP and BCI BNP.

Turnaround Time (TAT):
The assay time claimed by manufacture are 15 minutes on both Triage and UniCel® DxI 800. Most of the automation system is only suitable for using plasma, so the blood samples inevitably require centrifugation. Because of this, the true TAT is bound to extend. This study will retrieve the true TAT from LIS system. This TAT is calculated from sample submission to release the report to LIS.

* Results were calculated by EP Evaluator version 8 software to comply with the CLSI recommendation.

Results

Precision results of the BNP results on UniCel® DxI 800 are showed in Table 1 (See Table 1) . The CV is from 1.8% to 3.85%, displaying a well imprecision performance of BCI BNP.

Table 1. BCI BNP precision performance on Beckman Coulter UniCel® DxI 800. (Within Run: continuously 10 times of each level ； Between Day: 5 times per day, tested for 5 days)

Precision	Level	Mean	SD	%CV
Within Run	1	83.0	1.49	1.80%
	2	338.5	13.03	3.85%
	3	1227.1	30.92	2.52%
Between Day	1	82.4	2.00	2.43%
	2	326.4	11.89	3.64%
	3	1230.2	30.70	2.50%

The BNP results between Triage® MeterPro and UniCel® DxI 800 showed high correlation, 68 specimens were compared over a range of 5.0 to 4770.0 pg/mL. The correlation coefficient (R) was 0.9938, and the slop and intercept were 1.095 and -20.85 (See Figure 1). The difference between the two methods was within allowable error for 95.6% (65/68 specimens). The average Error Index was (Y-X)/TEa was 0.23 (-0.96 to 1.23, TEa was 20 pg/mL or 20%) (See Figure 2).

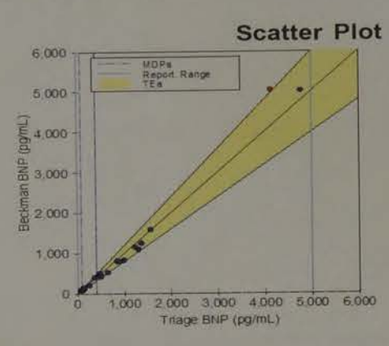


Figure 1. The BNP results between two method. X method was Triage BNP, and Y method was Beckman UniCel® DxI 800. The correlation was Y = 1.095 X - 20.85, R=0.9938. (MDP: Medical detection point ； Heart failure rule-out: <100 pg/mL, rule-in: >400 pg/mL)

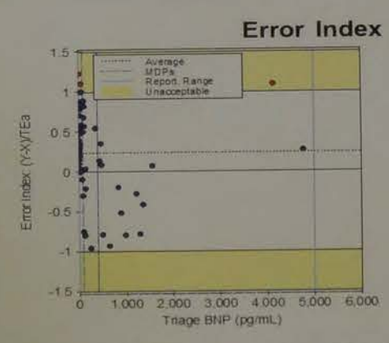


Figure 2. The Error Index of BNP between Triage and DxI 800. The difference between the two methods was within allowable error for 65 of 68 specimens (95.6%). The average Error Index (Y-X)/TEa was 0.23, with a range of -0.96 to 1.23. The largest Error Index occurred at a concentration of 20.5 pg/mL.

Although the correlation between Triage® and UniCel® DxI 800 was good, the TAT of UniCel® DxI 800 was 10 minutes longer than Triage® (See Figure 3).

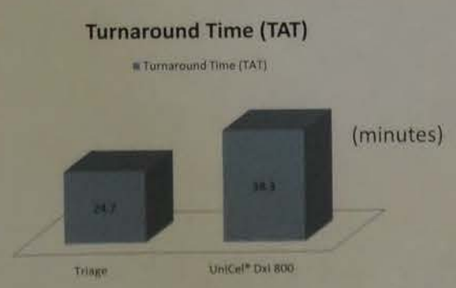


Figure 3. Turnaround time (TAT). The TAT results was retrieved from LIS system. The average TAT on Triage system was 24.7 mins, and the average TAT on DxI 800 was 38.3 mins .

Table 2. Comparison agreement between BNP and NT-proBNP on Triage® MeterPro system

		BNP		
		Neg	Pos	Total
NT-proBNP	Neg	35	0	35
	Pos	9	24	33
Agreement		79.50%	100%	86.80%

Conclusion

Triage® BNP is the only BNP testing which can perform on both POC system (Triage® MeterPro) and automatic system (Beckman UniCel® DxI 800) in the market. This study demonstrates the highly correlation and the different TAT between two systems. If we can properly use the characteristic of each system on the different needs, e.g. using POC BNP to diagnose emergency patients by short TAT, then using automatic BNP to monitor the inpatients by high-throughput batch, it will bring the greater benefit for patients and hospital. Although NT-proBNP can also aid to diagnose CHF, it seems might influence by other factors (e.g. renal failure, age...etc.). Therefore, NT-porBNP is more suitable for outpatient with known medical history.

Reference

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Clinical Chemistry

PD-05

Measure the Effects of Temperature on Blood Ketone Stability

Chao-Wei Liu[1], Chih-Hsuan Kuo[1], Tzu-I Chien[1], Mo Siu-Mei Lee[1]

[1] Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan

Background

Pre-analytical handling of blood samples can influence the laboratory results. With the increases in lab tests, sample collection time and various transported conditions can adversely affect the sample quality and accuracy of test results. Blood ketone traditionally requires to be collected separately from ammonia and lactate. In this study, we examine the possibility of combining blood ketone test with ammonia and lactate by measuring the stability of ketone under iced and room temperature conditions.

Method

Blood samples were collected in heparinized tubes and analyzed by electrochemical method using the MediSense Optium Xceed. Each sample was divided into 2 aliquot and stored at room or refrigerated (4^0C) temperature until time of test. To determine the effect of temperature on ketone stability, the heparinized whole blood was analyzed at 0, 30, 60, 120 and 180 min incubation time points.

Figure

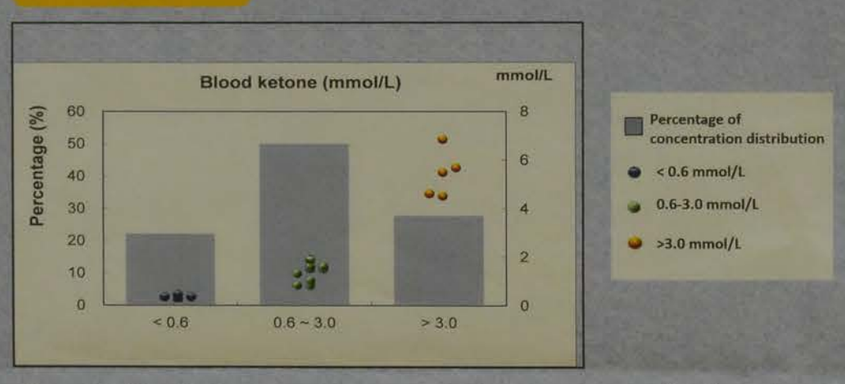


Figure 1. Blood ketone sample concentration percentage distributed (corresponds to the left Y-axis), individual specimen concentration shows by small circular dots (corresponds to the right Y-axis).

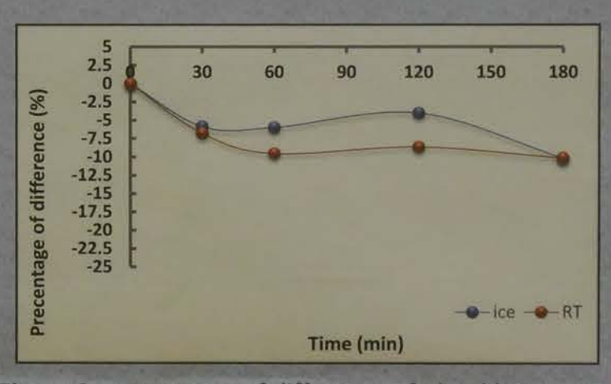


Figure 2. percentage of difference of Blood ketone in different incubation time and temperature between concentration at 0.6-3.0 mmol/L.

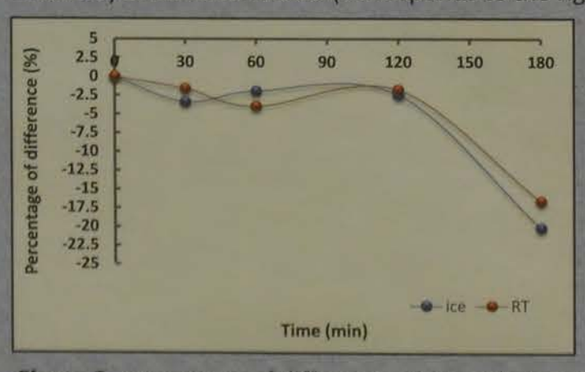


Figure 3. percentage of difference of Blood ketone in different incubation time and temperature at concentration greater than 3.0 mmol/L.

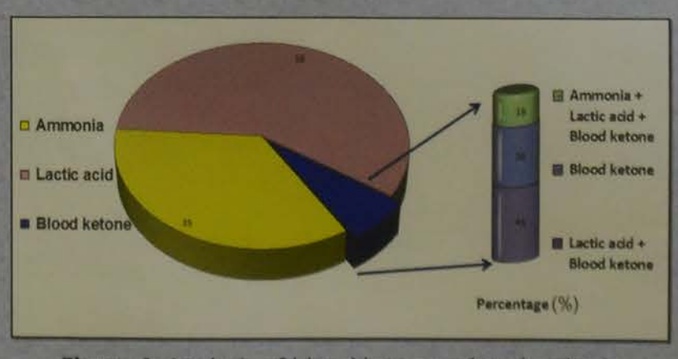


Figure 4. Analysis of blood ketone related order in emergency department.

Result

In median blood ketone level (0.6-3.0 mmol/L), the measurement difference was within 10% (7.0±6.6) for 120 min [Figure 2]. However, in high level (>3.0 mmol/L), the difference of blood ketone was less than 5% (2.7±2.0) within 120 min [Figure 3]. These results show that temperature has minimal effect on the blood ketone stability up to 120 min after specimen collection.

Conclusion

Analysis of the emergency department order proportion, which combine Lactic acid or Ammonia with blood ketone order were accounted for 64%, blood ketone order alone were accounted for 36% [Figure 4]. Given that ammonia and lactate levels are known to be stable on iced condition, our results suggest it is possible to include blood ketone with ammonia and lactate tests using heparinized tubes transferred on ice condition.

D-3 D-3

Clinical Chemistry

PD-06

From diagnostic biobank to research biobank, a pilot study

Kai Guttulsrød[1], Mette Bang Nyholm[1], Cecilie Okkenhaug[1,2]
Department of Medical Biochemistry, Diakonhjemmet Hospital AS, Oslo, Norway
[1]Biomedical Laboratory Scientist [2]Specialist in laboratory ethics

Background

A diagnostic biobank contains a lot of human material, which are normally stored for a week. Turned into a research biobank, it would become valuable for researchers because it rapidly would contain a large amount of samples from hospital patients with a variety of diagnoses and demography.
To convert the diagnostic biobank into a research biobank, we need a written consent from the patients.
In April 2013 the Regional Committees for Medical and Health Research Ethics in Norway, approved a consentform allowing transferal of surplus biological material into a research biobank at Diakonhjemmet Hospital. They also approved that a signed written consent gave possibility to collect information from the hospital journal and other health registries. We therefore wanted to find out if patients are willing to sign this consentform and experience how many samples and aliquots we could collect.

Methods

The study was performed as a pilot study from September 9th to December 9th 2015. We intended to ask all patients hospitalized at the Medical department to sign the consentform after given oral information by the nurses and the physicians.

The department of Medical Biochemistry collected the consentforms, used the laboratory information system to register the number of blood collections from the included patients and also to register the different patients diagnosis into an Excel sheet.

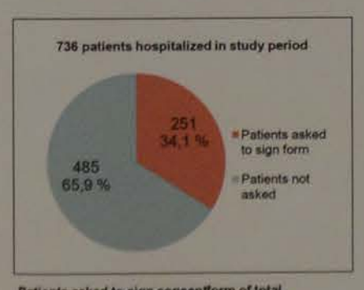
Then we calculated how much surplus biological material the number of blood collections generated. Divided into 0,5 mL aliquots we calculated the theoretical number of aliquots in our diagnostic biobank.

Result and discussion

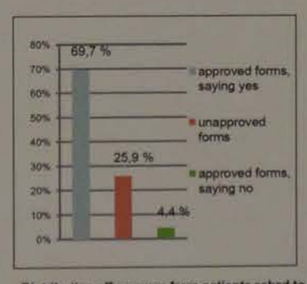
During the study period 736 patients were hospitalized at the ward. 34,1% of these patients were actually asked to sign the form. Of the collected forms 69,7% gave permission, 4,4% declined and 25,9% of the forms were not valid due to formal errors.

Of those who consented, 924 blood collections were performed. This generated a total number of 870 5mL serum-gel tubes, 765 4mL EDTA-tubes and 361 2,7mL Citrate-tubes. The potential volume for the research biobank, when divided into 0,5mL aliquots were 1740 serum aliquots, 3060 EDTA aliquots and 722 Citrate plasma aliquots.

Since only 34,1% of the hospitalized patients were asked, and 25,9% of the consentforms were unapproved, our study shows that there is a potential to improve the logistic in the process of collecting the consentforms. It is important that the nurses or the physicians ask all hospitalized patients and make sure that the consentforms are correctly completed.



Patients asked to sign consentform of total hospitalized patient in the study period



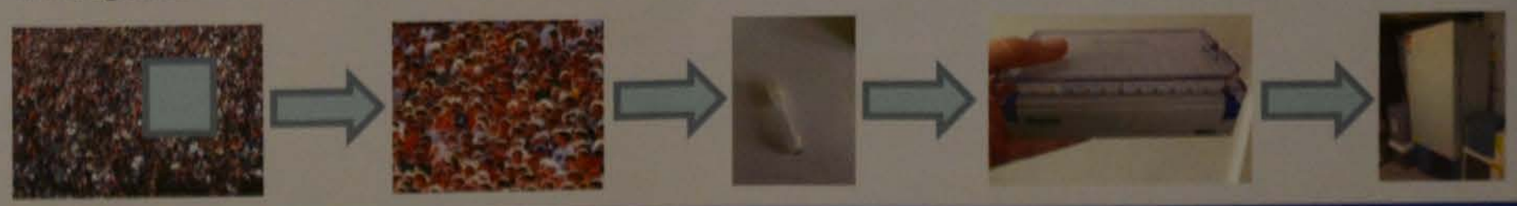
Distribution off answers from patients asked to sign consentform

	samples collected	surplus volume after analyzing	total 0,5 ml aliquots
Serum tubes collected	870	1 mL	1740
EDTA tubes collected	765	2 mL	3060
Citrate tubes collected	361	1 mL	722
Total	1996		5522

Samples collected from patients signing consentform

Conclusions

The pilot shows that patients are positive to contributing in a research biobank, only 4% decline to sign the consentform. Our study shows additionally that, in only three months, the research biobank would contain a large volume of aliquots. From the 175 patients with different diagnosis, we calculated 5522 aliquots for biobanking. This shows that the conversion of a diagnostic biobank into a research biobank can be of great value in future research.

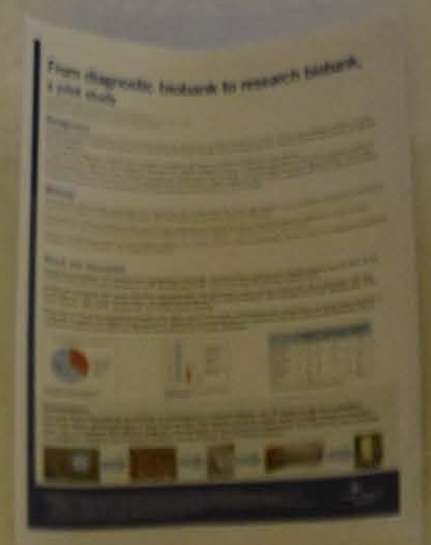


Diakonhjemmet Hospital is the local hospital for approxiamately 130 000 inhabitants living in the western sectors of Oslo.
The hospital provides medical services in the fields of internal medicine, surgery, intensive medicine, rheumatology and rheumatology surgery, as well as psychiatry.

- The hospital has extended responsibility for treating patients in the fields of rheumatology and rheumatology surgery form the whole health region
- The hospital is a competence center in the fields of rheumatology and psychopharmacology, and has an extended research activity - especially within these areas
- Health services for the elderly has a special focus at Diakonhjemmet. The hospital is responsible for the treatment of people 65 years of age or older with hip fractures, in an extended area of Oslo, as well as for elderly patients in need of psychiatric health services

The four core values of Diakonhjemmet Hospital are respect, quality, service and justice.

Diakonhjemmet Hospital



Clinical Chemistry

PD-07

The 32nd World Congress of Biomedical Laboratory Science
2nd of September, 2016

Lifestyle impact on serum of Vitamina D and PTH, in elderly

Ribeiro, Marta[1]; Caseiro, Armando[1]; Valado, Ana[1]; Osório, Nádia[1]; Mendes, Fernando[1]; Figueiredo, João [2]; Gabriel, António [1]

PD-07

1. Portugal, Polytechnic Institute of Coimbra, Coimbra Health School, Biomedical Science Department.
· Marta Ribeiro (martapereiraribeiro@gmail.com); Armando Caseiro (armandocaseiro@estescoimbra.pt); Ana Valadp (valado@estescoimbra.pt); Nádia Osório (nadia.osorio@estescoimbra.pt); Fernando Mendes (fjmendes@estescoimbra.pt); António Gabriel (agabriel@estescoimbra.pt).
2. Portugal, Polytechnic Institute of Coimbra, Coimbra Health School, Complementary Science Department
· João Figueiredo (jpfigueiredo1974@gmail.com)

Introduction

During the last few decades we have been witnessing the aging of global population. In Portugal, between the years 2009 and 2014, young people and population in active age have been decreasing at the same time that the older population increases. This are consequences of the birth rate decline and the increase of emigration and average life expectancy. The aging process is responsible for health decline and may lead to the dependence and consequent institutionalization. Bone metabolism involves serum calcium regulators, such as vitamin D and PTH.

Vitamin D is obtained through diet and cutaneous synthesis potentiated by the presence of ultraviolet radiation, by using 7-Dehydrocholesterol. UV radiation initiates the production of vitamin D_3, whose activation involves two hydroxylations. Is in the liver that vitamin D_3 is hydroxylated to 25– hydroxyvitamin D_3 (25(OH)D). This molecule goes to the kidney to suffer another hydroxylation in order to produced 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active form of vitamin D. The $1,25(OH)_2D_3$ acts in the intestine in order to absorb calcium. The decreasing of bone mineralization is a consequence of vitamin D deficiency. Hypovitaminosis D has become a main characteristic of developed countries, mainly because of dietary mistakes, low sun exposure and sedentary lifestyle. We assume that plasmatic concentration of vitamin D that is lower than 20 ng/ml reflect deficiency of hormone itself. In other hand, concentrations between 20 to 30 ng/ml are insufficient. Concentrations between 30 to 100 ng/ml are sufficient for an adult.

The parathyroid hormone (PTH) is produced by the parathyroid glands in order to stimulate calcium bone resorption, calcium reabsorption in renal tubules and $1,25(OH)_2D_3$ synthesis. The secretion of this hormone is regulated by calcium plasmatic concentration, in a inverse relation between iPTH and ion concentration. We assume that concentrations between 14 to 72 pg/ml of iPTH are sufficient for an healthy humans.

Aims

The following study evaluated and compared serum concentrations of 25(OH)D and iPTH in elderly people living in institutions and living in their homes (free-living), with active and independent life.

Material&Methods

Blood collection and measurement of serum concentrations of:

25(OH)D

iPTH

SAMPLE

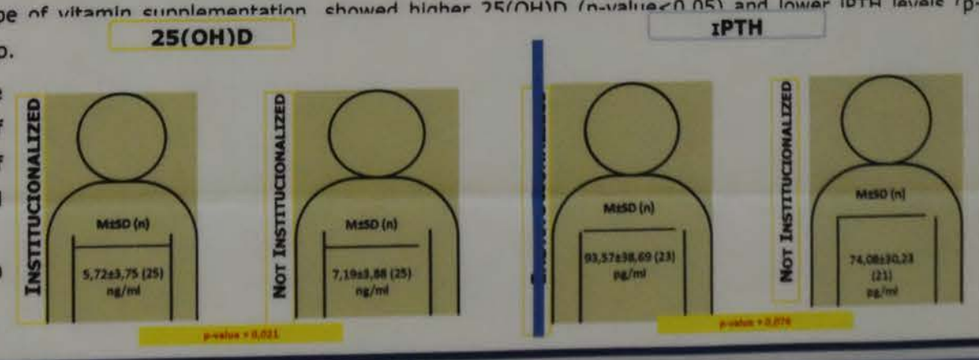
We evaluated 50 elderly divided in two groups (Figure 3). The first group was composed by 25 institutionalized elderly, aged between 63 and 98 years (19 females and 6 males), not completely immobilized, belonging to 3 nursing homes. The second group was composed by 25 elders living in their homes (not institutionalized), aged between 63 and 91 years (15 females and 10 males) with an active and independent life.

METHODS

1º Step—Individual questionnaire about lifestyle, general health and autonomy (Barthel Scale).
2º Step—Blood collection.
3º Step—Measurement of serum concentrations of iPTH and 25(OH)D using immunochemical methods (ADVIA Centaur, Siemens-Germany).

Results

- The free-living elderly showed higher 25(OH)D serum levels, comparing with institutionalized elderly (p-value<0,05). On the other hand, considering de medium values, institutionalized elders had higher iPTH concentrations than free-living elders (p-value=0,076).
- The serum concentration of 25(OH)D was inversely correlated with iPTH (r=-0,160).
- Calcium supplementation was correlated with higher serum levels of 25(OH)D (p-value<0,05) and lower concentrations of iPTH (p-value<0,05). However, the free-living elderly who didn't use to take any type of vitamin supplementation, showed higher 25(OH)D (p-value<0,05) and lower iPTH levels (p-value<0,05), comparing with the institutionalized group.
- The free-living elderly who practice three or more activities per day, had higher concentrations of 25(OH)D (p-value<0,05) and lower concentrations of iPTH (p-value<0,05), compared to the institutionalized elderly.
- Outdoor activities showed also correlation with serum concentrations of both hormones (p-value<0,05).



Discussion and Conclusion

We found a high prevalence of hypovitaminosis in the elderly used in this project, particularly on the institutionalized ones. The concentrations of 25(OH)D were substantially higher on the elderly with an active and independent life. This result might be explained with the higher sun exposure comparing with the institutionalized elderly. The iPTH presented an inverse relation with the 25(OH)D. This was expectable, meaning that the group with lower concentrations of 25(OH)D presented higher concentrations of iPTH. This is a physiological behavior with the goal of compensate the hypovitaminosis D. However this might be a result of secondary hyperparathyroidism.

We think that the incentive to sun exposure, vitamin supplementation for the calcium metabolism, food fortification and practice of daily activities is something to have in account in our country as well as the implementation of politics that encourage the Vitamin D ingestion directly to the ones with larger risk. This study corroborates the premise that the adoption of an active lifestyle, brings benefits to the age process.

Acknowledges

Labinsaúde: Co-funded by the QREN Project under the Program Mais Centro, Commission of the Central Region Coordination and the European Union through the European Regional Development Fund.

Clinical Chemistry

PD-08

Applying the information technology to building up laboratory quality control management system

Yu-Hsuan Yang[1], Chin-Mei,Chen[2], Li-Chuan Wang[1], Hsin-Yin Chou[1], Li-Ching Wu[1]

Division of Clinical Pathology[1], Department of Pathology, Chi Mei Medical Center, Tainan, Taiwan
Bio-Rad Naboratories Taiwan Ltd.Taiwan Branch(U.S.A)[2]

Background

Our hospital is actively expanding the health care system with patient-centered services. Due to the regional effect, the interoperability of patients between the two branches is frequent. Therefore, we hope to achieve the integration and analysis of quality control data between the laboratories in order to enhance data quality by customizing information technology software and implementing automation hardware.

Materials and Methods

With cross-team discussion, we integrate all laboratories from different branches by using the same instruments and quality control materials. Laboratory automation system releases data to the middleware, and build up better integral quality control system. Through the hospital intranet, the quality control data from different branches were uploaded automatically to the worldwide peer group comparison system. Six sigma quality control management systems was used to enhance workflow in laboratory. Authorized quality control commissioners were invited to do analysis for whole health care system.

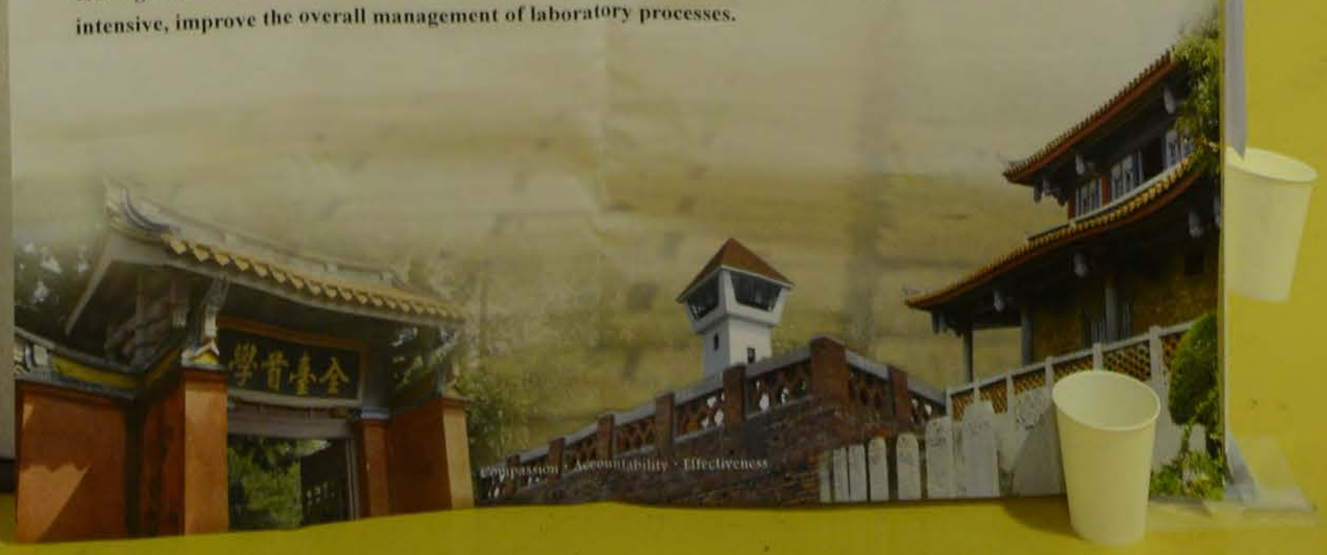
Results

Our hospital is the leading institution in Taiwan to implement customized software to build up an integral quality control system, and to lean laboratory quality control processes. The overall staff monthly operating time for quality control is reduced from 112 hours to 66 hours, which is 48.4% of reduction. This also means that approximately 552 hours were saved annually. At the same time, the laboratory was verified by the Westgard Sigma Verification program.

Westgard Sigma Verification

Discussion

The future goal of laboratory medicine will move toward personalized medicine. The experience of using IT software to monitor the quality helps to improve the quality of test reports. Also, simplify staff monthly operating time for quality control data analysis and organize report, it can significantly reduce the time and labor intensive, improve the overall management of laboratory processes.



Clinical Chemistry

PD-09

The 32nd World Congress of Biomedical Laboratory Science (IFBLS2016)

Kaohsiung Chang Gung Memorial Hospital ,Taiwain

Association of the DNA Repair Gene hOGG1 Polymorphisms with Nasopharyngeal Carcinoma

Tsai Chia-Jung[1]*, Chen Hsiang-Lan[2]*, Jian Da-Jhih[3], Teng Hsiu-Chen[3], Chen Chung-Chi[3], Chen Chih-Wei[4], Chih Yu-Chia[3], Tang Kung-Sheng[3#]

[1]Department of Laboratory Medicine, Kaohsiung Chang Gung Memorial Hospital,Taiwain.
[2] Department of Laboratory ,Kaohsiung Municipal of Kai-Syuan Psychiatric Hospital ,Taiwain.
[3] Department of Medical Laboratory Science and Biotecnology,Fooyin University, Taiwain.
[4] Department of Biomedical Science,Fooyin University,Taiwain.

INTRODUCTION

The DNA repair enzyme OGG1 is a DNA glycosylase/AP lyase that has been hypothesized to play an important role in preventing carcinogenesis by repairing oxidative damage to DNA . Specifically, glycosylase/AP lyase can efficiently repair 8-OH-G a major base lesion produced by ROS, formed as a byproduct of endogenous metabolism or exposure to environmental oxidizing agents, such as ionizing radiation or chemical genotoxic compounds. 8-OH-G is highly mutagenic and, if not excised on DNA replication, can cause GC to TA transversions, which occur frequently in several oncogenes and tumor suppressor genes. Ser326Cys polymorphism in the hOGG1 gene is involved in the repair of 8-hydroxyguanine in oxidatively damaged DNA. Nasopharyngeal carcinoma has a striking geographic and ethnic distribution, with particularly high rates observed among southeast Chinese and other individuals of Chinese descent . Nasopharyngeal carcinoma is linked to EBV infection. In addition to EBV, numerous other environmental and host factors have been shown to be associated with the development of nasopharyngeal carcinoma. In particular, longterm cigarette smoking, consumption of salted fish and foods containing nitrosamine or nitrosamine precursors at an early age, and occupational exposure to wood dust have been shown to be consistently associated with this disease. In this study, hOGG1 genotyping was performed by PCR-restriction fragment length polymorphism analysis of genomic DNA isolated from 84 Taiwanese nasopharyngeal carcinoma cases and 345 individual healthy donors . We found that distribution of hOGG1 Ser326Cys genotype among controls (Ser/Ser,18.6%; Ser/Cys, 46.9%; and Cys/Cys 34.5%) was significantly different from that among nasopharyngeal carcinoma cases (9.5%, 60.7% and 29.8%,respectively)(p<0.05)

METHOD

Patients and Controls.

The study subjects consisted of 84 pathology-proven Taiwanese Nasopharyngeal Carcinoma patients and 345 healthy controls who attended physical examination. All the individuals gave their written consents to participate in this study.

Determination of HOGG1 genotypes.

The hOGG1 Ser326Cys polymorphism in exon 7. Amplification consisted of a 5-min denaturation at 95˚C followed by 30 cyclesof 95˚C for 30 s, 58˚C for 30 s, and 72˚C for 1 min. An incubation step at 72˚C was added at the end of the reaction for 7 min. We used a simple RFLP method to identify the Ser326Cys variant, because The C-to-G transversion creates a new Fnu4HI restriction site. The PCR product is 200 bp in length and is digested by the Fnu4HI restriction enzyme to two 100-bp fragments for the 326Cys allele and is undigested for the 326Ser allele. Fragments were separated on a 2% agarose gel and compared with genotype standards (confirmed by direct sequencing).

Statistical Analysis.

Student's t test and x2 test were used as appropriate to compare parameters between the case and control groups as well as between two groups. To evaluate the contribution of each genotype and each genetic allele, the simple and multivariate logistic regressions were used for the calculation of odds ratio (OR) and the 95% confidence interval (CI) of stomach cancer. A P value < 0.05 or a 95% CI of the OR above or below 1.0 was defined as statistically significant. All data were analyzed using SPSS version 16.0 software (SPSS, Chicago, IL, USA).

Table 1. Odds ratio(OR) and 95% confidence intervals (CI) of Nasopharyngeal Carcinoma with hOGG1 Genotypes

hOGG1 Genotypes	No. Cases N=84(%)	No. Controls N=345(%)	OR(95% CI)	P value
hOGG1 Ser/Ser				
ABSENT	76(90.5)	281(81.4)	1	
PRESENT	8(9.5)	64(18.6)	0.46(0.47~89)	< 0.05
hOGG1 Ser/Cys				
ABSENT	33(39.7)	183(53.1)	1	
PRESENT	51(60.7)	162(46.9)	1.75(1.29-1.81)	< 0.05
hOGG1 Cys/ Cys				
ABSENT	59(70.2)	226(65.5)	1	
PRESENT	25(29.8)	119 (34.5)	0.80(0.61-1.24)	> 0.05
hOGG1				
Ser/Ser	8(9.5)	64(18.6)	1	
Ser/Cys	51(60.7)	162(46.9)	2.52(1.50~2.42)	< 0.05
Cys /Cys	25(29.8)	119 (34.5)	1.69(1.28~2.16)	< 0.05
hOGG1				
Ser/Ser	8(9.5)	64(18.6)	1	
Ser/Cys or Cys /Cys	86(90.5)	281(81.4)	2.45(1.31~2.88)	< 0.05

Table 2. Adjusted Odds ratio(OR) and 95% confidence intervals (CI) of Nasopharyngeal Carcinoma with hOGG1 Genotypes

hOGG1 Genotypes	No. Cases N=84 (%)	No. Controls N=345(%)	AOR[a](95% CI)	P value
hOGG1				
Ser/ Ser	8(9.5)	64(18.6)	1	
Ser/ Cys	51(60.7)	162(46.9)	2.87(1.40–2.87)	< 0.05
Cys/ Cys	25(29.8)	119 (34.5)	1.93(1.31–2.61)	< 0.05

RESULTS

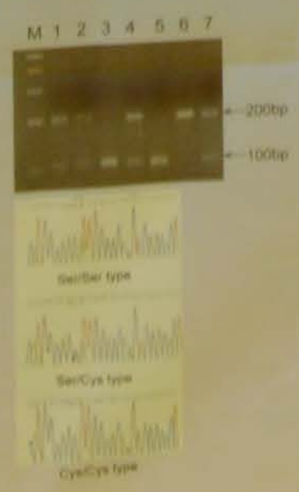


Figure 1 A:
The 200-bp PCR product is digested by Fnu4HI to two 100-bp fragments for the 326Cys variant and is undigested for the 326Ser allele in exon 7. Lane 6, a Ser/Ser homozygote; Lane 3,5, a Cys/Cys homozygote; Lane 1,2,4,7, a Ser/Cys heterozygote.

Figure 1 B:
DNA-sequence histogroam revealing the indicated sequence changes (underlined).In the 2nd histogram ,both the cytosine (blue line)and guanine (black line)are observes in the codon 326site (marked as N),indicating the heterozygous genotype.

CONCLUSIONS

1. *We found that the frequency of hOGG1 Ser/Ser, Ser/Cys, and Cys/Cys genotypes were 9.5, 60.7, and 60.7% in all cases, and 18.6, 46.9, and 34.5% in the controls.*
2. *Significantly increase risk for nasopharyngeal carcinoma was observed.*
3. *These results suggest that hOGG1Ser 326Cys, might have potential as a genetic marker for nasopharyngeal carcinoma susceptibility.*

Clinical Chemistry

PD-10

The 32nd World Congress of Biomedical Laboratory Science (IFBLS2016)

Association of the Multiple Drug Resistance 1(MDR1) gene 3435 Polymorphisms and bladder cancer

Chen Hsiang-Lan[1]* Teng Hsiu-Hsuan[2]* Cheng Hsiao-Tung[3] Teng Hsiu-Chen[3] Chen Chung-Chi[3] Chen Chih-Wei[4] Chih Yu-Chia[3] Tang Kung-Sheng[3#]

[1]Department of Laboratory ,Kaohsiung Municipal of Kai-Syuan Psychiatric Hospital ,Taiwain.
[2]Department of Pharmacy ,Tajen University Hospital,Taiwain.
[3]Department of Medical Laboratory Science and Biotecnology,Fooyin University, Taiwain.
[4]Department of Biomedical Science,Fooyin University,Taiwain.

INTRODUCTION

The P-glycoprotein, a product of multiple drug resistant 1 (MDR1) gene, is a membrane efflux pump localized in epithelial cells in the small and large intestine, a part of the gastrointestinal barrier that protects cells against xenobiotics from our diet, bacterial toxins, drugs and other biologically active compounds. possible carcinogen.Bladder cancer is strongly associated with smoking and occupational and environmental exposure to carcinogens. Tobacco smoking is estimated to be the main identifying risk factor for this cancer, which is responsible for 40–50% and 30% of all bladder cancer cases.The study population consisted of 108 subjects with bladder cancer and 227 healthy controls.The C3435T MDR1 gene polymorphisms was identified using the polymerase chain reaction–restriction fragment length polymorphism method.We found that distribution of MDR1 genotype among controls (CC, 41.1%; CT, 44.0%; and TT 14.7%) was significantly different from that among bladder cancer cases (24.0%, 53.7% and 22.3%, respectively) (p<0.05).Significantly increase risk for bladder cancer was observed.These results suggest that MDR1 3435 gene might have potential as a genetic marker for bladder cancer susceptibility.

METHODS

- Primer design and restriction enzyme choice
- DNA extraction (EDTA blood)
- PCR (Polymerase Chain Reaction)
- RFLP (Restriction Fragment Length polymorphism)
- Agarose gel electrophoresis
- DNA sequencing
- Statistics assay(SPSS)

DNA Extraction

1. Whole blood in EDTA tube
Put whole blood into eppendorf tubes
Add 3D-Isolation , Centrifuge , Discard the supernatant
Add 3D-Isolation , Centrifuge , Discard the supernatant
Add 3D-Isolation , Incubate
Add 3D-Isolation Centrifuge , Transfer the supernatants to new eppendorf tubes carefully
Add absolute ethanol Centrifuge , Discard the supernatant
Add 70˚ ethanol ethanol Centrifuge, Discard the supernatant
Air-dry
Add extraction dd H2O Incubate
Spin down and store at 4 ˚ for later uses

Polymerase Chain Reaction

Determination of MDR1 genotypes

DNA isolation kit
Amplified by PCR
Denaturation for 1 min at 94˚C
Annealing for 1 min at 57˚C
Primer extension for 1 min at 72˚C
40 cycles
Final extension for 10 min at 72˚C
Product was digested with the appropriate restriction enzyme
Analyzed on a 3 % agarose gel

RESULTS

Fig 1:Restriction Fragment Length Polymorphism (RFLP) in exon26 (C3435T)

1:T/T
2:C/T
3:C/C

Fig 2:DNA sequencing in exon26 (C3435)

C/C wild type homozygous
C/T heterozygous
T/T mutant homozygous

Table 1:Parameters of case and control groups

parameter	Case (N=108)	Control (N=227)	P value
Age	62.6±12.3	48.12.1	0.05
Age range	39-85	20-92	
Gender(%)			
Male	80(56%)	122(53.7%)	0.05
Female	48(44%)	105(46.3%)	

Table 2: Odds Ratios and 95% Confidence Intervals for bladder cancer with MDR1 3435

MDR1 3435 genotypes	Cases (N=108)	Controls (N=227)	OR(95%CI)	P
CC	25(24.0)	93(41.0)	1.00	
CT	58(53.7)	100(44.0)	2.26(1.32-3.61)	<0.05
TT	25(23.3)	34(15.0)	2.87(1.50-5.51)	<0.05
CC	25(24.0)	93(41.0)	1.00	
CT/TT	83(76.9)	134(59.0)	2.26(1.32-3.87)	<0.05

CONCLUSIONS

1. We found that distribution of MDR1 genotype among controls (CC, 41.1%; CT, 44.0%; and TT 14.7%) was significantly different from that among bladder cancer cases (24.0%, 53.7% and 22.3%, respectively) (p<0.05)
2. Significantly increase risk for bladder cancer was observed.
3. These results suggest that MDR1 3435 gene might have potential as a genetic marker for bladder cancer susceptibility.

Clinical Chemistry

PD-10

The 32th World Congress of Biomedical Laboratory Science 01~05, September 2016

PD10

Association of the Multiple Drug Resistance 1(MDR1) gene 3435 Polymorphisms and bladder cancer

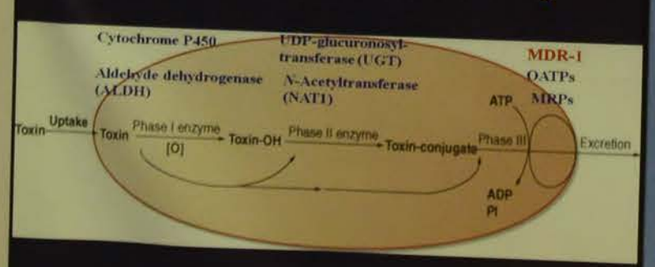
Chen Hsiang-Lan[1]*,Tang Hao-Hsuan[2]*, Cheng I-Han[3], Teng Hsiu-Chen[3], Chen Chung-Chi[3], Chen Chih-Wei[4], Chih Yu-Chia[3], Tang Kung-Sheng[3#]

Department of Laboratory ,Kaohsiung Municipal of Kai-Syuan Psychiatric Hospital,Taiwain[1].
Department of Pharmacy ,Tajen University,Taiwain[2].
Department of Medical Laboratory Science and Biotecnology,Fooyin University,Taiwain[3].
Department of Biomedical Science,Fooyin University,Taiwain[4].

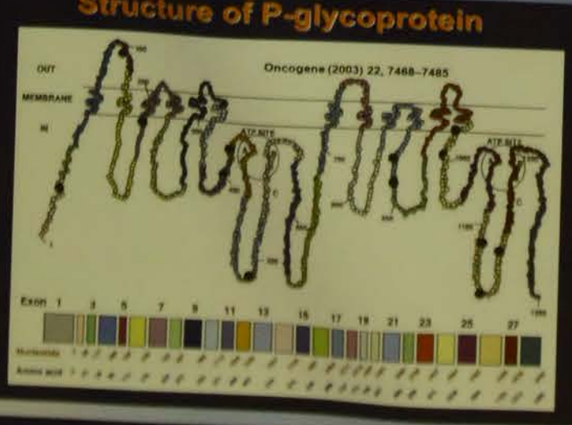
INTRODUCTION

The P-glycoprotein, a product of multiple drug resistance 1 (MDR1) gene, is a membrane efflux pump localized in epithelial cells in the small and large intestine, a part of the gastrointestinal barrier that protects cells against xenobiotics from our diet, bacterial toxins, drugs and other biologically active compounds, possibly carcinogens.Bladder cancer is strongly associated with smoking and occupational and environmental exposures to carcinogens.Tobacco smoking is estimated to be the main identifying risk factor for this cancer, which is responsible for 40–50% and 30% of all bladder cancer cases.The study population consisted of 108 subjects with bladder cancer and 227 healthy controls.The C3435T MDR1 gene polymorphisms was identified using the polymerase chain reaction- restriction fragment length polymorphism method.We found that distribution of MDR1 genotype among controls (CC, 41.1%; CT, 44.0%; and TT 14.7%) was significantly different from that among bladder cancer cases (24.0%, 53.7% and 22.3%, respectively) ($p<0.05$).Significantly increase risk for bladder cancer was observed.These results suggest that MDR1 3435 gene might have potential as a genetic marker for bladder cancer susceptibility.

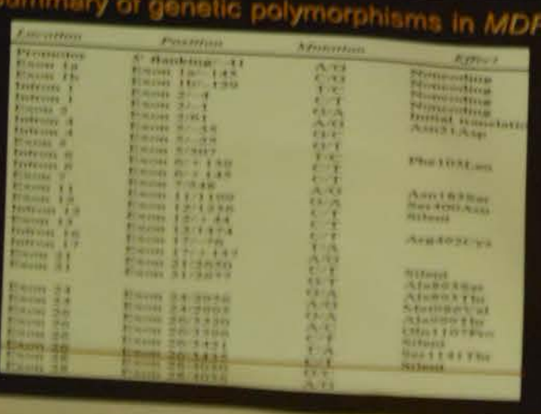
Detoxification Pathway



Structure of P-glycoprotein



Summary of genetic polymorphisms in *MDR1*



METHODS

Materials and Methods

- Primer design and restriction enzyme choice
- DNA extraction (EDTA blood)
- PCR(Polymerase Chain Reaction)
- RFLP (Restriction Fragment Length polymorphism)
- Agarose gel eletrophoresis
- DNA sequencing
- Statistics assay(SPSS)

DNA Extraction

- 收集 Whole blood in EDTA tube
- Put whole blood into eppendorf tubes
- Add BD-1solution , Centrifuge , Discard the supernatant
- Add BD-2solution , Centrifuge , Discard the supernatant
- Add BD-3solution , incubate
- Add BD-4solution , Centrifuge , Transfer the supernatants to new eppendorf tubes carefully
- Add absolute ethanol, Centrifuge , Discard the supernatant
- Add 70%ethanol ethanol, Centrifuge , Discard the supernatant
- Air-dry
- Add autoclave ddH2O , Incubate
- Spin down and store at 4℃ for later ues

Polymerase Chain Reaction

Determination of MDR1 genotypes

- DNA isolation kit
- Amplified by PCR
- Denaturation for 1 min at 94℃
- Annealing for 1 min at 57℃ } 40 cycles
- Primer extension for 1 min at 72 ℃
- Final extension for 10 min at 72 ℃
- Product was digested with the appropriate restriction enzyme
- Analyzed on a 3 % agarose gel

RESULTS

Fig 1:Restriction Fragment Length Polymorphism (RFLP) in exon26 (C3435T)

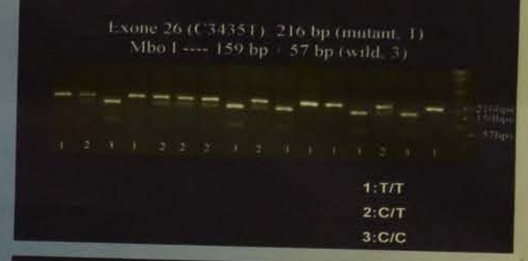


Fig 2:DNA sequencing in exon26 (C3435)

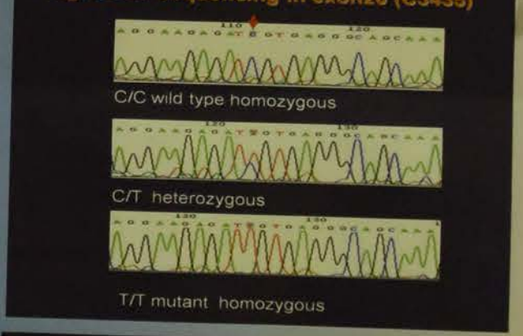


Table 1.Parameters of case and control groups

parameter	Case (N=108)	Control (N=227)	P value
Age	63.6±12.3	48±12.1	<0.05
Age range	39~85	20~92	
Gender(%)			
Male	60(56%)	122(53.7%)	>0.05
Female	48(44%)	105(46.3%)	

Table 2. Odds Ratios and 95% Confidence Intervals for bladder cancer with MDR1 3435

MDR1 3435 genotypes	Number (%) Cases (N=108)	Controls (N=227)	OR (95% CI)	P
CC	25(24.0)	46(43.8)	1.00	
CT	58(53.7)	46(43.8)	2.32(1.12-4.80)	<0.05
TT	24(22.3)	13(12.4)	3.19(1.03-9.82)	<0.05
CC	25(24.0)	46(43.8)	1.00	
CT+TT	82(76.0)	59(56.2)	2.66(1.08-6.52)	<0.05

CONCLUSIONS

1. *We found that distribution of MDR1 genotype among controls (CC, 41.1%; CT, 44.0%; and TT 14.7%) was significantly different from that among bladder cancer cases (24.0%, 53.7% and 22.3%, respectively) ($p<0.05$)*
2. *Significantly increase risk for bladder cancer was observed.*
3. *These results suggest that MDR1 3435 gene might have potential as a genetic marker for bladder cancer susceptibility.*

Clinical Chemistry

PD-11

Electrochemiluminescence immunoassay for cyclosporine and tacrolimus using Elecsys® Cyclosporine and Elecsys® Tacrolimus assays with cobas e411 analyzer

Maki Sasano [1], Shigeki Kimura [1], Ikuhiro Maeda [1], Yoh Hidaka [2]

[1] *Department of Medical Technology, Osaka University Hospital, Japan*
[2] *Laboratory for Clinical Investigation, Osaka University Hospital, Japan*

Clinical Chemistry PD-12

Introduction and Purpose

Cyclosporine (CsA) and tacrolimus (TAC) are immunosuppressant drugs that are often used to treat autoimmune diseases and as transplantation therapy.
Whole blood is recommended for measurements of CsA and TAC concentrations, because CsA and TAC are largely distributed in red blood cells and bound to proteins.
A one-step manual pretreatment is necessary to release the analytes from the proteins. This step is important for accurate measurements of CsA and TAC concentrations.
We evaluated the pretreatment condition and the analytical performance of Electrochemiluminescence immunoassay (ECLIA) .

Material and Method

Samples

200 residual CsA or TAC therapy patient whole blood samples

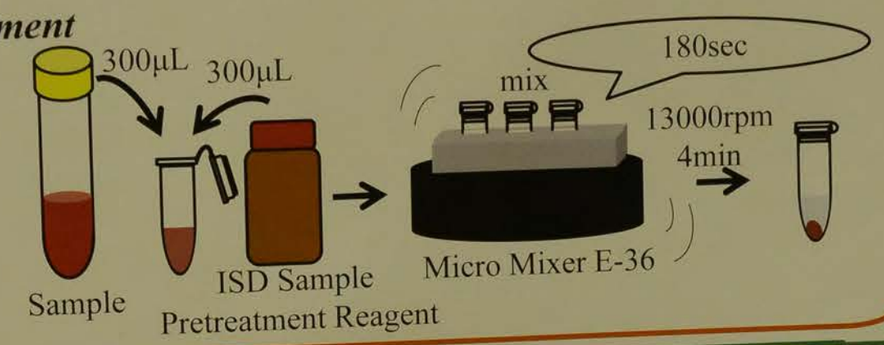
Apparatus

Cobas e411, Micro Mixer E-36, Electric pippette

Reagent

Elecsys® Cyclosporine assay kit, Elecsys® Tacrolimus assay kit
Elecsys ImmunoSuppressive Drug (ISD) SamplePretreatment Reagent

Pretreatment



Conclusion

We need to verify the mixing time when we use a new mixing apparatus because the efficiency of mixing depends on individual shaking apparatuses. The analytical performances of the Elecsys® Cyclosporine and Tacrolimus assays were acceptable.

Results

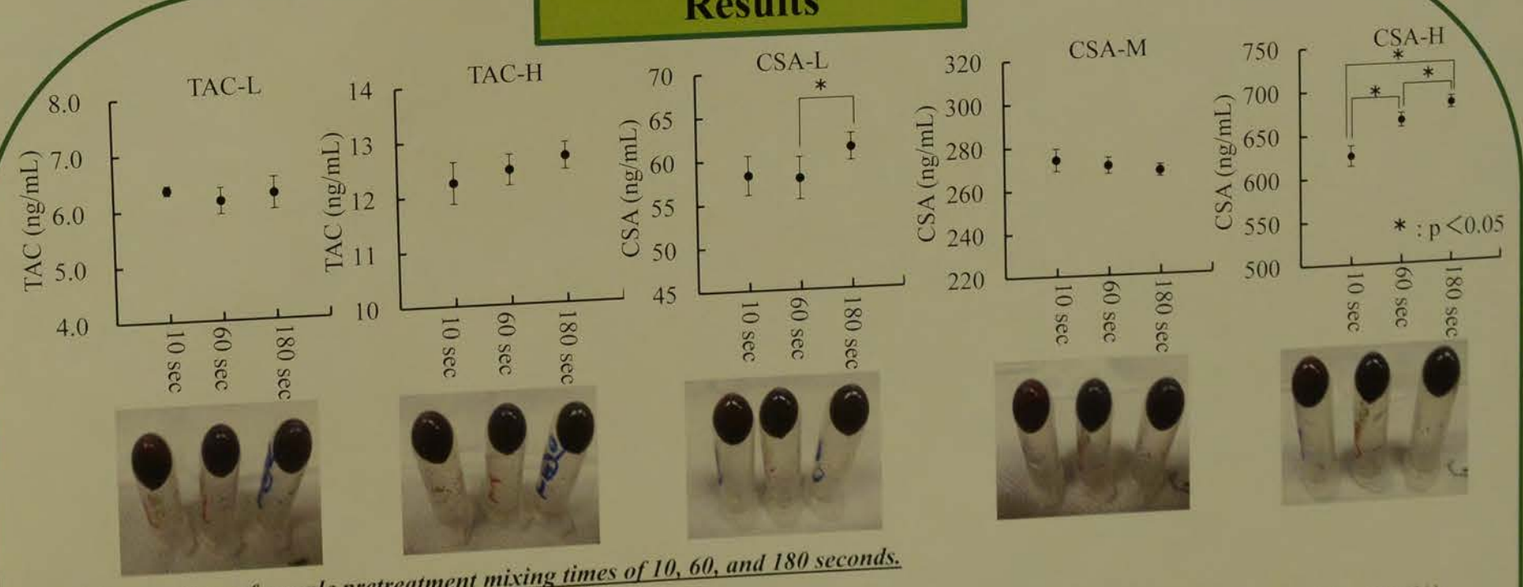


Fig. 1 Comparison of sample pretreatment mixing times of 10, 60, and 180 seconds.

Table 1 Assay Precision of Tacrolimus and Cyclosporine

TAC	Mean (ng/mL)	SD (ng/mL)	CV (%)
Within-assay (n=20)			
Control L	2.1	0.08	3.9
Control M	10.2	0.38	3.7
Control H	17.8	0.37	2.1
Day-to-day assay (n=10)			
Control L	2.0	0.08	3.9
Control M	10.0	0.29	2.9
Control H	17.5	0.50	2.8

CSA	Mean (ng/mL)	SD (ng/mL)	CV (%)
Within-assay (n=20)			
Control L	94.1	2.35	2.5
Control M	335.8	5.90	1.8
Control H	1237.6	44.66	3.6
Day-to-day assay (n=10)			
Control L	91.5	3.76	4.1
Control M	318.9	12.81	4.0
Control H	1239.8	37.43	3.0

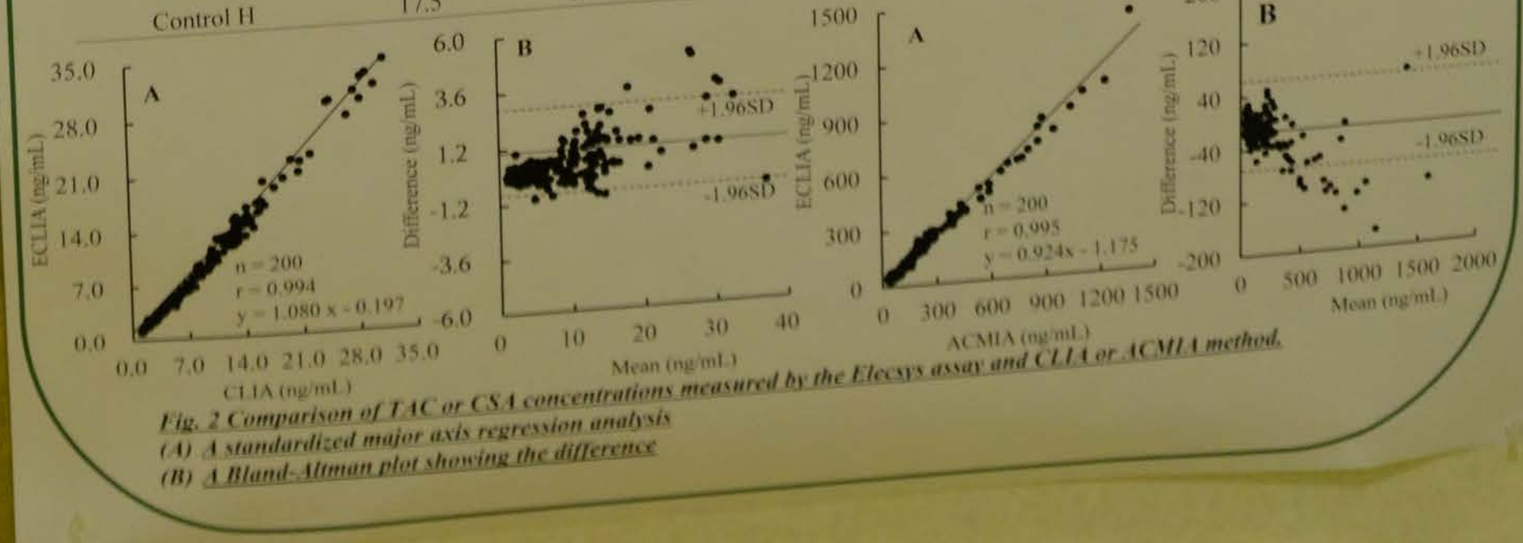
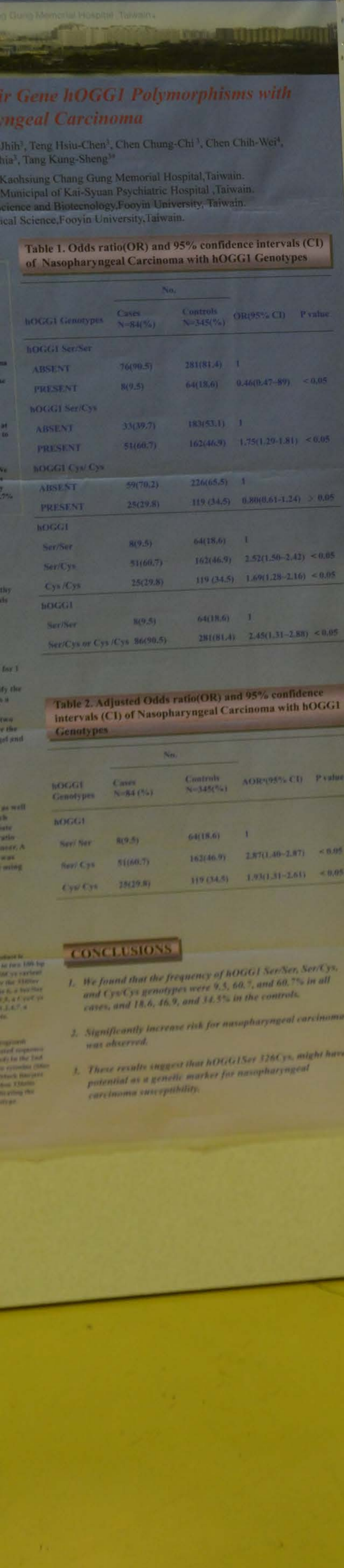


Fig. 2 Comparison of TAC or CSA concentrations measured by the Elecsys assay and CLIA or ACMIA method.
(A) A standardized major axis regression analysis
(B) A Bland–Altman plot showing the difference



ess of Biomedical Laboratory Science (IFBLS2016)

g Gung Memorial Hospital, Taiwain.

ir Gene hOGG1 Polymorphisms with ngeal Carcinoma

Jhih[3], Teng Hsiu-Chen[3], Chen Chung-Chi [3], Chen Chih-Wei[4], hia[5], Tang Kung-Sheng[3*]

Kaohsiung Chang Gung Memorial Hospital,Taiwain.
Municipal of Kai-Syuan Psychiatric Hospital ,Taiwain.
cience and Biotecnology,Fooyin University, Taiwain.
ical Science,Fooyin University,Taiwain.

Table 1. Odds ratio(OR) and 95% confidence intervals (CI) of Nasopharyngeal Carcinoma with hOGG1 Genotypes

hOGG1 Genotypes	No. Cases N=84(%)	No. Controls N=345(%)	OR(95% CI)	P value
hOGG1 Ser/Ser				
ABSENT	76(90.5)	281(81.4)	1	
PRESENT	8(9.5)	64(18.6)	0.46(0.47–89)	< 0.05
hOGG1 Ser/Cys				
ABSENT	33(39.7)	183(53.1)	1	
PRESENT	51(60.7)	162(46.9)	1.75(1.29-1.81)	< 0.05
hOGG1 Cys/ Cys				
ABSENT	59(70.2)	226(65.5)	1	
PRESENT	25(29.8)	119 (34.5)	0.80(0.61-1.24)	> 0.05
hOGG1				
Ser/Ser	8(9.5)	64(18.6)	1	
Ser/Cys	51(60.7)	162(46.9)	2.52(1.50–2.42)	< 0.05
Cys /Cys	25(29.8)	119 (34.5)	1.69(1.28–2.16)	< 0.05
hOGG1				
Ser/Ser	8(9.5)	64(18.6)	1	
Ser/Cys or Cys /Cys	86(90.5)	281(81.4)	2.45(1.31–2.88)	< 0.05

Table 2. Adjusted Odds ratio(OR) and 95% confidence intervals (CI) of Nasopharyngeal Carcinoma with hOGG1 Genotypes

hOGG1 Genotypes	No. Cases N=84 (%)	No. Controls N=345(%)	AOR(95% CI)	P value
hOGG1				
Ser/ Ser	8(9.5)	64(18.6)	1	
Ser/ Cys	51(60.7)	162(46.9)	2.87(1.40–2.87)	< 0.05
Cys/ Cys	25(29.8)	119 (34.5)	1.93(1.31–2.61)	< 0.05

CONCLUSIONS

1. *We found that the frequency of hOGG1 Ser/Ser, Ser/Cys, and Cys/Cys genotypes were 9.5, 60.7, and 60.7% in all cases, and 18.6, 46.9, and 34.5% in the controls.*
2. *Significantly increase risk for nasopharyngeal carcinoma was observed.*
3. *These results suggest that hOGG1Ser 326Cys, might have potential as a genetic marker for nasopharyngeal carcinoma susceptibility.*

Clinical Chemistry

PD-13

PD-13

Trace element levels in type 1 diabetes patients

Ching-Chiang Lin[a,b], **Guey-Ju Tsweng**[c], **Yeou-Lih Huang**[c,d*]

[a]Department of Laboratory Medicine, Fooyin University Hospital, Pingtung, Taiwan
[b]Department of Medical Laboratory Science and Biotechnology, Fooyin University, Kaohsiung, Taiwan
[c]Department of Medical Laboratory Science and biotechnology, Kaohsiung Medical University, Kaohsiung, Taiwan
[d]Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

Objective

Several trace elements are involved in insulin signal transduction and glucose metabolism. Present study aimed determine the levels of three important elements—magnesium, chromium, and zinc—as well as one oxidative stress marker—malondialdehyde (MDA)—in young type 1 diabetic patients at different periods of their growth, and to realize the relationships between trace elements, oxidative stress, and growth stages.

Methods

A total of 88 patients with type 1 diabetes mellitus in different growth stages and 76 gender- and age-matched healthy subjects were included in this study. The levels of MDA were measured through HPLC using a C-18 column. Zinc, magnesium, and chromium concentrations in serum were assessed using atomic absorption spectrophotometry.

Results

We found higher levels of blood malondialdehyde (MDA; $p < 0.001$), *significantly lower* levels of magnesium ($p < 0.001$), and no differences in zinc and chromium levels ($p = 0.153$ and 0.515, respectively) in younger type 1 diabetic subjects relative to those of control subjects. Only 3.4% (3/88) of younger diabetic subjects exhibited hypomagnesemia; similar results were obtained when comparing different subgroups: children, adolescents, and adults. We also observed no differences in the levels of the three elements between the genders and among the growth stages ($p > 0.05$) of the diabetic subjects. There were no correlations between the three trace elements and HbA1C, diabetes duration, and insulin dose/BMI (all $p > 0.05$), but there was a significant difference between zinc levels and insulin dose/BMI ($p = 0.043$) in the diabetic patients.

Table 1
Clinical characteristics and diabetes-related trace elements in type 1 diabetic patients and healthy subjects.

Parameter	Type 1 diabetic patients ($n = 88$)	Healthy subjects ($n = 76$)	P
Gender (male/female)	45/43	32/44	0.248
Age (years)	16.5 ± 5.4	17.1 ± 5.1	0.459
Diabetes duration (years)	7.4 ± 4.6	–	–
Cigarette smoking	0/88	0/76	–
Alcohol consumption habit	0/88	0/76	–
BMI (kg/m^2)	20.5 ± 3.8	20.5 ± 4.0	0.915
WBC (*10^3/uL)	5.5 ± 3.6	6.2 ± 3.4	0.242
HbAc (%)	8.6 ± 1.6	5.2 ± 0.2	<0.001
Blood MDA (μM)	1.67 ± 0.54	0.97 ± 0.27	<0.001
Magnesium (mg/L)	20.2 ± 2.1	22.2 ± 1.3	<0.001
Hypomagnesemia (<17 mg/L; %)	3.4 (3/88)	0 (0/76)	0.104
Zinc (mg/L)	0.91 ± 0.16	0.94 ± 0.16	0.153
Chromium (ng/mL)	0.79 ± 0.34	0.85 ± 0.59	0.515

Data are means ± SD. Student's t test was used for continuous variables. Chi-square test was used for categorical data.

Table 2
Clinical characteristics and diabetes-related trace elements in children with type 1 diabetes and healthy subjects.

Parameter	Type 1 diabetic patients ($n = 23$)	Healthy subjects ($n = 20$)	P
Gender (male/female)	08/15	05/15	0.486
Age (years)	9.8 ± 1.5	10.5 ± 1.1	0.114
Diabetes duration (years)	4.2 ± 2.8	–	–
Cigarette smoking	0/23	0/20	–
Alcohol consumption habit	0/23	0/20	–
BMI (kg/m^2)	16.8 ± 2.6	18.0 ± 3.5	0.223
WBC (*10^3/uL)	7.1 ± 3.4	7.2 ± 3.5	0.891
HbAc (%)	8.7 ± 1.1	5.2 ± 0.2	<0.001
Blood MDA (μM)	1.61 ± 0.46	1.08 ± 0.33	<0.001
Magnesium (mg/L)	20.6 ± 1.7	22.3 ± 1.3	0.009
Hypomagnesemia (<17 mg/L; %)	0 (0/23)	0 (0/20)	–
Zinc (mg/L)	0.89 ± 0.15	0.97 ± 0.14	0.073
Chromium (ng/mL)	0.62 ± 0.16	0.80 ± 0.45	0.278

Data are means ± SD. Student's t test was used for continuous variables. Chi-square test was used for categorical data.

Table 3
Clinical characteristics and diabetes-related trace elements in adolescents with type 1 diabetes and healthy subjects.

Parameter	Type 1 diabetic patients ($n = 38$)	Healthy subjects ($n = 31$)	P
Gender (male/female)	21/17	13/18	0.271
Age (years)	15.8 ± 2.0	16.7 ± 2.3	0.100
Diabetes duration (years)	6.6 ± 4.1	–	–
Cigarette smoking	0/38	0/31	–
Alcohol consumption habit	0/38	0/31	–
BMI (kg/m^2)	21.5 ± 3.6	21.3 ± 3.7	0.798
WBC (*10^3/uL)	4.8 ± 3.9	5.5 ± 3.8	0.434
HbAc (%)	8.6 ± 1.8	5.2 ± 0.2	<0.001
Blood MDA (μM)	1.61 ± 0.56	0.93 ± 0.22	<0.001
Magnesium (mg/L)	20.2 ± 2.0	22.0 ± 1.2	<0.001
Hypomagnesemia (<17 mg/L; %)	2.6 (1/38)	0 (0/31)	0.363
Zinc (mg/L)	0.91 ± 0.17	0.97 ± 0.17	0.171
Chromium (ng/mL)	0.81 ± 0.34	0.86 ± 0.61	0.746

Data are means ± SD. Student's t test was used for continuous variables. Chi-square test was used for categorical data.

Table 4
Clinical characteristics and diabetes-related trace elements in young adults with type 1 diabetes and healthy subjects.

Parameter	Type 1 diabetic patients ($n = 27$)	Healthy subjects ($n = 25$)	P
Gender (male/female)	16/11	14/11	0.812
Age (years)	23.1 ± 2.2	22.9 ± 1.8	0.731
Diabetes duration (years)	11.4 ± 4.0	–	–
Cigarette smoking	0/27	0/25	–
Alcohol consumption habit	0/27	0/25	–
BMI (kg/m^2)	22.3 ± 2.9	21.9 ± 3.9	0.657
WBC (*10^3/uL)	5.3 ± 3.2	6.2 ± 2.6±	0.271
HbAc (%)	8.5 ± 1.8	5.2 ± 0.2	<0.001
Blood MDA (μM)	1.80 ± 0.57	0.91 ± 0.24	<0.001
Magnesium (mg/L)	19.9 ± 2.4	22.5 ± 1.4	<0.001
Hypomagnesemia (<17 mg/L; %)	7.4 (2/27)	0 (0/25)	0.165
Zinc (mg/L)	0.91 ± 0.15	0.88 ± 0.15	0.498
Chromium (ng/mL)	0.81 ± 0.36	0.86 ± 0.64	0.745

Data are means ± SD. Student's t test was used for continuous variables. Chi-square test was used for categorical data.

Table 5
Levels of MDA and elements in type 1 diabetic patients, listed with respect to gender.

Parameter	Female ($n = 43$)	Male ($n = 45$)	P
Age (years)	15.5 ± 5.6	17.4 ± 5.2	0.100
Diabetes duration (years)	7.5 ± 4.7	7.4 ± 4.6	0.903
BMI (kg/m^2)	20.0 ± 4.2	21.0 ± 3.5	0.276
Insulin dose (IU/day)	54.3 ± 25.6	63.6 ± 27.6	0.169
Insulin dose/BMI (IU/day/kg/m^2)	2.6 ± 0.9	2.9 ± 1.1	0.269
WBC (*10^3/uL)	6.1 ± 3.6	5.1 ± 3.7	0.209
HbAc (%)	8.7 ± 1.4	8.5 ± 1.8	0.690
Blood MDA (μM)	1.64 ± 0.46	1.70 ± 0.62	0.639
Magnesium (mg/L)	20.3 ± 2.1	20.1 ± 2.2	0.634
Hypomagnesemia (<17 mg/L; %)	2.3 (1/43)	4.4 (2/45)	0.584
Zinc (mg/L)	0.88 ± 0.14	0.94 ± 0.17	0.077
Chromium (ng/mL)	0.88 ± 0.45	0.73 ± 0.17	0.059

Data are means ± SD.

Table 6
Levels of MDA and elements in children, adolescents, and young adults with type 1 diabetes.

Parameter	Pre-puberty ($n = 23$)	Puberty ($n = 38$)	Adulthood ($n = 27$)	P
Gender (male/female)	8/15	21/17	16/11	0.180
Age (years)	9.8 ± 1.5	15.8 ± 2.0	25.1 ± 2.2	<0.001
BMI (kg/m^2)	16.8 ± 2.6	21.5 ± 3.6	22.3 ± 2.9	<0.001
Diabetes duration (years)	4.2 ± 2.8	8.6 ± 4.1	11.4 ± 4.0	<0.001
Insulin dose (IU/day)	21.6 ± 16.9	59.0 ± 26.8	67.8 ± 23.3	<0.001
Insulin dose/BMI (IU/day/kg/m^2)	1.5 ± 0.6	2.8 ± 1.0	2.9 ± 0.8	0.005
WBC (*10^3/uL)	7.1 ± 3.4	4.8 ± 3.9	5.3 ± 3.2	0.003
HbAc (%)	8.7 ± 1.1	8.6 ± 1.8	8.5 ± 1.8	0.944
MDA (μM)	1.61 ± 0.48	1.61 ± 0.58	1.80 ± 0.37	0.343
Magnesium (mg/L)	20.6 ± 1.7	20.2 ± 2.0	19.9 ± 2.4	0.656
Hypomagnesemia (<17 mg/L; %)	0 (0/23)	2.6 (1/38)	7.4 (2/27)	0.334
Zinc (mg/L)	0.89 ± 0.15	0.91 ± 0.17	0.91 ± 0.15	0.814
Chromium (ng/mL)	0.62 ± 0.16	0.82 ± 0.34	0.81 ± 0.36	0.285

Data are means ± SD. ANOVA test was used for continuous variables. Chi-square test was used for categorical data.

Conclusions

We found elevated blood MDA, decreased magnesium, and no changes in zinc and chromium levels in younger type 1 diabetic subjects relative to those of control subjects. Only 3.4% of younger diabetic subjects exhibited hypomagnesemia. Whether magnesium supplementation is suitable for improving insulin sensitivity and decreasing oxidative stress and inflammation will require confirmation through additional studies.

Clinical Chemistry

PD-14

A microtubule inhibitor, ABT-751, induces autophagy through inhibition of the AKT/MTOR pathway and suppresses apoptosis in Huh-7 cells

Ren-Jie Wei[12], Su-Shuan Lin[1], Shang-Tao Chien[1], Yow-Ling Shiue[2]
[1]Pathology, Kaohsiung Armed Forces General Hospital
[2]Institute of Biomedical Sciences, National Sun Yat-Sen University

Background

Hepatocellular carcinoma (HCC) is the most common primary malignant neoplasm of the liver worldwide, accounting for approximately 6% of all human cancers annually. ABT-751 is an oral antimitotic sulfonamide that binds to the colchicine binding site on β-tubulin and inhibits polymerization of microtubules. As a single agent and in combination with standard cytotoxics and radiation, ABT-751 is active in multiple human tumor xenograft models, including fibrosarcoma, leukemia, breast, gastric, non-small cell lung and colon cancers.

Aim

We therefore aimed to study its apoptotic and autophagic effects on HCC-derived cell lines with distinct genetic background.

Results

a.

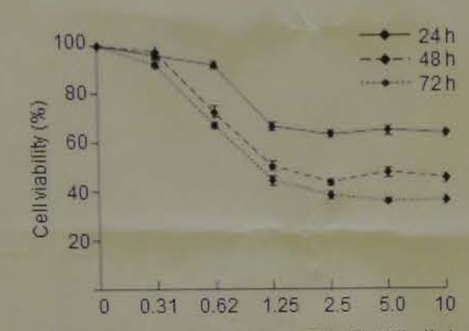


Figure 1. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay identified that IC_{50} of ABT-751 in Huh-7 cell were > 50 μM for 24 h; 1.5 μM for 48 h; 1 μM for 72 h.

a. b.

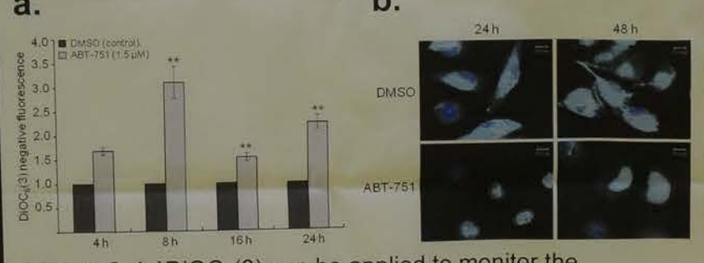
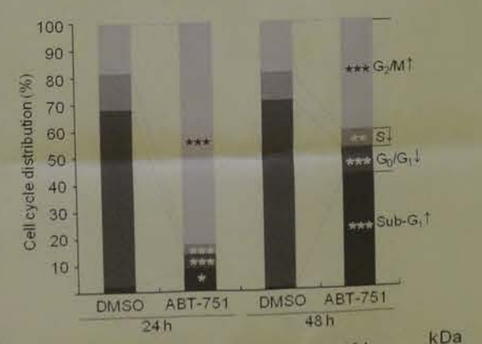


Figure 2. (a)$DiOC_6(3)$ can be applied to monitor the mitochondrial membrane potential were higher in HCC-derived cells treat with ABT-751 than control..: **(b)**Immunofluorescence (anti-α-tubulin) analyses further demonstrated that ABT-751 destabilized tubulin in Huh-7 cells.

a.



b.

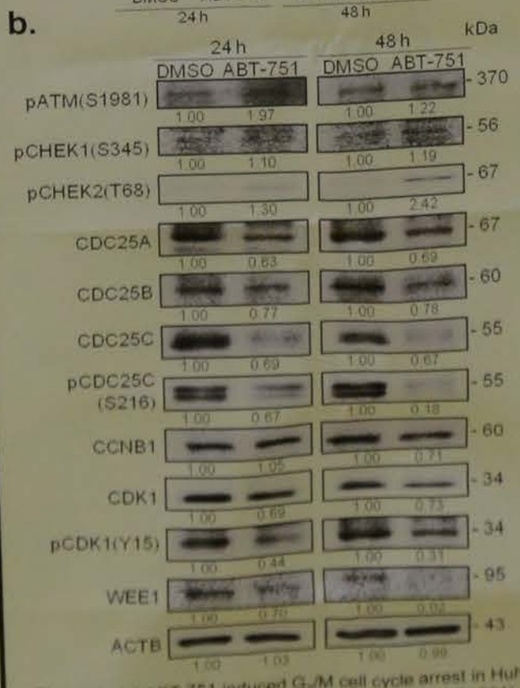
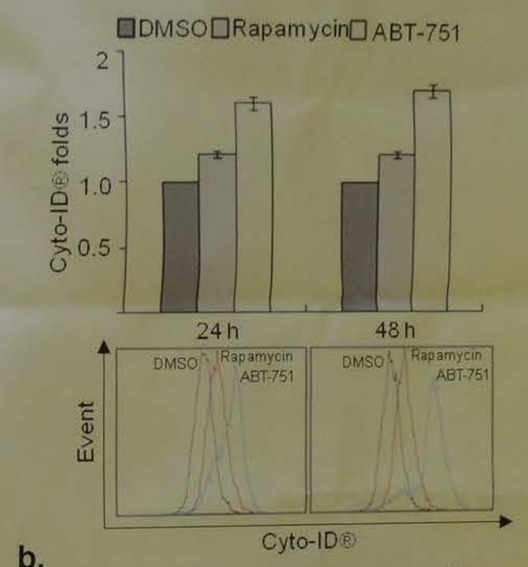


Figure 3. (a) ABT-751 induced G_2/M cell cycle arrest in Huh-7 cells. **(b)** Immunoblotting analysis further showed that pATM(S1981), pCHEK2(T68) and pTP53(S15) protein levels were upregulated, while CDC25A, CDC25B, CDC25C, pCDC25C(S216), CDK1, pCDK1(Y15) and WEE1 were notably downregulated in Huh-7 cells after treatments with ABT-751 for 24 and 48 h. All experiments were triplicated and results are expressed as mean ± SEM. One representative immunoblotting image was shown. The actin, beta (ACTB) was served as a loading control. Statistical significance: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

a.



b.

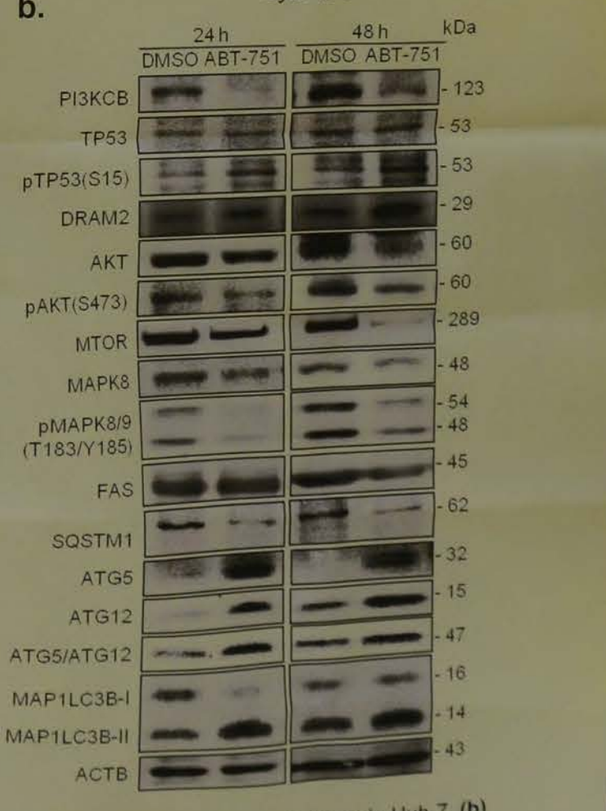


Figure 4. (a) ABT-751 induced autophagy in Huh-7. **(b)** upregulated MAP1LC3B-II/-I/ACTB folds and downregulated of SQSTM1 protein levels.

a.

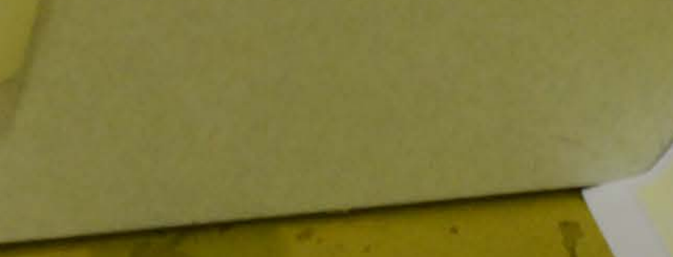


Figure 5. Inhibition of autophagy enhanced ABT-751-induced apoptosis.

Conclusion

Above results indicated that ABT-751 could dysregulated microtubules and induces autophagy through inhibition of the AKT/MTOR pathway. *Inhibition of autophagy significantly enhanced apoptotic cells.*

Clinical Chemistry

PD-15

Abstract

Bilirubin is considered as the ma
pigment. This study was performe
(34) pooled serum samples were
were divided for exposure in an
bilirubin levels were measured usi
values taken prior to exposure. T
bilirubin values since P-values of
p=0.990), and Indirect Bilirubin [F
and indirect bilirubin [F
significant difference when values

Introduction

Serum bilirubin determination has alwa
hemolytic disease and liver disease. G
population, and about 0-5% in Southea
preserve the integrity of the specimen
Previous studies have shown that bilirub

Objectives

General Objective: To determine the ef

Specific Objectives:

1. To determine the changes in the value
different time intervals .
2. To correlate the changes in bilirubin lev

Methodology

1. Pooled serum samples (normal and icteric) n = 34, stored at 3° to 5 °C
2.
3. Division of mother tubes for different set-ups and subsequent light exposure of varying intensity
4.

Results and Discussio

Figure 1b. Percentage Change of Direc

Mean Direct Bilirubin Change (%)
Normal
Icteric
Time

Interpretation: The joint effect of light intensity fo
significant difference [F

Table 1. Number

Level		Time
Icteric		1 hour
		2 hours
Normal	24 hrs	3 hours
		4 hours
Icteric		1 hour
		2 hours
Normal	48 hrs	3 hours
		4 hours
Icteric		1 hour
		2 hours
Normal	72 hrs	3 hours
		4 hours

Conclusion and Recomm

References

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2. Bhatt, A. Chua, A. Eggert, D. ... & Schwartz, C. ... Bilirubin Levels at Different Time Intervals

Clinical Chemistry

PD-15

The Effects of Varying Intensities of Light in Serum Bilirubin

Baquiran, C., Basa, A., Constantino, A., Garcia, A., Genuino, J., Lazatin, M., Quevedo, D., Martin, G.

Department of Medical Technology - Faculty of Pharmacy

Abstract

Bilirubin is considered as the major heme waste product from the destruction of red blood cells. This is a photosensitive bile pigment. This study was performed in order to determine the effects of varying intensities of light in serum bilirubin. Thirty-four (34) pooled serum samples were collected, seventeen (17) normal samples and seventeen (17) icteric samples. The specimens were divided for exposure in an enclosed set-up at 100-lux, 150-lux, and 200-lux and had a fix distance of 1.5 meters. The bilirubin levels were measured using *Prestige Chem Analyzer* at different time interval (1 hour, 2 hours and 3 hours) with baseline values taken prior to exposure. The results of the experiment showed that light intensity in relation to time did not affect the bilirubin values since p-values of percentage change for Total Bilirubin [$F_{4,128}$=0.265, p=0.900], Direct Bilirubin [$F_{4,128}$=0.074, p=0.990], and Indirect Bilirubin [$F_{4,128}$=0.607, p=0.658] were greater than 0.05. Further, the joint effect of intensity to total, direct, and indirect bilirubin [$F_{4,128}$=0.140, p=0.967] was not significant. However, the number of samples (normal & icteric) showed significant difference when values were compared with the baseline.

Introduction

Serum bilirubin determination has always been done in order to diagnose disease. High levels of bilirubin have been associated with hemolytic disease and liver disease. Gilbert's syndrome, a hepatic defect in the ability to take up bilirubin, affects 5-10% of the world's population, and about 0-5% in Southeast Asia[1] . In the clinical laboratory, medical technologists take precautionary measures in order to preserve the integrity of the specimens they receive especially for tests such as determination of bilirubin, a photosensitive pigment. Previous studies have shown that bilirubin degradation varies depending on the distance of the specimens to the source of light.[2]

Objectives

General Objective: To determine the effects of varying intensities of light in serum bilirubin

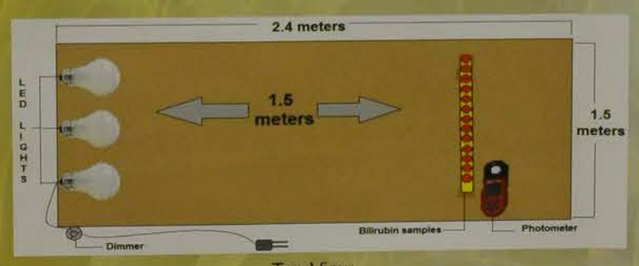
Specific Objectives:

1. To determine the changes in the values of direct, indirect and total bilirubin when exposed to varying intensities of light and taken at different time intervals .
2. To correlate the changes in bilirubin levels to light intensity and length of serum exposure.

Methodology

1. Pooled serum samples (normal and icteric): n = 34, stored at 3° to 5 °C
2. Baseline bilirubin measurement of pre-exposed sera
3. Division of mother tubes for different set-ups and subsequent light exposure of varying intensity
4. Bilirubin assay of post-exposure sera taken at different time interval: 1-hour, 2-hour, 3-hour.

Experimentation Set-up



Results and Discussion

Figure 1b. Percentage Change of Direct Bilirubin

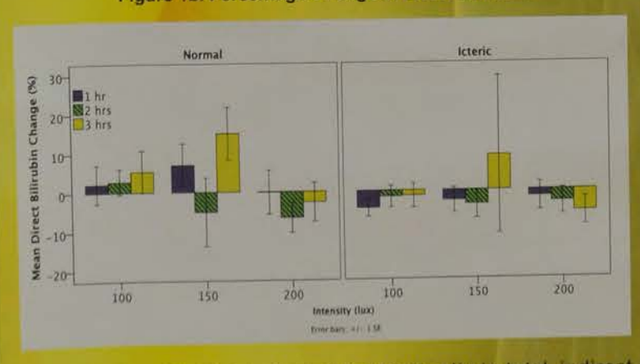
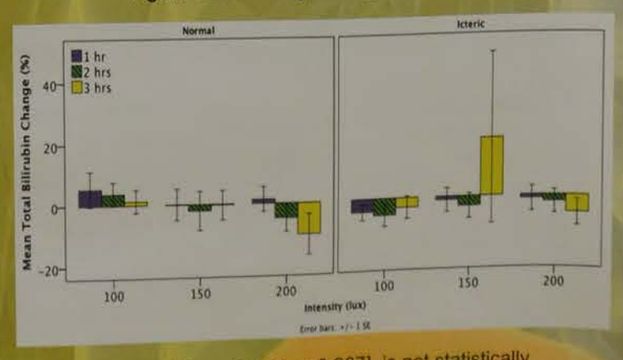


Figure 1a. Percentage Change of Total Bilirubin



Interpretation: The joint effect of light intensity to total, indirect and direct bilirubin [$F_{4,128}$=0.140, p=0.967] is not statistically significant. Likewise, no significant difference [$F_{2,64}$=0.273, p=0.762] was observed in their mean percentage change from the baseline.

Table 1. Number of samples with decreased bilirubin

Level		Time	Intensity (lux) 100	150	200	p-value
Icteric	Total	1 hour	11 (64.7%)	12 (70.6%)	10 (58.8%)	0.651
		2 hours	12 (70.6%)	12 (70.6%)	13 (76.5%)	0.819
		3 hours	11 (64.7%)	13 (76.5%)	13 (76.5%)	0.513
Normal		1 hour	7 (41.2%)	9 (52.9%)	8 (47.1%)	0.867
		2 hours	7 (41.2%)	8 (47.1%)	10 (58.8%)	0.497
		3 hours	6 (35.3%)		9 (52.9%)	0.459
Icteric	Direct	1 hour	11 (64.7%)	13 (76.5%)	10 (58.8%)	0.417
		2 hours	11 (64.7%)	10 (58.8%)	13 (76.5%)	0.368
		3 hours	10 (58.8%)	12 (70.6%)	13 (76.5%)	0.417
Normal		1 hour	8 (47.1%)	4 (23.5%)	10 (58.8%)	0.078
		2 hours	7 (41.2%)	8 (47.1%)	9 (52.9%)	0.651
		3 hours	**8 (47.1%)**	**4 (23.5%)**	**10 (58.8%)**	**0.045**
Icteric	Indirect	1 hour	7 (41.2%)	8 (47.1%)	9 (52.9%)	0.741
		2 hours	10 (58.8%)	10 (58.8%)	11 (64.7%)	0.895
		3 hours	10 (58.8%)	12 (70.6%)	10 (58.8%)	0.264
Normal		1 hour	7 (41.2%)	8 (47.1%)	7 (41.2%)	0.867
		2 hours	8 (47.1%)	7 (41.2%)	7 (41.2%)	0.882
		3 hours	8 (47.1%)	10 (58.8%)	12 (70.6%)	0.607

Interpretation: Generally, the number of samples (normal or icteric) showing decrease in the values as compared with the baseline increases over time of exposure and intensity of light. This may not be statistically significant but evident in direct bilirubin from normal serum exposed for 3-hour at 200-lux (p=0.045).

Conclusion and Recommendation

Based on the gathered data and statistical analyses used, the researchers conclude that as the light intensity increases, the greater the effect on the bilirubin concentration especially for direct bilirubin. The researchers recommend using longer time interval in the exposure of serum in different types of light, increasing light intensity and ensuring control over the whole automated procedure.

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Clinical Chemistry

PD-16

EFFECTS OF THE USE OF DIFFERENT COVERING ON SERUM SAMPLES FOR BILIRUBIN DETERMINATION

Bansil, N.J.[1], Bautista, K.[1], Cruz, C.[1], Eltanal, E.J.[1], Maralit, L.[1], Taton, R.M.[1], Trinidad, M.J.[1], Martin I, G.L.[1]

[1]University of Santo Tomas - Faculty of Pharmacy, Department of Medical Technology
Manila, Philippines

Abstract

The effects of the use of different covering materials on serum bilirubin were determined. Seventeen pooled sera each of icteric and normal samples were covered using bond, carbon, and aluminum foil. Aliquoits of the collected sera were exposed to fluorescent light at a predetermined distance of 2.1 meters for 3 time intervals of 1-hour, 2-hour, and 3-hour. Baseline values of the sera were measured prior to exposure. Values of the total, direct, and indirect bilirubin after exposure were compared to the established baseline values. Results for both icteric and normal samples showed that joint effect of covering material and time of total bilirubin is not significant with (p=0.417 and p=0.400) values respectively. Similarly, the mean direct bilirubin using the four covering material for both icteric and normal samples do not show any significance with (p=0.500 and p=0.900) values respectively. Moreover, the mean bilirubin do not depend on the covering material. This indicates that the use of different covering materials on both normal and icteric samples do not have significant effect with (p=0.227 and p=0.555) values respectively. These imply that any of the four covering materials in the study may be used for serum bilirubin determination.

KEYWORDS: *Bilirubin, Icteric, Jaundice*

Introduction

Large amounts of bilirubin in the blood can lead to jaundice or liver diseases. Therefore, bilirubin levels should be measured accurately and should not be acted upon by factors that could affect its accuracy. Other studies show that environmental factors such as temperature and light could affect bilirubin levels. However, there is inadequate study regarding the specific covering materials and their effect on bilirubin determination. Thus, this study aimed to determine the effects of various covering materials to serum bilirubin levels.

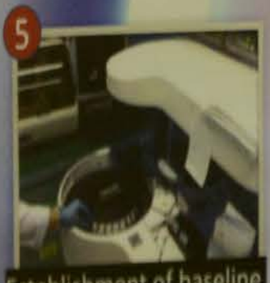
Methodology


1 Preparation of sample tubes for collection


2 Sample Collection


3 Sample Storage


4 Cover tubes using different covering materials


5 Establishment of baseline values


6 Introduction to exposure setup


7 Measurement of bilirubin levels

Results and Discussion

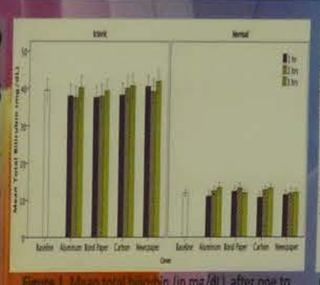


Figure 1. Mean total bilirubin (in mg/dL) after one to three hours at different cover materials

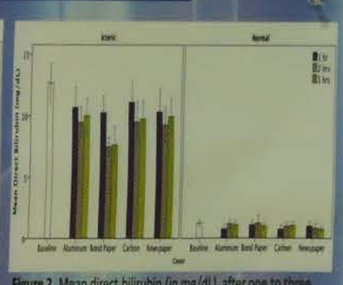


Figure 2. Mean direct bilirubin (in mg/dL) after one to three hours at different cover materials

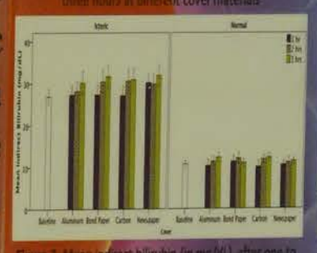


Figure 3. Mean indirect bilirubin (in mg/dL) after one to three hours at different cover materials

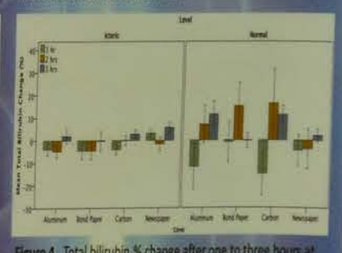


Figure 4. Total bilirubin % change after one to three hours at different materials

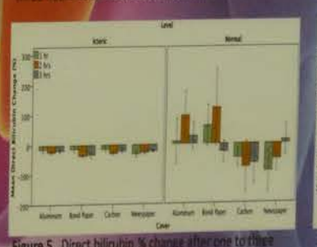


Figure 5. Direct bilirubin % change after one to three hours at different materials

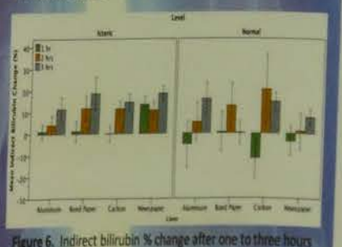


Figure 6. Indirect bilirubin % change after one to three hours at different materials

After gathering all the data needed and formulating statistical analyses, the researchers were able to formulate three conclusions. First, the direct bilirubin concentrations of icteric samples had a significant decrease after 2 hours and 3 hours of light exposure. Unlike direct bilirubin, total and indirect bilirubin levels of icteric samples had a significant increase after the 3 hours of light exposure. Second, there was no significant change to the total, direct and indirect bilirubin concentrations of the normal serum samples after light exposure. Lastly, the effect of the use of the different covering materials utilized in the study was insignificant to the bilirubin levels of both normal and icteric sera.

Conclusion and Recommendation

From the findings obtained in the experiment, the researchers recommend future researchers to make an experiment comparing the results of the direct, indirect and total bilirubin of serum samples using the various covering materials exposed to different light intensities. Moreover, future researchers may compare the bilirubin levels of sera covered with different materials that were stored for a long period of time from freshly extracted samples.

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Clinical Chemistry

PD-17

Assessing the diagnostic characteristics of procalcitonin assay in patients suspected bacterial sepsis

Wang Chiou-Huey [1], Lai Pei-Ling [1], Chen Jun-he [1], Yu Suan-Hung [2]
Department of Clinical Pathology [1] and Emergency Department [2], Tungs' Taichung MetroHarbor Hospital, Taiwan

Background:

Procalcitonin (PCT) is the prohormone of the hormone calcitonin, and PCT can be produced by several cell types and many organs in response to proinflammatory stimuli, in particular by bacterial products. The induction of PCT can be caused by different stimuli both in vitro and in vivo. Bacterial endotoxins and pro-inflammatory cytokines are powerful stimuli for the production of PCT. The exact biological role of PCT remains largely unknown, however, recent experimental studies suggest that PCT may play a pathogenic role in sepsis. The PCT assay is a useful screening test for diagnosis of bacterial sepsis. The expected value of PCT (<0.1 ng/mL) in reagent instruction (Siemen) was obtained from 465 normal subjects. However, the diagnostic characteristics of PCT assay in patient suspected bacterial sepsis were not clear. The aim of the study was to assess the diagnostic characteristics of PCT assay in patient suspected bacterial sepsis.

Materials and Methods:

Two hundred and sixteen patients suspected with bacterial sepsis were recruited in the study from October to December in 2015. Blood from the patients was collected to conduct both of PCT test and blood culture. Positive results of blood culture were considered as standard method for diagnosis of bacterial sepsis. Sensitivity, specificity, positive predictive value (PPV), negative predicative value (NPV) with 95% confidence intervals and receiver operating characteristic (ROC) curve were obtained by SPSS Statistics 16.0 (IBM Corporation, Armonk, NY, USA).

Results:

Blood from the 216 patients was performed for PCT test in Advia centur CP system (Siemen) and the results of blood culture were monitored by BACTEC™ Instrumented Blood Culture Systems (BD). In healthy people, plasma PCT concentrations are found to be below 0.05 ng/mL. Usually, PCT concentrations exceeding 0.5 ng/mL are interpreted as abnormal values suggestive of a sepsis syndrome. PCT values in the range of 0.5 and 2 ng/mL represent a"grey" zone with uncertainty as far as the diagnosis of sepsis is concerned. Therefore, we calculated the cut-off value of blood PCT in 0.1 ng/ml and 2 ng/ml to evaluate the diagnostic characteristics of PCT assay in patients suspected bacterial sepsis. The diagnostic characteristics of PCT cut-off value in 0.1 ng/ml and 2 ng/ml were summarized in Table 1 and Table2, respectively. Of the 216 blood sample, positive results and negative results in blood culture were 48 specimens and 168 specimens, respectively. The cut-off value of blood PCT was set in 0.1 ng/ml according to reagent instruction. We found that the sensitivity, specificity, PPV, and NPV of PCT assay were 97.9%, 8.3%, 23.4%, and 93.3%, respectively (Table 1). Furthermore, the cut-off value of blood PCT was set in 2 ng/ml as an indication for sepsis diagnosis. We found that the sensitivity, specificity, PPV, and NPV of PCT assay were 45.8%, 85.1%, 46.8%, and 84.6%, respectively in Table 2. The ROC curve was performed in Figure 1 and the area under the curve was 0.683.

Table 1. The diagnostic characteristics of PCT cut-off value in 0.1 ng/ml.

		Blood culture		
		positive	negative	Total
PCT (ng/ml)	>0.1	47	154	201
	<0.1	1	14	15
	Total	48	168	

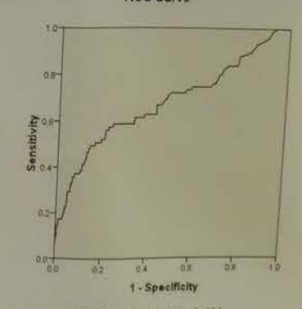
Sensitivity: 97.9%, specificity: 8.3%, PPV: 23.4%, and NPV: 93.3%.

Table 2. The diagnostic characteristics of PCT cut-off value in 2 ng/ml

		Blood culture		
		positive	negative	Total
PCT (ng/ml)	>2	22	25	47
	<2	26	143	169
	Total	48	168	

Sensitivity: 45.8%, specificity: 85.1%, PPV: 46.8%, and NPV: 84.6%.

Figure 1. ROC curve analysis to assess diagnostic characteristics of procalcitonin assay.



Area under curve was 0.683.

Conclusion:

The PCT level at 0.1 ng/mL performed high sensitivity and NPV as a screening test for diagnosis of bacterial sepsis and the PCT level at 2 ng/mL performed high specificity as a diagnostic marker for bacterial sepsis. Therefore, plasma PCT is a useful marker of the inflammatory response of the human body to a non-viral infection and elevated values are highly suggestive of a bacterial infection with systemic consequences.

童綜合醫院社團法人 童綜合醫院
Tungs' Taichung MetroHarbor Hospital

D-1 D-1

Clinical Chemistry
PD-18

IgA/λ Plasma Cell Myeloma with λ Light Chain Amyloidosis : A Case Report

Kai-Pei Huang, Yen-Chuan Hsieh, Ting-Chung Hung, Li-Ching Wu, Shih-Sung Chuang

Department of Pathology, Chi Mei Medical Center, Tainan, Taiwan

Introduction

Amyloidosis refers to a variety of conditions wherein amyloid proteins are abnormally deposited in organ or tissues and cause harm. Among the several forms of amyloidosis, the principal types of that in inpatient medical services are the AL amyloidosis (primary) and AA amyloidois (secondary). AL Amyloidois is due to deposition of protein derived from overproduction of immunoglobulin light chain in plasma cell myeloma. Furthermore, it is a systemic disorder that can present with a variety of symptoms, including heavy proteinemia and edema, heptosplenomegaly, otherwise unexplained heart failure.[1,2,3]

Case report

This 78 year-old female has history of hypertension, hypertrophic obstructive cardiomyopathy and CKD stage 2 for many years. She complained she suffered from severe pain during urination, dysuria, burning urination, oliguria, general malaise, fatigue, poor appetite, leg edema for several months. Regular nephro OPD follow-up and scheduled admission for renal biopsy due to glomerulonephritis. Related laboratory examination data arranged by nephrology and hematology & oncology department are as follows.

- Urine routine : Mild pyurai and proteinuria [urine Protein (2+)].
- Biochemistry : Serum BUN[39 mg/dL], Creatinine[0.76 mg/dL], ALB[2.3mg/dL], Ca[8.5mg/dL], UPCR[1679.8 mg/g]
- Immunology : Serum IgG: 748 mg/dL, IgA: 635 mg/dL, IgM: 63 mg/dL, β2-microglobulin 6404 ug/L. Free light chain in serum: (Kappa: 18.80 mg/L, lambda: 110.00 mg/L), Kappa/lambda ratio: 0.17 (0.26-1.65).
- CBC : WBC 10.2x 10^3/uL , Hb 10.9 g/dL , MCV 91.4 fL, Plt 344x 10^3/uL, Band 0%, Seg 56.7%, Lym 27.9%, Mono 12.5%, Eos 1.9%, Baso 0%, atypical lymphocyte 1%.
- Serum protein electrophoresis : A major monoclonal gammaglobulin peak is present in β2 region, and a minor monoclonal gammaglobulin peak is present in γ region (Figure.1).
- Serum protein immunoelectrophoresis : M protein, IgA lambda (Figure 2).
- Kidney needle biopsy : Presence of amyloid fibrils in glomerulus. The Congo red stain highlights amyloid deposition in arterial wall and glomeruli, withapple-green birefringence. Additional immunofluorescent stain show lambda light chain deposition along glomerular capillary wall. Staining for Kappa light chain is negative.
- Bone marrow biopsy(IHC) : These plasma cells show monotypic for lambda light chain and are positive for IgA. Nearly no kappa light chain-positive cells are present (Figure 3).

Discussion

The amyloidosis ara a group of disorders in which soluble proteins aggregate and deposit extracellularly in tissue as insoluble fibrils, causing progressive organ dysfunction.[4]

Secreated IgA are often dimeric with two immunoglobulin monomer. To clarify two IgA/λ found in serum immunoelectrophoresis are secreted from the same plasma cell clone in bone marrow, we treated sample with beta-mercaptoethanol (BME) which reduces the polymerized immunoglobulin and the result showed the small peak in gamma region disappeared after BME treated (Figure 4), which reveals IgA polymer are reduced to IgA monomer.

Besides, Bortezomib, melphalan, and prednisolone combination chemotherapy (VMP) was applied to this patient, and there were no paraprotein peak found in these follow-up protein electrophoresis pattern(Figure 5~7).

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Figure 1. Serum protein electrophoresis.

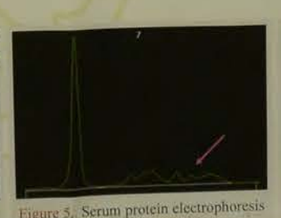


Figure 5. Serum protein electrophoresis analyzed on Dec.2015

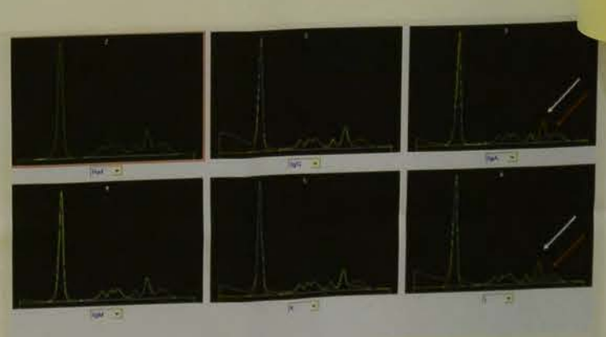


Figure 2. Serum protein immunoelectrophoresis.

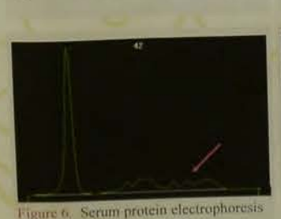


Figure 6. Serum protein electrophoresis analyzed on Feb.2016

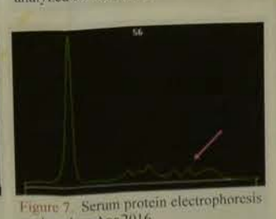


Figure 7. Serum protein electrophoresis analyzed on Apr.2016

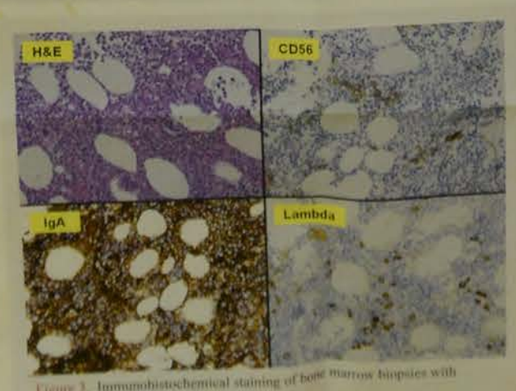


Figure 3. Immunohistochemical staining of bone marrow biopsies with monoclonal antibodies.

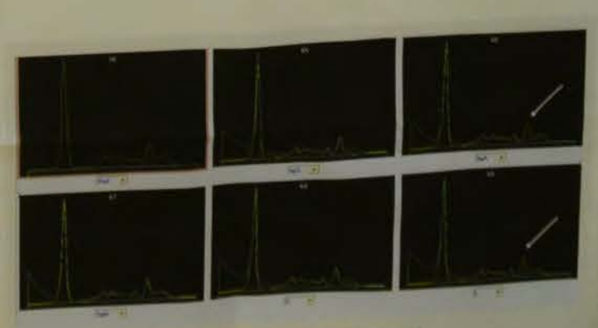
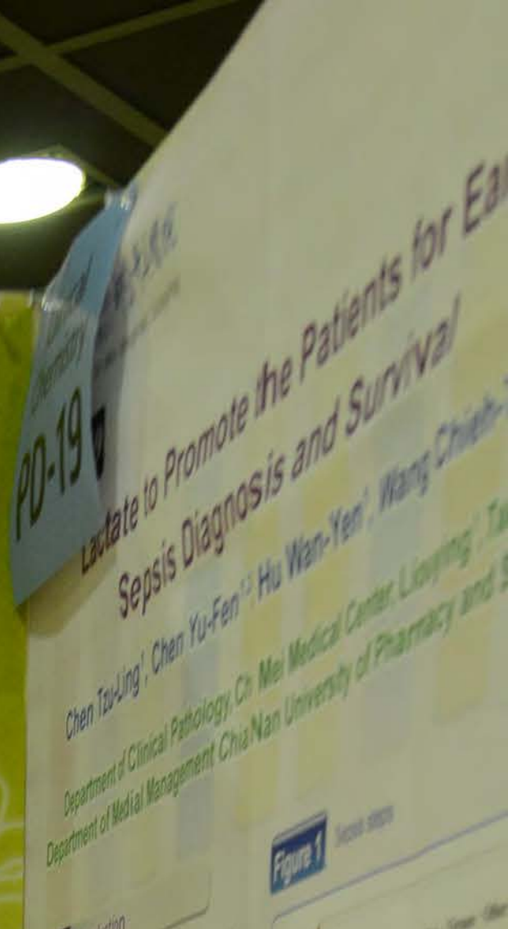


Figure 4. Serum protein immunoelectrophores after 1% BME treated.



Clinical Chemistry

PD-19

Lactate to Promote the Patients for Early Sepsis Diagnosis and Survival

Chen Tzu-Ling[1], Chen Yu-Fen[1,2], Hu Wan-Yen[1], Wang Chieh-Tien[1]

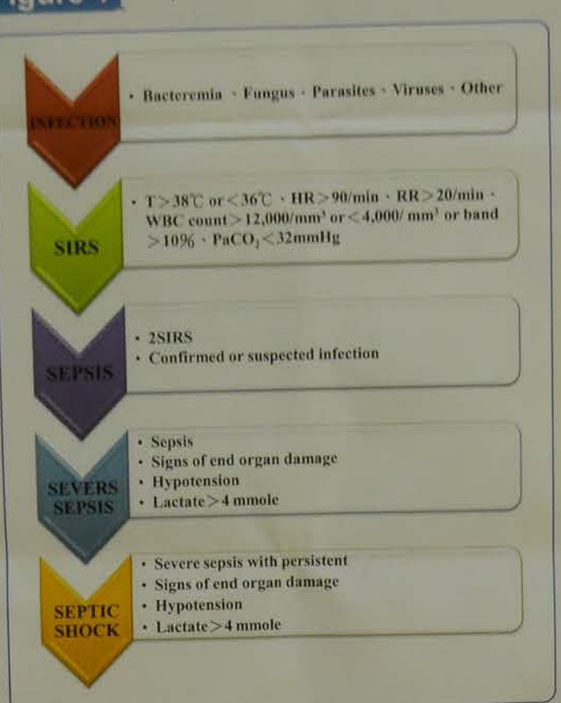
Department of Clinical Pathology, Chi Mei Medical Center, Liouying[1], Taiwan
Department of Medial Management Chia Nan University of Pharmacy and Science[2]

Introduction

Sepsis is a leading cause of illness worldwide and multifaceted host response to an infecting pathogen that may be significantly amplified by endogenous factors. The original conceptualization of sepsis as infection with at least 2 of the 4 SIRS criteria focused solely on inflammatory excess.Sepsis is now recognized to involve early activation of both before and anti-inflammatory responses.Recently, the incidence of sepsis continues to increase tendency. The patient who is immunocompromised and is under aging process has more possibility to get sepsis. Sepsis is used to define as systemic inflammatory response syndrome (SIRS) and causes many symptoms such as fever, hypotension and leukocytosis. Furthermore, severe septic shock is in a high risk of mortality.The clinical and research settings are PCT and lactate combined measurement to improve prognostic utility improve prognostic utility and survival.SIRS definition have two or more reaction of temperature＞38℃ or＜36 ℃, heart rate＞90/min,reapiratory rate＞20/min or $PaCO_2$ ＜32mmHg,WBC count＞12,000/mm³ or＜4,000/ mm³ or band＞10% (Figure 1).

It has been known that lactate increases during apoptosis, but the link between lactate and sepsis is uncertainty. Nevertheless, procalcitonin (PCT) is an important and automated in vitro diagnostic bacteremia test for the management of patients with sepsis.Clinical application of PCT assays have defined golden standard to a high degree. PCT biomarker for detection of sepsis and septic shock. In this study, we aimed to investigate if the combination of PCT and lactate both biomarkers helps to improve the sensitivity for the diagnosis of sepsis, achieving the purpose of early treatment.

Figure 1 Sepsis steps



Materials and methods

The study comprised 261 patients who had already been diagnosed with sepsis in 2015. The serum samples were tested for PCT and NaF plasma samples were tested for lactate respectively.Using multivariate analysis the presence of increase lactate and PCT level.Pearson correlation statistics and binary regression statistics method was used to analyze significant changes between PCT and lactate.

Result

The result showed that both PCT and lactate level elevated in 261 sepsis - diagnosed patients in early-stage of disease.Descriptive Statistics Lactate Mean 2.53 ; SD 2.70, PCT Mean 13.97 ; SD 35.94 (Table 1). As Pearson correlation coefficients, PCT and Lactate positively statistical correlation was found between PCT and lactate ($r=0.439$, $r^2=0.190$). Moreover, binary regression statistics gave p values< 0.01 (Table 2), indicating significant positive association between the increase of both PCT and lactate level and sepsis. In addition, by using the combined measurement of PCT and lactate, patient who was early diagnosis of sepsis was received appropriate management timely. There were 94 of 117 patients surviving from sepsis by early investigation and then receiving early treatment. The survival rate was 80.34%.

Conclusion

In the present study, it was found that PCT level rise in early stage of sepsis accompanied with elevation of lactate level. In summary, when patient is with suspected sepsis,PCT and lactate - combined measurement provides a tool for early diagnosis of sepsis and beneficial to timely and Appropriate Treatment, even to reduce mortality from sepsis.

Table 1 PCT and Lactate Level Descriptive Statistics

	Mean	SD	Number
Lactate	2.5330	2.70152	261
PCT	13.9712	35.93531	261

Table 2 PCT and Lactate SPSS Statistics Related

Test	explain	PCT	Lactate
PCT	Pearson Related	1	0.439(**)
	significance (Two-tailed)		0.000
	Number	261	261
Lactate	Pearson Related	0.439(**)	1
	significance (Two-tailed)	0.000	
	Number	261	261

** $p<0.01$ (*Two-tailed*), *Significant differences*

Mode Summary (b)

Mode	R	R^2	Revision R^2	Standard error	Change Statistics				
					Change R^2	Change F	Numerator degrees of freedom	Denominator degrees of freedom	Significance Change F
1	0.439(a)	0.193	0.190	2.43167	0.193	61.907	1	259	0.000

a : Predictive variables : (constant), PCT
b : Variable : Lactate

Analysis of variance (b)

Mode		Sum of square	DOF	The average sum of squares	F test	Significance
1	Restitute	366.058	1	366.058	61.907	0.000(a)
	Residuals	1531.478	259	5.913		
	Sum	1897.537	260			

a : Predictive variables : (constant), PCT
b : Variable : Lactate

p of Phosphorus, Parathyroid Hormone Phosphatase in Hemodialysis Patients

ui Chen, Xian Lang Fang, Hen Li Chao, Shun Liang Chen
Tungs' Taichung MetroHarbor Hospital, Taiwan

f the serious complications of long-term dialysis patients. It affects the life quality of waste of medical resources. It is therefore needed to monitor and prevent long-term phosphatemia. When the glomerular filtration rate is reduced to 30 mL / min, emia will ensue and parathyroid hormone (PTH) release will be increased, resulting in nd many complications such as renal osteodystrophy with subsequent rise of alkaline r to control the complications, patients should obey the NKF-K / DOQI ™ specification. : 8.4 ~ 9.5 mg / dL ; phosphorus value : 3.5 ~ 5.5 mg / dL ; calcium and phosphorus act parathyroid hormone (intact PTH ; i-PTH) values : 150 ~ 300 pg / mL. In this study, phorus value, i-PTH and Alkaline-Phosphatase were analyzed to monitor and prevent d complications in patients on chronic hemodialysis.

of 80 patients were collected in December 2015. The patients had been on hemodialysis the last six months or longer. The blood samples were collected before hemodialysis and 5 minutes. Biochemical analyses were performed by using a Hitachi 7170 automatic mulite 2000 luminescence inspection analyzer. We divided hemodialysis patients into two .5 mg/dL and the other ≥ 5.5 mg/dL. The phosphorus values of the two groups were ne phosphatase data. The p value by t-test was calculated, with $p < 0.05$ regarded as

th serum phosphorus ≥ 5.5 mg / dL, their average alkaline phosphatase was g / mL. In contrast, the patients with serum phosphorus < 5.5 mg / dL, their U / L and a mean i-PTH at 107.7 pg / mL. Our data showed that the phosphorus er alkaline phosphatase and i-PTH levels than the phosphorus ≥ 5.5 mg / dL

tion rate was decreased to 30 ml / min or less, a condition of persistent hyperphosphatemia portant pathogenic mechanism of hyperparathyroidism. Many studies have noted s and increased mortality in dialysis patients. NKF-K / DOQI ™ guidelines tion. However, whether the guidelines are applicable to the population of ta showed that when the phosphorus was < 5.5 mg / dL, the i-PTH value could aline-P was significantly lower. It indicated that when the serum phosphorus was , the secondary hyperparathyroidism and renal osteodystrophy might be well the parathyroid gland will not be needed. Thus, the serum phosphorus level can ing the patient's condition as well as a guideline for prognosis.

Clinical Chemistry

PD-21

The Relationship of Phosphorus, Parathyroid Hormone and Alkaline Phosphatase in Hemodialysis Patients

Ming Hui Chen, Xian Lang Fang, Hen Li Chao, Shun Liang Chen
Tungs' Taichung MetroHarbor Hospital, Taiwan

Preface :

Hyperphosphatemia is one of the serious complications of long-term dialysis patients. It affects the life quality of patients and causes unnecessary waste of medical resources. It is therefore needed to monitor and prevent long-term hemodialysis patients from hyperphosphatemia. When the glomerular filtration rate is reduced to 30 mL / min, hyperphosphatemia and hypocalcemia will ensue and parathyroid hormone (PTH) release will be increased, resulting in secondary hyperparathyroidism and many complications such as renal osteodystrophy with subsequent rise of alkaline phosphatase in the blood. In order to control the complications, patients should obey the NKF-K / DOQI ™ specification. The indicators are: calcium value: 8.4 ~ 9.5 mg / dL ; phosphorus value : 3.5 ~ 5.5 mg / dL ; calcium and phosphorus multiplied : < 55 mg2 / dL2 ; intact parathyroid hormone (intact PTH ; i-PTH) values : 150 ~ 300 pg / mL. In this study, the relevance among serum phosphorus value, i-PTH and Alkaline-Phosphatase were analyzed to monitor and prevent hyperphosphatemia and its related complications in patients on chronic hemodialysis.

Methods and Materials:

Biochemical data in a total of 80 patients were collected in December 2015. The patients had been on hemodialysis for two to three times a week in the last six months or longer. The blood samples were collected before hemodialysis and then centrifuged at 3000 rpm for 5 minutes. Biochemical analyses were performed by using a Hitachi 7170 automatic biochemical analyzer and an Immulite 2000 luminescence inspection analyzer. We divided hemodialysis patients into two groups, one with phosphorus < 5.5 mg/dL and the other ≥ 5.5 mg/dL. The phosphorus values of the two groups were compared with i-PTH and alkaline phosphatase data. The p value by t-test was calculated, with $p < 0.05$ regarded as significant.

Results:

The results showed that for patients with serum phosphorus ≥ 5.5 mg / dL, their average alkaline phosphatase was 126.4 IU / L, with a mean i-PTH at 841.1 pg / mL. In contrast, the patients with serum phosphorus < 5.5 mg / dL, their alkaline phosphatase average was at 80.3 IU / L and a mean i-PTH at 107.7 pg / mL. Our data showed that the phosphorus < 5.5 mg / dL group had significantly lower alkaline phosphatase and i-PTH levels than the phosphorus ≥ 5.5 mg / dL group ($p < 0.05$). (Figure 1, Figure 2)

Discussion:

When the glomerular filtration rate was decreased to 30 ml / min or less, a condition of persistent hyperphosphatemia was present. Hyperphosphatemia is an important pathogenic mechanism of hyperparathyroidism. Many studies have noted a correlation between high blood phosphorus and increased mortality in dialysis patients. NKF-K / DOQI ™ guidelines were proposed by National Kidney Foundation. However, whether the guidelines are applicable to the population of Taiwan remained to be confirmed. Our data showed that when the phosphorus was < 5.5 mg / dL, the i-PTH value could be maintained at < 300 pg / mL, and Alkaline-P was significantly lower. It indicated that when the serum phosphorus was controlled to be lower than 5.5 mg / dL, the secondary hyperparathyroidism and renal osteodystrophy might be well prevented. Further surgical removal of the parathyroid gland will not be needed. Thus, the serum phosphorus level can be used as an index for monitoring the patient's condition as well as a guideline for prognosis.

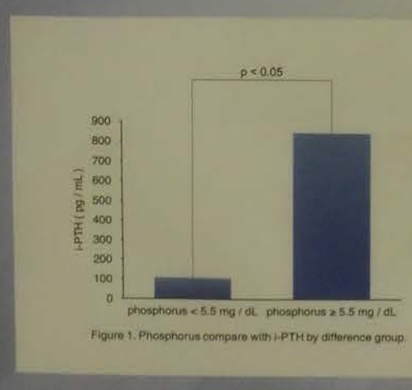


Figure 1. Phosphorus compare with i-PTH by difference group.

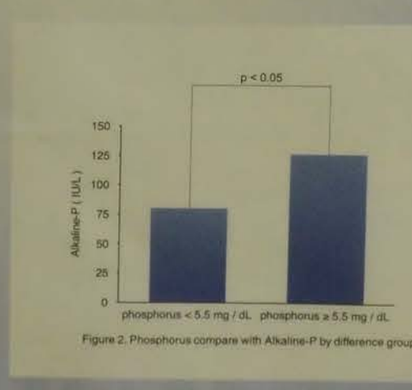


Figure 2. Phosphorus compare with Alkaline-P by difference group.

Tungs' Taichung MetroHarbor Hospital

Clinical Chemistry

PD-22

PD-22 The 32nd World Congress of Biomedical Laboratory Science 2nd of September, 2019

MMP-10 levels in serum and saliva of patients with different body-mass index

Fernando Mendes[1], Ana Freitas[1], Frederic Mota[1], Lisa Antunes[1], Carlos Tavares[2], Rui Gonçalves[2], João Figueiredo[3], Nádia Osório[1], Ana Valado[1], António Gabriel[1] and Armando Caseiro[1,4]

[1] Instituto Politécnico de Coimbra, ESTESC-Coimbra Health School, Ciências Biomédicas Laboratoriais, Portugal;
[2] Instituto Politécnico de Coimbra, ESTESC-Coimbra Health School, Fisioterapia, Portugal;
[3] Instituto Politécnico de Coimbra, ESTESC- Coimbra Health School, Ciências Complementares, Portugal;
[4] Unidade I&D Química-Física Molecular, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3000 Coimbra, Portugal.

Introduction

Obesity is a multifactorial chronic disease and its incidence has been increasing dramatically, being considered a global epidemic by World Health Organization. It's characterized by increased adipose tissue that results from hyperplasia and hypertrophy. This excess weight increases the risk of multiple conditions, such cardiovascular and cerebrovascular disease, insulin resistance, type 2 diabetes, dyslipidemia and certain types of cancer. Their development is associated with extensive modifications in adipose tissue involving adipogenesis, angiogenesis, and extracellular matrix (ECM) remodelling. The infiltration of macrophages in adipose tissue comes from adipocytes killed by hypoxia resulting from the hypertrophy of adipose tissue and blood supply to cells, while not processed the angiogenesis. Moreover, the high adipokines production induces an immune response, stimulate the adhesion and phagocytosis of macrophages, and T cell proliferation. The inflammatory condition in obesity, which occurs after infiltration of macrophages in adipose tissue, leads to an extensive remodeling of ECM by Matrix Metalloproteinases (MMPs).

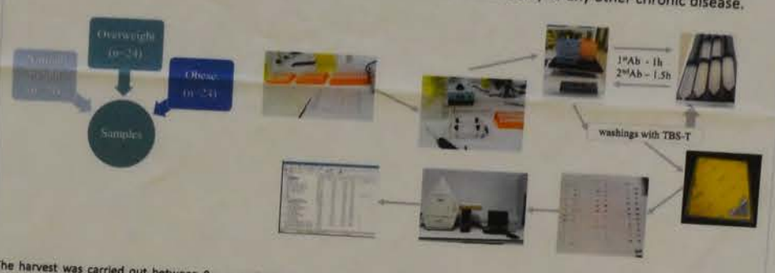
The MMPs are proteolytic enzymes that are collectively able to cleave all of the ECM components and they are responsible of tissues remodelling. Moreover, it's able to cleave several non-ECM proteins, such as cytokines, hormones and other MMPs. The MMP-10 degrades several ECM components such as proteoglycans, gelatin, elastin, fibonectin and collagen (type III, IV and V). It's activated by serine proteases such as plasmin and neutrophil elastase and its active form is able to activate other MMPs like MMP-1 and MMP-8. MMP-10 has been studied in other inflammatory diseases such as diabetes mellitus, however, studies of MMP-10 in obesity don't exist. MMPs which are involved in inflammatory process may contribute to the development of new perspectives in terms of disease monitoring. Therefore, it becomes important to prematurely understand the potential consequences and damage of the obesity, through the evaluation of biomarkers that can reflect the individual inflammatory state.

Aims

- Assess the levels of MMP-10 in the serum and saliva;
- Correlation of MMP-10 levels in individuals with different body-mass index in both fluids.

Material and Methods

The participants in this study are aged between 18 and 35 years. All participants encompassed in the study were non-smokers and had no symptoms of inflammation, diseases of the oral cavity or any other chronic disease.



The harvest was carried out between 8 a.m and 10 a.m in clinical biochemistry laboratory of Coimbra Health School (L0.1). Thereafter, we performed separation and processing of specimens of samples, which are then centrifuged and stored at -20ºC. Before performing the quantification method by slot blot, was executed one biochemical serum protein characterization and quantification of both fluids. Analysis by slot blot was performed in accordance with Caseiro *et al.* Statistical calculations were performed using the GraphPad Prism software. Values are presented as mean ± standard deviation and optical density values (OD) are expressed in arbitrary units.

Results

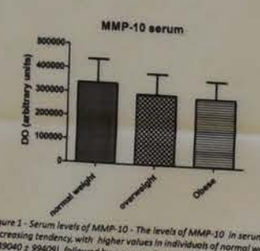


Figure 1 - Serum levels of MMP-10 - The levels of MMP-10 in serum showed a decreasing tendency, with higher values in individuals of normal weight (339040 ± 99406), followed by a group of overweight individuals (296080 ± 88090), and obese subjects had the lower values (288,301 ± 74,985).

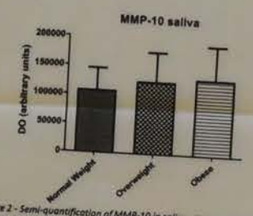


Figure 2 - Semi-quantification of MMP-10 in saliva - It was observed a growing tendency, unlike that observed in the serum, wherein the normal weight had lower MMP-10 values (108400 ± 37416), followed by the group of individuals with overweight (125182 ± 49620) and the group of obese individuals with higher values (131829 ± 56560).

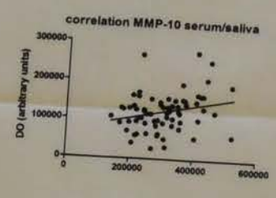


Figure 3 - Correlation of MMP-10 levels in serum and saliva - The results showed a weak positive correlation between MMP-10 levels in serum and in saliva (r = 0.2891, p ≤ 0.05).

- The individuals with the highest percentage of body fat (overweight and obese) showed higher MMP-10 serum levels. The individuals with normal weight presented higher MMP-10 saliva levels. A statistically significant correlations between the levels achieved in serum and in saliva was observed.

Conclusion

In summary, the results suggest that MMP-10 is altered in obesity and appear to be implicated in ECM remodeling. However, it would be good conduct new studies to evaluate these proteases in a population with a greater disease duration and more participants per group (normal weight, overweight and obese). Saliva showed to be a fluid with a greater ability to monitor the remodeling of ECM and with a better diagnostic potential than serum.

Acknowledgment

Labinsaúde: Co-funded by the QREN Project under the Program Mais Centro, Commission of the Central Region Coordination and the European Union through the European Regional Development Fund.

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Pathology PE-39

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Improvement of Patient Safety and Pa

Lin Shu-Wen; Li Ya-Ching; Wan

Department of Pathology and Laboratory Medicine, Taip

Taiwan, R.O.C

Introduction

Results

Discussion

Conclusion

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The 32nd World Congress of Biomedical Laboratory Science
2nd of September, 2018

MMP-7 LEVELS IN SERUM AND SALIVA OF OBESE PATIENTS

Fernando Mendes[1], Fréderic Mota[1], Ana Freitas[1], Lisa Antunes[1], Carlos Tavares[2], Rui Gonçalves[2], João Paulo Figueiredo[3], Nádia Osório[1], Ana Valado[1], António Gabriel[1], Armando Caseiro[1,4]

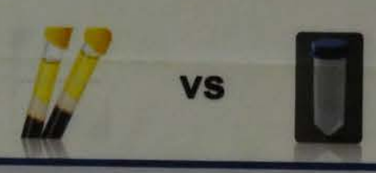
[1] Instituto Politécnico de Coimbra, ESTeSC – Coimbra Health School, Ciências Biomédicas Laboratoriais, Portugal.
[2] Instituto Politécnico de Coimbra, ESTeSC – Coimbra Health School, Departamento de Fisioterapia, Portugal.
[3] Instituto Politécnico de Coimbra, ESTeSC – Coimbra Health School, Departamento de Ciências Complementares, Portugal.
[4] Unidade I&D Química-Física Molecular, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3000 Coimbra, Portugal.

Introduction

Obesity is a metabolic disease of pandemic proportion. The World Health Organization estimates that 300 million of adults worldwide are obese and more than 1 billion are overweight. This disease is chronic, resulting in expansion of adipocytes and extracellular matrix remodelling, performed by matrix metalloproteinases (MMPs) and regulated by its tissue inhibitors (TIMPs). Studies in recent years have shown the role of white adipose tissue as a producer of certain substances with endocrine, paracrine, and autocrine action. These bioactive substances are denominated adipokines, among which are found tumor necrosis factor-alpha (TNF-α), interleukin-6, leptin, and adiponectin. Some secondary injuries are caused by chronic inflammation related to obesity, through the break of the homeostasis between MMPs and TIMPs. Recently, it was shown that MMP-7 and MMP-8 may exhibit down-regulation in obesity, and reflect an unexpected anti-inflammatory or defensive potential in human inflammatory disease.

Aims

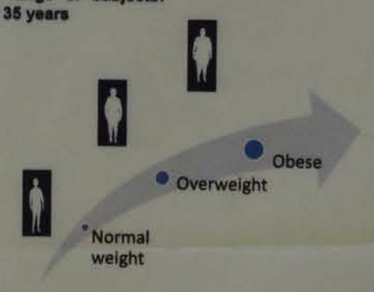
- Determine the levels of MMP-7, MMP-8 and TIMP-3 in serum and saliva samples of normal weight, overweight and obese young adults.
- Compare MMP-7 results in serum and saliva.



Methods

- The study included 72 subjects classified by body fat percentage.

Age range of subjects: 18 – 35 years



- The semi-quantification of MMP-7, MMP-8 and TIMP-3 were performed by slot blot.

Results

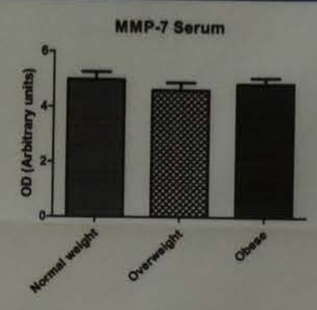


Figure 1:
Normal weight's levels slightly higher, lower in overweight's levels and slightly higher in obese individuals.

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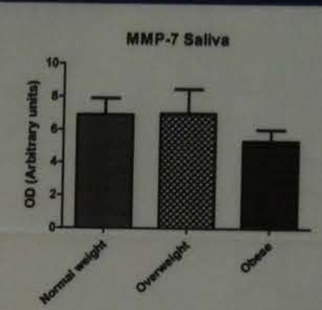

Figure 2:
Decreasing tendency of MMP-7 levels in individuals with higher % of fat mass.

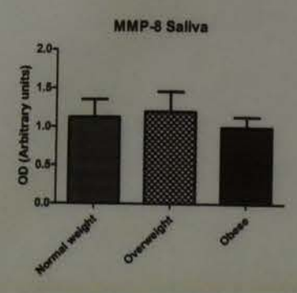

Figure 3:
Decreased MMP-8 levels in individuals with higher % of fat mass.

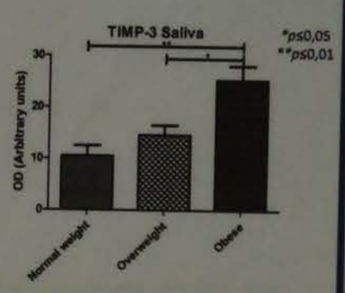

Figure 4:
Increased TIMP-3 levels in subjects with with higher % of fat mass.

- The ratio of MMP-7/TIMP-3 were superior in the group of normal weight, comparing with the obese adults. On the other hand, it occured a decreasing tendency of MMP-8/TIMP-3 ratio in obese subjects.

Discussion and Conclusion

The tendentious higher levels of MMP-7 and MMP-8 in normal weight adults compared with obese adults, in saliva, suggest that these MMPs could play a protective role, being obesity associated with specific changes in the MMP/TIMP balance. The higher levels of TIMP-3 in obese adults suggest that it plays an important role in the pathophysiology of obesity. Salivary results show higher sensibility in relation to serum and allows us to propose saliva sample as an alternative to serum with great potential in early diagnosis.

Acknowledges

Labinsaúde: Co-funded by the QREN Project under the Program Mais Centro, Commission of the Central Region Coordination and the European Union through the European Regional Development Fund.

Clinical Chemistry

PD-24

Analysis for Stat Biochemistry Laboratory Specimen Rejection Rate Improvement of Patient Safety and Patient Care Quality

Lin Shu-Wen; Li Ya-Ching; Wang Fang-Yu

Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taiwan, R.O.C

Introduction

Physicians rely on accurate laboratory test results for proper disease diagnosis and for guiding therapy. During laboratory practice, error rates for pre-analytic performance measures were about 46 to 68.2%. Specimen rejection rate is one of the important pre-analytic quality indicators for laboratory quality and patient safety. When the quality of a blood specimen is poor, it cannot be processed by the laboratory. This leads to a second request for blood specimen and therefore to an increased turnaround time for the laboratory, which is positively correlated with the delay in diagnosis. The CAP Q-Probes and Q-Tracks studies have reported aggregated specimen rejection rates ranging from 0.30% to 0.83%. In our Laboratory Medicine department, the overall specimen rejection rate was 0.39% in 2015. The rejection rates between different kinds of specimens were different, for example, the rejection rates between the STAT and non-STAT biochemistry specimens were significant different. And, the rejection rate of emergency department was usually higher than that of inpatient services and outpatient services. In this study, we intended to analyze the causes of rejection for STAT biochemistry specimens. The analytic items included specimen collection sites and laboratory personnel rejecting the samples.

Materials & Methods

The specimen rejection rate was calculated based on the number of specimen rejection and the total number of specimen that we intended to evaluate from our laboratory information system (LIS). In 2015, the non-STAT biochemistry had rejected 2,288 specimens and the total specimens during this period were 1,515,203. The STAT biochemistry had rejected 4,227 specimens and the total specimen number was 398,241. Items such as clinical personnel who collected the sample, laboratory personnel rejecting the sample, and the reason(s) for rejection of all the rejected specimens were retrieved from LIS and analyzed.

Results

The rejection rate of STAT biochemistry, 1.06% (0.81%~1.35%), was 7 times higher than that of non-STAT biochemistry, 0.15% (0.12%~ 0.20%). (Figure 1)

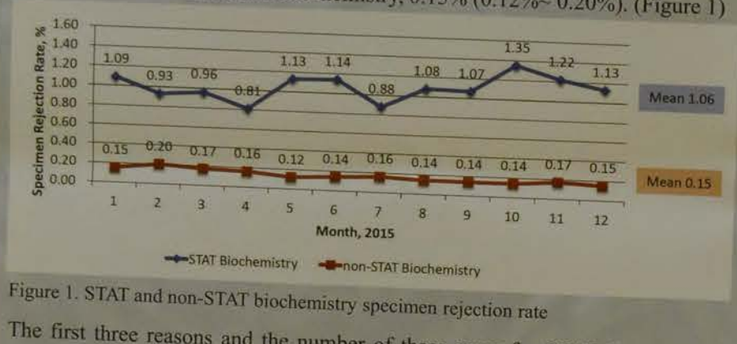

Figure 1. STAT and non-STAT biochemistry specimen rejection rate

The first three reasons and the number of these cases for STAT biochemistry rejection were severe hemolysis (n=3,136, 74.19%), insufficient specimen (n=541, 12.80%), and specimen contamination (n=157, 3.71%). The reasons for the rest of the cases (n=393, 9.3%) were recheck inquired by physician, no sample, unable add-on request, improper container, unlabelled specimen, and clotting of the specimen. Of note, severe hemolysis was the most common cause of rejection, which was 5.8 times more commonly encountered than the second reason of rejection, insufficient specimen. (Table 1)

Table 1. Proportions and Reasons for Rejection of STAT Biochemistry Specimens

Rejection reason	Rejection No.	Percentage
Severe hemolysis	3,136	**74.19 %**
Insufficient specimen	541	**12.80 %**
Specimen contamination	157	**3.71 %**
Recheck inquired by physician	128	3.03 %
No sample	99	2.34 %
Unable add-on request	97	2.29 %
Improper container	48	1.14 %
Unlabelled specimen	14	0.33 %
Specimen clotted	7	0.17 %
Total	**4,227**	**100.00 %**

Table 1. Proportions and reasons for rejection of STAT biochemistry specimens

Figure 2 shows the reasons and numbers of specimens that were rejected by the 5 main laboratory staffs (A~E) working in the STAT biochemistry laboratory, who were responsible for handling 80% of specimens. The rest of the specimens were handed by staffs rotating from the other work stations and these specimens were not analyzed. The number one cause of rejection was severe hemolysis, and the highest number of rejection was 552 by staff D, which was 120 more than the last, 430, by staff E.

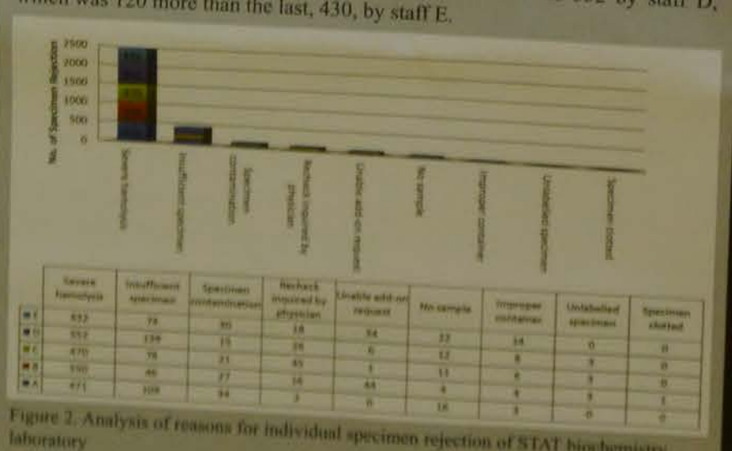

Figure 2. Analysis of reasons for individual specimen rejection of STAT biochemistry laboratory

To realize the difference between laboratory personnel rejecting the samples, we compare the specimen rejection rate and hemolyzed specimen rejection rate between these 5 staffs. Both the highest rates of rejected specimens and rejected hemolyzed specimens were 1.38% and 1.00% by staff D, followed by staff B (1.13%, 0.90%), and the last by staff C (0.91%, 0.68%). (Figure 3)

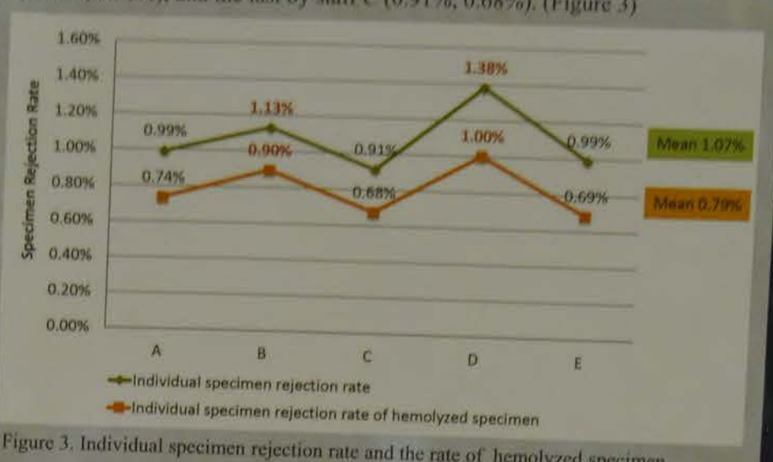

Figure 3. Individual specimen rejection rate and the rate of hemolyzed specimen

Figure 4 shows the number of rejected hemolyzed specimens from different specimen collection sites in October 2015. The total number of rejected hemolyzed specimens were 330. The collection sites with more than 10 rejected specimens were ED, ICUA, ICUB, NCUA, CCU, A191, and RCUB. In these 7 units, only A191 is a general ward, the others were emergent or critical units. The number (%) of rejected specimens from the first 3 collection sites (ED-NS, ICUA, ICUB) were 55 (16.7%), 23 (7.0%) and 22 (6.7%), respectively. Except for ED-MT, all the samples were drawn by nursing staffs. Samples ED-MT were blood from the ED drawn by medical technologists, which consisted of 55% samples from the ED. Of these specimens, only 7 samples were rejected because of severe hemolysis.

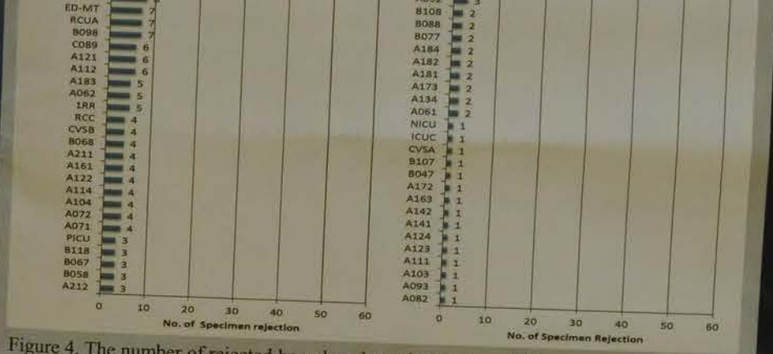

Figure 4. The number of rejected hemolyzed specimens from different specimen collection sites in October 2015

Discussion

This study showed that the rejection rate of STAT biochemistry was 7 times higher than that of non-STAT biochemistry. Therefore, reduce specimen rejection rate of STAT biochemistry is to improve the overall rejection rate. Up to 74.19% rejections of STAT biochemistry were because of severe hemolysis, and the collection sites of these samples were mainly in ED and critical care units. In ED, the number of rejections between nursing staffs (ED-NS) and medical technologists (ED-MT) were significant different, which were 55 and 7, respectively. That is because nursing staffs in ED were mainly responsible for the patients belongs to triage level I (Resuscitation) and leve II (Emergent). These kind of patients were in critical and emergent health conditions and difficult to draw the blood. The patients in ICU were also have the same characteristics. In this situation, nurses is easily to ignore the details for proper blood collection. We could communicate with the first 3 high rejection units to reduce rejection rate by continuous training. Besides, we found the rates of rejected hemolyzed specimen by Staff D and staff B were higher than other 3 staffs. We could through review the rejections for these 2 staffs to realize the criteria for hemolyzed specimen rejection. Keep the consistency for rejected hemolyzed specimens between ours laboratory staffs is to help us reduce unnecessary rejections.

Conclusion

In this study, we found severe hemolysis was the most common cause of rejections. We can reduce the rate of rejected hemolyzed specimens in two ways. One is to improve the quality of specimens, especially from the beginning of specimen collection, it can effectively reduce the rate of specimen rejection. The other is to improve the consistent judgment for rejected hemolyzed specimens between ours laboratory staffs to avoid too strict judgment for rejections. Therefore, that could not only provide clinicians some useful lab results for diagnosis or treatment in a critical situation first, but also reduce the delayed TAT from recollecting samples.

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Clinical Chemistry

PD-25

MacKay Memorial Hospital

Quantitative analysis of free/total carnitine in plasma by Liquid Chromatography/tandem mass spectrometry assay -A method detect carnitine deficiency

Sung Fa Huang[a*], Tuen Jen Wang [a], Chih Kuang Chuang[b], Tzu Lin Chen[a], Chi Kuan Chen[a,c]

[a]Dept. of Laboratory Medicine, [b]Division of Genetics and Metabolism, Dept of Medical Research, and [c]Dept of Pathology , Mackay Memorial Hospital, Taipei, Taiwan.

Background :

Carnitine is a key substance for transportation of long-chain acyl-CoA esters across mitochondria membrane for subsequent fatty acid oxidation. Carnitine can be obtained from exogenous meat supply and endogenous synthesis in liver. Defects of carnitine may eventually result in carnitine deficiency. The conventional method for quantitative analysis of plasma free/total carnitine is carnitine acetyltransferase (CAT) spectrophotometric assay. However, the CAT method is highly affected by hemolysis and any compound in physiological fluids containing SH functional group. In addition, a larger volume of blood sample is required which makes the measurement more unfeasible. In this study, we intended to establish a liquid chromatography/tandem massspectrometry method for clinical diagnostic purpose.

Methods:

By using multiple reaction mornitoring (MRM) of tandem mass analysis, the mass to charge (m/z) of the precursor and the product ions of carnitine was 162.2/85.0. The internal standard, a stable radio-isotope D3-carnitine, was used for the calibration and basis of quantitative measurement. The m/z of D3-carnitine was 165.2/103.0.

Results:

According to the preliminary results, the within-run and between-run precisions of the method were 4.1% and 5.5% , respectively.The recovery of this tandem mass assay was 94.5% and the subsequent regression analysis showed good correction with the CAT method (r2=0.957).The reference values of free and total carnitine in normal control were were 42.3 and 52.4 μ mol/L, respectively, with a mean standard deviation of 42.3 ± 8.01(Free carnitine, n=130);52.4 ± 8.80(Total carnitine, n=130), and the samples showed a normal distribution.

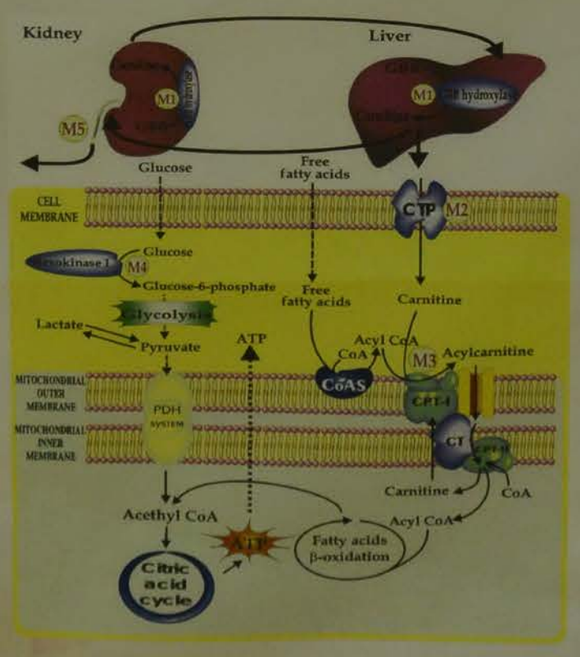


Figure1 Pathway of carnitine production in the liver and its role in the mitochondria (From Flaws of a Vegan Diet –The Most Important Article You Will Ever Read About veganism.)

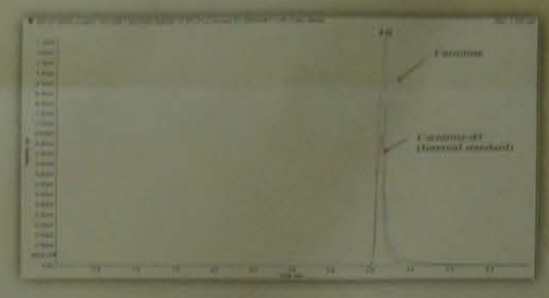


Figure 2 MRM chromatography of carnitine and its internal standard (carnitine-D3)

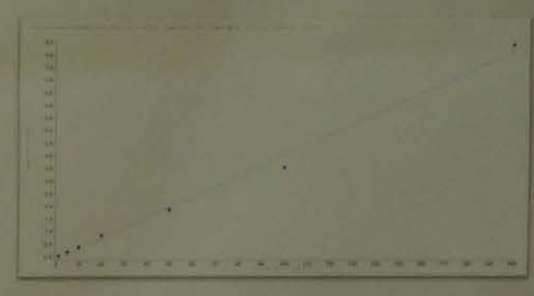
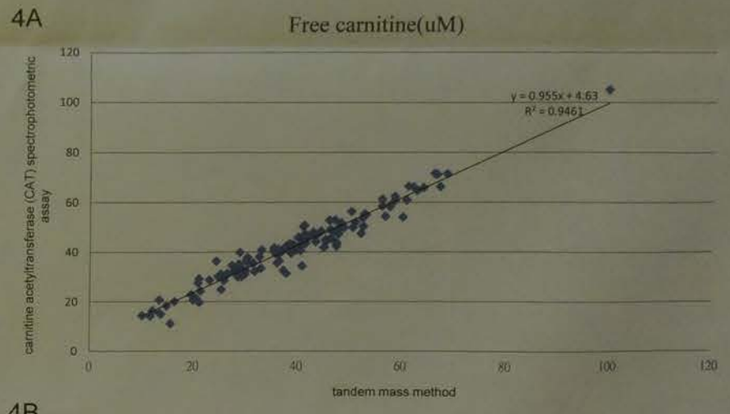


Figure 3 Calibration of the carnitine.
Linear regression y=0.041x-0.0941 , R^2=0.993

4A



4B

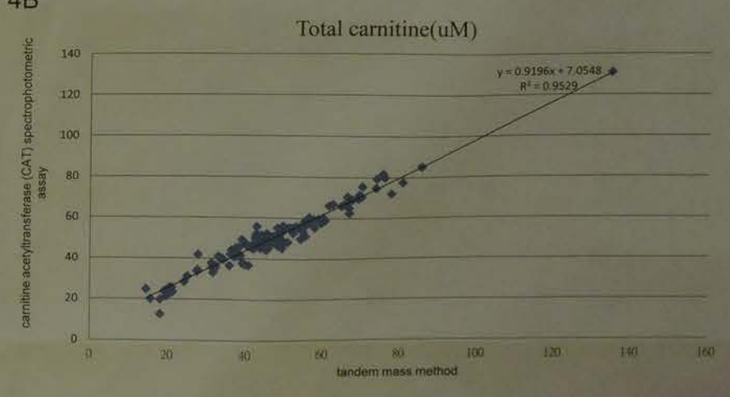


Figure 4A Correlation between two methods of free carnitine
4B Correlation between two methods of total carnitine

Conclusions:

The developed LC-MS/MS method for plasma free/total carnitine determination is specific, sensitive, validated, and accurate. The method is applicable and a great benefit particularly to the patients from pediatric department when a small volume of plasma sample is avaiable. This method is feasible and can be applied for clinical diagnostic services.

Clinical Chemistry

PD-26

PD-26

Globulin levels and hypertension are related with brachial-ankle pulse wave velocity in type 2 diabetic patients with dabetic autonomic neuropathy

Wang Yi-Mei
Department of Neurology, National Taiwan University Hospital, Yun-Lin Branch, Taiwan

Background

Dabetic autonomic neuropathy (DAN) is a common complication of type 2 diabetes mellitus (T2DM), and represents a significant cause of cardiovascular morbidity and mortality in diabetic patients.

Objective

Brachial-ankle pulse wave velocity (baPWV) is known to be a good surrogate marker of vascular damages. The goal of this study was to investigate the relationship between baPWV in T2DM with DAN.

Methods

This was a cross-sectional study. A total 93 T2DM patients (42 men and 51 women, age 46-76 years) without history of cardiovascular disease (CVD) who visited our hospital from March 2011 to April 2014 were assessed. After accurate clinical examinations and biochemical evaluations, the enrolled subjects underwent automatic waveform analyzer to assess baPWV. DAN was assessed by the electrocardiograms to assess coefficient of variation in the R-R intervals (CVR-R), the CVR-R related indexes, including CVR-R at rest ($CVR\text{-}R_{rest}$), CVR-R with deep breaths ($CVR\text{-}R_{breath}$) and their difference (CVR-R breath minus CVR-R rest: $CVR\text{-}R_{dif}$ (reference range: >2%)), were defined.

Results

Table 1. Baseline characteristics of T2DM subjects

Characteristics	T2DM			DAN vs. no DAN
	Total (n=93)	DAN (n=15)	No DAN (n=78)	*p* value
Age (years)	61.5 ± 7.8	62.0 ± 11.0	61.4 ± 7.1	0.78
Males, n (%)	42 (45.0)	5 (33.0)	37 (47.0)	0.32
BMI (kg/m²)	26.5 ± 4.0	27.1 ± 3.1	26.3 ± 4.1	0.53
Waist (cm)	91.4 ± 9.9	94.0 ± 7.0	91.0 ± 10.3	0.27
Alcohol, current/past, n (%)	27 (29.3)	3 (20.0)	24 (31.1)	0.39
Smoker, current/past, n (%)	26 (28.0)	3 (20.0)	23 (29.5)	0.46
Regular exercise, n (%)	54 (58.1)	8 (53.3)	46 (59.0)	0.69
DM duration (years)	11.7 ± 6.9	13.3 ± 7.4	11.4 ± 6.8	0.33
Autonomic neuropathy, n (%)	*15 (16.1)*	*15 (100)*	*0 (0)*	
$CVR\text{-}R_{rest}$	*2.3 ±0.9*	*1.0 ±0.3*	*2.5 ±0.8*	*<0.001*
$CVR\text{-}R_{breath}$	*3.7 ±1.8*	*1.4 ±0.5*	*4.1 ±1.6*	*<0.001*
$CVR\text{-}R_{dif}$	*1.4 ±1.3*	*0.4 ±0.3*	*1.6 ±1.3*	*<0.001*
Anti-hypoglycemic drugs, n (%)	88 (94.6)	14 (93.3)	74 (94.9)	0.81
Anti-platelet treatment, n (%)	30 (32.0)	7 (47.0)	23 (29.0)	0.20
Use of anti-hypertensive drugs, n (%)	65 (69.9)	11 (73.3)	54 (69.2)	0.75
Use of statins, n (%)	48 (51.6)	9 (60.0)	39 (50.0)	0.48
ba PWV (m/s)	*15.5 ±2.0*	*16.6 ±2.6*	*15.2 ±1.8*	*0.01*
Systolic blood pressure (SBP) (mmHg)	133 ± 15	148 ± 18	130 ± 13	*<0.001*
Diastolic BP (mmHg)	81 ± 9	88 ± 11	79 ± 8	*<0.001*
Pulse pressure (PP) (mmHg)	54 ± 10	62 ± 11	53 ± 10	*<0.001*
Mean arterial pressure (MAP) (mmHg)	101 ± 12	112 ± 15	99 ± 10	*<0.001*
White blood cell count (10^3/μl)	6.14 ± 1.53	6.15 ± 1.87	6.14 ± 1.48	0.98
Red blood cell count (10^6/μl)	4.54 ± 0.53	4.23 ± 0.62	4.6 ± 0.49	*0.01*
Hemoglobin (g/dl)	13.4 ± 1.5	12.7 ± 1.8	13.6 ± 1.4	*0.04*
Hematocrit (%)	40.1 ± 4.0	37.9 ± 5.1	40.6 ± 3.7	*0.02*
Platelet (10^3/μl)	235 ± 65	253 ± 60	232 ± 66	0.26
HbA1c (%)	7.6 ± 1.5	8.5 ± 2.5	7.4 ± 1.2	*<0.01*
Fasting glucose (mg/dl)	149 ± 49	169 ± 88	145 ± 37	*0.09*
Creatinine (mg/dl)	0.97 ± 0.36	1.1 ± 0.51	0.94 ± 0.33	0.13
Uric acid (mg/dl)	6.1 ± 1.4	5.9 ± 1.3	6.1 ± 1.5	0.62
AST (U/L)	26 ± 14	20 ± 4	27 ± 15	0.12
ALT (U/L)	30 ± 22	23 ± 10	31 ± 23	0.17
TG (mg/dl)	131 ± 60	154 ± 64	127 ± 59	0.11
T-CHO (mg/dl)	160 ± 34	167 ± 29	159 ± 35	0.39
HDL-C (mg/dl)	42 ± 11	41 ± 10	42 ± 11	0.80
LDL-C (mg/dl)	91 ± 26	99 ± 26	89 ± 26	0.16
Albumin (g/dl)	4.62 ± 0.34	4.47 ± 0.33	4.65 ± 0.34	*0.06*
Globulin (g/dl)	2.74 ± 0.38	3.04 ± 0.32	2.69 ± 0.37	*<0.001*
hs-CRP (mg/dl)	0.22 ± 0.26	0.33 ± 0.28	0.20 ± 0.25	*0.09*
Log hs-CRP	-0.88 ± 0.46	-0.62 ± 0.36	-0.93 ± 0.46	*0.02*
D-Dimer (mg/L)	0.42 ± 0.45	0.84 ± 0.83	0.33 ± 0.28	*<0.001*
Fibrinogen (mg/dl)	300 ± 54	342 ± 56	292 ± 50	*<0.001*
Urine microalbumin (mg/dl)	137 ± 459	426± 965	82± 251	*<0.01*
Urine creatinine (mg/dl)	125± 75	101± 80	130± 74	0.18
Urine albumin/creatinine ratio (UACR)	106± 264	289± 399	70± 216	*<0.005*
Log Urine albumin/creatinine ratio	1.14± 0.80	1.96± 0.77	0.99± 0.72	*<0.001*

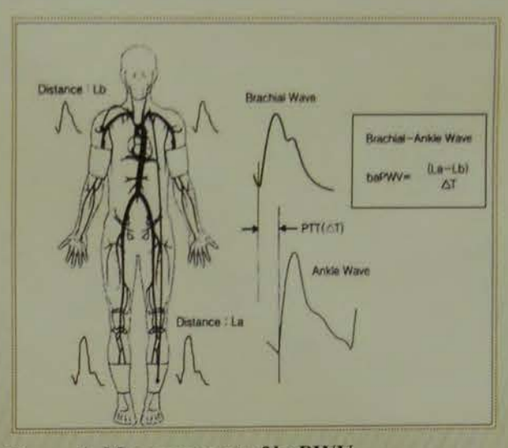


Figure 1. Measurement of baPWV.

Source from: http://www.monews.co.kr/news/articleView.html?idxno=56405

Table 2. Multiple linear regression analysis of the determinants of baPWV

Parameter	T2DM with DAN		
	β	SE	*p* value
Age (year)	0.122	0.044	*0.07*
Sex (male vs. female)	7.73	2.834	*0.07*
MAP (per mmHg)	0.151	0.038	*0.03*
Hematocrit (1% increase)	-0.523	0.184	*0.07*
HbA1c (1% increase)	-0.405	0.374	0.36
Albumin (per g/dl)	2.507	1.754	0.25
Globulin (per g/dl)	9.195	2.687	*0.04*
D-Dimer (per mg/l)	9.194	0.730	0.30
Fibrinogen (per mg/dl)	-0.059	0.024	*0.09*
log hs-CRP	3.103	1.757	0.18
log UACR	-1.152	0.902	0.29

Conclusion

Independently of other cardiovascular risk factors, serum globulin levels and hypertension are associated with baPWV even in T2DM patients with DAN, factors known to increase the risk of CVD.

Reference

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Bagherzadeh A, Nejati-Afkham A, Tajallizade-Khoob Y, Shafiee A, Sharifi F, Esfahani MA, et al. Association of cardiac autonomic neuropathy with arterial stiffness in type 2 diabetes mellitus patients. *J Diabetes Metab Disord.* 2013 Dec 20;12(1):55. doi: 10.1186/2251-6581-12-55.

Clinical Chemistry
PD-27

Effects of potassium in the platelets on serum levels of potassium

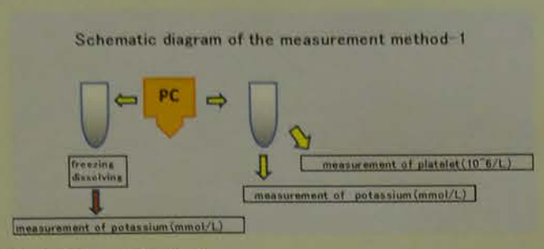
Change value of serum potassium in the case of adding the frozen platelets in whole blood

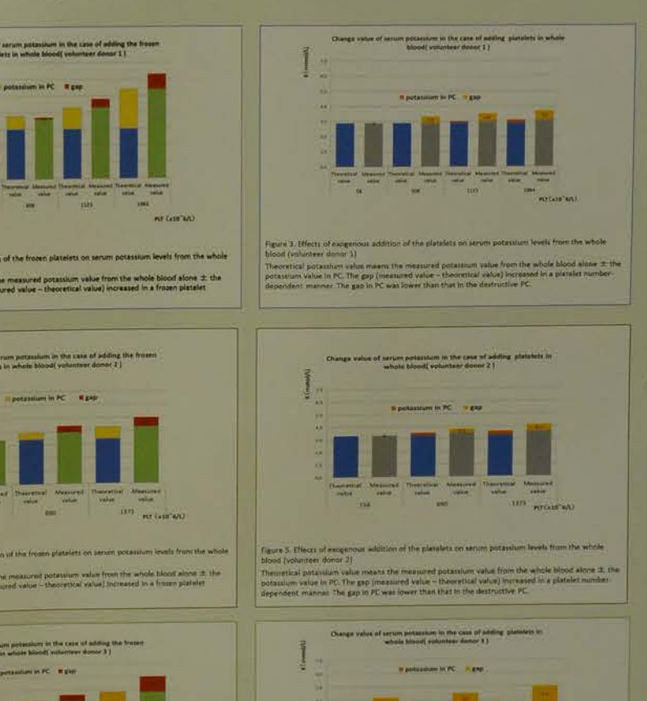
Mutsuko Hosoya Hiroshi Fujita Yoko Shiotani Yuko Takada Tomoko Igarashi
Shizuka Hasegawa Ayane Koto Yoshio Sato

Tokyo Metropolitan Bokuto Hospital

Introduction

Pseudo-hyperkalemia is known to occur in patients with essential thrombocytosis. Increased potassium release from platelets during *in vitro* coagulation in venipuncture tubes results in high serum potassium levels.
We have recently reported the occurrence of pseudo-hyperkalemia in Japanese polycythemia vera (PV) patients.
In this study, we analyzed the role of erythrocytes in pseudo-hyperkalemia, and examined the effects of damaged platelet-released potassium on serum potassium levels. Damaged platelets are considered equivalent to platelets activated during *in vitro* blood coagulation.





Materials and Methods

Platelet concentrates (PCs, n = 8) were obtained from Japanese Red Cross Society, and stored at our hospital for a maximum of 4 days (undamaged platelets). Plasma potassium levels in the PC samples were measured by EA07U Electrolyte Analyzer (A&T Co. Ltd.) . The PCs were then frozen for 8 hours at -20° C to yield damaged platelets (Fig.1), and their plasma potassium levels were measured. Whole blood was obtained from normal volunteers after they provided informed consents.
In one set of experiments, damaged platelets were exogenously added to whole blood at varying doses (platelet numbers determined by Coulter ACT), and serum potassium levels were measured for these whole blood samples (Fig. 2, 4, and 6). Addition of a similar volume of saline to the whole blood served as control. In another set of experiments, various doses of undamaged platelets were added to the whole blood, and serum potassium levels were measured (Fig. 3, 5, and 7).
To examine the effect of coagulation factors present in the plasma, the red blood cells were washed to remove the plasma, and similar numbers of damaged or undamaged platelets were added to them (Fig. 8 and 10).

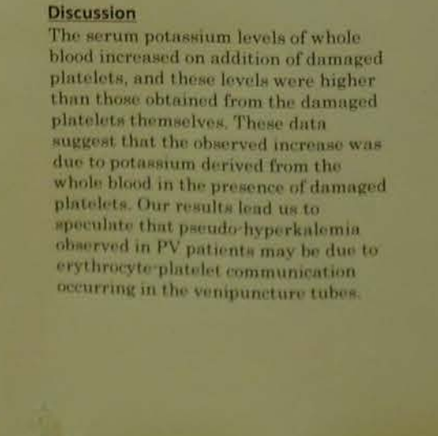


Figure 1. Plasma levels of potassium in platelets concentrates from eight donors (A~H).
The gap represented the difference between the potassium from the destructive PC and the potassium from PC. The platelet number containing each bag of PC was shown here (248~907 million/L). The potassium levels from the destructive PC, PC or gap were not platelet number-dependent.

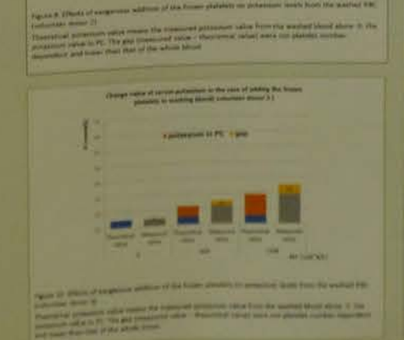


Figure 2. Effects of exogenous addition of the frozen platelets on serum potassium levels from the whole blood (volunteer donor 1)
Theoretical potassium value means the measured potassium value from the whole blood alone ± the potassium value in PC. The gap (measured value – theoretical value) increased in a frozen platelet number-dependent manner.

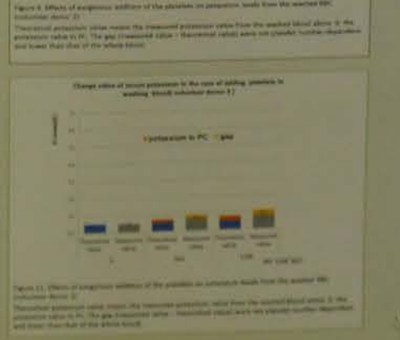


Figure 3. Effects of exogenous addition of the platelets on serum potassium levels from the whole blood (volunteer donor 1)
Theoretical potassium value means the measured potassium value from the whole blood alone ± the potassium value in PC. The gap (measured value – theoretical value) increased in a platelet number-dependent manner. The gap in PC was lower than that in the destructive PC.

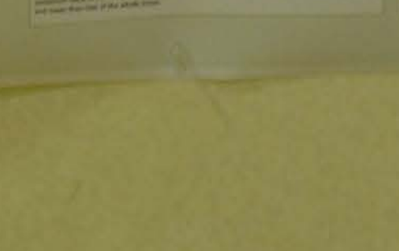


Figure 4. Effects of exogenous addition of the frozen platelets on serum potassium levels from the whole blood (volunteer donor 2)
Theoretical potassium value means the measured potassium value from the whole blood alone ± the potassium value in PC. The gap (measured value – theoretical value) increased in a frozen platelet number-dependent manner.

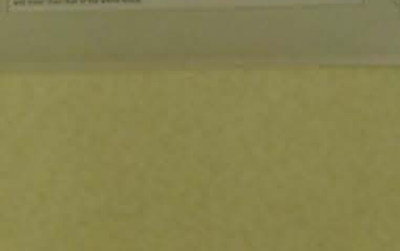


Figure 5. Effects of exogenous addition of the platelets on serum potassium levels from the whole blood (volunteer donor 2)
Theoretical potassium value means the measured potassium value from the whole blood alone ± the potassium value in PC. The gap (measured value – theoretical value) increased in a platelet number-dependent manner. The gap in PC was lower than that in the destructive PC.

Figure 6. Effects of exogenous addition of the frozen platelets on serum potassium levels from the whole blood (volunteer donor 3)
Theoretical potassium value means the measured potassium value from the whole blood alone ± the potassium value in PC. The gap (measured value – theoretical value) increased in a frozen platelet number-dependent manner.

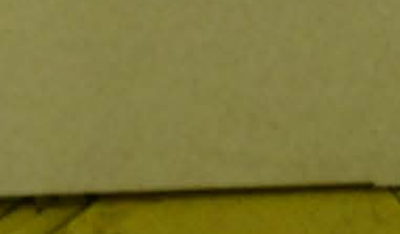


Figure 7. Effects of exogenous addition of the platelets on serum potassium levels from the whole blood (volunteer donor 3)
Theoretical potassium value means the measured potassium value from the whole blood alone ± the potassium value in PC. The gap (measured value – theoretical value) increased in a platelet number-dependent manner. The gap in PC was lower than that in the destructive PC.

Figure 8. Effects of exogenous addition of the frozen platelets on potassium levels from the washed RBC (volunteer donor 3)
Theoretical potassium value means the measured potassium value from the washed blood alone ± the potassium value in PC. The gap (measured value – theoretical value) were not platelet number-dependent and lower than that of the whole blood.

Figure 9. Effects of exogenous addition of the platelets on potassium levels from the washed RBC (volunteer donor 3)
Theoretical potassium value means the measured potassium value from the washed blood alone ± the potassium value in PC. The gap (measured value – theoretical value) were not platelet number-dependent and lower than that of the whole blood.

Figure 10. Effects of exogenous addition of the frozen platelets on potassium levels from the washed RBC (volunteer donor 4)
Theoretical potassium value means the measured potassium value from the washed blood alone ± the potassium value in PC. The gap (measured value – theoretical value) were not platelet number-dependent and lower than that of the whole blood.

Figure 11. Effects of exogenous addition of the platelets on potassium levels from the washed RBC (volunteer donor 4)
Theoretical potassium value means the measured potassium value from the washed blood alone ± the potassium value in PC. The gap (measured value – theoretical value) were not platelet number-dependent and lower than that of the whole blood.

Results

The plasma potassium levels was higher in platelets following freezing-induced damage (damaged platelets: 0.3–0.8 mmol/L). The addition of damaged platelets to the whole blood increased serum potassium level in a dose dependent manner (experimental group with damaged platelets: 3.0–6.3 mmol/L, control group with saline : 0–2.4 mmol/L). Theoretical potassium value is the potassium level measured in whole blood (or washed red blood cells without plasma) alone ± the potassium level in the PC. The difference between the theoretical values and the actual measured values are shown in Fig. 2-11. This difference in the potassium level was higher when damaged platelets were added to the blood, as compared to undamaged platelets (Fig. 2-7). Further, the difference between the theoretical and the actual measured potassium levels were lower in experiments using washed red blood cells (no plasma), than in the ones using whole blood . (Fig. 8-11).

Discussion

The serum potassium levels of whole blood increased on addition of damaged platelets, and these levels were higher than those obtained from the damaged platelets themselves. These data suggest that the observed increase was due to potassium derived from the whole blood in the presence of damaged platelets. Our results lead us to speculate that pseudo-hyperkalemia observed in PV patients may be due to erythrocyte-platelet communication occurring in the venipuncture tubes.

Clinical Chemistry
PD-28

Renal function is the most important factor for the high-sensitivity cardiac troponin measurements

Yoshio Sato[1], Tomoyasu Kitaoka[2], Yuko Takada[1], Kazumasa Watanabe[1], Kazuyuki Fujita[1], Hiroshi Fujita[1], Yoko Shiotani[1], Mutsuko Hosoya[1], Tomoko Igarashi[1], Shizuka Hasegawa[1], Ayane Koto[1]
[1]Department of Medical Laboratory and Blood Transfusion, Tokyo Metropolitan Bokuto Hospital, [2]Department of Laboratory Medicine, Graduate School of Medicine, Juntendo University

Purpose

High-sensitivity cardiac troponin is useful for diagnosing ACS

Do CKD or other factors affects the values of hs-cTnI & T ?

Kidney Dysfunction, Hypertension, Diabetes Mellitus, Heart Disease, Urinary protein, Eth, Other factors

Introduction

High-sensitivity cTnI (hs-cTnI) and cTnT (hs-cTnT) are useful for diagnosing acute myocardial infarction (AMI) within 3 hours from onset. Cardiac troponin, cTnT in particular, is elevated in patients with renal dysfunction. To determine the effect of renal dysfunction on hs-cTnI and hs-cTnT assay, 194 patients with chronic kidney disease (CKD) were classified into 6 stages based on eGFR was calculated. Their G1, G2, G3a, G3b, G4, G5 and effects of CKD on hs-cTnI and hs-cTnT were evaluated.
In addition, because cardiac troponin values are affected by other factors such as cardiac diseases, diabetes mellitus, heart diseases and so on, we analyzed the factors affecting the values of hs-cTnI and hs-cTnT using multiple regression analysis.

Results High-sensitivity cardiac troponin values are affected by renal function.

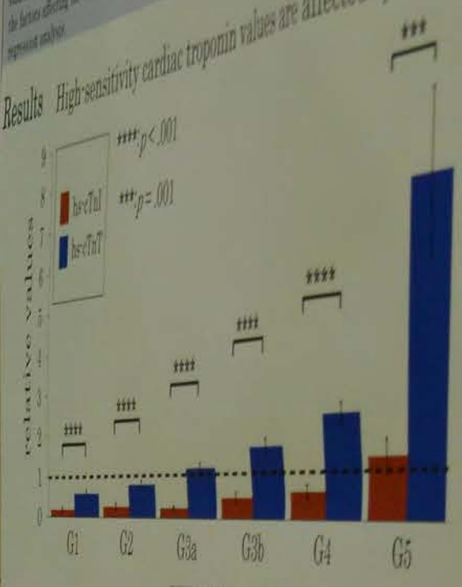


Figure 1. Comparison of relative values of hs-cTnI and hs-cTnT at each CKD stage.
Relative values of hs-cTnT were higher than hs-cTnI in all CKD stages.
Dot line means cut off value.

Table 1. Multiple regression analysis of hs-cTnI and hs-cTnT in Untransformed model and Log-transformed model.

Significant in Untransformed model
hs-cTnI: NT-proBNP
hs-cTnT: eGFR, NT-proBNP

Untransformed model	hs-cTnI		hs-cTnT	
Explanatory variable	β	r	β	r
Gender	.175**	.192**	.192**	.183**
Age	.355**	.309**	.070	-.067
eGFR$_{MDRD}$	-.196**	-.078	-.233**	-.295**
Diabetes Mellitus	.048	.093	.051	.055
Heart Disease	.045	.070	-.091	-.096
Urinary protein	.046	.095	.041	.043
Eth concentration	.165**	.188**	.123**	.107**
LnNT-proBNP	.322**	.317**	.385**	.381**

β: standardized partial regression coefficient, r: partial correlation coefficient

Significant in Log-transformed model
hs-cTnI: Gender (Man), Age, DM, NT-proBNP
hs-cTnT: Gender (Man), Age, eGFR, NT-proBNP

Log-transformed model	Ln(hs-cTnI)		Ln(hs-cTnT)	
Explanatory variable	β	r	β	r
Gender	.185**	.246**	.093*	.142*
Age	.273**	.328**	.113*	.158*
eGFR$_{MDRD}$	-.096	-.110	-.294**	-.354**
Diabetes Mellitus	.101*	.142*	.058	.091
Heart Disease	.053	.072	-.063	-.095
Urinary protein	.042	.057	.058	.069
Eth concentration	.078	.087	-.095	-.118
LnNT-proBNP	.493**	.477**	.481**	.504**

**: p< .01, *: p< .05

Conclusion

This study convincingly showed that
(1) high-sensitivity cardiac troponin values are affected by renal function.
(2) hs-cTnI is less sensitive than hs-cTnT to renal function.

Materials and methods

Comparison of relative values of hs-cTnI and hs-cTnT at each CKD stage using t test
Relative values of hs-cTnT over cut-off value of hs-cTnI (CKD stage 1-5 and overall)

Multiple regression analysis of Untransformed model and Log-transformed model
Explanatory variables (Gender (man=1, woman=0), Age, eGFR$_{MDRD}$, Diabetes Mellitus (yes=1, no=0), Heart Disease (yes=1, no=0), Urinary protein (yes=1, no=0), Eth concentration, NT-proBNP, LnNT-proBNP)
In the Untransformed model, all variables were adjusted to natural logarithm.

Clinical Chemistry

PD-28

Renal function is the major influencing factor for the high-sensitivity cardiac troponin measurements

○Atsushi Ito[1,2], Noriyasu Niizeki[1], Yutaka Tomoda[1], Kazumi Akasaka[1,2], Satoshi Fujii[1,2]

[1]Department of Medical Laboratory and Blood Center, Asahikawa Medical University Hospital
[2]Department of Laboratory Medicine, Asahikawa Medical University Graduate School of Medicine

Introduction

High sensitive cTnI (hs-cTnI) and cTnT (hs-cTnT) are useful for diagnosing acute myocardial infarction (AMI) within 2 hours from onset. Cardiac troponin, cTnT in particular, is elevated in patients with renal dysfunction. To determine the effect of renal dysfunction on hs-cTnI and hs-cTnT assay, 244 patients with chronic kidney disease (CKD) were classified into 6 stages based on $eGFR_{creat}$ (mL/min/1.73m^2, G1: 42, G2: 45, G3a: 48, G3b: 40, G4: 35, G5: 34) and effects of CKD on hs-cTnI and hs-cTnT were evaluated.
In addition, because cardiac troponin values are affected by other factors such as anemia, diabetes mellitus, heart diseases and so on, we analyzed the factors affecting the values of hs-cTnI and hs-cTnT using multiple regression analysis.

Purpose

High-sensitive cardiac troponin is useful for diagnosing AMI

↓

Do CKD or other factors affects the values of hs-cTnI/T ?

Kidney Dysfunction, Diabetes Mellitus, Heart Disease, Hb↓, age, man etc…

Other factors

Results

High-sensitivity cardiac troponin values are affected by renal function.

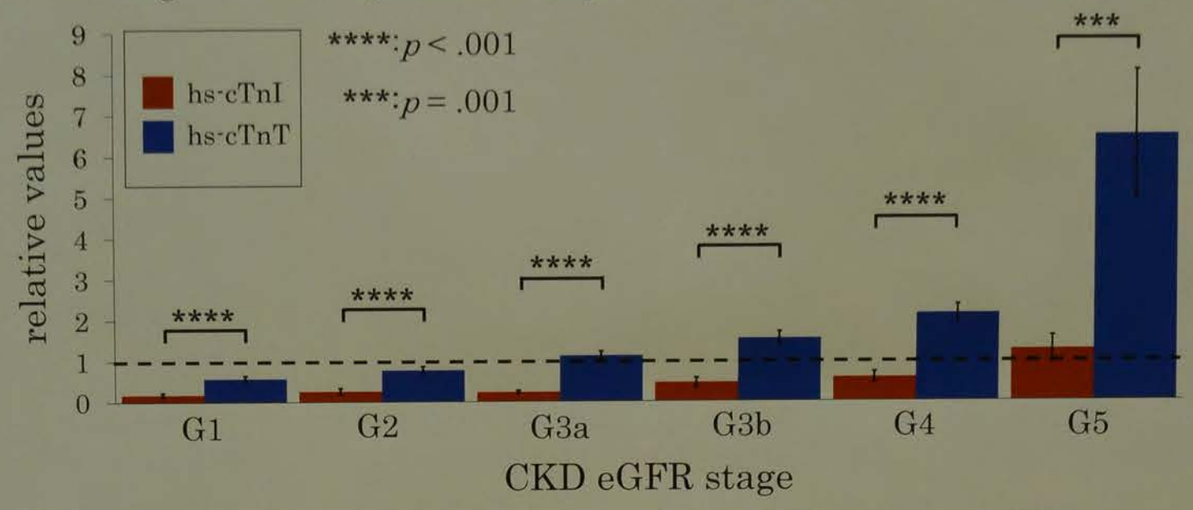


Figure 1. Comparison of relative values of hs-cTnI and hs-cTnT at each CKD stage.
Relative values of hs-cTnT were higher than hs-cTnI in all CKD stages.
Dot line means cut off value.

Table 1. Multiple regression analysis of hs-cTnI and hs-cTnT in Untransformed model and Log-transformed model.

Significant in Untransformed model
hs-cTnI: NT-proBNP
hs-cTnT: eGFR, NT-proBNP

Significant in Log-transformed model
hs-cTnI: Gender (Man), Age, DM, NT-proBNP
hs-cTnT: Gender (Man), Age, eGFR, NT-proBNP

Untransformed model	hs-cTnI		hs-cTnT	
Explanatory variable	β	r	β	r
Gender	.078	.082	.032	-.033
Age	.095	.092	-.070	-.067
$eGFR_{creat}$	-.085	-.078	-.233**	-.205**
Diabetes Mellitus	.046	.050	.051	.055
Heart Disease	.065	.070	-.091	-.096
Urinary protein	.005	.005	.041	.043
Hb concentration	-.065	-.059	-.121	-.107
NT-proBNP	.321**	.311**	.205**	.201**

Log-transformed model	Ln(hs-cTnI)		Ln(hs-cTnT)	
Explanatory variable	β	r	β	r
Gender	.185**	.246**	.093*	.142*
Age	.273**	.328**	.113*	.158*
$eGFR_{creat}$	-.096	-.110	-.294**	-.354**
Diabetes Mellitus	.101*	.142*	.058	.091
Heart Disease	.053	.072	-.063	-.095
Urinary protein	.042	.057	.058	.069
Hb concentration	.078	.087	-.095	-.118
Ln(NT-proBNP)	.499**	.477**	.481**	.504**

β : standardized partial regression coefficient, r: partial colleration coefficient
**: $p < .01$, *: $p < .05$

Conclusion

This study convincingly showed that
(1) high-sensitivity cardiac troponin values are affected by renal function.
(2) hs-cTnI is less sensitive than hs-cTnT to renal function.

Materials and methods

Patients with CKD (n=244)
Patients setting: no history of overt ischemic heart disease in the past 3 years.

classified into G1-G5 based on $eGFR_{creat}$ (mL/min/1.73m^2)

Comparison of relative values of hs-cTnI and hs-cTnT at each CKD stage using t test
※Relative values = the value of hs-cTnI/hs-cTnT over cut off value of hs-cTnI(=26 pg/mL)/hs-cTnT(=14 pg/mL)

Multiple regression analysis of Untransformed model and Log-transformed model
age, gender, $eGFR_{creat}$, Hb, urinary protein(qualitative test), NT-proBNP, history of heart disease, diabetes mellitus as determinants
In the distribution of data was not normal, the data was subjected to natural logarithmic transformation in Log-transformed model.

※$eGFR_{creat}$ (mL/min/1.73m^2)
$=194 \times [Cr]^{-1.094} \times [Age]^{-0.287}$ ($\times 0.739$ in women)

C-1 C-1

Clinical Chemistry

PD-29

Factors affecting blood high sensitive troponin T concentrations in patients without cardiac diseases

Takeo Yuno[1)], Tomoko Takayama[1)], Michiko Nagano[1)], Misuzu Sakai[1)], Mikio Nagahara[1)], Mika Mori[1)], Kenshi Sakai[1)], Yoshio Sakai[1)], Takashi Wada[1), 2)]

1) Clinical Laboratory, Kanazawa University Hospital, Kanazawa, Japan

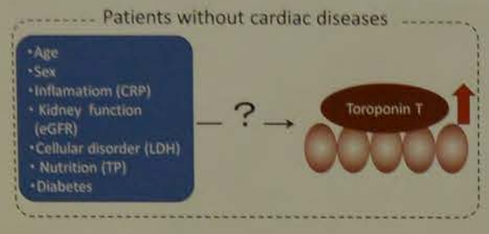
2) Department of Nephrology and Laboratory Medicine, Kanazawa University, Kanazawa, Japan

Background

The cardiac troponin T (TnT) is considered to be a specific biomarker of a myocardial injury.

On the other hand, elevations of TnT have been described in patients with kidney dysfunction.

However, it is not fully investigated whether there are any factors influencing on TnT levels in patients without cardiac diseases.



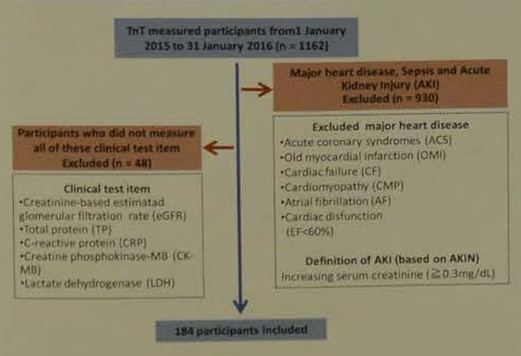
Object

The aim of this study was to investigate factors related to the value of TnT levels in blood of patients without cardiac diseases.

Methods

The data of TnT and several clinical indices were assessed using database at our laboratory from 1 January 2015 through 31 January 2016. Single and multiple linear regression analysis were used to examine relationships between TnT density and clinical indices (Age, Sex, Diabetes, TP (Total protein), CRP (C-reactive protein), eGFR (creatinine-based estimated glomerular filtration rate), CK-MB (creatine phosphokinase-MB), LDH (Lactate dehydrogenase)).

Inclusion and exclusion criteria

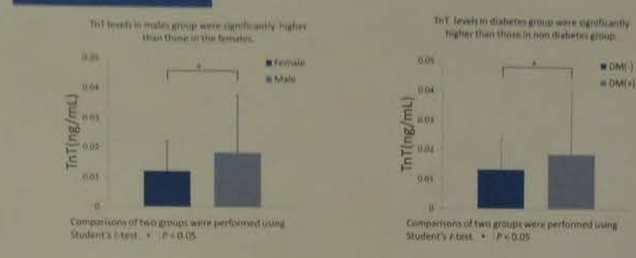


Patients background

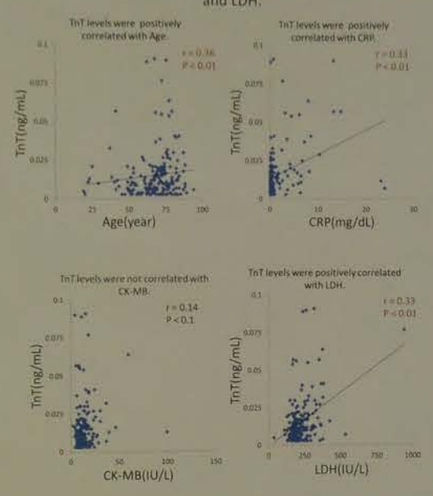
	ALL	TnT		P
		Normal group (0.01ng/mL ≧)	Increased group (0.01≦ng/mL <)	
N	184	118	66	
Age(yaer)	67.3±14.1	65.2±14.2	70.9±13.4	<0.01
Sex(% of male)	51.1	46.6	59.1	N.S.
TP(g/dL)	6.72±0.77	6.89±0.59	6.43±0.94	<0.01
CRP(mg/dL)	1.45±3.41	0.93±3.20	2.38±3.60	<0.01
eGFR(ml/min/1.73m²)	78.4±27.6	84.8±20.5	67.0±34.5	<0.01
CK-MB(IU/L)	13.3±9.9	12.4±10.1	14.7±9.4	N.S.
LDH(IU/L)	220.4±85.4	205.7±63.8	246.7±110.1	<0.01
Diabetes(%)	39.1	38.1	40.9	<0.05

The data are presented means ± standard deviasion(SD).
N.S. not significant. Student's *t*-test was used to compare Age, TP, CRP, eGFR,CK-MB and LDH. χ^2 test was used to compare gender difference and the presence or absence of diabetes.

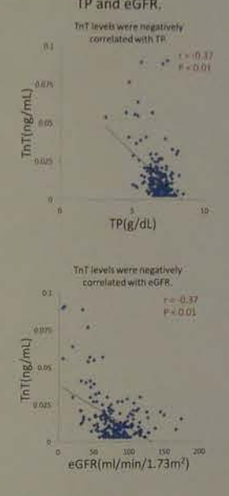
Results



TnT levels were positively correlated with Age, CRP and LDH.



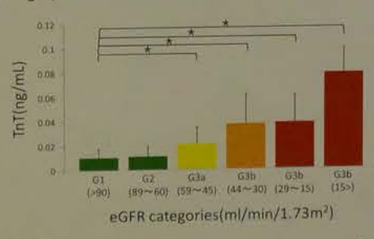
TnT levels were negatively correlated with levels of TP and eGFR.



TnT levels were associated with levels of TP, eGFR and LDH.

Coefficient model	Partial regression coefficient		Standard partial regression coefficient	
	B	SE(B)	β	P
Age(yaers)	0.967×10^{-4}	0.532×10^{-4}	0.086	N.S.
Sex(male)	0.307×10^{-2}	0.149×10^{-2}	0.097	N.S.
TP(g/dL)	-0.593×10^{-2}	0.129×10^{-2}	-0.288	<0.01
CRP(mg/dL)	0.747×10^{-3}	0.572×10^{-3}	0.161	N.S.
eGFR(mL/min/1.73m²)	-0.201×10^{-3}	0.485×10^{-5}	-0.351	<0.01
LDH(IU/L)	0.543×10^{-4}	0.917×10^{-5}	0.293	<0.01
Diabetes(Yes)	0.179×10^{-2}	0.176×10^{-2}	0.055	N.S.

TnT levels in category G3a, category G3b category G4 and category G5 were significantly higher than those in category G1.



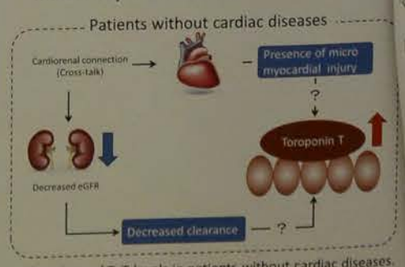
One-way analysis of variance (ANOVA) and Dunnett's post hoc test was used to compare TnT levels in category G1 to those in other categories. * : P < 0.05

Summary

- TnT levels were significantly higher in men than those in female.
- TnT levels were significantly higher in diabetes group than those in non diabetes group.
- TnT levels were positively correlated with levels of Age, CRP and LDH.
- TnT levels were negatively correlated with levels of TP and eGFR.
- TnT levels were associated with levels of TP, eGFR and LDH.
- TnT levels were significantly increased in the category G3a or later.

Discussion

TnT levels are increased in patients reduced renal function (eGFR:59mL/min/1.73m²>) without major heart disease.



Elevations of TnT levels in patients without cardiac diseases.

↓

This was thought to be due to decrease clearance or presence of micro myocardial injury.

↓

It is important to use clinical symptoms and other biomarkers, in interpreting the increase of TnT in patients reduced renal function.

Conclusion

This study suggests that TnT levels are associated with clinical index of kidney function.

Clinical Chemistry

PD-30

Usefulness of abnormal reaction data-detecting function of automated biochemical analyzer

Saori Honda[1],Masanori Seimiya[2],Yoshitake Suzuki[1],Toshihiko Yoshida[1],
Mari Watanabe[1],Haruna Asano[1],Yuji Sawabe[1],Kazuyuki Matushita[1]
[1]Division of Laboratory Medicine,Chiba University Hospital.
[2]International University of health and Welfare

【Background】

In laboratory tests using an automated biochemical analyzer, unexpected problems lead to erroneous test values. Recently, to detect abnormal reactions and failures of the device, a reaction data monitoring system has been provided for analyzers.

【Objective】

we aimed to investigate the usefulness of this system to prevent errors in routine tests.

【Materials and methods】

Sera were from patients who visited our hospital. Written informed consent was obtained from all participants prior to blood sampling, and the study protocol was approved by the Ethics Committee of Chiba University Graduate School of Medicine.

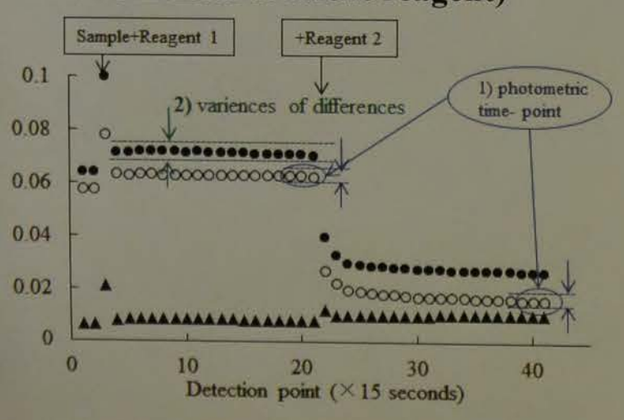
We used the analyzers, BM-2250 (supplied by JEOL Inc. in Japan, and supplied as ADVIA 2400 by Siemens Healthcare Diagnostics Inc. in other countries) and BM-8040 automated biochemical analyzers (JEOL Inc., supplied only in Japan) .

The absorbance of various test items measured during a 5-day period from July 13, 2010 (7,283 samples in total) was summed, and the following items were calculated:

1) variances of operated absorbance at photometric time-points to calculate the measurement result

2) variances of differences in absorbance after mixing the sample and reagent between adjacent photometric points. When an abnormality was detected by the system in a routine test, the person in charge confirmed the reaction data in the reaction monitor of the analyzer.

The reaction detection parameters (Total Bilirubin measurement reagent)

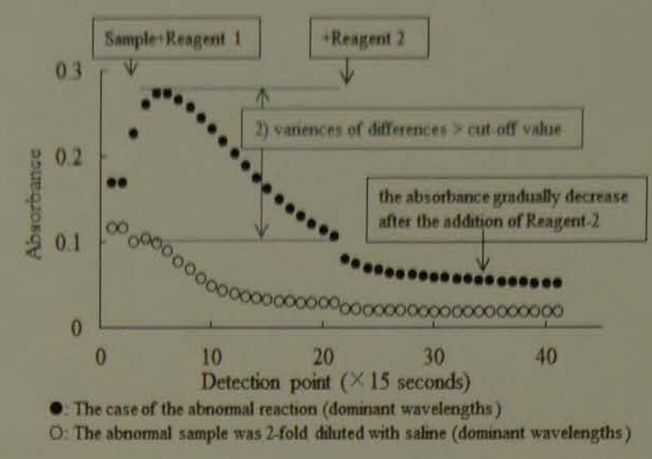


The reagents of total bilirubin, HDL-cholesterol and LDL-cholesterol increase in transient absorbance were detected after the mixing of sample and first reagent . These samples two fold dilution and enforced a protein electrophoresis and immunofixation law to confirm existence of the abnormal protein.

【Results】

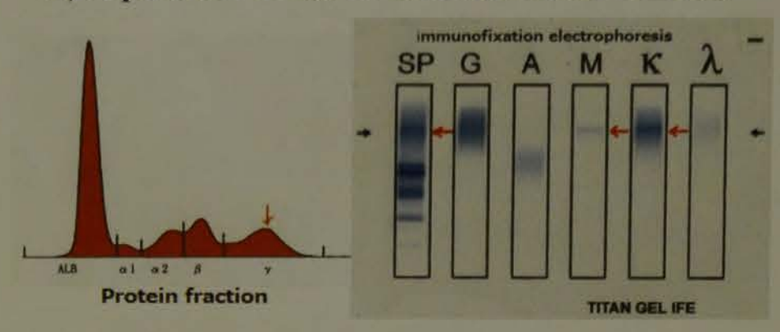
When samples in which clouding was caused were subjected to protein electrophoresis, 22 cases of monoclonal gammopathy were detected in 20 months.

1) The Case of T-bilbin's abnormal reaction



●: The case of the abnormal reaction (dominant wavelengths)
○: The abnormal sample was 2-fold diluted with saline (dominant wavelengths)

2) A possible cause of the abnormal reaction



When this particular serum sample was subjected to immunofixation electrophoresis, the presence of a monoclonal protein was confirmed (IgM-κ type).

3) Similar Case of T-bilbin's abnormal reaction

	T-Bil Undiluted	T-Bil 2-fold diluted	Pathlogy
1	0.4	0.5	Primary macroglobulinemia(IgM-κ)
2	0.6	0.7	Without scrutiny(IgG-κ)
3	0.4	0.4	MGUS (IgM-λ)
4	0.9	0.9	MGUS (IgM-κ)
5	1.2	0.9	Premary macroglobur inemai (IgM-κ)
6	0.6	0.6	Multiple Myloma(IgG-λ)
7	1.8	1.8	Cirrhosis of the liver(IgG-)
8	1.4	1.1	MGUS(IgM-λ)
9	0.9	0.9	Malignant lymphoma(IgG-κ)
10	0.4	0.4	MGUS(IgM-κ)×2
11	1.8	0.8	MGUS(IgM-λ)×2
12	1.1	1.1	Multiple MyelomaIgG-κ)
13	1.3	1.1	Sjogen, Interstitial pneumonia(IgM-polyclonal)
14	0.7	0.7	Unknown(IgG-κ)
15	0.6	0.5	Malignant lymphoma(IgM-λ)
16	0.3	0.3	Lymphoma(IgG-polyclonal)
17	1.7	1.7	Cirrhosis of the liver(IgG-polyclonal)
18	0.9	1.0	Chronic lymphatic lekemia(IgM-κ)×2
19	0.6	0.7	Multipe Myeloma(IgG-κ)
20	1.4	1.4	MGUS(IgM-κ)

【Conclusions】

The reaction data monitoring system of the automated biochemical analyzers was useful to prevent false reports due to unexpected problems. When a false reaction with a reagent is detected, a new pathology, such as monoclonal gammopathy, may be identified by close and careful examination of the sample.

Clinical Chemistry

PD-32

Introduction

A variety of factors, such as sex, age, and diet, influence laboratory determina
Growth hormone (GH) and testosterc
age-dependent and responsible for s
little is known about the weekly variatio
and GH.

Aims/Objectives

The purpose of this study was to asse
variation of hormones and other factors
supplements, on influencing FT and GH

Methods

Participants:
We recruited 9 volunteers (4 men and
years) who had no physical signs of dise
any medications.

Methods:
A 5-mL blood sample was obtained at 5
month from each participant to measure
Luteinizing hormone (LH), follicle stim
estradiol (E2), and progesterone (PG)
determine ovulatory stages. We also mea
factor-1 (IGF-1) to clarify the GH ac
polypeptide. 75% of serum IGF-1 is se
GH stimulation, so the amount of IGF-1 s
secretion of GH. IGF-1 also mediates th
GH.
A zinc supplement (15mg/day) was admin
Muscle training was performed at 70%
follows: 1 set, 8 repetitions, with 2 differ
workouts for 3 sets total.

Statistics:
The Pearson moment correlation coe
determine correlations among the quantita

Result 1

Fig.1 shows the weekly changes of horm
participants. It is well known that some h
FSH, E2, and PG, have ovarian cycles.
showed weekly changes. At the pre-ovulat
stages, the levels of T and GH increased re

Table 1 shows that GH levels were signif
FT levels and total testosterone (TT) lev
correlated with LH levels in women, s
secretion of LH may regulate release of the

Clinical Chemistry

PD-31

Comparison of fetal hemoglobin levels in infants by days after conception and days after birth.

Yuki Watanabe[1], Kayo Osawa[2], Itsuko Sato[1], Ruri Kono[1], Ikuyo Hayakawa[1], Nobuhide Hayashi[1], Ichiro Morioka[3], Jun Saegusa[1]

1) Department of Clinical Laboratory, Kobe University Hospital
2) Department of Biophysics, Kobe University Graduate School of Health Sciences
3) Department of Pediatrics, Kobe University Graduate School of Medicine

Background

- Fetal hemoglobin (HbF) is predominant in a fetal erythrocyte and structurally different from adult hemoglobin (HbA). The binding capacity of HbF is highly affinity for oxygen than HbA.
- HbF to HbA switching is necessary to facilitate trans-placental oxygen exchange in the blood. The switching was noted to take place after birth, but the mechanism is unclear. But, HbF is known to be a high level in the preterm infants.
- HbF is known to be a high level in the preterm infants. It has been reported that HbF showed a high level in a patients with bronchopulmonary dysplasia and might be a prospective marker for some infants at risk for sudden infant death syndrome (SIDS).
- However, there is no report focusing on the change of HbF with increase in gestational age (GA).

Aim

This study was aimed to evaluate whether there is an association between the HbF levels and days after birth, or days after conception according to GA.

Materials and Methods

【Subjects】
The blood samples (n=1,095) were obtained from 407 infant patients at birth to 364 days excluding 18 patients with hereditary disease or post-transfusion.

【Measuring instrument】
The HbF levels were measured based on high-performance liquid chromatography using by ADAMS A1c HA-8180T (ARKRAY,Inc).

【Study method】
The samples divided into 2 groups as follows: preterm infants group (<37 weeks' GA, n=491) and term infants group (≥37 weeks' GA, n=604). We compared the HbF levels between 2 groups by days after birth (6 categories) or days after conception (6 categories).

【Statistical analysis】
To compare between preterm and term infants groups, statistical analysis was performed using the nonparametric Mann-Whitney U-test.

This study was approved by the ethics committee of Kobe University Graduate School of Medicine.

Results

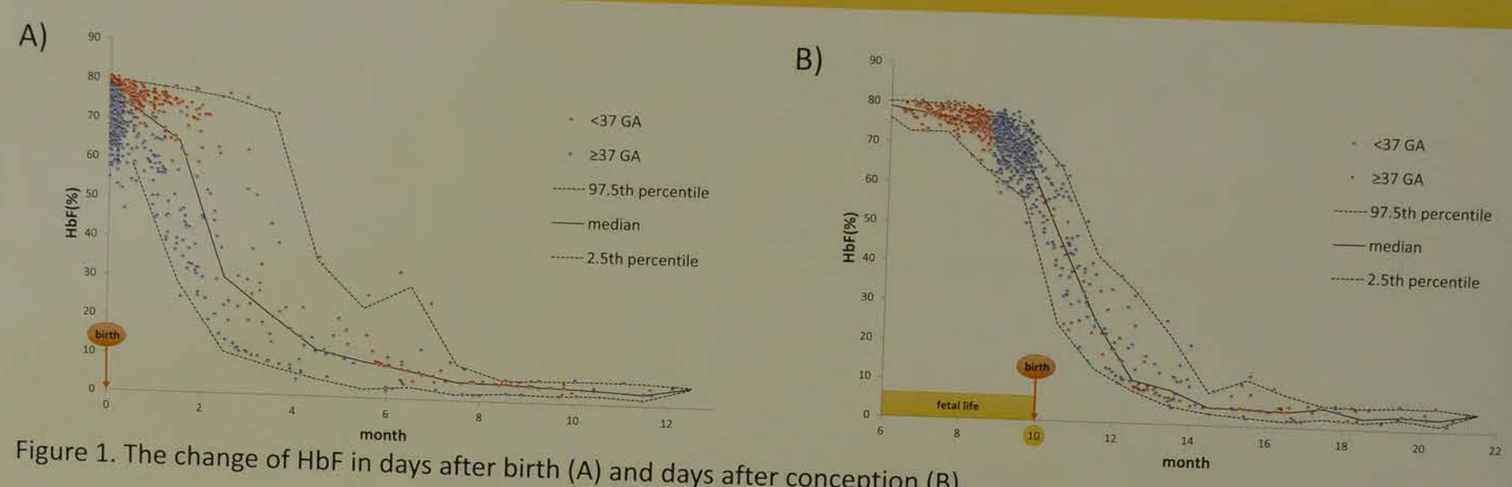


Figure 1. The change of HbF in days after birth (A) and days after conception (B).

Table 1. The comparison of HbF in days after birth

month	all HbF (%)	all n	<37 GA HbF (%)	<37 GA n	≥37 GA HbF (%)	≥37 GA n	
0-1	72.0 (44.6-80.3)	825	75.5 (64.5-80.3)	344	69.5 (44.6-78.5)	481	p<0.0001
1-2	60.0 (24.6-78.1)	105	73.2 (55.2-78.1)	58	43.6 (24.6-64.7)	47	p<0.0001
2-3	37.5 (10.7-76.7)	36	62.7 (30.2-76.7)	14	21.4 (10.7-50.9)	22	p<0.0001
3-4	24.8 (6.7-72.8)	26	40.0 (21.5-72.8)	12	11.8 (6.7-37.3)	14	p<0.0001
4-5	15.0 (4.1-35.6)	17	28.2 (15.7-35.6)	4	10.9 (4.1-20.1)	13	p=0.0017
>5	4.6 (1.3-32.3)	86	5.1 (2.0-32.3)	59	3.6 (1.3-12.7)	27	p=0.0021

Table 2. The comparison of HbF in days after conception

month	all HbF (%)	all n	<37 GA HbF (%)	<37 GA n	≥37 GA HbF (%)	≥37 GA n	
≤9	74.5 (57.5-80.3)	551	75.3 (62.6-80.3)	384	72.5 (57.5-78.5)	167	p<0.0001
9-10	68.1 (46.8-78.0)	333	68.2 (52.4-75.7)	29	68.1 (46.8-78.0)	304	p=0.7459
10-11	46.3 (23.2-64.8)	60	43.4 (23.2-55.2)	8	46.8 (24.6-64.8)	52	p=0.3844
11-12	25.6 (12.9-50.9)	33	26.6 (13.6-35.6)	11	25.1 (12.9-50.9)	22	p=0.8937
12-13	10.7 (8.2-37.3)	29	9.3 (8.2-32.3)	13	11.8 (8.4-37.3)	16	p=0.2318
>13	5.0 (1.3-26.2)	89	4.6 (2.0-26.2)	46	5.5 (1.3-20.1)	43	p=0.6078

- In days after birth, the HbF levels of preterm infants group were higher than those of term infants group (Figure 1A). The medians of HbF levels between 2 groups were statistically significant different from preterm and term infants in 6 categories of days after birth (Table 1).
- On the other hand, the distribution of HbF was matched between preterm and term infants groups in days after conception (Figure 1B). In days after conception, the medians of HbF levels between 2 groups were a difference in a category (under 9 month, $p<0.0001$), while those were closely matched in the other categories (Table 2).

Discussion

- Previous studies reported that HbF levels of the preterm infants were higher than those of term infants.
- In this study, we revealed that the HbF levels were regulated with days after conception rather than days after birth regardless of GA.
- Our results provide clues to recognize the mechanism of Hb switching and may help to establish age-specific reference values of HbF.

Clinical Chemistry

PD-32

Weekly variation of free testosterone and growth hormone levels in men and women

Chie Hirayama-Negishi[1], Kazumasa Isobe[2], Keiko Inoue[3], Michikuni Isijima[1], Hitoshi Oikawa[1], Toru Nanmoku[1], Yasushi Kawakami[2]

1: Division of Laboratory, University of Tsukuba Hospital, Japan (e-mail: chie-tuk@umin.ac.jp)
2: Department of Laboratory Medicine, University of Tsukuba, Japan
3: Tsukuba Medical Laboratory of Education and Research, Japan

Introduction

A variety of factors, such as sex, age, diurnal variation, exercise, and diet, influence laboratory determinations of hormones.
Growth hormone (GH) and testosterone (T) are known to be age-dependent and responsible for sex differences. However, little is known about the weekly variation of free testosterone (FT) and GH.

Aims/Objectives

The purpose of this study was to assess weekhly and seasonal variation of hormones and other factors such as exercise, or zinc supplements, on influencing FT and GH levels.

Methods

Participants:
We recruited 9 volunteers (4 men and 5 women, aged 20-59 years) who had no physical signs of disease and were not taking any medications.

Methods:
A 5-mL blood sample was obtained at 5 pm once per week for a month from each participant to measure the levels of FT and GH. Luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E2), and progesterone (PG) were also measured to determine ovulatory stages. We also measured insulin-like growth factor-1 (IGF-1) to clarify the GH action. IGF-1 is a basic polypeptide. 75% of serum IGF-1 is secreted by the liver upon GH stimulation, so the amount of IGF-1 secretion reflects the total secretion of GH. IGF-1 also mediates the anabolic properties of GH.
A zinc supplement (15mg/day) was administered for 7 days.
Muscle training was performed at 70% maximal strength as follows: 1 set, 8 repetitions, with 2 different types of muscular workouts for 3 sets total.

Statistics:
The Pearson moment correlation coefficient was used to determine correlations among the quantitative variables.

Result 1

Fig.1 shows the weekly changes of hormone levels in female participants. It is well known that some hormones, such as LH, FSH, E2, and PG, have ovarian cycles. GH and FT levels also showed weekly changes. At the pre-ovulatory and post-ovulatory stages, the levels of T and GH increased respectively.

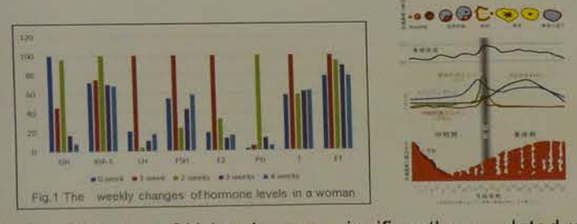


Fig.1 The weekly changes of hormone levels in a woman

Table 1 shows that GH levels were significantly correlated with FT levels and total testosterone (TT) levels were significantly correlated with LH levels in women, suggesting that cyclic secretion of LH may regulate release of these hormones.

Table 1 Correlations among GH, LH, FSH, estradiol, progesterone, free testosterone, and total testosterone in 25 specimens from 5 women

	GH	LH	FSH	estradiol	progesterone	total testosterone	free testosterone
GH	1.000	-0.137	-0.292	0.105	0.078	-0.279	0.438+
LH		1.000	0.794++	0.611+	-0.194	0.673++	-0.170
FSH			1.000	0.114	-0.377	0.257	-0.153
estradiol				1.000	0.385	0.265	0.101
progesterone					1.000	-0.040	-0.137
total testosterone						1.000	0.071
free testosterone							1.000

Abbreviations: GH, growth hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone.
+P<.05, ++P<.01

Result 2

Fig. 2 shows the weekly changes of hormone levels in male participants. In men, a weekly variation of T and GH were observed, even though the increase is smaller than that in women.

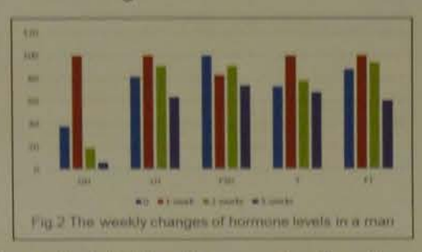


Fig.2 The weekly changes of hormone levels in a man

Table 2 shows that LH levels were significantly correlated with both TT and FT, and that TT levels were significantly correlated with FT levels.

Table 2 Correlations among GH, LH, FSH, free testosterone, and total testosterone in 14 specimens from 3 men

	GH	LH	FSH	total testosterone	free testosterone
GH	1.000	-0.095	0.091	-0.1252	-0.043
LH		1.000	0.523	0.674++	0.881++
FSH			1.000	0.257	-0.153
total testosterone				1.000	0.899++
free testosterone					1.000

Abbreviations: GH, growth hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone.
++P<.01

Result 3

Table 3 shows seasonal changes in FT levels in 3 persons. FT levels might be high in winter.

Table 3. Seasonal changes in free testosterone levels in 3 persons

	Jan	Feb	Mar	Apr	May	Jun
KI (24yrs,♂) n=4	25.4 ±1.1		14.6 ±0.9↓ **			
MA (30yrs, ♀) n=4		1.6 ±0.2			1.1 ±0.3↓ **	
GH (47yrs,♂) n=4			5.0 ±0.6			6.0 ±0.8 ↑**

Result 4

Table 4 shows the effect of the zinc supplement intake on the hormone levels in men. Seven days intake of zinc supplement remarkably increased the levels of LH, TT and FT levels.

Table4. Effect of zinc supplement intake on the hormones levels in a man

(%)

	GH ng/ml	LH mIU/ml	FSH mIU/ml	T ng/ml	FT pg/ml	Zn μg/dl
before intake	4.1 (100)	1.1 (100)	3.7 (100)	1.65 (100)	5 (100)	59 (100)
7 days intake	1.7 (41)	3 (273)	4.7 (127)	4.36 (264)	10.9 (218)	76 (129)

Result 5

Table 5 shows the effect of muscle training on GH and FT levels over 1 year in male participants. After the first week FT levels maximally increased and was maintained over a year.

Table5. Effects of muscle training on the levels of the hormones in a man

(%)

time	0 day	7 days	14 days	21 days	365 days
GH ng/ml	0.1 (100)	0.5 (500)	0.1 (100)	0.1 (100)	0.1 (100)
free T pg/ml	7.5 (100)	13.6 (181)	11.6 (155)	10.6 (141)	11.5 (153)

Conclusion

GH and T levels changed weekly, and FT levels have seasonal variations. Zinc supplementation and exercise increased the levels of T. In order to address pre-analytical problems, future studies will more closely address variability in testing subjects.

Clinical Chemistry

PD-33

Development of equation to calculate true kalium levels in hemolyzed blood samples.

Noritaka HANDA[1)] Kesae HATAGUCHI[1)] Emi YAGASAKI[1)] Youki NAKAJIMA[1)] Waki KANAI[1)] Toshiyuki OZAWA[1)]

1)Department of Clinical Laboratory, Saku Central Hospital Advanced Care Center

Background

Hemolysis increases serum kalium (K) levels. Determination of K levels using non-hemolyzed blood samples is recommended, but sometimes blood collection is difficult. Our purpose is to develop a K-compensating equation to estimate the true K levels using hemolyzed blood samples.

Materials

Heparinized blood samples from fifty patients were used.
K levels and hemolysis index were determined using BM6050 (JEOL).
Hemolysis index is levels of hemolysis. It is distinctive value of BM series.

Methods

Ⅰ. Manufacture of the hemolysis reproduction reagent.

1. Washed the heparinized blood with saline (0.9% NaCl) three times and diluted with saline.
2. Adjusted hemoglobin (Hb) levels to 2,500 mg/dL.
3. Hemolyzed by freezing at -30 ℃.
4. This solution was referred to as the hemolysis reproduction reagent.

Ⅱ. Development of K-compensating equation.

1. Mixed hemolysis reproduction reagent with saline and pooled serum.
2. Adjusted Hb levels to 0, 100, 200, 300, 400 and 500 mg/dL (table1).
3. Determined K levels and hemolysis index.

Table 1. Mix rate of hemolysis reproduction reagent, saline and pooled serum

Hb levels (mg/dL)	0	100	200	300	400	500
Hemolysis reproduction reagent (μL)	0	10	20	30	40	50
Saline (μL)	50	40	30	20	10	10
Pooled serum (μL)	200	200	200	200	200	200

Ⅲ. Verification test of the accuracy of the equation.

1. Serum samples from forty patients whose blood was collected twice because the first sample was hemolyzed were used to verify the accuracy of the equation.
2. Compare K levels of non-hemolyzed samples with compensated K levels of hemolyzed samples.

Results

1. Correlation between hemolysis index and Hb levels.

We observed good correlation between hemolysis index (x) and Hb levels (y):
$y = 39.487x - 1.6325$, $r=0.99$.

2. Correlation between hemolysis index and increases in K levels.

Correlation between hemolysis index (x) and increases in K levels (y) is shown as equation 1: $y = 0.125x + 0.0027$ (Fig. 1).

3. Correlation between hemolysis index and increase rate of K levels.

Correlation between hemolysis index (x) and increase rate (y) is shown as equation 2: $y = 3.623x + 0.0767$ (Fig. 2).

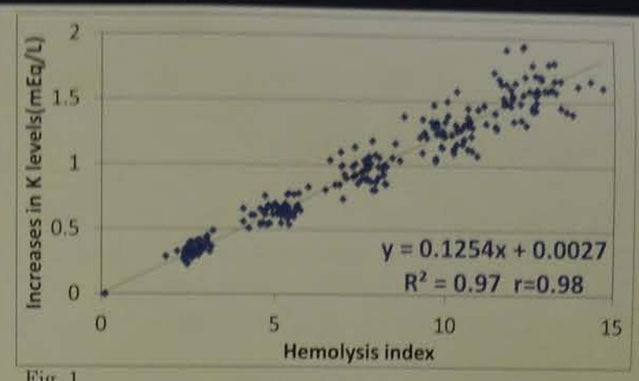


Fig. 1.
Correlation between hemolysis index and increases in K levels.

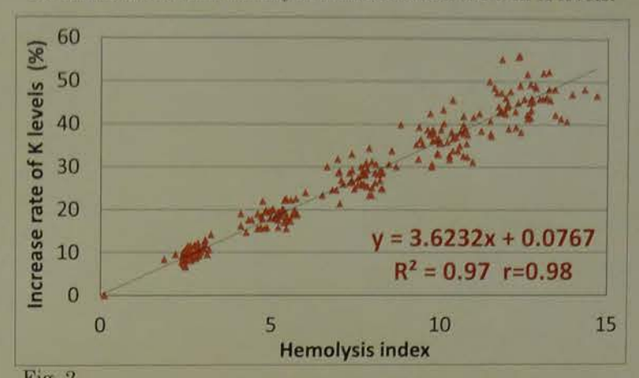


Fig. 2.
Correlation between hemolysis index and increase rate of K levels.

4. Development of K-compensating equations.

We developed two compensating equations.
Equation 3: $y = A - (0.125x + 0.0027)$
Equation 4:
$y = A \times 100 / (100 + (3.623x + 0.0767))$
* Hemolysis index (x), Compensated K levels (y), K levels of hemolyzed samples (A).

Equation 3 was developed using equation 1.
Equation 4 was developed using equation 2.

5. Verification test of the accuracy of the equation.

Detailed results are shown in table 2.
Using equation 3, the differences in K levels of non-hemolyzed samples and compensated K levels of hemolyzed samples are shown as 0.60 ± 0.44 mEq/L.
Using equation 4, these differences are shown as 0.34 ± 0.36 mEq/L.

Table 2. Differences in K levels of non-hemolyzed samples and compemnsated K levels of hemolyzed samples

	Equation 3				Equation 4		
n	40	Max	1.72	n	40	Max	1.10
Mean	0.60	Min	-0.19	Mean	0.34	Min	-0.30
SD	0.44	Median	0.49	SD	0.36	Median	0.23

Discussion

We found a correlation between the hemolysis index and increases in K levels. Using equation 3, compensated K levels of hemolyzed samples are not useful because differences are large. Using equation 4, differences are smaller than using equation 3. Equation 4 is useful to estimate of true K levels. We consider that the one of the factor of the differences is release of K from muscles and cells.

Conclusion

We developed K-compensating equation:
$y = A \times 100 / (100 + (3.623x + 0.0767))$.
If we use this equation, we can compensate K levels and estimate the true K levels in hemolyzed blood samples.
We wish this study contribute to decrease of re-collection of blood samples because first sample is hemolysed.

Clinical Chemistry

PD-34

Influence of red blood cells in the pseudo-hyperkalemia

Involvements of kinds of blood collecting tubes, the number of red blood cells and underlying diseases.

Yoko Shiotani Hiroshi Fujita Mutsuko Hosoya Shizuka Hasegawa
Ayane Kato Miyoko Kato Hiroe Oriuchi Yoshio Sato
Tokyo Metropolitan Bokutoh Hospital

Abstract

【Introduction】Pseudo-hyperkalemia can occur in patients with thrombocytosis or leukocytosis. We performed a study to determine the mechanisms by which red blood cells cause pseudo-hyperkalemia.
【Method】 We first compared the differences in serum and plasma potassium in patients with myeloproliferative neoplasms (MPN) and non-hematological disease (NHD). Next, we prepared samples with various hematocrits using phlebotomized blood. Samples in various venipuncture tubes were measured by using the full volume of blood and 1 mL of blood.
【Results】 Results: Serum potassium, average plasma potassium (K), and serum potassium – plasma potassium (ΔK) values were 4.77 mmol/L, 4.04 mmol/L, and 0.73 mmol/L in polycythemia vera; 5.59 mmol/L, 4.37 mmol/L, and 1.21 mmol/L in essential thrombocytosis; and 4.09 mmol/L, 3.83 mmol/L, and 0.26 mmol/L in NHD patients, respectively. In experiments using samples of phlebotomized blood, hemolysis in 1 mL of blood was greater than that in the full volume of blood. Potassium levels increased in a hematocrit-dependent manner from 50%-70%.
【Conclusions】Serum potassium increases depending on the number of red blood cells. We also examined the differences according to venipuncture tube and blood sample volume, and found that the type of tube and sample volume could also affect the results.

Introduction

【Introduction】 Pseudo-hyperkalemia occurs in patients with thrombocytosis or leukocytosis, because potassium is released from platelets and white blood cells (WBCs) during clotting in the venipuncture tube (VT).
Thus, in patients with thrombocytosis and leukocytosis, the plasma potassium level should be measured.
In addition, we recently reported the occurrence of pseudo-hyperkalemia in Japanese polycythemia vera (PV) patients. We examined the effects of red blood cells (RBCs) on pseudo-hyperkalemia with regard to hemolysis caused by blood sampling technique.

method

【Clinical settings】 Serum and plasma potassium were measured in patients with myeloproliferative neoplasms (MPN, N=23) or non-hematological disease (NHD, N=76) in our hospital from 2013 to 2015.
【Method】
1. We compared the differences of serum, plasma potassium (ΔK=serum potassium- plasma potassium) between the MPN and NHD.
2. We examined the relationship of the numbers of red blood cell (RBC) and white blood cell (WBC), platelet (PLT), the serum potassium level in the blood with high hematocrit and the ΔK.
3. We prepared samples with various hematocrits using phlebotomized blood (Fig.1).

figure1. Procedure of adjusting the various hematocrit.

Full volume samples and 1 mL samples of blood were placed in a variety of venipuncture tubes (Fig.2). The VT top was removed to release the vacuum. Blood was dispensed with a dropper. Potassium and lactate dehydrogenase (LDH) in simulated blood were also measured.

	A	B	C
Blood Clotting Accelerant	○ strong type	○	○
Serum Aeparating Gel	○	○	×
Defined Amount (mL)	8.0	5.5	5.0

Insepack™ (Sekisui medical Co. Ltd.)

figure2. venipuncture tube.

Results 1

Table1. Serum K , plasma K and ΔK in the patients with MPD and NHD.

	PV (n=7)			ET (n=6)			NHD (n=76)		
	serum	plasma	ΔK	serum	plasma	ΔK	serum	plasma	ΔK
min	4.2	3.8	0.3	4.2	3.7	0.2	2.8	2.6	0.0
max	5.7	4.6	1.1	7.4	4.8	3.0	5.4	5.4	0.6
ave	4.78	4.04	0.73	5.59	4.37	1.21	4.09	3.83	0.26

(mmol/L)

Results 2

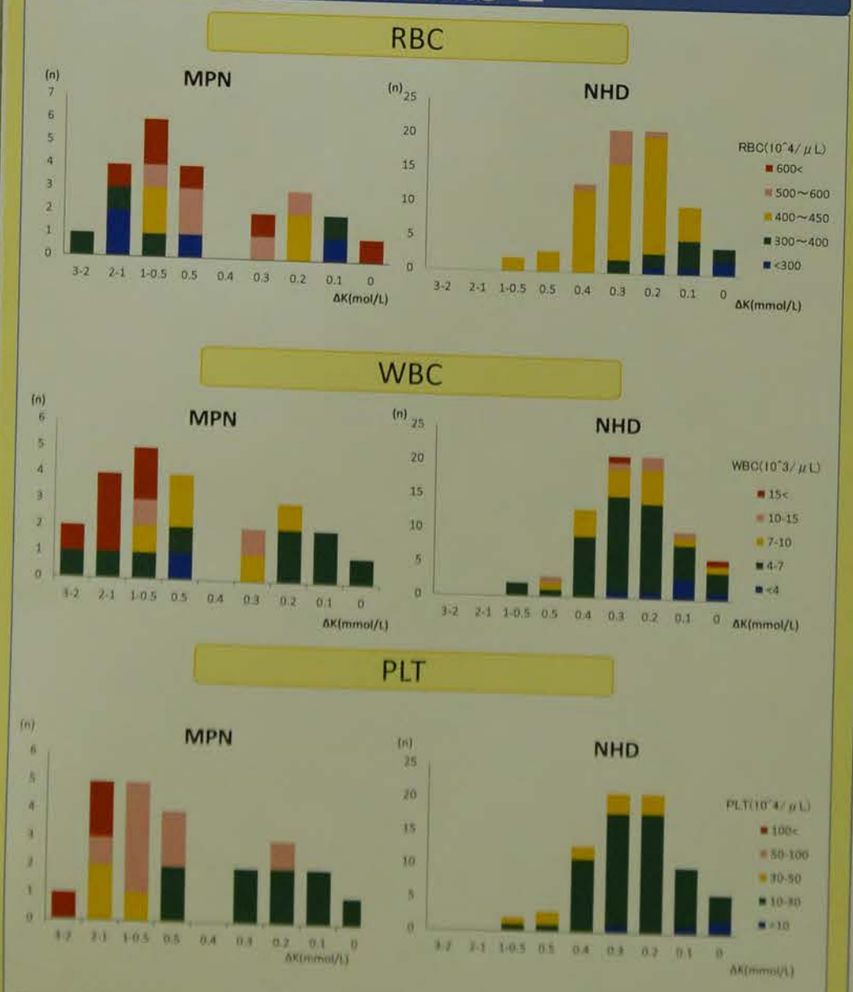


Figure3.The relationship of each blood cell count and ΔK in the patients with MPN and NHD.

Results 3

Table2. Effects of VT on the potassium level in the blood with various of hematocrit.(30～70%) Arrow showed % of control.

(%)

		A			B			C		
		min	max	ave	min	max	ave	min	max	ave
FULL volume	30%	100	109	102	100	103	101	➡100	100	100
	40%	101	118	105	100	106	102	100	106	102
	50%	102	128	109	102	112	104	100	112	104
	60%	105	146	116	103	121	109	103	121	109
	70%	110	131	121	107	121	113	108	119	113
1mL	30%	99	110	104	100	103	101	100	104	101
	40%	102	119	109	102	109	104	100	109	103
	50%	105	129	114	103	117	108	102	115	106
	60%	110	146	121	105	126	113	105	125	112
	70%	113	138	126	111	135	122	111	126	120

Table3. Effects of VT on the LDH level in the blood with various of hematocrit.(30～70%)

(%)

		A			B			C		
		min	max	ave	min	max	ave	min	max	ave
FULL volume	30%	100	103	101	100	103	101	➡100	102	100
	40%	101	108	104	102	106	104	101	106	103
	50%	105	116	109	103	116	109	99	113	107
	60%	115	140	123	112	132	119	108	128	116
	70%	133	188	157	125	179	148	113	190	142
1mL	30%	109	129	118	103	111	106	103	109	106
	40%	113	186	143	110	116	113	105	114	109
	50%	130	225	157	118	137	129	114	161	126
	60%	158	229	182	128	174	150	124	170	143
	70%	186	295	231	159	303	204	154	288	207

Conclusion

- In patients with MPN, RBC concentration was not associated with increased serum potassium levels. However, Serum potassium in NHD is not only PLT and WBC, there is a possibility to increase depending on the number of RBC.
- The concentration of serum potassium and LDH rose in a hematocrit-dependent manner. In particular, serum potassium levels were significantly higher in blood with high hematocrit (≥50%).
- The difference between serum and plasma potassium levels was dependent on the type of VT; this might be due to the differences in separating gel and accelerant contained in each type of VT. Since the difference was dependent on hematocrit, cautious interpretation is required in reactive or secondary polycythemia, as well as in PV.

A-1 A-1

Clinical Chemistry

PD-35

Influence of In Vitro Hemolysis on 80 Different Laboratory Tests

Yasuhiro Yanagisawa[1], Kazumasa Isobe[2], Michikuni Ishijima[3], Toru Nanmoku[3], Takayuki Yamamoto[1], Etsu Suzuki1 Yasushi Kawakami[2]

1.Tsukuba Medical Laboratory of Education and Research Tsukuba i-Laboratory LLP,
2.Department of Laboratory Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan
3.Division of Laboratory, University of Tsukuba Hospital, Tsukuba, Japan

Introduction

In vitro hemolysis is probably the most common pre-analytic problem in laboratory medicine.: it influences many laboratory tests in many ways. The purpose of this study was to assess the quantitative effects of hemolysis on 80 routine different laboratory tests. Hospital laboratories often use basic techniques of immunology and biochemistry to automatically analyze clinical samples from patients. Although these tests have been developed and improved to perform rapid and sensitive assays, hemolysis has been a common issue for laboratory test accuracy. Most causes of in vitro hemolysis are related to specimen collections, difficult collections, unsecure line connections, as well as improper tube mixing, due to the effects of the mechanical processing of blood.

Hemolysis is qualitatively evaluated by a laboratory autoanalyzer measuring the free plasma hemoglobin concentration. In many cases it is difficult to recollect the samples, since hemolysis is found after analysis of the samples.
In this study, we assessed the quantitative effects of in vitro hemolysis on 80 biochemical and immunochemical tests.

Methods

In vitro hemolysis frequency

We counted hemolysis samples obtained in Tsukuba University Hospital for 3 months (from January 1th to March 31th, 2015). The hemolysis was calculated and classified automatically into 5 degrees of hemolysis, none, 1+, 2+, 3+, 4+, based on Hb absorption.

Hemolysed samples

Hemolysed samples were prepared as follows: 450 µl serum or plasma mixed with 50 µl of concentrated hemolysate to give hemoglobin concentrations of 90 to 1400 mg/dL. Hemolysate was obtained by subjecting a plasma red blood cell pellet to one freezing-thawing cycle. The degree of hemolysis was quantified by measuring the hemoglobin absorbance. Hemolysis values were expressed in g/L of hemoglobin and referred to the hemoglobin concentrations.Then, 80 different laboratory tests were determined with Hitachi 7700 for biochemical tests and with AIA 2000, Cobas 6000, and Lumipulus G1200 for other tests.

Results 1

In vitro hemolysis frequency

We counted hemolysis samples obtained in Tsukuba University Hospital for 3 months (from January 1st to March 31st, 2015). As shown in Table 1, hemolysis occurred in 8.6% of all the specimens, 6.2% of outpatients and 12.4% of inpatients. The frequency was high in the pediatric and emergency departments. The frequency was high in ages under 16 years or over 90 years.

Table. 1 Hemolysis insidents at Tsukuba University Hospital from Jan 1th to March 31th 201

hemolysis	mean Hb g/dL	numbers	percent %	person	average times
no hemolysis		43961	91.4	27030	
hemolysis level	0.18	3929	8.2	2278	1.72
level 2	0.36	152	0.32	126	1.21
level 3	0.54	15	0.03	14	1.07
level 4	0.72	11	0.02	9	1.22
total		48068	100	29457	

Results 2

Quantitative effects on the laboratory tests

Among the 80 laboratory tests we examined, exactly 11 test levels increased and 7 test levels decreased due to hemolysis as described below. Levels of K, IP, AST, LD, TTT and ZTT increased proportionally; levels of Fe and HA increased exponentially; increase of LIP levels declined at a higher level of hemolysis.

Electrolyte and cation

Table 2A summarizes the serum electrolyte values. Among 9 electrolytes tests, potassium and iron were extremely increased by hemolysis as previously well reported. Inorganic phosphate also moderately increased. The increases of K and IP were additive and proportional, whereas the increase of Fe was synergetic and exponential. The estimated increases of K and IP against 0.1 g Hb were 0.248 mEq/L and 0.117 mEq/L respectively.
On the other hand, sodium and chloride were slightly decreased by hemolysis at Hb 540 mg/dL (3+), seeming to show the volume dilution effect of hemolysis on these electrolytes.

Biochemical tests

Table 2B summarizes the serum biochemical substance values. Among 27 tests, the levels of aspertate aminotransferase, lactate dehydrogenase, hyaluronic acid and TTT were extremely increased by hemolysis. Lipase, TBA, and ZTT levels were also moderately increased. On the other hand, total bilirubin, glycoalbumin and 1,5AG levels were decreased by hemolysis.
The increases in ASP, LD, TTT, ZTT and hyaluronic acid levels were additive and proportional, whereas the increase of LIP declined at a higher level of hemolysis. The estimated increases of ASP, LD, TTT, ZTT, and hyaluronic acid against 0.1 mg Hb were 5.9 IU/L, 81.8 IU/L, 0.16 U, 0.24 U and 8.88 ng/mL respectively.

Immunochemical tests

Table 2C summarizes the serum immunochemical substance values. Among 12 tests, no constituent level was changed by hemolysis.

Tumor markers, KL-6 and TNT assays

Table 2D summarizes the serum tumor markers and Troponin T values. Among 15 tests, neuronspecific enolase, NSE extremely increased by hemolysis. On the otherhands, Troponin T value was decreased by hemolysis.

Endocrine assays

Table 2E summarizes the endocrine tests values. Among 17 tests, ACTH, IRI and BNP levels were remarkably decreased by hemolysis.

Table.2A Effects of hemolysis on serum electrolytes some cations

Hb(mg/dL)	0	0.09	0.18	0.27	0.36	0.45	0.54	0.63	0.72	0.81
Na	142.9(100)	140.8(99)	137.7(96)	135.3(95)	133(93)	130.3(91)	128.4(90)	126.4(88)	122.1(85)	121(85)
K	3.68(100)	3.9(106)	4.11(112)	4.33(118)	4.57(124)	4.78(130)	5.02(136)	5.27(143)	5.42(147)	5.69(155)
Cl	111.7(100)	109.5(98)	106.8(95)	103.8(93)	101.6(91)	99.3(89)	96.7(87)	94.8(85)	91.4(82)	90.1(81)
Ca	8.12(100)	7.99(98)	8.1(100)	7.91(97)	7.98(98)	7.7(95)	7.87(97)	7.88(97)	7.85(97)	7.58(93)
IP	3.25(100)	3.33(102)	3.48(107)	3.53(109)	3.79(116)	3.82(118)	3.88(119)	4(123)	4.23(130)	4.29(132)
Mg	1.7(100)	1.7(100)	1.79(105)	1.73(102)	1.78(105)	1.78(105)	1.76(104)	1.81(106)	1.83(108)	1.9(11)
Fe	47.6(100)	49.5(104)	50.7(107)	57.4(121)	79.7(167)	95.4(200)	108.4(228)	120.1(252)	129.8(272)	142.2(299)
UIBC	209.7(100)	209.6(100)	216.8(103)	216.7(103)	224.7(107)	233.5(111)	228.6(109)	232.7(111)	237.6(113)	239.7(114)
ZN	63.9(100)	64.1(100)	64.1(100)	63.6(100)	62.3(97)	60.5(5)	62.1(97)	62(97)	61.2(96)	62.5(98)

Table.2B Effects of hemolysis on biochemical tests

Hb(mg/dL)	0	0.09	0.18	0.27	0.36	0.45	0.54	0.63	0.72	0.81
AST	17(100)	23(135)	28(165)	33(194)	37(218)	41(241)	49(288)	55(324)	56(329)	62(365)
ALT	16(100)	15(94)	15(94)	15(94)	15(94)	16(100)	15(94)	16(100)	16(100)	17(106)
LD	157(100)	229(146)	308(196)	380(242)	455(290)	527(336)	599(382)	671(427)	738(470)	807(511)
ALP	222(100)	214(96)	209(94)	209(94)	206(93)	204(92)	203(91)	199(90)	202(91)	200(90)
GGT	74(100)	75(101)	75(101)	75(101)	75(101)	75(101)	73(97)	68(92)	72(97)	74(100)
AMY	69(100)	68(99)	69(100)	69(100)	69(100)	67(97)	68(99)	68(99)	68(99)	68(99)
LIP	92(100)	94(106)	98(119)	99(122)	41(128)	42(131)	42(131)	43(134)	42(131)	43(134)
CK	63(100)	64(102)	62(98)	63(100)	62(98)	66(105)	61(97)	64(102)	60(95)	64(102)
CHE	203(100)	201(99)	203(100)	203(100)	209(103)	214(105)	209(103)	207(102)	216(106)	217(107)
LAP	51(100)	51(100)	51(100)	51(100)	53(104)	47(92)	49(96)	48(94)	50(98)	49(96)
TP	5.72(100)	5.62(98)	5.63(98)	5.69(99)	5.78(101)	5.91(103)	5.9(103)	5.86(102)	5.9(103)	5.9(103)
Alb	3.24(100)	3.22(99)	3.2(99)	3.19(98)	3.23(100)	3.31(102)	3.3(102)	3.3(102)	3.32(102)	3.29(102)
UA	4.41(100)	4.39(100)	4.32(98)	4.31(98)	4.34(98)	4.28(97)	4.38(99)	4.2(95)	4.27(97)	4.21(95)
CRE	0.951(100)	0.927(97)	0.895(94)	0.877(92)	0.881(93)	0.891(94)	0.874(92)	0.87(91)	0.855(90)	0.828(87)
UN	16.5(100)	16.7(101)	16.9(102)	16.9(102)	16.8(102)	16.5(100)	16.9(102)	16.6(101)	16.7(101)	16.9(102)
TBIL	0.32(100)	0.28(88)	0.27(84)	0.25(78)	0.26(81)	0.25(78)	0.27(84)	0.27(84)	0.28(88)	0.28(88)
TG	85.3(100)	82.7(97)	83.4(98)	82.3(96)	85.7(100)	85(100)	84.4(99)	85.9(101)	84.2(99)	85.9(101)
TCHO	143.8(100)	141.8(99)	141.9(99)	140.2(98)	144.9(101)	141.3(98)	143.7(100)	141.3(98)	139.8(97)	142.8(99)
HDL	40.2(100)	39.6(99)	39.4(98)	39.2(98)	39.4(98)	38.6(96)	39.1(92)	38.5(96)	38.6(96)	39(97)
PL	157.2(100)	156.9(100)	158.7(101)	157.1(100)	157.2(100)	155.2(99)	155(99)	154.3(98)	155.8(99)	158(101)
LDL	86.9(100)	85.4(98)	86.4(99)	84.8(98)	85.9(99)	86.2(99)	86(99)	84.5(97)	83.8(96)	84.4(97)
TBA	4.1(100)	3.8(93)	4(98)	4.3(105)	4.7(115)	5.5(134)	5.9(144)	5.4(132)	6.3(154)	6.7(163)
GLU	74(100)	75(101)	75(101)	75(101)	75(101)	74(100)	75(101)	75(101)	75(101)	76(103)
GA	0.571(100)	0.55(96)	0.521(91)	0.506(89)	0.458(80)	0.461(81)	0.431(75)	0.404(71)	0.375(66)	0.382(67)
1 5AG	12.64(100)	12.12(96)	11.51(91)	11.42(90)	10.81(86)	10.2(81)	10.1(80)	9.6(76)	9.5(75)	9.5(75)
TTT	0.32(100)	0.48(150)	0.58(181)	0.75(234)	0.91(284)	1.05(328)	1.22(381)	1.33(416)	1.5(467)	1.65(516)
ZTT	2.43(100)	2.52(104)	2.83(116)	3.16(130)	3.29(135)	3.45(142)	3.71(153)	3.77(155)	4.01(165)	4.05(167)
HA	46(100)	48(104)	57(124)	65(141)	71(154)	81(176)	94(204)	84(204)	106(230)	112(243)

Table.2C Effects of hemolysis on immunochemical assays

Hb(mg/dL)	0	0.09	0.18	0.27	0.36	0.45	0.54	0.63	0.72	0.81
IgG	674.6(100)	661.8(98)	660.9(98)	662.7(98)	660.2(98)	672.9(100)	674.3(100)	672.9(100)	676(100)	679.8(101)
IgA	218.2(100)	214.1(98)	214.3(98)	217.9(100)	217.8(100)	214.8(98)	215.4(99)	217.3(100)	215.9(99)	217.2(100)
IgM	85.3(100)	85.9(100)	83.4(98)	84.6(99)	86.9(102)	82.5(97)	79.1(93)	82.3(96)	79(93)	79.4(93)
CH50	28.5(100)	24.6(86)	29(102)	24.3(85)	28.7(104)	30.4(107)	23.2(81)	27.7(97)	29.3(103)	29(102)
C3	111.3(100)	111.3(100)	108.1(97)	110.4(99)	109.5(98)	107.2(96)	109.1(98)	108.4(97)	108.6(98)	109.6(98)
C4	24.5(100)	25(102)	24.5(100)	24.7(101)	24.4(100)	23.6(96)	24.3(99)	23.9(98)	24.1(98)	23.8(97)
CRP	0.345(100)	0.331(96)	0.334(97)	0.326(94)	0.326(94)	0.32(93)	0.317(92)	0.32(93)	0.321(93)	0.32(97)
ASO	22.9(100)	20.8(90)	21.8(95)	21.4(93)	23.5(103)	22.7(99)	24.7(108)	26.5(116)	25.1(110)	26.4(115)
RF	29.6(100)	30.7(104)	29.3(99)	31.5(106)	30.4(103)	31.5(106)	30.6(103)	31.2(105)	29.4(99)	26.4(89)
β2MG	1.4(100)	1.5(107)	1.5(107)	1.4(100)	1.4(100)	1.5(107)	1.5(107)	1.6(114)	1.5(107)	1.6(114)
MMP-3	78.9(100)	74.7(95)	71.8(91)	74.7(95)	68.8(87)	75.7(96)	80.8(102)	81.4(103)	78.9(100)	73.4(93)

Table.2D Effects of hemolysis on tumor marker assays KL-6 and troponinT

Hb(mg/dL)	0	0.28	0.42	0.56	0.7	0.84	0.98	1.12	1.26	1.4
AFP	2.81(100)	2.89(103)	2.79(99)	2.67(95)	2.66(95)	2.55(91)	2.64(94)	2.48(88)	2.6(93)	2.57(91)
CEA	11.89(100)	11.38(96)	11.94(100)	11.63(98)	11.98(101)	11.34(95)	11.71(98)	11.54(97)	11.92(100)	11.97(101)
CA19-9	109(100)	108.2(99)	106.5(98)	108(99)	108.5(100)	108.7(100)	109.3(100)	108.7(100)	109.1(100)	111.1(102)
CA125	55.74(100)	56.04(101)	55.53(100)	55.82(100)	54.4(98)	55.54(100)	54.44(98)	56.4(101)	55.98(100)	55.67(100)
CA72-4	3.5(100)	3.52(101)	3.5(100)	3.57(102)	3.51(100)	3.52(101)	3.55(101)	3.52(101)	3.57(102)	3.52(101)
CA15-3	7.5(100)	7.7(96)	7.5(100)	7.5(100)	7.5(100)	7.5(100)	7.3(97)	7.6(101)	7.4(99)	7.5(100)
FER	57.7(100)	57.2(99)	58(105)	59.4(103)	61(106)	58.6(102)	59.6(103)	61(106)	60.9(106)	60.4(105)
HCG	0.9(100)	0.9(100)	0.9(100)	0.8(89)	0.8(89)	0.8(89)	0.8(89)	0.9(100)	0.8(89)	0.9(100)
HCG(+β)	0.685(100)	0.68(99)	0.67(98)	0.684(100)	0.639(93)	0.643(94)	0.638(93)	0.662(97)	0.702(102)	0.645(94)
PSA	0.659(100)	0.655(99)	0.657(100)	0.653(99)	0.657(100)	0.656(100)	0.649(98)	0.658(99)	0.651(99)	0.664(101)
NSE	6.5(100)	29.81(459)	54.24(834)	77.36(1190)	99.42(1530)	121.4(1868)	143.7(2211)	162.7(2503)	186.6(2871)	206.2(3172)
CYFRA	2(100)	1.99(100)	2.01(101)	2.01(101)	2.02(101)	1.99(100)	2.04(102)	2.01(101)	2(100)	2.04(102)
SCC	1.4026(100)	1.3726(98)	1.3667(97)	1.4761(105)	1.3833(99)	1.4281(102)	1.506(107)	1.4259(102)	1.4968(107)	1.4703(105)
TNT	0.113(100)	0.101(89)	0.093(82)	0.086(76)	0.084(74)	0.081(72)	0.079(70)	0.077(68)	0.076(67)	0.075(66)
KL-6	130(100)	131(101)	137(105)	132(102)	128(98)	130(100)	127(98)	130(100)	130(100)	130(100)

Table.2E Effects of hemolysis on endocrine assays

Hb(mg/dL)	0	0.28	0.42	0.56	0.7	0.84	0.98	1.12	1.26	1.4
GH	1.41(100)	1.37(97)	1.33(94)	1.37(97)	1.39(99)	1.4(99)	1.4(99)	1.41(100)	1.4(99)	1.35(96)
LH	15.9(100)	15.7(99)	16.1(101)	15.8(99)	15.7(99)	15.7(99)	16.1(101)	15.8(99)	15.4(97)	16.1(101)
FSH	53.7(100)	53.9(100)	53.1(99)	54.1(101)	54.9(102)	53.5(97)	53.9(100)	55(102)	53.9(100)	52.7(98)
PRL	13(100)	12.9(99)	12.6(97)	12.7(98)	12.8(98)	12.8(98)	12.7(98)	12.8(98)	12.8(98)	12.3(95)
ACTH	8.6(100)	6.96(81)	5.29(62)	4.01(47)	3.23(38)	2.57(30)	1.98(23)	1.8(21)	1.38(16)	1.18(14)
TSH	1.8(100)	1.8(100)	1.59(99)	1.56(98)	1.56(98)	1.57(98)	1.57(98)	1.55(97)	1.56(98)	1.53(96)
FT3	2.56(100)	2.66(104)	2.42(95)	2.73(107)	2.61(102)	2.67(104)	2.66(104)	2.64(103)	2.66(104)	2.56(100)
FT4	1.22(100)	1.21(99)	1.26(103)	1.22(100)	1.24(102)	1.21(99)	1.24(102)	1.22(100)	1.26(103)	1.25(102)
Tg	10.58(100)	10.46(99)	10.37(98)	10.36(98)	10.25(97)	10.16(96)	9.95(94)	10.23(97)	10.19(96)	9.97(94)
TgAb	160.5(100)	156.9(98)	158.8(99)	158.7(99)	156.7(98)	162(101)	153.6(96)	159.4(99)	155.9(97)	160.5(100)
TPOAb	35.9(100)	36(100)	35.5(99)	36(100)	34.3(96)	34.3(96)	35.6(99)	36.1(101)	35.7(99)	34.7(97)
PCT	0.55(100)	0.515(94)	0.514(93)	0.521(95)	0.51(93)	0.49(89)	0.487(89)	0.484(88)	0.466(85)	0.452(82)
CORT	9.6(100)	9.57(100)	9.47(99)	9.33(97)	9.34(97)	9.54(99)	9.52(96)	9.69(101)	9.33(97)	9.52(99)
IRI	17.3(100)	14.3(83)	11.5(66)	9.3(54)	9.8(57)	8.3(48)	7.6(44)	6.9(40)	6.4(37)	6.2(36)
CPR	2.5(100)	2.5(100)	2.5(100)	2.5(100)	2.5(100)	2.5(100)	2.5(100)	2.5(100)	2.5(100)	2.4(96)
BNP	189.82(100)	168.02(89)	157.49(83)	146.14(77)	135.21(71)	126.1(66)	120.87(64)	113.01(60)	104.94(55)	102.98(54)
Pro-BNP	1514(100)	1529(101)	1488(98)	1481(98)	1481(98)	1504(99)	1505(99)	1485(98)	1482(98)	1507(99)

Discussion

Hemolysis is common in blood specimens: in this study 8.6% of all specimens yielded hemolysis. Even though the frequency of hemolysis is highly variable among institutions, our data seems much higher than other reports, suggesting that criteria for hemolysis vary.
We examined in vitro hemolysis's quantitative effect on 80 laboratory tests that are routinely done in the laboratory. At level 2 hemolysis, 11 test levels were increased and 7 test levels were decreased by hemolysis.
First, hemolysis can falsely increase blood constituents, such as K, AST, LD, Fe, and NSE, by cellular release because of their high content in red blood cells.Second, Hb released by hemolysis can falsely increase colorimetric results for substances such as TTT, ZTT and hyarulonic acid measured by turbidity and falsely decrease by inhibiting biochemical reactions substances such as TBIL, GA, 1,5AG, TNT and MMP3. Third, protease released by hemolysis has effects on endocrine tests such as BNP, ACTH and IRI.If a reliable correction factor existed for hemolyzed specimens, we could provide accurate test results.
Because hemolysis influences many laboratory tests in many ways, we should know the extent of the influence of hemolysis and report the test results with more proper comments about hemolysis.
More reports like ours may eventually contribute to avoiding misdiagnosis by reducing the hemolysis rate, by instigating more precise and detailed test results, and by raising awareness of the extensive influence of in vitro hemolysis.

Study for possibility of medi
at disaster using the i-STAT
point-of-care analyzer
- Second report: measurement in th

Koyo Shirai[1], Chitoshi Sato[1], Kosuke Amano[1], Kazuhiro Hayashi[1], Osamu Yamada[1], Mitsuhiro Hori[1]
[1]Department of Clinical Laboratory, Okazaki city Hospital
[2]Department of Virology and Parasitology, Hamamatsu University School of M

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e compared laboratory data acquired by i-STAT1
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cold agents, 3) or the outside of the building
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nainly used to analyze blood gas, electrolyte
d samples, because these items were thought
medical treatment in the disaster situation.
e collected from authors and volunteers.
od gas and electrolyte, were simultaneously
ab 1265 (Siemens, Germany) which is used as
laboratory, except for glucose being analyzed
an). The acquired data by the different
compared.

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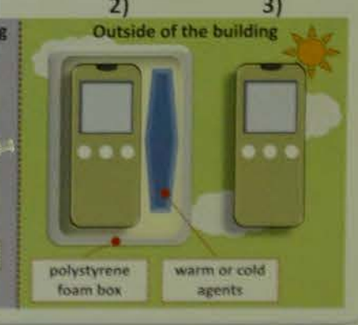

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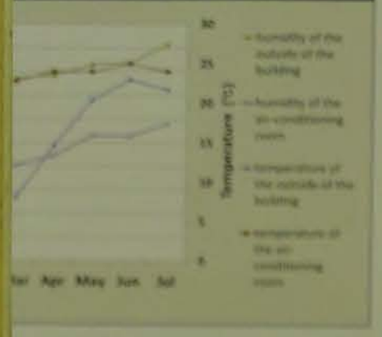

ta by i-STAT1 to corresponding data by
GA-08 were examined to clarify the
using polystyrene foam box containing
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air-conditioning room, the inside of the
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the building and were shown in Fig.2,
pectively.

Fig. 2 The ratios of data by i
Rapidlab1265 or GA-08,

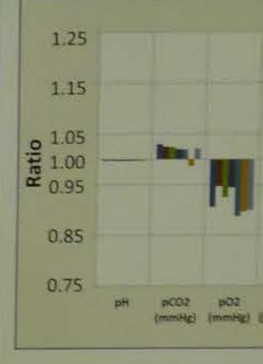


Fig. 3 The ratios of data by i-
Rapidlab12
in the polystyrene foam box

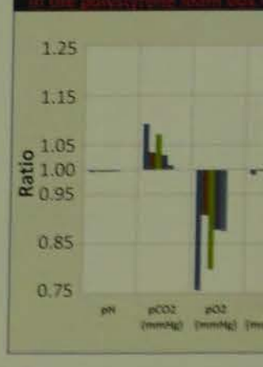


Fig. 4 The ratios of data by i-S
Rapidlab1265 or GA-08,

Discussion

This study revealed that there
between measurement meth
pO2, electrolyte and glucose
previous report in the 32nd W
Biomedical Laboratory Scien
change of the humidity did n
measurement in the air-cond
The polystyrene foam box c
agents which is our original i
environmental temperature
We thus suggest that POCT e
could obtain reliable results
the disaster.

Acknowled

This work was partly supp
organizatios of J.O.C.V. and

Clinical Chemistry

PD-36

The effect of water quality degradation for automatic analyzer

Mixture mechanism of calcium and consideration of the influential amount

Shigeo Sueyoshi[1)] Masashi Amemiya[2)] Yoshihito Ito[3)] Yasuhiro Yoshikawa[4)] Masanori Kanazawa[5)]

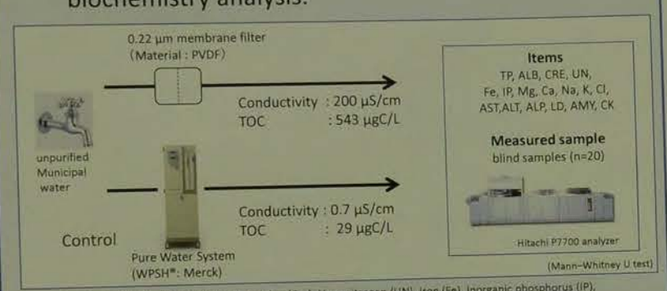
1) Chiba Cancer Center 2) Chiba Children's Hospital 3) Sanritsu Co.,Ltd.
4) Kameda Medical Center 5) Merck Ltd.

Introduction

We have observed that the results of 7 items out of 31 are affected if unpurified municipal water is used to feed automatic analyzers. Ca and Mg were the most affected items. We investigate the data shift and wider dispersion mechanism of 2 electrolytes, it proved water mixed into the reaction cuvette through the reagent pipette.

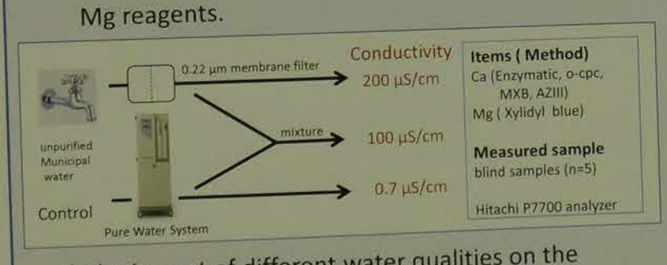
Methods

1) The impact of different water qualities on the biochemistry analysis.

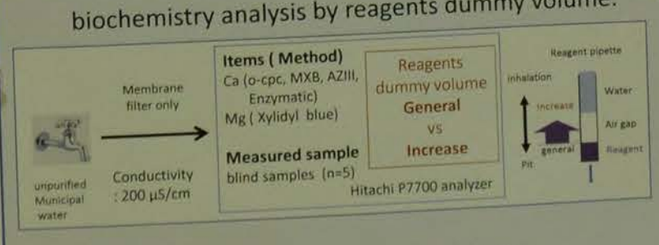


Protein, total (TP), Albumin (Alb), Creatinine (Cre), Urea nitrogen (UN), Iron (Fe), Inorganic phosphorus (IP), Magnesium (Mg), Calcium (Ca), Sodium (Na), Potassium (K), Chloride (Cl),Aspartate aminotransferase(AST), Alanine aminotransferase(ALT), Alkaline phosphatase(ALP), Lactate dehydrogenase(LD), Amylase(AMY), Creatine kinase(CK)

2) The impact of different water qualities by Ca and Mg reagents.



3) The impact of different water qualities on the biochemistry analysis by reagents dummy volume.



4) Investigation by the contamination on the biochemistry analysis.

Measured sample and reagent : Orange G (absorbance=1.027)
Automatic analyzer : Hitachi P7700 P module

Condition
1. S.V. = 3.5 μL, R.V.1 = 120 μL, R.V.2 = 40 μL (parameter of Ca enzymatic)
2. S.V. = 2.0 μL, R.V.1 = 240 μL (parameter of Mg xylidyl blue)

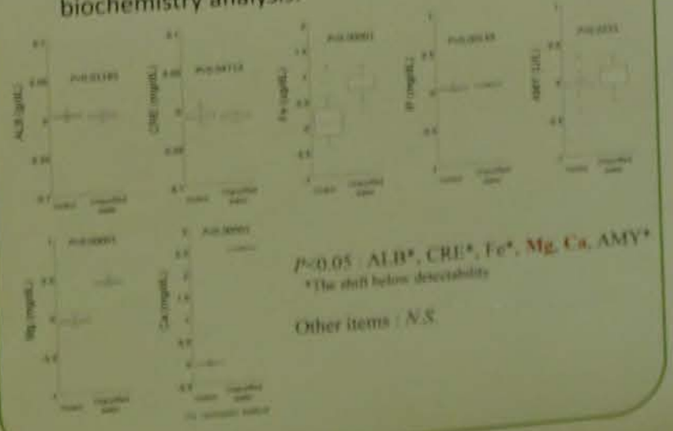
The solutions were manually sampled from the reaction cuvette at each step of the operation.
a) sample injection → reagent injection → mixing → STOP
b) sample injection → reagent injection → mixing **(remove)** → STOP
c) sample injection **(remove)** → reagent injection → mixing **(remove)** → STOP
d) sample injection **(remove)** → reagent injection **(remove)** → mixing **(remove)** → STOP
e) sample injection **(remove)** → reagent injection **(remove)** → mixing **(remove)** → STOP

These solutions were measured with a spectrophotometer. (n=4)

sample volume (S.V.), Reagent volume(R.V.)

Results and Discussions

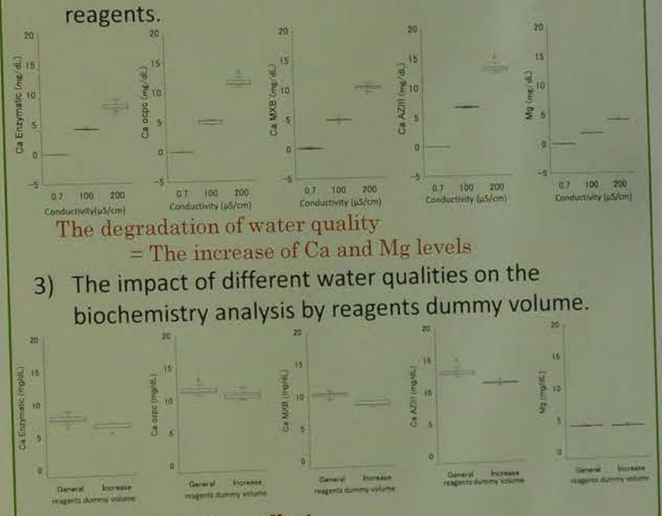
1) The impact of different water qualities on the biochemistry analysis.



$P<0.05$: ALB*, CRE*, Fe*, **Mg**, **Ca**, AMY*
*The shift below detectability

Other items : *N.S.*

2) The impact of different water qualities by Ca and Mg reagents.

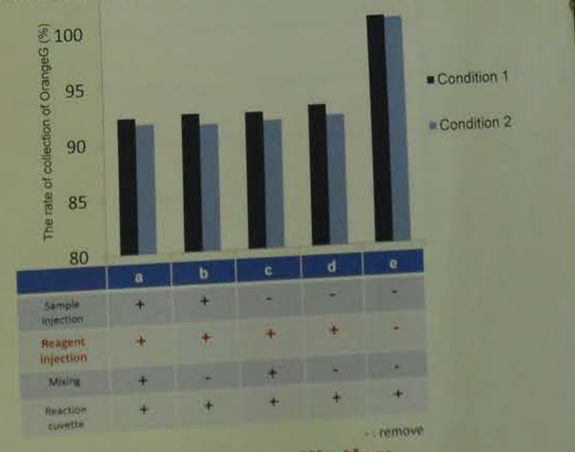


The degradation of water quality
= The increase of Ca and Mg levels

3) The impact of different water qualities on the biochemistry analysis by reagents dummy volume.

Reagent injection : **effect**

4) Investigation by the contamination on the biochemistry analysis.



	a	b	c	d	e
Sample injection	+	+	-	-	-
Reagent injection	+	+	+	+	-
Mixing	+	-	+	-	-
Reaction cuvette	+	+	+	+	+

- : remove

Reagent injection → dilution

water mixed into the reaction cuvette through the reagent pipette.

The relation of ion content to Conductivity

Conductivity (μS/cm)	Ca (mg/dL)
0.0548	0
0.1	0.0039
0.5	0.039
1	0.082
5	0.43
10	0.87

The influence of mixing water to the Ca measurement
(In case of S.V. =2.0 μL, R.V. =180 μL, Mixing water = 12 μL)

	Volume (μL)	Ca Conductivity (mg/dL)	Ca content (μg)	Mixing Ratio
sample	2	10	0.2	-
Mixing water : Conductivity (μS/cm)				
0.1	12	0.0039	0.0005	0.2 %
0.5		0.039	0.0047	2.3 %
1		0.082	0.01	4.9 %
5		0.43	0.05	25.8 %
10		0.87	0.10	52.0 %

Calculated : $CaNO_3$ from water conductivity
The 4th edition electrochemistry Handbook, Maruzen Co., Ltd. P2, 1985

Conclusion

Although Clinical Laboratory Reagent Water (CLRW), as defined by the CLSI, should have a conductivity below 0.1 μS/cm, some analyzer manufacturers still recommend less than 1 μS/cm. 1 μs/cm is equivalent to a maximum of 0.082mg/dL Ca. Assuming a target sample contains 10 mg/dL, Maximum 4% Ca is added into a reaction cuvette. In conclusion, water quality is an important parameter in the measurement of electrolytes. 1 μs/cm water is too high conductivity for reliable results.

Clinical Chemistry

PD-37

Study for possibility of medical technology at disaster using the i-STAT1 portable point-of-care analyzer

- Second report: measurement in the field -

Koyo Shirai[1], Chitoshi Sato[1], Kosuke Amano[1], Kazuhiro Hayashi[1], Akira Ishih[2], Osamu Yamada[1], Mitsuhiro Hori[1]

[1]Department of Clinical Laboratory, Okazaki city Hospital

[2]Department of Virology and Parasitology, Hamamatsu University School of Medicine

Purpose

Operating temperature of i-STAT1 is recommended between 16 and 30 degrees Celsius, and thus it seems to be difficult to use this equipment under severe environment in the field of tropical or cold area in the world as well as severe seasons in Japan.
We have already reported some device keeping the optimum measurement environment for working i-STAT1 analyzer elsewhere. Then we compared laboratory data acquired by i-STAT1 analyzer kept under three different measurement environments.

Methods

Three i-STAT1 analyzers introduced for a disaster and/or emergency in our hospital were used. Each analyzer was put in the 1) air-conditioning room, 2) the inside of the polystyrene foam box containing warm or cold agents, 3) or the outside of the building (Figure 1). To examine the influence of measurement environment, clinical laboratory data of the same sample were acquired monthly from each machine. We selected CG8+ cartridge which is mainly used to analyze blood gas, electrolyte and glucose in blood samples, because these items were thought to be important for medical treatment in the disaster situation. Blood samples were collected from authors and volunteers.
The test items, blood gas and electrolyte, were simultaneously analyzed by Rapidlab 1265 (Siemens, Germany) which is used as routine work in our laboratory, except for glucose being analyzed by GA-08 (A&T, Japan). The acquired data by the different analyzers were also compared.

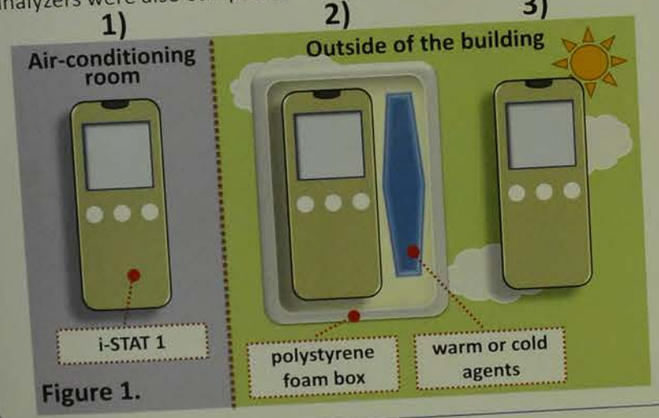
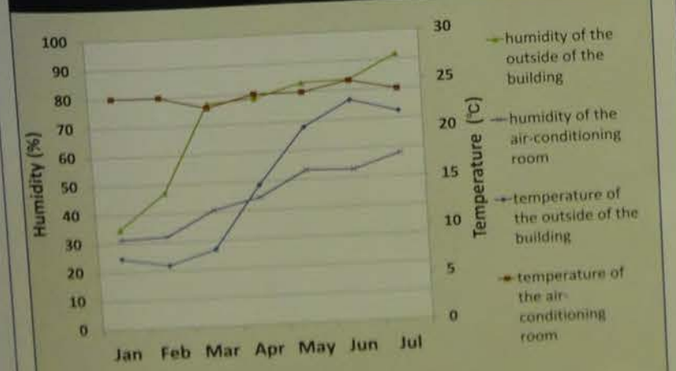


Figure 1.

Result

The figure 1 shows that the changes of temperature and humidity of the air-conditioning room and the outside of the building, from January to July in Japan.

Fig.1 The changes of temperature and humidity from January to July in 2016



The ratios of data by i-STAT1 to corresponding data by Rapidlab1265 or GA-08 were examined to clarify the validity of device using polystyrene foam box containing warm or cold agents.
The results of the air-conditioning room, the inside of the polystyrene foam box containing warm or cold agents, or the outside of the building and were shown in Fig2, Fig3 and Fig4, respectively.

Fig. 2 The ratios of data by i-STAT1 to corresponding data by Rapidlab1265 or GA-08, in the air-conditioning room

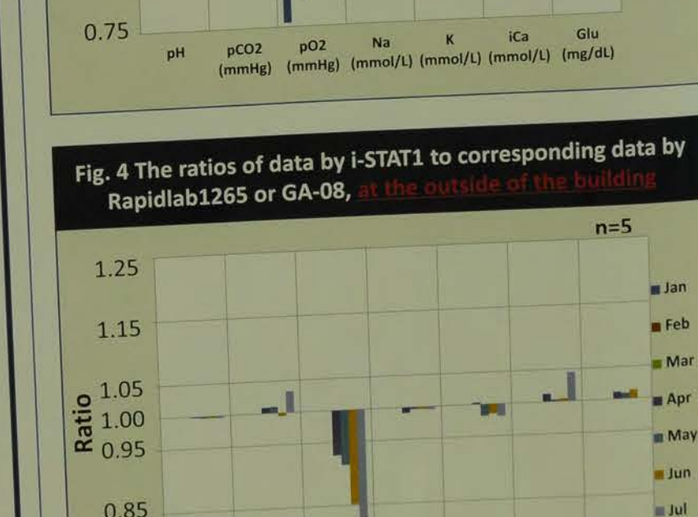


Fig. 3 The ratios of data by i-STAT1 to corresponding data by Rapidlab1265 or GA-08, in the polystyrene foam box containing warm or cold agents

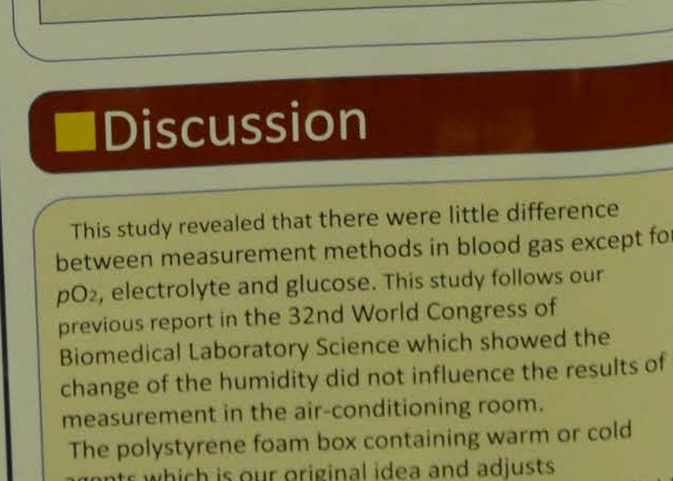


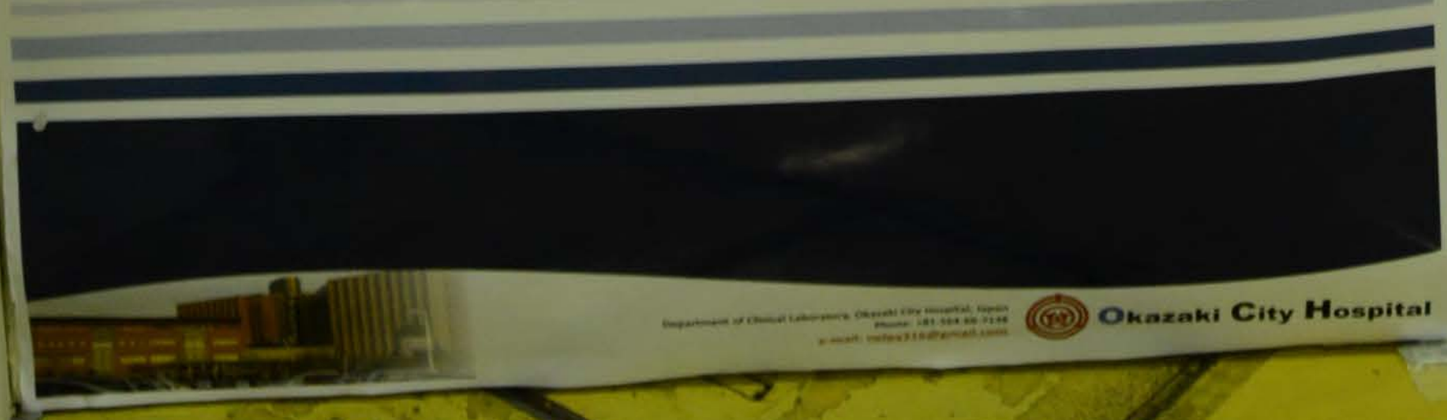
Fig. 4 The ratios of data by i-STAT1 to corresponding data by Rapidlab1265 or GA-08, at the outside of the building

Discussion

This study revealed that there were little difference between measurement methods in blood gas except for pO_2, electrolyte and glucose. This study follows our previous report in the 32nd World Congress of Biomedical Laboratory Science which showed the change of the humidity did not influence the results of measurement in the air-conditioning room.
The polystyrene foam box containing warm or cold agents which is our original idea and adjusts environmental temperature might be useful in the field. **We thus suggest that POCT equipment inside of device could obtain reliable results of measurement under the disaster.**

Acknowledgments

This work was partly supported by the supporting organization of J.O.C.V. and Mitsubishi UFJ Foundation



Okazaki City Hospital

B-1

Clinical Chemistry
PD-38

Possibility of Introduction of i-STAT1 Portable Point-of-care Analyzer for Acquiring Clinical Laboratory Data at Disaster

-First report: Optimum measurement environment for usingi-STAT1 analyzer-

Kosuke Amano[1], Chitoshi Sato[1], Koyo Shirai[1], Kazuhiro Hayashi[1], Akira Ishih[2], Osamu Yamada[1], Mitsuhiro Hori[1]

[1]Department of Clinical Laboratory, Okazaki city Hospital

[2]Department of Virology and Parasitology, Hamamatsu University School of Medicine

Purpose

At the situation of disaster, we are often forced to do laboratory testing under severe environment such as high/low temperature or high humidity – for example, the temporary tent without air conditioner in JMTDR Pakistan mission (Picture 1), and the working in the squall in JMTDR Philippine mission (Picture 2).

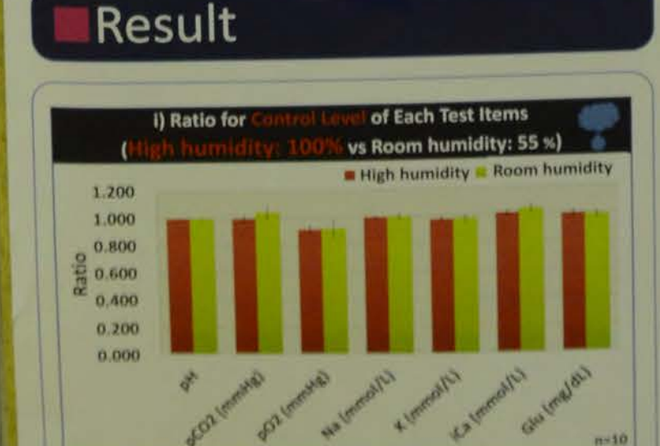
In this project, effects of temperature and/or humidity changes on laboratory data were examined inside the air-conditional room producing severe conditions, using i-STAT1 portable point-of-care analyzer.

Picture 1:
JMTDR Pakistan mission

Picture 2:
JMTDR Philippine mission

Methods

A) We made the device, which was the polystyrene foam box containing warm or cold agents (Picture 3), to keep i-STAT1 (Abott, Japan) appropriate temperature.



Picture 3: The device we made

B) We produced environments as follows;
i) high humidity (100%) ,
ii) low humidity (7%) ,
iii) high temperature (37℃) ,
iv) low temperature (6℃),
and examined whether or not i-STAT1 analyzed accurately blood gas, electrolyte and glucose in blood samples under these environments.

Rapidlab 1265 (Siemens, Germany) for blood gas and electrolyte and GA-08 (A&T, Japan) for glucose were used to get control data.

Blood samples were collected from authors and volunteers.

Result

Figure 1

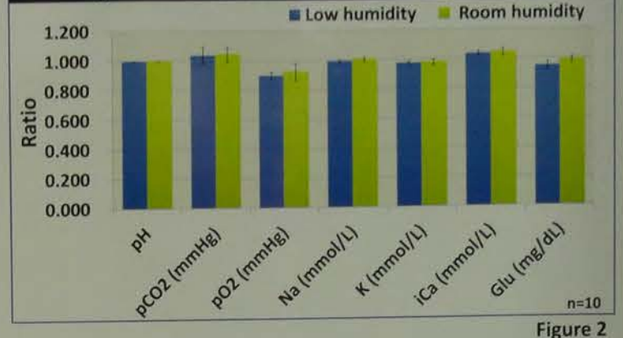


Figure 2

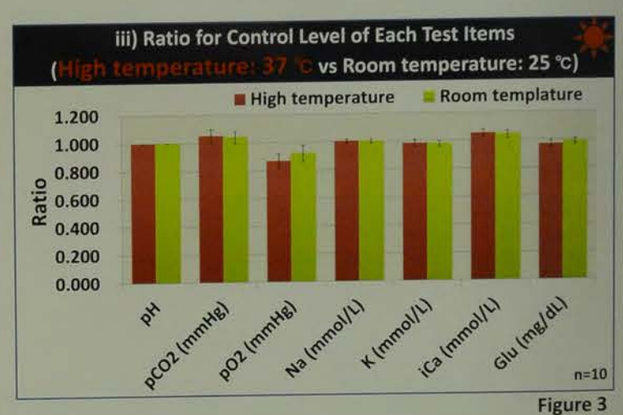


Figure 3

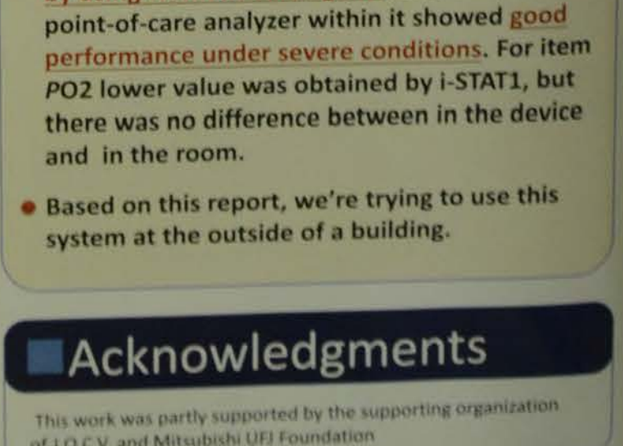


Figure 4

Taken together, i-STAT1 kept under the environment of i)～iv) showed good performance which was corresponding to that of the equipment used in laboratory.

Conclusion

- **In this study, there was no difference between results in laboratory environment and high/low humidity.**
- **The device could keep appropriate environment by using warm or cold agents, and the portable point-of-care analyzer within it showed good performance under severe conditions. For item *P*O2 lower value was obtained by i-STAT1, but there was no difference between in the device and in the room.**
- **Based on this report, we're trying to use this system at the outside of a building.**

Acknowledgments

This work was partly supported by the supporting organization of J.O.C.V. and Mitsubishi UFJ Foundation

Okazaki City Hospital

Clinical Chemistry

PD-39

Evaluation of Immunobiochemical Hybrid-type Automatic Analyzer cobas8000

Junya Takahashi[1], Noriyasu Niizeki[1], Hitomi Kurose[2], Keisuke Nozawa[1], Naomi Onodera[1], Mineji Tachibana[1], Yutaka Tomoda[1], Satoshi Fujii[1]

1) Department of Medical Laboratory and Blood Center, Asahikawa Medical University Hospital
2) Sapporo Medical Technology , Welfare and Dentistry Professional Training College of Nishino Gakuen School Foundation

Introduction

We obtain the opportunity to evaluate the basic performance of cobas8000 (Roche Diagnostics) composed of photometric measuring unit c702 and electrochemiluminescence technology measuring unit e602. We confirm cobas8000 is useful for routine examination.
In addition, we evaluate carry-over using high-level HBsAg samples to measure biochemistry items and immune test items.

Purpose

To evaluate the basic performance of cobas8000 and confirm whether cobas8000 can prevent carry-over.

Results

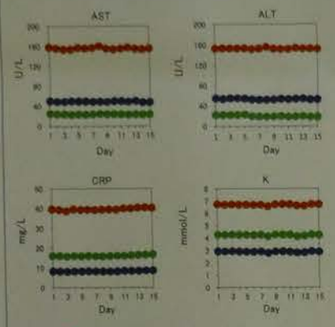
(1) Coefficient of variance (CV) of within-run repeatability is 0.16-21.30%. CV of between-run repeatability is 0.29-28.48%.
(2) Difference from target value is -3.07-2.80%.
(3) The linearity of up to 3,007.5 U/L in ALP, 1,390 U/L in AST, 1,286 U/L in ALT, 127g/L in ALB, 1,398.8 mg/L in CRE, 11,800 mg/L in GLU and 204.2 mmol/L in Na were confirmed.
(4) Hemolysis hemoglobin affords positive error in TP, AST, LD and IP and negative error in DBIL.
Free bilirubin affords positive error in CKMB. Chyle and ascorbic acid afford no effects on all items.
(5) Correlation coefficient (r) between contrastive reagents is 0.910-0.999.
(6) LoQ is 5.5 U/L in AST, 3.6 U/L in ALT, 0.7 g/L in ALB, 0.48 mg/L in CRE, 3.5 mg/L in GLU, and 13.7 mg/L in BUN.
(7) With probe cleaning system, carry-over is absent.

Discussion and conclusion

Basic performance of cobas8000 and correlation with the contrastive reagents is satisfactory. But, in study of effects of interfering substances, hemolysis hemoglobin and free bilirubin affect more than 10% error to some of the items (Fig.4). Although the cause is unknown, we consider these errors due to concentrations of sample. Because of using low concentrations sample, the ratio of errors is large. All of the detailed data is shown in the lower right of this poster.
Probe cleaning system is considered to prevent carry-over so we can measure immune and biochemistry items at the same time.
Thus, cobas8000 may reduce reporting time by selecting shortest measure time of immune and biochemistry items and added measure of immune items.
From the results of this studies, we evaluate cobas8000 is useful for routine examination.

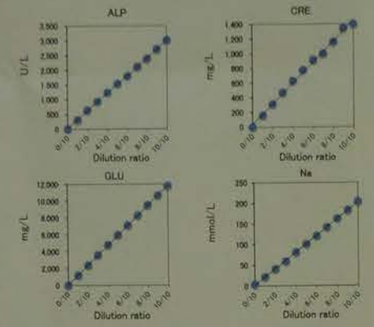
Basic performance of cobas8000

Fig.1 Between-run repeatability.



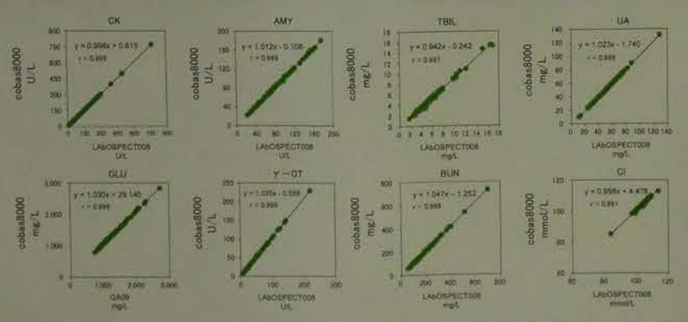
Between-run repeatability of four items (AST, ALT, CRP and K) are shown.
We confirm good between-run repeatability.

Fig.2 Linearity.



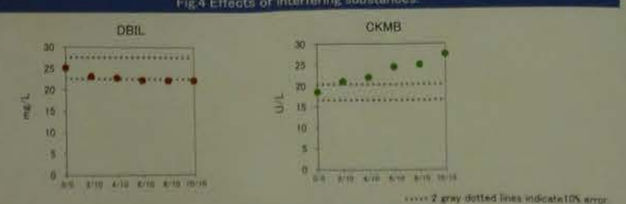
Linearity of four items (ALP, CRE, Na and GLU) are shown. We confirm better linearity than values written on the reagent instructions.

Fig.3 Correlation with contrastive reagents.



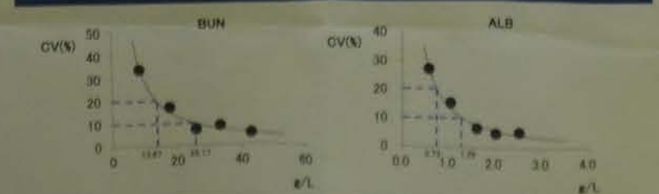
Correlation with contrastive reagents of eight items (CK, AMY, TBIL, UA, GLU, γ-GT, BUN and Cl) are shown. We confirm good correlations versus LAbOSPECT08 and GA09.

Fig.4 Effects of interfering substances.



Effect of hemolysis hemoglobin for DBIL is shown as ●.
Effect of free bilirubin for CKMB is shown as ●.
Concentrations of hemolysis hemoglobin is 0-5200 mg/L (0/0-10/10). Concentrations of free bilirubin is 0-201 mg/L (0/0-10/10). Items affected more than 10% by interfering substances are shown.

Fig.5 Sensitivity test.



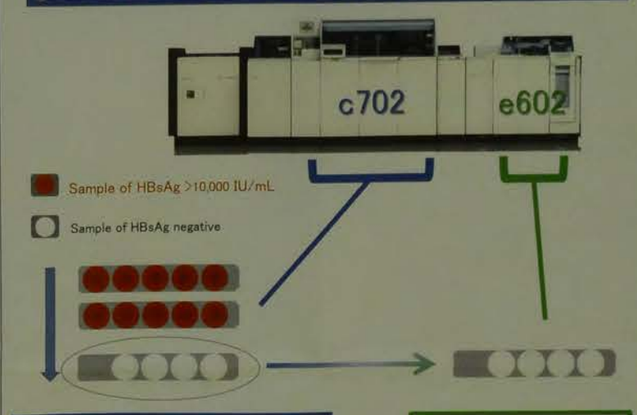
BUN	Sample	n	Unit : g/L
LoB	Saline	80	4.23
LoD	Low concentrations sample	60	5.18
LoQ	Low concentrations sample	60	CV 10% 15.17 CV 20% 13.61

ALB	Sample	n	Unit : g/L
LoB	Saline	80	0.39
LoD	Low concentrations sample	60	0.80
LoQ	Low concentrations sample	60	CV 10% 1.28 CV 20% 0.75

Precision curves calculated from LoB and LoD of some of the items (BUN, ALB) are shown.
In addition, values of study are shown in tables.
Cobas8000 is reliable to measure low concentrations sample.

Effect of carry-over

Sample probe of c702 can be permanently with cleaning system.
If cleaning of sample probe is inadequate, carry-over may occur through sample probe by high concentrations of samples.
Thus, we evaluate probe cleaning system using high concentrations samples of HBs antigen (HBsAg). These sample's concentrations are over 10,000 IU/mL.
The study method is shown below. We evaluate the carry-over from high concentrations samples of HBsAg to negative samples using 3 patterns of the probe cleaning system (①: Water and Water ②:Water and alkaline solution ③: Alkaline solution and alkaline solution) in c702.



Measuring some biochemical items 10 times in samples with HBsAg concentrations >10,000 IU/mL in c702. Then, measuring same items 4 times in samples with HBsAg negative in c702.

Measuring HBsAg concentrations of HBsAg negative samples in e602.

Is there carry-over of HBsAg?

Table.1 HBsAg measurement of HBsAg negative samples in e602.

Cleaning pattern	① Water + Water	② Water + Alkaline solution	③ Alkaline solution + Alkaline solution
HBsAg >10,000 IU/mL samples	11,992 IU/mL	11,992 IU/mL	14,473 IU/mL
HBsAg negative samples	<0.05 IU/mL	<0.05 IU/mL	<0.05 IU/mL
	<0.05 IU/mL	<0.05 IU/mL	<0.05 IU/mL
	<0.05 IU/mL	<0.05 IU/mL	<0.05 IU/mL
	<0.05 IU/mL	<0.05 IU/mL	<0.05 IU/mL

There is no carry-over in all cleaning systems.

Data of study

Item	Repeatability		Accuracy	Linearity	Correlation	Sensitivity	
	CV (%) within-run reproducibility	CV (%) Between-run reproducibility	relative error (%)		r	LoB	LoD
AST	1.02 - 3.44	1.74 - 2.48	-0.83 - 3.29	1,390.0 U/L	0.999	1.34 U/L	3.05 U/L
ALT	0.70 - 4.03	0.74 - 8.45	0.60 - 3.78	1,286.0 U/L	0.998	1.03 U/L	2.00 U/L
ALP	0.85 - 0.88	1.15 - 2.24	2.15 - 2.98	3,007.5 U/L	0.999	1.99 U/L	3.89 U/L
γ-GT	1.03 - 1.18	0.59 - 1.51	0.34 - 2.64	2,874.5 U/L	0.999	0.48 U/L	1.99 U/L
LD	0.73 - 0.86	1.15 - 1.55	-0.69 - 3.00	3,473.5 U/L	0.999	0.24 U/L	0.81 U/L
CK	0.49 - 0.81	0.65 - 1.84	-0.17 - 2.61	5,186.5 U/L	0.999	1.44 U/L	3.17 U/L
CKMB	2.04 - 21.30	1.81 - 28.48	1.81 - 3.29	2,290.0 U/L	0.910	0.13 U/L	4.09 U/L
AMY	0.81 - 1.07	0.85 - 1.40	0.18 - 2.54	3,329.5 U/L	0.999	1.50 U/L	3.89 U/L
LIPA	0.91 - 0.98	0.49 - 0.99	-0.78 - 3.28	884.5 U/L	0.999	0.91 U/L	0.97 U/L
ChE	0.50 - 0.71	0.71 - 1.42	-1.07 - 2.90	1,491.5 U/L	0.999	0.95 U/L	4.17 U/L
TP	0.53 - 0.83	0.59 - 1.02	0.51 - 2.58	220.1 g/L	0.999	0.99 g/L	0.99 g/L
ALB	0.85 - 0.91	1.34 - 1.78	0.14 - 2.79	127.0 g/L	0.997	0.35 g/L	0.80 g/L
TBIL	0.71 - 1.45	1.41 - 5.48	-0.79 - 3.34	851.8 mg/dL	0.997	0.33 mg/dL	0.92 mg/dL
DBIL	0.70 - 3.89	3.42 - 4.65	-0.49 - 3.84	327.6 mg/dL	0.991	0.32 mg/dL	0.54 mg/dL
CHE	0.82 - 1.05	0.82 - 1.38	-0.10 - 3.02	1,256.5 mg/dL	0.999	0.19 mg/dL	0.56 mg/dL
UA	0.48 - 1.33	0.74 - 1.84	-0.15 - 1.05	1,134.0 mg/dL	0.999	0.40 mg/dL	0.96 mg/dL
BUN	0.66 - 0.84	1.24 - 1.69	0.10 - 2.96	5,186.5 mg/dL	0.999	4.23 mg/dL	5.18 mg/dL
GLU	0.43 - 0.66	1.00 - 1.95	1.00 - 4.19	1,398.8 mg/dL	0.999	0.96 mg/dL	3.26 mg/dL
Ca	0.66 - 0.82	0.76 - 1.04	-0.16 - 3.01	272.8 mg/dL	0.991	0.91 mg/dL	1.78 mg/dL
IP	0.56 - 0.82	1.10 - 1.43	0.41 - 3.01	429.1 mg/dL	0.999	0.74 mg/dL	0.91 mg/dL
CRP	0.82 - 1.74	1.01 - 1.96	0.05 - 3.43	951.8 mg/dL	0.999	0.00 mg/dL	0.00 mg/dL
Mg	0.14 - 0.24	0.29 - 0.52	-1.14 - 4.08	1,458.1 mmol/L	0.999		
K	0.34 - 0.54	0.74 - 1.56	0.51 - 0.95	19.5 mmol/L	0.999		
Cl	0.17 - 0.50	0.48 - 0.91	-1.01 - 3.80	2,144.5 mmol/L	0.991		

Materials and methods

We use sera from patients. We use cobas reagent in all study.
Test items are AST, ALT, ALP, γ-GT, LD, CK, CKMB, AMY, LIPA, ChE, TP, ALB, TBIL, DBIL, CRE, UA, BUN, GLU, Ca, IP, CRP , Na, K and Cl.
The following parameters are assessed.
(1) Repeatability : We use 2 concentrations of control sera and pooled sera. We evaluate within-run repeatability (n=20) and between-run repeatability (n=15).
(2) Accuracy : We measure calibrators of each items and evaluate difference from target value.
(3) Linearity : We dilute each concentrated samples and evaluate linearity of each items.
(4) Effects of interfering substances : We use Interfering Check A Plus (Sysmex). We evaluate effects of interfering substances in each items.
(5) Correlation : We evaluate correlation with contrastive reagents with LAbOSPECT008 (HITACHI) and GA09 (A&T).
(6) Sensitivity test : We evaluate Limit of Blank (LoB) by measuring saline. We evaluate Limit of Detection (LoD) by measuring low concentrations samples. Limit of Quantitation (LoQ) is calculated by LoB and LoD.
(7) Effect of carry-over : We evaluate carry-over from high-level HBs antigen samples on antigen-negative samples using 3 patterns of the probe cleaning system in c702.

Clinical Chemistry

PD-40

Evaluation of Free and Total Prostate-specific Antigen Assay on the AIA-CL2400 Analyzer

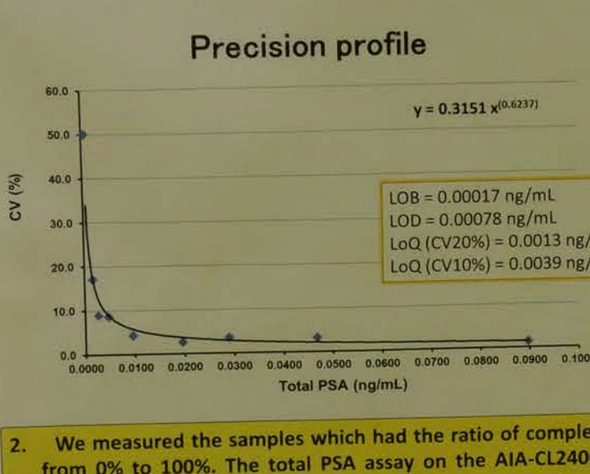
Mika Iwai[1] Kiyomi Nakajima[1] Keita Kamiyama[1] Sakie Fujii[1] Tetsuo Machida[1], Kazuto Ito[2], Masami Murakami[3]

[1]Department of Clinical Laboratory, Gunma University Hospital
[2]Department of Urology, Gunma University Graduate School of Medicine
[3]Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine

Introduction

Prostate-specific antigen (PSA) is a protein that is produced from epithelium of prostate gland and is widely used as a marker of prostate cancer. We evaluated the performance of the newly developed free and total PSA reagents based on chemiluminescence enzyme immunoassay (CLEIA).

Principle of the assay



Methods

Both free and total PSA were measured with a two-step sandwich CLEIA on the fully automated chemiluminescence enzyme immunoassay analyzer AIA-CL2400 (TOSOH Corporation). We used control samples and residual serum samples after routine testing. Correlation studies were performed with ST AIA-PACK free PSA and ST AIA-PACK PSA II on the AIA-2000 analyzer (TOSOH Corporation), respectively.

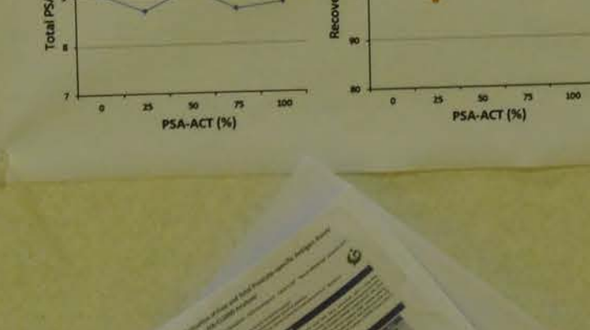
Kits and Analyzers

	AIA-CL2400	AIA-2000
Analyzer	AIA-CL2400	AIA-2000
Principle	CLEIA	FEIA
sample volume	10 μL	20 μL
Reporting time	15min.	20min.
Measurement range (ng/mL)	0.003～100	0.01～100

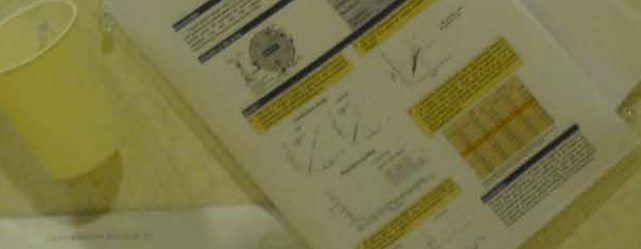
Results

1. Both free and total PSA assays showed good performance in imprecision, dilution linearity, interference and correlation study. Based on the imprecision profile, functional sensitivity at CV20% of the CLEIA PSA was 0.0013 ng / mL.

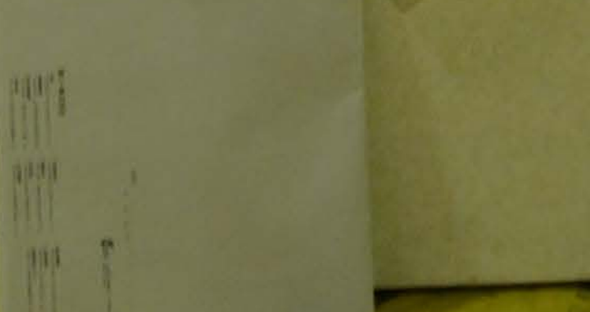
Correlation study



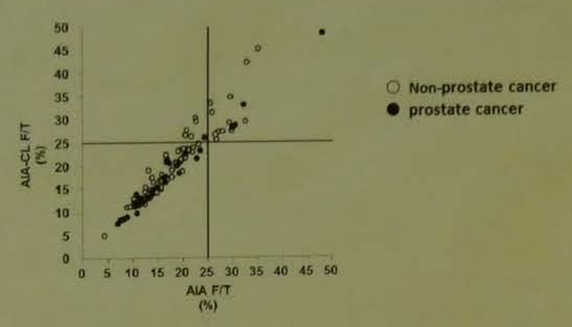
Precision profile



2. We measured the samples which had the ratio of complex PSA from 0% to 100%. The total PSA assay on the AIA-CL2400 was confirmed to have an adequate equimolar-response to report precise PSA concentration.



3. As a result of having examined the correlation of the free/total PSA ratio (F/T) about 95 patient samples of PSA level 4 - 10ng/mL.



4. A comparison of free/total PSA ratio was performed between both assays to evaluate their ability to detect prostate cancer in 34 patients with prostate cancer and 61 patients without prostate cancer in men with PSA levels of 4 to 10 ng / mL. At 85 % sensitivity, free/total PSA ratio obtained by CLEIA showed greater specificity for prostate cancer as compared with that obtained by AIA-2000.

	AIA		AIA-CL	
sensitivity	specificity	F/T	specificity	F/T
100%	0%	47.9%	0%	48.6%
95%	5%	32.2%	7%	33.2%
90%	5%	29.9%	15%	28.3%
85%	20%	23.5%	38%	23.3%
80%	21%	22.8%	41%	22.6%
75%	48%	19.3%	48%	21.3%
70%	49%	18.9%	51%	20.6%
65%	54%	17.4%	51%	20.5%
60%	56%	16.8%	56%	18.4%

34 patients with prostate cancer and 61 patients without prostate cancer were retrospectively selected in men with PSA levels of 4 to 10ng/mL

Conclusions

The newly developed free and total PSA reagents based on CLEIA showed good performance. Clinical specificity of AIA-CL2400 was greater than that of AIA-2000. In addition, the new assays on the AIA-CL2400 are able to report in 15 minutes, show high sensitivity, and need less volume of specimens. Thus, these assays are useful for diagnosis of prostate cancer, monitoring the course of treatment and detection of prostate cancer reoccurrence in clinical practice.

Clinical Chemistry

PD-41

Evaluation of the L-FABP assay using a latex turbidimetric immunoassay and stability of urinary L-FABP

Takaki Iiduka[1] Tomoaki Tsukushi[1] Hitomi Yamaguchi[1] Hiroko Nakazaki[1]
Kazuhiro Uchida[1] Shin-ichi Munekata[1] Yuhsaku Kanoh[1,2]
[1]Department of Clinical Laboratory, Kitasato University Hospital
[2]Department of Laboratory Medicine, Kitasato University School of Medicine

Introduction

Liver-type fatty acid binding protein (L-FABP) is a 14-kDa protein expressed in proximal tubular epithelial cells. Urinary L-FABP is associated with the severity of tubulointerstitial injury. In the present study, we evaluated the performance of an L-FABP kit, "Nordia L-FABP" (Sekisui Medical Co., Ltd.) using a latex turbidimetric immunoassay (LTIA). We also investigated the stability of L-FABP in urine.

Methods

The analytical parameters assessed included within-run and between-day precision, limit of detection, dilution linearity, and interference studies, and the correlation between LTIA and an enzyme-linked immunosorbent assay (ELISA) (L-FABP ELISA kit, CMIC Co., Ltd., Tokyo, Japan). To evaluate the stability of urinary L-FABP over 24 hours, we investigated the effects of storage at room temperature(25° C) , refrigeration (4° C), and freezing (−20° C). In addition, we investigated the effects of pH adjustment and shading on the stability of urinary L-FABP.

Results ① (performance of Nordia L-FABP)

within-run(n=20)

	control L	control H	Pooled urine
Mean(ng/ml)	12.3	55.0	9.2
SD(ng/ml)	0.75	1.56	0.73
CV(%)	6.10	2.83	7.96

between-day(30days)

	control L	control H
Mean(ng/ml)	11.4	53.7
SD(ng/ml)	0.25	1.29
CV(%)	2.19	2.41

Table1 .Within-run and between-day precision
Within-run precision was determined using controls and pooled urine. Between-day precision was determined using controls every 3 days for 30 days.

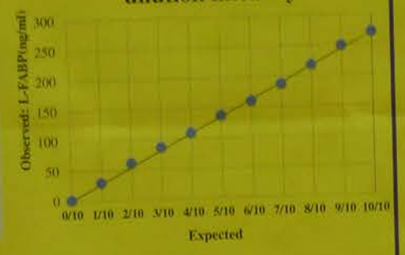


Fig1.Dilution linearity
Each sample was diluted 1:10 with saline solution, in 10 consecutive steps, and measured 3 times. The linearity of the Nordia L-FABP system was confirmed up to ~270 ng/mL.

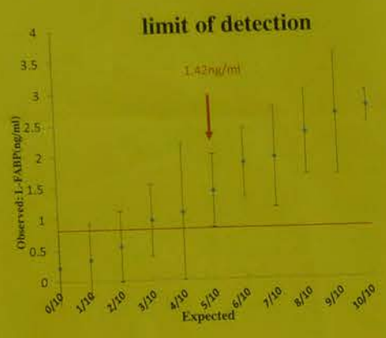


Fig2.Limit of detection
Each sample was diluted 1:10 with saline solution, in 10 consecutive steps, and measured 10 times. The limit of detection was 1.42 ng/mL.

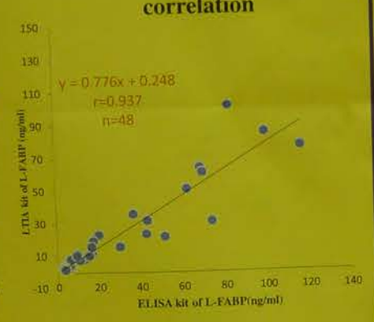


Fig4. Correlation
Passing-Bablok regression analysis revealed a linear correlation (Y=0.776X+0.248, R=0.937, N=48). Samples were used within 2 hours of submission to the laboratory.

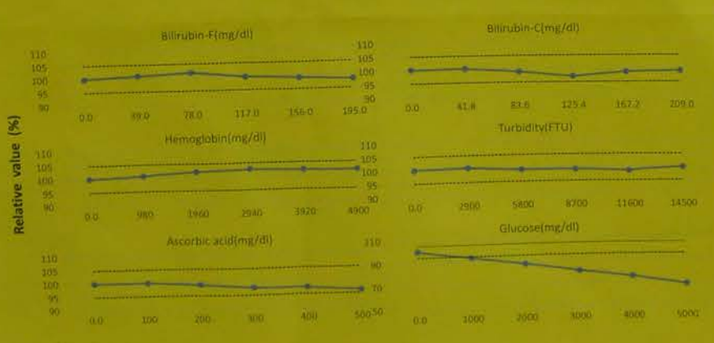


Fig3.Interference
None of the following conditions interfered with the assay: bilirubin F (195 mg/dL), bilirubin C (209 mg/dL), hemoglobin (4900 mg/dL), ascorbic acid (500 mg/dL), or turbidity (14500 FTU). However, glucose interfered with the assay above 1000 mg/dL, showing an interference of 31.6% at 5000 mg/dL. We defined interference as a change in the measurements value exceeding the expected concentration by 5%.

Results ② (stability)

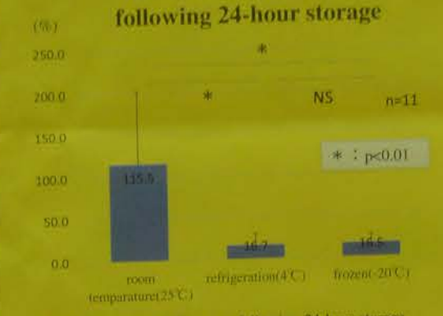


Fig5 .Measurement differences following 24-hour storage
The L-FABP value measured following 24-hour storage at room temperature (25° C) was significantly higher than that measured following 24-hour storage at 4° C (refrigeration) or −20° C (freezing). However, the measurements of the refrigerated and frozen samples did not differ sigmificantly.

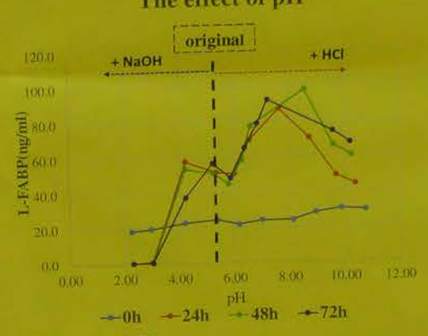


Fig6 The effect of pH
Using 0.1N HCl and 0.1N NaOH, we examined the effect of pH on stability of urinary L-FABP. The original pH was 5.37; urinary L-FABP was used at a concentration of 24.6 ng/mL. There was no immediate effect on pH; however, effects were seen after 24 hours. This graph depicts the result showing that the stability of measured value was not good at either high or low pH. Furthermore, a similar tendency was confirmed in other two cases.

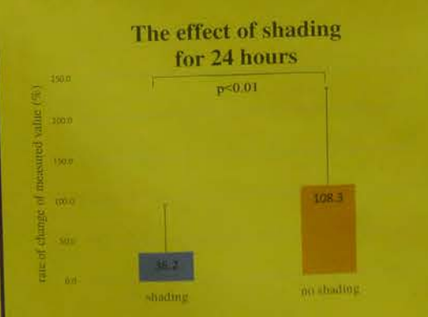


Fig7 The effect of shading for 24 hours
This test compared shaded and unshaded samples. The rate of change of measured value was 108.3% in the unshaded sample. In contrast, the rate of change of measured value was only 36.2% in the shaded sample. Therefore, the effect of shading on samples is significant.

conclusion

The "Nordia L-FABP" system performed well, providing a rapid LITA-based assay. However, highly concentrated urinary glucose can interfere with this system. Urinary glucose often reaches high concentrations in patients with diabetic nephropathy. It has been reported that urinary L-FABP is effective for early diagnosis of diabetic nephropathy. Consequently, particular care must be taken when evaluating measurements in samples from patients with diabetic nephropathy.

The stability of urinary L-FABP was poor at room temperature. Therefore, it is inappropriate to measure L-FABP in urine samples stored at room temperature once 24 hours have elapsed. In this study, we found that shading and refrigeration were effective at maintaining the stability of urinary L-FABP. As 24-hour urine samples are important for quantitative urinalysis, we recommend that they be refrigerated and shaded.

The authors declare no conflict of interest associated with this manuscript.

B-3 B-3

Clinical Chemistry

PD-42

The influence of Hydroxyurea on the Measurement for Glucose by glucose oxidase hydrogen peroxide electrode method.

Yukio Kume[1], Megumi Takahashi[1], Yoshikazu Ono[1], Masahiro Jyona[1], Sigeo Okubo[1], Makoto Kurano[1,2], Yutaka Yatomi[1,2]

1) Department of Clinical Laboratory Medicine, The University of Tokyo
2) Department of Clinical Laboratory Medicine, Graduate School of Medicine,The University of Tokyo

Background: Hydroxyure (HU) is one of the common clinical medicines for the treatment of essential thrombocythaemia (ET). It is well known that some reagents, such as ascorbic acid, interfere with laboratory testing, however the interference of HU with laboratory testing is not common. We have experienced a case of a possible positive interference of HU with glucose oxidase (GOD) hydrogen peroxide electrode reduction method. Therefore, in this study, we investigated whether HU interferes this method.

Methods: We measured plasma glucose with two glucose assay; GOD immobilized enzyme membrane/ hydrogen peroxide reduction electrode method (GOD electrode reduction method, chemical reagents and ADAMS Glucose GA-1171 form ARKRAY Inc, Kyoto, Japan) and Hexokinase Assay method (HK-method, Quick Auto Neo GLU-HK from SHINO-TEST CORPORATION , Kanagawa, Japan).

(1) We measured glucose concentrations of the plasma samples obtained from 4 subjects taking HU with these methods.

(2) We investigated the interference of HU with glucose values determined with each method by adding HU to glucose standard solution. (fig 1.)

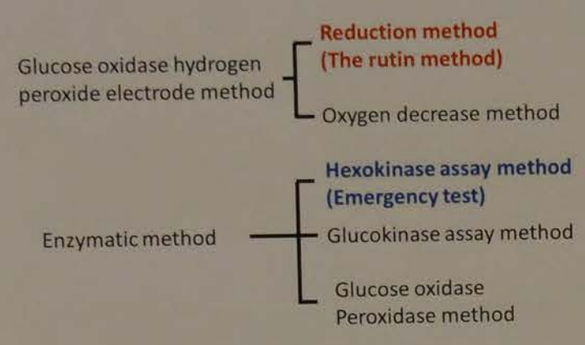


Fig.1. Classification of glucose assays

(3) We determined how much HU passed through the GOD immobilized enzyme membrane with newly developed HPLC-UV detection assay.

Results:

(1) the positive deviation of glucose values determined with GOD electrode reduction method from those determined with HK-method was 5 ～ 8%. (table 1.)

	The rutin method (mg/dL)			Emergency test (mg/dL)
	Electrode Reduction method			Hexokinase Assay
	Analyzer No1.	Analyzer No2.	Analyzer No3.	
Case 1 1.5CP/day	88 (+5)	99 (+16)	85 (+2)	83
Case 2 1CP/day	95 (+2)	100 (+7)	92 (+1)	93
Case 3 1.5CP/day	243 (+7)	249 (+13)	238 (+2)	236
Case 4 1CP/day	192 (+3)	195 (+6)	189 (0)	189

Correlation in the normal plasma y=1.014x-0.312 r=0.998

Table 1. The influence of hydroxyurea on the glucose concentrations determined by glucose oxidase hydrogen peroxide electrode reduction -method.

(2) The addition of HU did not affect the glucose values determined with HK-method, while the addition of HU elevated those determined with GOD electrode reduction method.(table 2,3.)

Hydroxyurea concentration (μg/mL)	Electrode Reduction method Glucose Bias (mg/dl)	Hexokinase assay Glucose Bias (mg/dl)	Glucokinase assay Glucose Bias (mg/dl)	Glucose oxidase Peroxidase Glucose Bias (mg/dl)	Electrode oxygen decrease method Glucose Bias (mg/dl)
0	-	-	-	-	-
50	6	1	0	4	0
100	8	0	0	3	-4
150	18	0	-1	4	-1
200	24	1	0	3	-1
250	29	-1	0	1	-1
300	34	0	0	0	1
350	39	0	0	1	0
400	44	-1	-1	-1	0
450	49	1	1	4	0
500	55	0	0	-2	0

Table 2. Influence of hydroxyurea on each glucose assay
* When patients take 800 mg/m^{2}q4hr of hydroxyurea, the highest blood concentratioin is estimated to rise to 173 μg/mL .

Hydroxyurea concentration (μg/mL)	Electrode Reduction method Glucose concentration (mg/dL)	Bias (mg/dL)
0	149	-
10	150	1
20	151	2
30	153	4
40	154	5
50	155	6
60	156	7
70	158	9
80	159	10
90	160	11
100	161	12

Table 3. Influence of hydroxyurea on the GOD electrode reduction -method at low concentrations.
* When patients take 1000 mg of hydroxyurea, the blood concentratioin is estimated to rise to 20 - 30 μg/mL at 1 ～ 3 hours after the administration.

(3) When we challenged the membrane with 10 mg/ml HU solution, we observed that 60 % of the HU had passed through the membrane.

Conclusions: The medication with HU influences the glucose concentrations determined with GOD electrode reduction -method, but not those determined with HK-method. When we measured glucose with GOD electrode reduction method, we should consider the interference of HU with glucose concentration. (fig 2.)

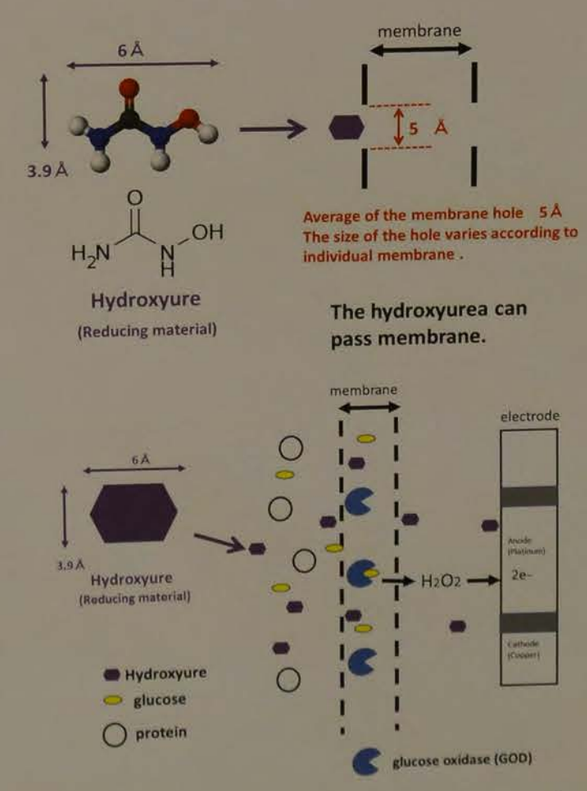


Fig. 2. The mechanism that hydroxyurea cause potivie errots in the GOD electrode reduction - method.

Usefulness of eGFRcre corrected by mus… creatinine in the …

Takahashi Youhei [a], Ukida Minoru [b]

[a] Department of Clinical Laboratory, National Organization for Aute… Okayama Medi-Care Center, Okayama, Japan
[b] Department of Central Clinical Laboratory, Okayama Saiseikai G…

: Creatinine, Cystatin-C, eGFR, muscle-mass-volume, …

…f bedridden patients, muscle-mass-volume …eatinine value (CRE) decrease because of …xercise or the activity. It is hard to use …ar filtration rate (eGFR) using CRE …/aluation of renal function in such condition. …e (Cys-C) which is not affected by mv has …the suspicious case of the renal impairment. …dy is to investigate the usefulness of eGFR …E (eGFRmvcre) compared to eGFR using …

METHODS】

…ere 42 patients of persistent vegetative state …ital.

says:
…ic method
(manufactured by SINOTEST CO.,LTD)
…urbidimetric immunoassay *
…according to the IRMM commutability study
(manufactured by LSI Medience CO.,LTD)
…re analyzed by H7180.
…ed by Hitachi High-Technologies CO.,LTD)

E Corrected By mv":
…nv of each patient using body composition …0), and calculated the correction value from …rd mv adjusted by height and sex, male = …dy weight, female = 72.7% of ideal body …orrected by mv was calculated by CRE and …(CRE corrected by mv = CRE × (standard …

…;
…alculated by method of Japanese Society …tients under eighteen years of age, were used …Society Pediatric Nephrology.

…d that evaluation of calculated result by …mpared eGFRmvcre with eGFRcys using …and correlation.

…s the baseline, clinical data and calculated …ents. The average mv of the patient was …ed with the standard mv of 44.6±8.7kg. The …value was 1.37±0.12. Each of eGFR value …94.0±23.5, eGFRcre = 139.5±38.5 and …±22.9. Figure 1 shows the distribution of …ference range. In the Cys-C and the CRE …e majority of the patients were within the …ut, in the CRE, it showed low value trend …ange. Figure 2 shows the each of distribution …FRcre value was significantly higher than …0.000). There was no significant difference …e value and eGFRcys value (p=0.242). The …of eGFRcre and eGFRcys was y=1.63x-14.2, …coefficient was r=0.268. The regression …vcre and eGFRcys was y=0.97x+7.2, and the …nt was r=0.367 (figure 3). The results of test …een two correlation coefficient were no …e (p=0.059).

…baseline, clinical data, calculated results

…ristic	Male Mean(SD)	Female Mean(SD)	Total Mean(SD)
	27	15	42
	42.2(17.8)	42.4(25.7)	42.3(20.5)
	168.5(7.8)	152.7(7.3)	162.8(10.7)
	53.3(7.0)	46.3(7.8)	50.8(7.8)
	18.7(1.8)	19.8(2.7)	19.1(2.2)
…ss, kg	36.1(5.2)	26.8(3.3)	32.8(6.4)
	49.9(5.3)	35.1(4.5)	44.6(8.7)
	1.39(0.11)	1.22(0.12)	1.35*(0.12)
	0.88(0.12)	0.89(0.27)	0.89(0.19)
…nge	0.81-1.00	0.51-0.82	0.53-0.96
	0.55(0.13)	0.40(0.09)	0.49(0.14)
…ss, mg/dL	0.75(0.14)	0.53(0.10)	0.67(0.17)
…nge	0.65-1.07	0.46-0.79	-
…n²	94.4(19.6)	93.4(28.8)	94.0(23.5)
…n²	137.2(34.8)	143.8(45.4)	139.5(36.5)
…l/m²	94.8(19.6)	106.0(28.6)	99.8(22.8)

…ts according to table 1 in our presentation

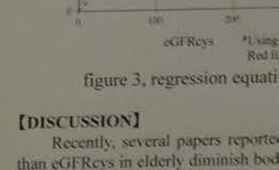
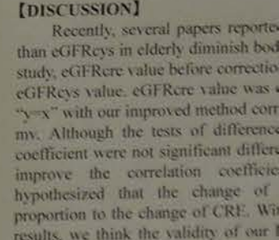


figure 1, distribution of clinic…



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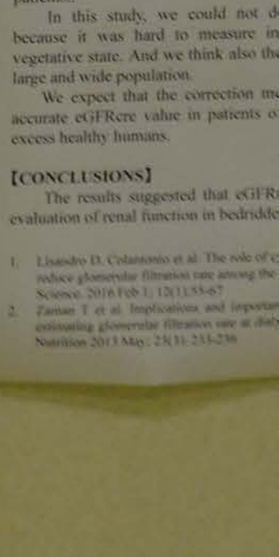


figure 3, regression equati…

【DISCUSSION】

Recently, several papers reporte… than eGFRcys in elderly diminish bod… study, eGFRcre value before correctio… eGFRcys value. eGFRcre value was … "y=x" with our improved method corr… mv. Although the tests of differenc… coefficient were not significant differ… improve the correlation coefficie… hypothesized that the change of … proportion to the change of CRE. Wi… results, we think the validity of our … patients..

In this study, we could not d… because it was hard to measure in… vegetative state. And we think also th… large and wide population.

We expect that the correction m… accurate eGFRcre value in patients o… excess healthy humans.

【CONCLUSIONS】

The results suggested that eGFR… evaluation of renal function in bedridde…

1. Lisandro D. Colantonio et al. The role of e… reduces glomerular filtration rate among the … Science. 2016 Feb 1; 12(1):55-67
2. Zaman T et al. Implications and importan… estimating glomerular filtration rate at dis… Nutrition 2013 May; 23(3): 233-236

Clinical Chemistry

PD-43

Evaluation of the improved Dimension-TAC assay for the determination of tacrolimus concentrations in whole blood

Tomoaki Tsukushi[1] Minami Katsumata[1] Hitomi Yamaguchi[1] Hiroko Nakazaki[1] Kazuhiro Uchida[1] Shin-ichi Munekata[1] Yuhsaku Kanoh[1,2]

[1]Department of Clinical Laboratory, Kitasato University Hospital

[2]Department of Laboratory Medicine, School of Medicine, Kitasato University

Background

Tacrolimus is macrolide immunosuppressant derived from the actinomycetes. Because of its narrow therapeutic index, large inter- and intrapatient variability in pharmacokinetics, and poor correlation between dose and trough blood concentrations, tacrolimus concentrations in the blood must be monitored regularly. The Dimension RxL Tacrolimus assay (TACR), using the antibody-conjugated magnetic immunoassay (ACMIA), was reported to show a relatively large variation in the low concentration range. In present study, we evaluated the analytical performance of the improved Dimension tacrolimus assay (TAC).

Methods

The analytical parameters assessed included within-run and between-day precision, limit of quantitation, dilution linearity, interference studies, and assay kit comparisons TAC and TACR.

Results

Within-run and between-day precision

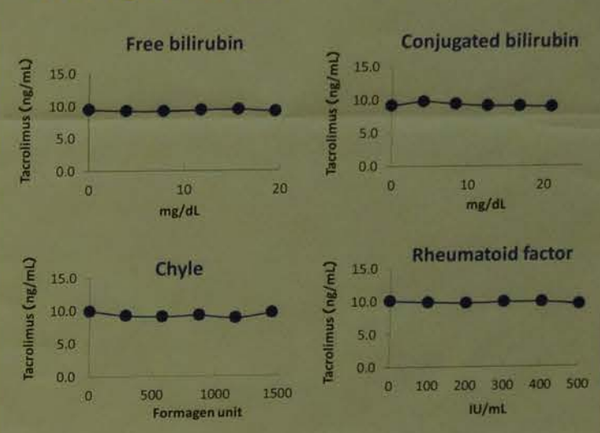
	TAC			TACR		
	Mean (ng/dL)	Within-run CV (%)	Between-day CV (%)	Mean (ng/dL)	Within-run CV (%)	Between-day CV (%)
MoreQC1	3.5	7.4	5.7	3.4	11.1	10.2
MoreQC2	10.4	5.8	2.8	10.4	4.3	5.6
MoreQC3	17.2	5.2	3.2	17.3	2.9	4.0

The within-run and between-day precision were obtained with 5.2-7.4% and 3.2-5.7%, respectively.

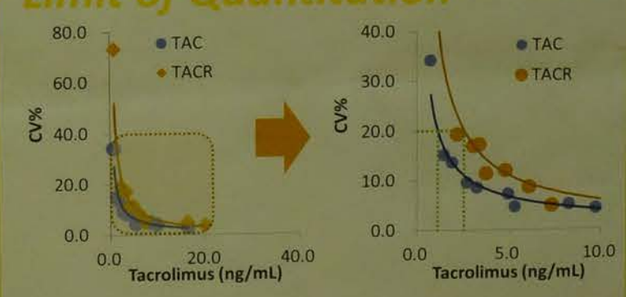
Dilution linearity

Dilution Linearity was found up to nearly 30.0ng/mL.

Interference

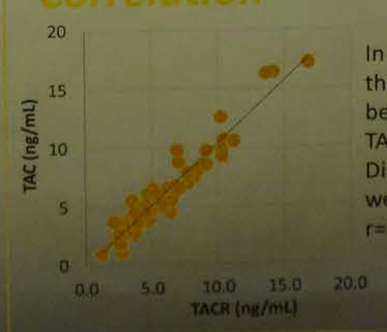


Limit of Quantitation

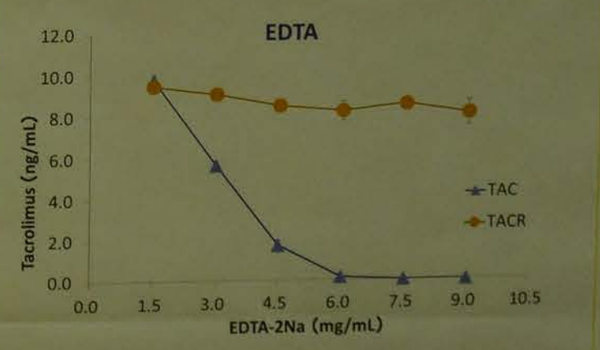


The limit of quantitation with between TAC and TACR assay were determined to be 1.1ng/mL and 2.6ng/mL, respectively.

Correlation



In comparison study, the correlations between the Dimension TAC assay and the Dimension TACR assay were as follows: n=79, r=0.959, y=1.06x - 0.1.



No interference was observed in the testing of specimens containing potential interfering substances such as free bilirubin (≦19.5mg/dL), conjugated bilirubin (≦20.9mg/dL), chyle (≦1450 formazin turbidity), rheumatoid factor (≦500IU/mL) and hematocrit. However in the EDTA interference study, tacrolimus concentrations was decreased 57.7% at EDTA-2Na 3.0mg/mL and the influence was observed in a concentration-dependent manner.

Results

The Dimension TAC assay showed good analytical performance. In limit of quantitation study, it admitted to improve of performance with low concentration range in comparison with the Dimension TACR. However, it is necessary to pay attention to the amount of collected blood, because of influence of EDTA concentrations on tacrolimus.

• Conflict of Interest (COI) of the Principal Presenter : No potential COI to disclose

B-3 B-3

Clinical Chemistry

PD-44

Usefulness of eGFRcre corrected by muscle-mass-volume creatinine in the bedridden patients

Takahashi Youhei [a], Ukida Minoru [b]

[a] Department of Clinical Laboratory, National Organization for Automotive Safety & Victim's Aid Okayama Medi-Care Center, Okayama, Japan
[b] Department of Central Clinical Laboratory, Okayama Saiseikai General Hospital

Key words: Creatinine, Cystatin-C, eGFR, muscle-mass-volume, Correction method

【BUCKGROUND】

For the most of bedridden patients, muscle-mass-volume (mv) and serum creatinine value (CRE) decrease because of reduction of the exercise or the activity. It is hard to use estimated glomerular filtration rate (eGFR) using CRE (eGFRcre) for the evaluation of renal function in such condition. So, Cystatin-C value (Cys-C) which is not affected by mv has been used usually in the suspicious case of the renal impairment. The aim of this study is to investigate the usefulness of eGFR corrected by mv CRE (eGFRmvcre) compared to eGFR using Cys-C (eGFRcys).

【SUBJECTS AND METHODS】

Subjects:

The subjects were 42 patients of persistent vegetative state admitted to our hospital.

CRE and Cys-C Assays:

CRE : Enzymatic method
(manufactured by SINOTEST CO.,LTD)
Cys-C : Latex turbidimetric immunoassay *
* standardized according to the IRMM commutability study
(manufactured by LSI Medience CO.,LTD)
Both assays were analyzed by H7180.
(manufactured by Hitachi High-Technologies CO.,LTD)

Calculation of "CRE Corrected By mv":

We measured mv of each patient using body composition analyzer (InBody S10), and calculated the correction value from standard mv (standard mv adjusted by height and sex, male = 80.2% of ideal body weight, female = 72.7% of ideal body weight). The CRE corrected by mv was calculated by CRE and the correction value (CRE corrected by mv = CRE × (standard mv / measured mv)).

Estimation of GFR;

eGFR was calculated by method of Japanese Society Nephrology. Also patients under eighteen years of age, were used method of Japanese Society Pediatric Nephrology.

We investigated that evaluation of calculated result by correction and compared eGFRmvcre with eGFRcys using regression equation and correlation.

【RESULTS】

Table 1 shows the baseline, clinical data and calculated results of the patients. The average mv of the patient was 32.8±6.4kg compared with the standard mv of 44.6±8.7kg. The average correction value was 1.37±0.12. Each of eGFR value were eGFRcys = 94.0±23.5, eGFRcre = 139.5±38.5 and eGFRmvcre = 98.8±22.9. Figure 1 shows the distribution of clinical data and reference range. In the Cys-C and the CRE corrected by mv, the majority of the patients were within the reference range. But, in the CRE, it showed low value trend than the reference range. Figure 2 shows the each of distribution of eGFR. The eGFRcre value was significantly higher than eGFRcys value (p=0.000). There was no significant difference between eGFRmvcre value and eGFRcys value (p=0.242). The regression equation of eGFRcre and eGFRcys was y=1.63x-14.2, and the correlation coefficient was r=0.268. The regression equation of eGFRmvcre and eGFRcys was y=0.97x+7.2, and the correlation coefficient was r=0.367 (figure 3). The results of test of difference between two correlation coefficient were no significant difference (p=0.059).

table 1, patient baseline, clinical data, calculated results

Patient Characteristic	Male Mean(SD)	Female Mean(SD)	Total Mean(SD)
n	27	15	42
Mean age, years	42.2(17.5)	42.4(25.7)	42.3(20.5)
Height, cm	168.5(7.8)	152.7(7.3)	162.8(10.7)
Weight, kg	53.3(7.0)	46.3(7.8)	50.8(7.8)
BMI, kg/m²	18.7(1.8)	19.8(2.7)	19.1(2.2)
Muscle-mass-volume(mv), kg	36.1(5.2)	26.8(3.3)	32.8(6.4)
Standard mv, kg	49.9(5.3)	35.1(4.5)	44.6(8.7)
Corrected Value by mv	1.39(0.11)	1.32(0.12)	1.37(0.12)
Cystatin C, mg/L	0.88(0.12)	0.89(0.27)	0.89(0.19)
Cystatin C reference range	0.61-1.00	0.51-0.82	0.53-0.96
Creatinine, mg/dL	0.55(0.13)	0.40(0.09)	0.49(0.14)
Creatinine corrected by mv, mg/dL	0.75(0.14)	0.52(0.10)	0.67(0.17)
Creatinine reference range	0.65-1.07	0.46-0.79	-
eGFRcys, ml/min./1.73m²	94.4(19.8)	93.4(29.9)	94.0(23.5)
eGFRcre, ml/min./1.73m²	137.2(34.8)	143.8(45.4)	139.5(38.5)
eGFRmvcre, ml/min./1.73m²	94.8(19.9)	106.0(26.9)	98.8(22.9)

*notation of units according to table 1 in our presentation

figure 1, distribution of clinical data and reference range

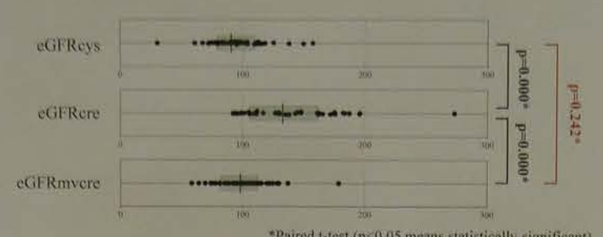


figure 2, each of distribution of eGFR

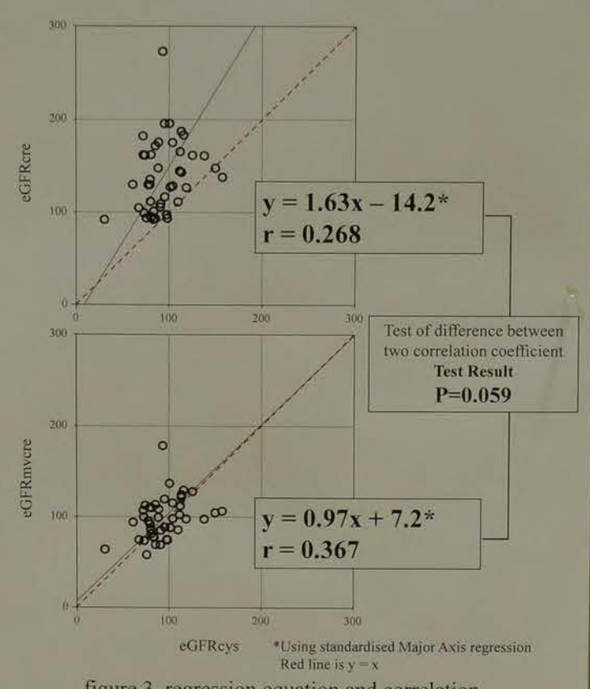


figure 3, regression equation and correlation

【DISCUSSION】

Recently, several papers reported that eGFRcre was higher than eGFRcys in elderly diminish body mass persons[1,2]. In our study, eGFRcre value before correction was 1.6 times higher than eGFRcys value. eGFRcre value was closed near the equation of "y=x" with our improved method corrected by standardization of mv. Although the tests of difference between two correlation coefficient were not significant difference, there were trended to improve the correlation coefficient. In this study, we hypothesized that the change of mv were related direct proportion to the change of CRE. With the above relatively fair results, we think the validity of our hypothesis in mv decrease patients..

In this study, we could not do the "inulin clearance", because it was hard to measure in patients of persistent vegetative state. And we think also the investigation needs more large and wide population.

We expect that the correction method may obtained more accurate eGFRcre value in patients of skinny, obesity, and mv excess healthy humans.

【CONCLUSIONS】

The results suggested that eGFRmvcre was useful for the evaluation of renal function in bedridden patients.

1. Lisandro D. Colantonio et al. The role of cystatin-C in the confirmation of reduce glomerular filtration rate among the oldest old. Archives of Medical Science. 2016 Feb 1; 12(1):55-67
2. Zaman T et al. Implications and importance of skeletal muscle mass in estimating glomerular filtration rate at dialysis initiation. Journal of Renal Nutrition 2013 May; 23(3): 233-236

Clinical Chemistry

D-46

The case that a result of HbA1c and GA became estranged

OAi Mutou, Hiroki Yanagida, Hiroki Iio, Shinsuke Ueno, Hiroshi Okada, Kumiko Kouchi, Yutaka Yoshimura
Dept. of Central Clinical Laboratory, Nara Prefecture General Medical Center, Nara, Japan

Introduction

Objective
Because the abnormal hemoglobin blood sympton that HbA1c and GA became estranged this time experienced a doubted case , I report it .

Case
44 years old woman
Weight loss (5 kg decrease in 6 months) She is introduced hyperglycosemia than a nearby doctor for main complaint . It becomes the diabetes educatuon hospitalization in an insulin introduction purpose in our House.

Anamnesis and family career
Type 2 diabetes , diabetes retinnopathy. Doubt of hemolytic anemia (in other hospitals) Her grandmother is diabetes. She takes a contraceptive for a long term.

Medical views

Hospitalization views
Blood sugar level : 380 mg/dl , Hba1c : 6.9% , HbF : 2.5% , GA : 42.4% Hb : 13.3g/dl , MCV : 111.4fl , target cell (+) , Indirect Bil : 1.67mg/dl , Reticulocyte : 6.9% , Blood Insulin : 3.0μU/ml , Blood C Peputid : 1.3ng/ml , Urine C peputid : 92.6μg/day , 75OGTT Fasting Value : 247mg/dl , One houer value : 428mg/dl , Two houer value : 503mg/dl , Reduced blood sugar level : 147mg/dl , Reduced HbA1c : 12.3%

Reduced blood sugar level
= (HbA1c) − 2 × 30

Reduced HbA1c
= (GA ÷ 4) − 2 + 1.73

Medical views

The other measurement
HbA1c of enzymatic test : 4.1%

HbA1c of enzymatic
This method uses proteolytic enzyme that estranges βchain n-terminus of hemoglobin, and leads to color by FPOX. This is suitable to process mass sample using automated analyzer.

Medical views

It is views after a discharge
Blood sugar level : 166mg/dl , HbA1c : 3.8% , HbF 2.7% , GA : 16.1% , Blood C peputid : 1.9ng/ml , Reduced blood sugar level : 54mg/dl , Reduced HbA1c : 5.7%

Chart of the HPLC method of HbA1c in 3.8%
A1A : 1.0% , A1B : 0.3% , F : 2.7% , LA1C+ : 1.7% , SA1C:3.8% , A0 : 91.4% , Abnormal compartmentation does not accept.

Progress

Blood sugar control with the insulin treatment was started after education hospitalization . I was doubted , and Hb abnormality performed a close inspection and gene analysis from HbF high price hemolytic anemia from blood test views again. A drop of the blood sugar level was seen by an examination for outpatient after the discharge , and blood sugar conntrol was good . But , I measured an HPLC chart abnormality compartmentation close inspection and the enzyme method because HbA1c was 3.8% and conversion HbA1c 5.7% and estrangement were seen. The clinical report merged conversion HbA1c 5.7%. The gene analysis did not lead to identification of the Hb abnormality and became the follow up as an unstable Hb symptom.

Conssideration

There was the weight loss in the short term and thought from 380mg/dl and it was supposed , and it was thougt that it was a chronicity sustained hyperglycosemia state with the numerical value that more than 10% were desirable for the HbA1c level at hospitalization. Estrangement was seen in conversion HbA1c in HbA1c/GA in 12.3%. Estrangement was seen in conversion HbA1c5.7% in HbA1c at outpatient department time. The abnormal fraction was not able to identify the cause of the HbA1c set price without being detected, but it was thought that it was proper for measurement. I coped by reporting a conversion HbA1c level this time.

clusion

ected the HbA1c abnormality which I could not detect in the HPLC this time. It is thought to be available for a case like this time by ng conversion HbA1c using a GA level.

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Clinical Chemistry

PD-43

Evaluation of the improved Dimension-TAC assay for th determination of tacrolimus concentrations in whole bl

Tomoaki Tsukushi[1)] Minami Katsumata[1)] Hitomi Yamaguchi[1)] Hiroko Nakazaki[1)] Kazuhiro Uch Shin-ichi Munekata[1)] Yuhsaku Kanoh[1)2)]
[1)]Department of Clinical Laboratory, Kitasato University Hospital
[2)]Department of Laboratory Medicine, School of Medicine, Kitasato University

Background

crolimus is macrolide immunosuppressant derived from the actinomycetes. Because of its narrow erapeutic index, large inter- and intrapatient variability in pharmacokinetics, and poor correlation be se and trough blood concentrations, tacrolimus concentrations in the blood must be monitored regu e Dimension RxL Tacrolimus assay (TACR), using the antibody-conjugated magnetic immunoassay (A as reported to show a relatively large variation in the low concentration range. In present study, we aluated the analytical performance of the improved Dimension tacrolimus assay (TAC).

Methods

ne analytical parameters assessed included within-run and between-day precision, limit of quantitati lution linearity, interference studies, and assay kit comparisons TAC and TACR.

Results

Within-run and between-day precision

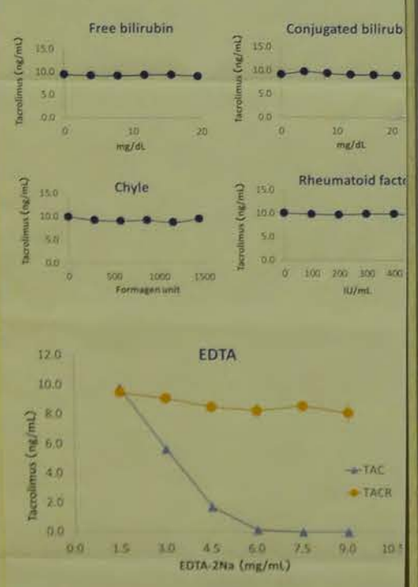
	TAC			TACR		
	Mean (ng/dL)	Within-run CV (%)	Between-day CV (%)	Mean (ng/dL)	Within-run CV (%)	Between-day CV (%)
MoreQC1	3.5	7.4	5.7	3.4	11.1	10.2
MoreQC2	10.4	5.8	2.8	10.4	4.3	5.6
MoreQC3	17.2	5.2	3.2	17.3	2.9	4.0

The within-run and between-day precision were obtained with 5.2-7.4% and 3.2-5.7%, respectively.

Dilution linearity

Dilution Linearity was found up to nearly 30.0ng/mL.

Interference

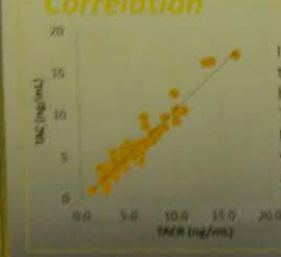


No interference was observed in the testing of specin containing potential interfering substances such as fr bilirubin (≦19.5mg/dL), conjugated bilirubin (≦20.9mg/dL), chyle (≦1450 formazin turbidity), rheumatoid factor (≦500IU/mL) and hematocrit. However in the EDTA interference study, tacrolimus concentrations was decreased 57.7% at EDTA-2Na 3.0mg/mL and the influence was observed in a concentration-dependent manner.

Limit of Quantitation

The limit of quantitation with between TAC and TACR assay were determined to be 1.1ng/mL and 2.6ng/mL, respectively.

Correlation



In comparison study, the correlations between the Dimension TAC assay and the Dimension TACR assay were as follows: n=79, r=0.959, y=1.06x - 0.1.

Results

The Dimension TAC assay showed good analytical performance. In limit of quantitation study, it admit improve of performance with low concentration range in comparison with the Dimension TACR. How is necessary to pay attention to the amount of collected blood, because of influence of EDTA concent on tacrolimus.

* Conflict of Interest (COI) of the Principal Presenter : No potential COI to disclose

Clinical Chemistry

PD-45

Markedly high levels of glycated albumin in a case with subtotal pancreatectomy

Shinobu Doi [a], Tomoko Kobayashi [a], Izumi Mori [a], Masafumi Koga [b], Takuji Kohzuma [c], Midori Ishibashi [d], Ikki Shimizu [e], Kazuyuki Akesaka [f]

[a] Department of Clinical Laboratory, Ehime Prefectural Central Hospital, Ehime, Japan
[b] Department of Internal Medicine, Hakuhokai Central Hospital, Hyogo, Japan
[c] Medical Affairs Group, Diagnostic Department, Asahikasei Phama Corporation, Tokyo, Japan
[d] Department of Laboratory Medicine, New Tokyo Hospital, Chiba, Japan
[e] Department of Diabetes, The Sakakibara Heart Institute of Okayama, Okayama, Japan
[f] Department of Diabetes and Endocrinology, Ehime Prefectural Central Hospital, Ehime, Japan

Background

Pancreatectomy is associated with pancreatic exocrine and endocrine hypofunction, which may cause in metabolism nutrition hypofunctions, such as glucose metabolism, digestion and absorption[1,2]. We encountered a patient with a markedly high glycated albumin (GA) level at 97.8%, who had a Subtotal Stomach-Preserving Pancreaticoduodenectomy (SSPPD). To investigate the causes of the markedly high GA, we analyzed the glycation sites of albumin, self-monitoring of blood glucose (SMBG), HbA1c and GA.

Patient

- A 77-year-old male patient
  Height: 155.7cm, Weight: 45.2kg, BMI: 18.6
- Past Medical History
  - At 60 years old, type 2 diabetes mellitus
  - At 74 years old, acute myocardial infarction
  - At 77 years old, pancreatic head tumor
- History of Present Illness
  Six months after SSPPD, weakness of the lower limbs, general malaise, dry mouth, polydipsia, and polyuria were noted during follow-up at the Department of Gastrointestinal Surgery.
  On the same day, the patient was referred to the Department of Diabetes, and a blood test showed that the Plasma Glucose (PG), HbA1c, and GA values were 589 mg/dL, 15.8%, and 97.8%, respectively, suggesting deterioration of glycemic control. He was hospitalized, and started intensive insulin therapy.

Methods

We measured SMBG, PG, HbA1c, and GA during the treatment in hospitalization.

1. PG: GOD / H_2O_2 Amperometric method (GA-1171, ARKRAY, Inc., Japan).
   GA: Enzymatic method (Lucica GA-L kit, Asahi Kasei Pharma Corp., Japan).
   HbA1c: HPLC method (HA-8181, ARKRAY, Inc., Japan).
   SMBG: GOD / POD colorimetric method (Medisafe FIT, Terumo Corporation., Japan).
2. To determine the glycation sites of albumin, samples were reduced and S-carboxymethylated and digested by Glu-C endoprotease, and peptides were analyzed using liquid chromatography/mass spectrometry (LC / MS) [3].

Results and Discussion

Diabetic ketosis rapidly deteriorated six months after surgery (Table 1). PG was decreased without delay after the start of intensive insulin therapy (Fig. 1). Preprandial blood glucose level was improved after the day of glargine addition (Fig. 2).
HbA1c and GA levels also decreased almost linearly. However, the GA/HbA1c ratios were ≥4 after the glycemic control improved (Fig. 1). Therefore, Based on the mean blood glucose level, respective estimated values were calculated, and compared with the actual values. The measured HbA1c value was consistent with the estimated values calculated from the 6 times/day SMBG measurements, but the GA value was falsely high (Table 2). Although the cause of the falsely high GA value is unclear, it may have been associated with a delay in albumin catabolism.
The glycation sites of albumin of the pre-treatment patient were extended to various sites in addition to the major glycation site of Lys-525 (Table 3). This is consistent with the results of a study published by Kisugi, et al. (Jikei University Hospital)[3].
If there is an elevation of blood glucose changes and a delay in albumin catabolism, as demonstrated in the present case, albumin glycation may be advanced. Strict glycemic control using HbA1c may be necessary.

Conclusions

The GA value and GA/HbA1c of this case were markedly high. This was possibly related to the rapid deterioration of glycemic control and a delay in albumin catabolism for the following reasons: no abnormal glycation site was found compare to previous report; and the measured HbA1c value was consistent with the estimated values calculated from the 6 times/day SMBG measurements, but the GA value was falsely high.

Results

Table 1 Patient Demographics at Baseline

CBC		Blood Chemistry				Endocrinology	
WBC	6800 /μL	TP	6.6 g/dL	BUN	21.6 mg/dL	TSH	5.062 μIU/mL
RBC	389 10^4/μL	ALB	3.2 g/dL	CREA	0.88 mg/dL	FT3	1.10 pg/mL
Hb	11.2 g/dL	AST	28 U/L	eGFR	64 mL/min/1.73m²	FT4	0.89 ng/dL
Ht	31.3 %	ALT	24 U/L	Na	128 mEq/L	CPR (before)	0.7 ng/mL
MCV	80.5 fL	ALP	419 U/L	K	4.4 mEq/L		
MCH	28.8 pg	T-Bil	0.4 mg/dL	Cl	89 mEq/L	CPR (after)	1.0 ng/mL
MCHC	35.8 %	LDH	157 U/L	Ca	9.0 mg/dL		
Plt	37.1 10^4/μL	ChE	177 U/L	iP	3.5 mg/dL	CPR (urine)	21.5 μg/day
		rGTP	108 U/L	CRP	0.47 mg/dL		
		AMY	192 U/L	S-Osm	300 mOsm		
Metabolic							
HbA1c	15.8 %	T-CHO	167 mg/dL	Total-ketone	938 μmol/L		
PG	589 mg/dL	T-G	169 mg/dL				
GA	97.8 %	HDL-c	61 mg/dL	AcAc	246 μmol/L		
		LDL-c	74 mg/dL	3-OHBA	692 μmol/L		

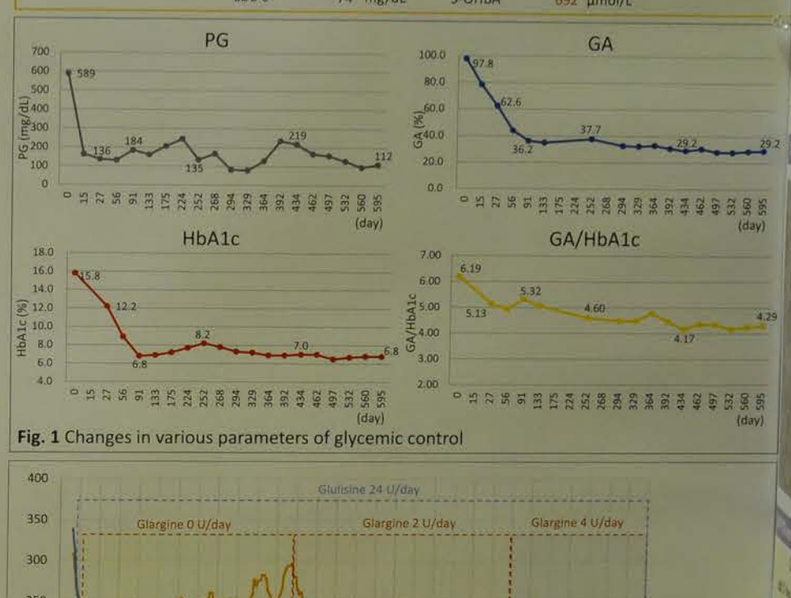


Fig. 1 Changes in various parameters of glycemic control

Fig. 2 14-day Simple Moving Average in preprandial blood glucose level (SMBG)

Table 2 Comparison of the actual values with the estimated value

	SMBG								
day	before breakfast	after breakfast	before lunch	after lunch	before dinner	after dinner	mean (mg/dL)	estimated HbA1c*(%)	estimate GA*(%)
873	122	164	172	214	159	144	162.5	7.2	19
880	149	185	177	190	170	241	185.3	7.9	21
887	130	177	184	216	138	251	182.7	7.9	21.
							Mean	7.67	20.7

day	HbA1c (%)	GA (%)
868	7.9	37.9
896	7.9	36.2
Mean	7.90	37.05

Hb1c/eHb1c (%)	GA/eGA (%)
103.00	178.73

*eHbA1c (%)= Mean BG(mg/dL) × 1.11 / 35.6 + 2.17
*eGA (%)= A1C × 2.7

Table 3 Percentage of various putative glycation sites on albumin, and Lys-525 glycation in all glycation sites

Fragment	F57	F22	F25	F35	F37	F47	F58	%525 of Total	GA(%)
Lysine site	525	199	233	317	351	439	534		
Pre-treatment (Day 0)	28.42	10.72	11.82	5.52	3.58	7.67	2.95	40.2	97.8
Post-treatment 1 (Day 15)	24.75	5.81	9.34	4.76	3.13	4.92	2.6	44.7	78.3
Post-treatment 2 (Day 252)	16.16	2.62	3.76	1.91	1.91	3.11	1.19	54.5	37.7
Healthy Control	6.71	1.35	1.29	0.69	0.96	0.87	0.59	53.9	14.8
Diabetic Patient	12.82	2.44	2.74	1.37	1.73	1.94	0.89	53.6	29.5

Reference

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3. Kisugi R, et al. Clin Chim Acta 2007; 382: 59-64
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Further information

Ehime Prefectural Central Hospital
83 Kasugamachi, Matsuyama city, Ehime 790-0024, Japan
Tel : +81 89-947-1111 Fax: +81 89-943-4136
E-mail address: c-sdoi@epch.pref.ehime.jp (S. Doi)

A-

The case that I could cut HbA1c and GA became estranged

Tell 0742-46-6001 Ex 2358

muscle-mass-volume
the bedridden patients

for Automotive Safety & Victim's Aid

seikai General Hospital

ume, Correction method

Female

ion of clinical data and reference range

*Paired t-test ($p<0.05$ means statistically significant)

each of distribution of eGFR

$y = 1.63x - 14.2^*$
$r = 0.268$

Test of difference between two correlation coefficient
Test Result
P=0.059

$y = 0.97x + 7.2^*$
$r = 0.367$

eGFRcys *Using standardised Major Axis regression
Red line is y = x

3, regression equation and correlation

]

everal papers reported that eGFRcre was higher
n elderly diminish body mass persons[1,2]. In our
value before correction was 1.6 times higher than
. eGFRcre value was closed near the equation of
improved method corrected by standardization of
the tests of difference between two correlation
e not significant difference, there were trended to
correlation coefficient. In this study, we
that the change of mv were related direct
he change of CRE. With the above relatively fair
nk the validity of our hypothesis in mv decrease

tudy, we could not do the "inulin clearance",
as hard to measure in patients of persistent
e. And we think also the investigation needs more
population.

t that the correction method may obtained more
Rcre value in patients of skinny, obesity, and mv
humans.

IONS]

lts suggested that eGFRmvcre was useful for the
renal function in bedridden patients.

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016 Feb 1; 12(1):55-67
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2013 May; 23(3): 233-236

Clinical Chemistry

PD-46

The case that a result of HbA1c and GA became estranged

OAi Mutou, Hiroki Yanagida, Hiroki Iio, Shinsuke Ueno, Hiroshi Okada, Kumiko Kouchi, Yutaka Yoshimura

Dept. of Central Clinical Laboratory, Nara Prefecture General Medical Center, Nara, Japan

Abstract

Background

HbA1c which is saccharification hemoglobin reflects 2-month blood sugar level from the past 1. We measure it by the HPLCmethod which is the stanndard method.

Introduction

Objective

Because the abnormal hemoglobin blood sympton that HbA1c and GA became estranged this time experienced a doubted case , I report it

Case

44 years old woman.
Weight loss (5 kg decrease in 6 months). She is introduced hyperglycosemia than a nearby doctor for main complaint . It becomes the diabetes educatuon hospitalization in an insulin introduction purpose in our House.

Anamnesis and family career

Type 2 diabetes , diabetes retinnopathy. Doubt of hemolytic anemia (in other hospitals). Her grandmother is diabetes. She takes a contraceptive for a long term.

Medical views

Hospitalization views

Blood sugar level : 380 mg/dl , Hba1c : 6.9% , HbF : 2.5% , GA : 42.4% Hb : 13.3g/dl , MCV : 111.4fl , target cell (+) , Indirect Bil : 1.67mg/dl , Reticulocyte : 6.9% , Blood Insulin : 3.0μU/ml , Blood C Peputid : 1.3ng/ml , Urine C peputid : 92.6μg/day , 75OGTT Fasting Value : 247mg/dl , One houer value : 428mg/dl , Two houer value : 503mg/dl , Reduced blood sugar level : 147mg/dl , Reduced HbA1c : 12.3%

Reduceed blood sugar level
= (HbA1c) − 2 × 30

Redueced HbA1c
= (GA ÷ 4) − 2+1.73

Medical views

Close inspection views

CGM inspection : Hyperglycosemia after a meal , Red blood cell resistannce examination : The osmolar fragility does not accept , Examination of outside order : GLT50 does not accept , HbH inclusion body component red blood cell does not accept . Isoprofessional Nord test negative , Drop of the third band of erythrocyte membrane does not accept . An resalt of genetic analysis by GAP-PCR : αglulobin gene-3.7type and anti3.7type does not accept . An resalt of genetic analysis by sequencing : β and α1 and α2-globin does not accept.

Medical views

It is views after a discharge

Blood sugar level : 166mg/dl , HbA1c : 3.8% , HbF 2.7% , GA : 16.1% , Blood C peputid : 1.9ng/ml , Reduced blood sugar level : 54mg/dl , Reduced HbA1c : 5.7%

Chart of the HPLC method of HbA1c in 3.8%

A1A : 1.0% , A1B : 0.3% , F : 2.7% , LA1C+ : 1.7% , SA1C:3.8%, A0 : 91.4% , Abnormal compartmentation does not accept

Medical views

The other measurement

HbA1c of enzymatic test : 4.1%

HbA1c of enzymatic

This method uses proteolytic enzyme that estranges βchain n-terminus of hemoglobin, and leads to color by FPOX. This is suitable to process mass sample using automated analyzer.

Target cell view

HbA1c HPLC Method chart

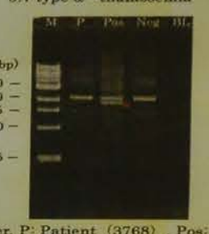
GAP-PCR view

GAP-PCR
anti 3.7 type (triplicate α-globin)

M: Marker, P: Patient (3768) , Pos: Positive control, Neg: Negative control, BL: PCR Blank, *: anti 3.7 Positive product

GAP-PCR view

GAP-PCR
·3.7 type α*–thalassemia



M: Marker, P: Patient (3768) , Pos: Positive control, Neg: Negative control, BL: PCR Blank, *: -3.7 Positive product

Progress

Blood sugar control with the insulin treatment was started after education hospitalization , I was doubted , and Hb abnormality performed a close inspection and gene analysis from HbF high price hemolytic anemia from blood test views again. A drop of the blood sugar level was seen by an examination for outpatient after the discharge , and blood sugar conntrol was good . But , I measured an HPLC chart abnormality compartmentation close inspection and the enzyme method because HbA1c was 3.8% , and conversion HbA1c 5.7% and estrangement were seen. The clinical report merged conversion HbA1c 5.7%. The gene analysis did not lead to identification of the Hb abnormality and became the follow up as an unstable Hb symptom.

Conssideration

There was the weight loss in the short term and thought from 380mg/dl , and it was supposed , and it was thougt that it was a chronicity sustained hyperglycosemia state with the numerical value that more than 10% were desirable for the HbA1c level at hospitalization. Estrangement was seen in conversion HbA1c in HbA1ctGA in 12.3%. Estrangement was seen in conversion HbA1c5.7% in HbA1c at outpatient department time. The abnormal fraction was not able to identify the cause of the HbA1c set price without being detected, but it was thought that it was proper for measurement. I coped by reporting a conversion HbA1c level this time.

Conclusion

I expected the HbA1c abnormality which I could not detect in the HPLC chart this time. It is thought to be available for a case like this time by utilizing conversion HbA1c using a GA level.

Tell 0742-46-6001
Ex 2358

C-3

al
try
48

Development of a
for L-hydroxypro

Kazuaki Yamamoto[1], Seiya W

[1]Clinical Laboratory, Medical Hospi
[2]Graduate School of Agriculture, Eh

d
roline (L-Hyp) is one of the
included in the collagen. It was
at L-Hyp was reduced in a non-
acute coronary syndrome[1],
he association of arteriosclerosis
It is considered that L-Hyp can
binding of LDL-cholesterol to
a) deposited in the vascular wall[2].
L-Hyp concentration is measured
but this method takes time for
nt. Therefore, we developed the
simple enzymatic measurement
L-Hyp using a new enzyme, and
s basic performance.

)
ted analyzer LABOSPECT 008
(HITACHI)
ent principle)
ypE *1
D-Hyp
etoxy PMS + WST-3
D-HypDH *2
y-4-hydroxy-2-carboxylate +
DS(Red.) + WST-3 formazan dye
roline epimerase
roline dehydrogenase
433 nm
mpositions)
set the reagent composition as
nd the enzymatic reaction for 10

agent
.1 mol/L HEPES buffer
ding 1 mmol/L EDTA)
L L-HypE
L 1-methoxy PMS
reagent
.1 mol/L HEPES buffer
ding 1 mmol/L EDTA)
L D-HypDH
, WST-3.

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ve. Anal Bioanal Chem. 394, 2009, 1517-1524.
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Supplements. Journal of Orthomolecular
, 153-162.

Measured value [μmol/L]

A-3

A-2

Clinical Chemistry

PD-47

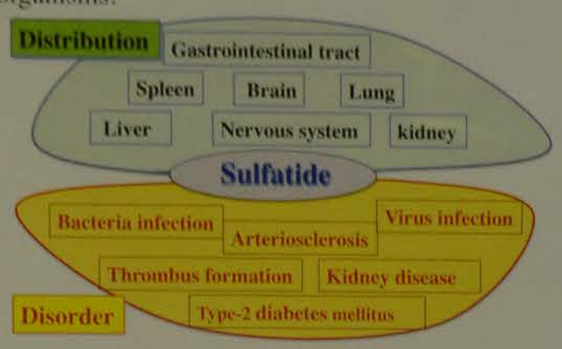
Simple and sensitive identification of molecular species of human serum sulfatide using MALDI-TOF mass spectrometry

Atsushi Hori [1,2], Makoto Yamaura [1], Sunao Morita [1], Kenji Kawasaki [2], Mtsutoshi Sugano [2], Takayuki Honda [3], Hiroya Hidaka [1]

1) Department of Biomedical and Laboratory Sciences, Shinshu University School of Medicine, Japan
2) Department of Laboratory Medicine, Shinshu University Hospital, Japan
3) Department of Laboratory Medicine, Shinshu University School of Medicine, Japan

【Background and Aim】

❖Galactosylsulfatide (SM4s) is an important molecule that is involved in processes such as myelination and insulin secretion and serves as a ligand for micro-organisms.



❖Some studies of serum SM4s have evaluated its total level but not its molecular species.

❖In this study, we aimed to assign of the structure of SM4s species and then to profile serum SM4s species in healthy young people.

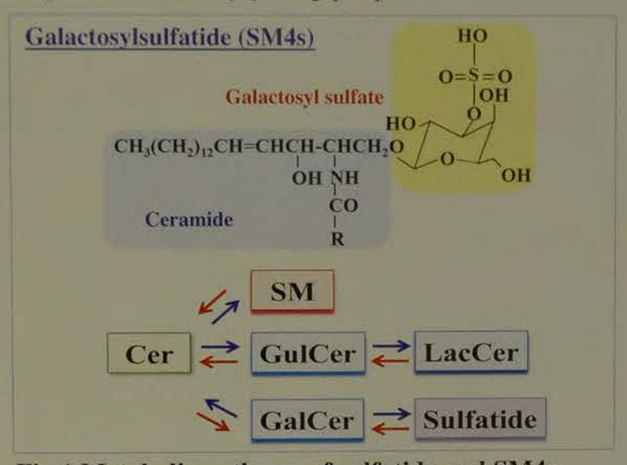


Fig.1 Metabolic pathway of sulfatide and SM4s
Cer;Ceramide, SM;Sphingomyeline, GulCer;Glucosyl-ceramide, LacCer; Lactosylceramide, GalCer; Galactosyl- ceramide.

【Methods】

- Serum was collected from healthy young volunteers, and 500-μL samples were treated with lipid hydrolases.
- Lipids were extracted after adding an internal standard (SM4s[d18:1 C12:0]). The SM4s-rich fraction was partially purified using Phenyl-Sepharose gel chromatography and mixed with a matrix (9-AA) .
- SM4s was analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and TOF/TOF-MS (AB SCIEX) in negative ion mode.

【Results】

1. Mass ion peaks

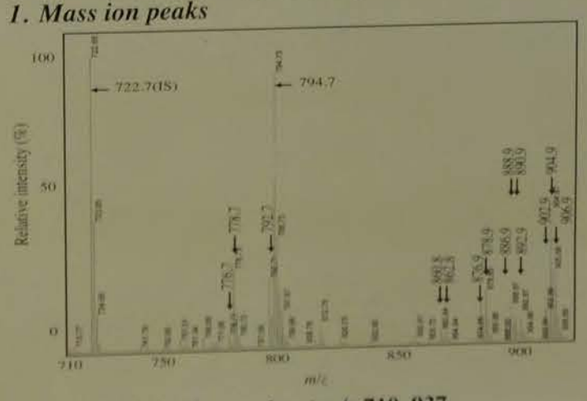


Fig. 2 Mass ion peaks at *m/z* 710–937.
IS: an internal standard material

2. Product ion analysis

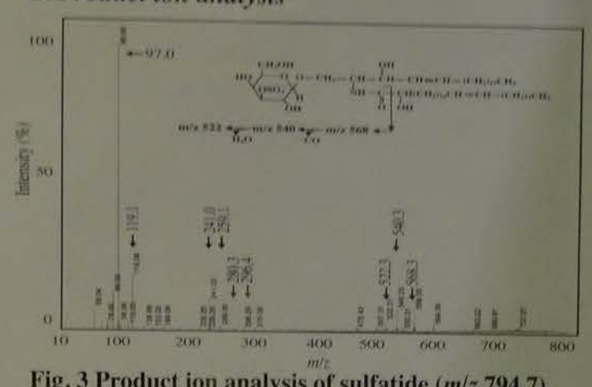


Fig. 3 Product ion analysis of sulfatide (*m/z* 794.7)

3. Assignment of sulfatide species (SM4s [d18:1])

Table. 1 Assignment of sulfatide species

m/z	d18:1	*m/z*	d18:1	*m/z*	d18:1
750.6	C14:0	822.7	**C18:0h**	888.8	**C24:1**
764.6	C15:0/C14:1h	832.7	C20:1	890.8	C24:0/**C23:1h**
766.6	**C14:0h**	834.7	C20:0/C19:1h	892.8	**C23:0h**
776.7	C16:1	836.7	C19:0h	902.8	C25:1/**C24:2h**
778.7	**C16:0**/C15:1h	846.7	C21:1	904.8	C25:0/**C24:1h**
780.7	C15:0h	848.7	C21:0/**C20:1h**	906.8	**C24:0h**
790.7	C17:1	850.7	**C20:0h**	916.8	C26:1
792.7	C17:0/**C16:1h**	860.8	C22:1	918.8	C26:0/**C25:1h**
794.7	**C16:0h**	862.8	**C22:0**/C21:1h	920.8	**C25:0h**
804.7	C18:1	864.8	C21:0h	930.8	C27:1
806.7	C18:0/**C17:1h**	874.8	C23:1	932.8	C27:0/C26:1h
808.7	**C17:0h**	876.8	C23:0/**C22:1h**	934.8	C26:0h
818.7	C19:1	878.8	**C22:0h**		
820.7	C19:0/**C18:1h**	886.8	C24:2		

The bold characters represent the assignment of SM4s species based on both sulfatide ion *m/z* data and product ion data. The non-bold characters represent the assignment of SM4s species based on sulfatide ion *m/z* data alone.
C14:0h – C25:0h : C14:0 – C25:0 hydroxy fatty acids.

4. Sulfatide species profile of healthy young subjects

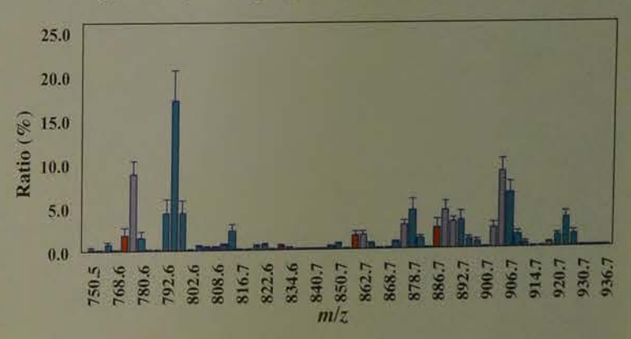


Fig. 4 Sulfatide species in health young subjects
Red column: normal fatty acids, blue column: hydroxy fatty acids, and purple column: mix of normal and hydroxy fatty acids; $n = 8$.

【Discussion】

This study showed that the simple and sensitive identification of human serum sulfatide species could be performed using MALDI-TOF-MS. Furthermore we could estimate the SM4s profile in serum from healthy young subjects. Our screening of numerous species of sulfatides in human serum may help to determine the sulfatide species present and their associations with sulfatide metabolic disorders.

The variation of serum sulfatide species including hydroxy fatty acid may be useful to understand sulfatide metabolism. However, the function of hydroxy fatty acid species is not clear.

【Conclusion】

This study presented a simple and sensitive method for analyzing sulfatide species in human serum using MALDI-TOF MS.

Clinical Chemistry

PD-48

Development of a novel measurement method for L-hydroxyproline using a new enzyme

Kazuaki Yamamoto[1], Seiya Watanabe[2], Michio Hagihara[1], Shuji Tohda[1]

[1]Clinical Laboratory, Medical Hospital, Tokyo Medical and Dental University
[2]Graduate School of Agriculture, Ehime University

Background

L-hydroxyproline (L-Hyp) is one of the amino acid included in the collagen. It was reported that L-Hyp was reduced in a non-ST-elevation acute coronary syndrome[1], suggesting the association of arteriosclerosis and L-Hyp. It is considered that L-Hyp can prevent the binding of LDL-cholesterol to lipoprotein(a) deposited in the vascular wall[2]. Currently, L-Hyp concentration is measured by HPLC, but this method takes time for measurement. Therefore, we developed the quick and simple enzymatic measurement method for L-Hyp using a new enzyme, and evaluated its basic performance.

Method

〈Equipment〉

an automated analyzer LABOSPECT 008 (HITACHI)

〈Measurement principle〉

L-Hyp $\xrightarrow{\text{L-HypE }※1}$ D-Hyp

D-Hyp + Metoxy PMS + WST-3 $\xrightarrow{\text{D-HypDH }※2}$ Δ^1-pyrroline-4-hydroxy-2-carboxylate + Methoxy PMS(Red.) + WST-3 formazan dye

※1 : L-hydroxyproline epimerase
※2 : D-hydroxyproline dehydrogenase

433 nm

〈Reagent compositions〉

We have set the reagent composition as follows to end the enzymatic reaction for 10 minute.

The first reagent

- pH 8.0, 0.1 mol/L HEPES buffer (including 1 mmol/L EDTA)
- 0.18 U/mL L-HypE
- 5.5 µmol/L 1-methoxy PMS

The second reagent

- pH 8.0, 0.1 mol/L HEPES buffer (including 1 mmol/L EDTA)
- 0.074 U/mL D-HypDH
- 55 µmol/L WST-3.

References
1) M. Vallejo, et al , Plasma fingerprinting with GC-MS in acute coronary syndrome. Anal Bioanal Chem, 394, 2009, 1517-1524.
2) Rath, Matthias. Reducing the Risk for Cardiovascular Disease with Nutritional Supplements. Journal of Orthomolecular Medicine, 7, 1992, 153-162.

Results

1) The within-run reproducibility

We measured L-Hyp standard solution (25 µmol/L and 50 µmol/L), and evaluated the within-run reproducibility (n=20). The coefficient of variation (CV%) was 0.96 % for 25 µmol/L L-Hyp, and 1.33 % for 50 µmol/L L-Hyp.

2) The linearity

We made the dilution series of L-Hyp standard solution, and obtained the linearity by measuring each three times. The linearity was linear up to 500 µmol/L (Figure 1).

3) The detection limit

We made the dilution series of L-Hyp standard solution, and obtained the detection limit by measuring each five times.The detection limit of L-Hyp, of which range of 2.6 SD did not cross 0, was 2 µmol/L (Figure 2).

Conclusion

We showed that our novel method was able to measure L-Hyp concentration quickly. Its within-run reproducibility, linearity and the detection limit were good enough. We are planning to measure L-Hyp concentration in healthy and patient specimens, and investigate the relevance of L-Hyp, triglyceride, LDL-cholesterol, and HDL-cholesterol.

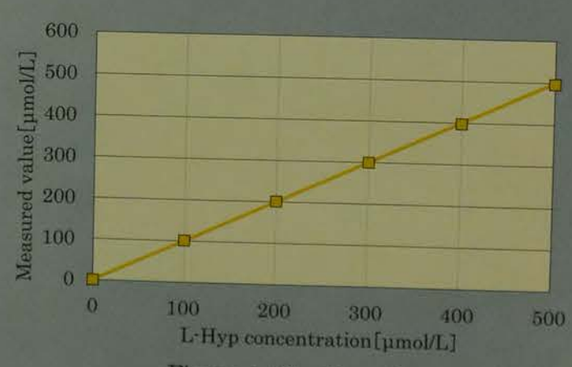


Figure 1. The linearity

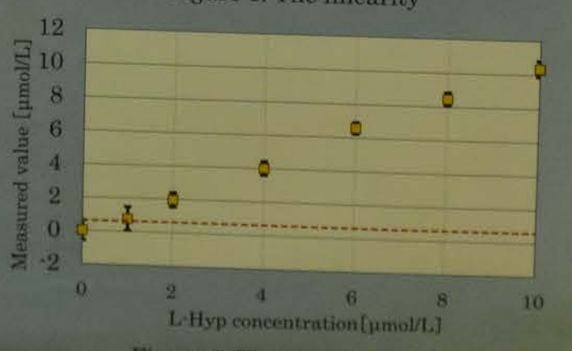


Figure 2. The detection limit

Clinical Chemistry

PD-49

Setting reference intervals of serum analytes (serum proteins) in Japanese elderly populations

Noriaki Harada[1], Juon Lee[1], Kiyoshi Ichihara[2], Yasuo Yano[1], Nobuko Ikeda[1], Yoshihisa Itoh[1].

[1]Clinical Laboratory, Eiju General Hospital, Life Extension Research Institute, Tokyo,
[2]Department of Medical Science, Yamaguchi University, Japan.

Background

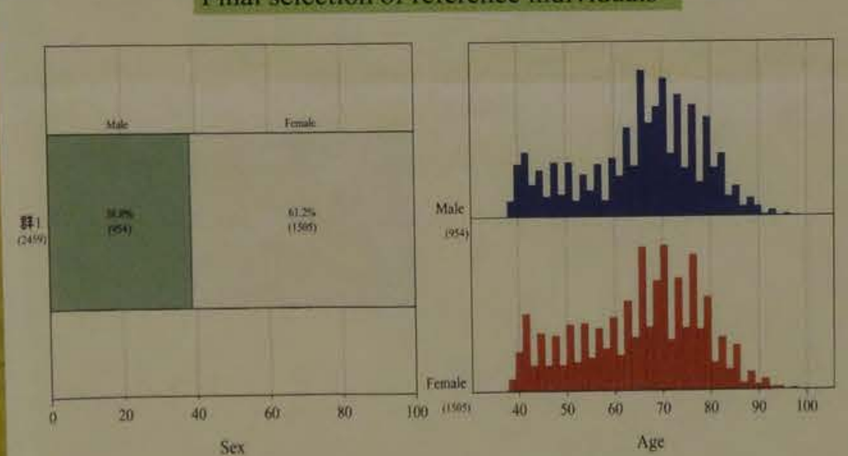
Setting reference intervals (RI) of serum proteins started in 1993 when CRM470, first international reference material for serum proteins was prepared and thus introduced to Japan (1) . While promoting standardization of immunoassays with the material, Ichihara et al. have developed the statistical procedure for RI and thus set them initially in Japanese, then Asian and global populations (2, 3).
Now second lot of the material, ERM-DA470k/IFCC, is available. This is an initial report to set RIs in Japanese elderly populations in association with age,sex, life style, and BMI.

Materials and Methods

Sample: After informed consent, total 2459 sera were collected from November 2013 to January 2015 at Taito Ward health checkup. It consisted of 924 males and 1565 females aged 39 to 94 years old.
Selection of Reference Individuals: RI's were determined by the established method (3). Apparently healthy individuals was selected based on questionnaires without remarkable present/past history of diseases (CVD, DM, malignancy and others), operation, and pregnancy. Those with hypertension and hyperlipidemia under good control were included for reference individuals. A LAVE (Latent abnormal values exclusion) method was further used for selecting them statistically, in which only one analyte in which value is out of RIs was allowed for selection among 10 routine analytes.
Independently of above method, we chose individuals completely fulfilled with the laboratory data in various clinical guidelines. We tried to find age-related analytes among all analytes.
Measurement of proteins: CBC was determined on Ruby (Abott). Total protein and albumin were measured by Buret method and a modified BCP method (Kainos), respectively. CRP, cystatin C, C3, TTR, RBP (Nittobo, Tokyo) and pepsinogen I, II (Eiken, Tokyo) were measured by turbidimetry on Hitachi Analyzer (LAbOSPECT 006; Hitachi, Tokyo). ProBio-S, secondary RM in which value was transferred from ERM-DA470k/IFCC was used as control.
Setting reference intervals:
Using defined reference individuals, RIs of serum proteins were finally determined by Box-Cox transformation (3).

Results

Final selection of reference individuals



Assay performance

Analyte	TP	Alb	CRP	IgG	IgA	IgM
Unit	g/dL	g/dL	mg/dL	mg/dL	mg/dL	mg/dL
Number of Measurement	43	43	43	43	43	43
Mean	5.7	3.5	4.108	1046	205	57
SD	0.1	0.1	0.1	24	7	4
CV	0.1%	1.4%	1.4%	2.2%	3.4%	6.2%

Analyte	C3	TTR	RBP	cysC	Pep I	Pep II	Type IV Collagen
Unit	mg/dL	mg/dL	mg/dL	mg/L	mg/dL	mg/dL	mg/dL
Number of Measurement	43	43	43	43	43	43	14
Mean	120	20.1	2.4	4.70	49.4	12.1	93.1
SD	3	0.3	0.1	0.09	3.3	1.0	6.1
CV	2.3%	1.8%	2.4%	2.0%	6.7%	7.9%	6.8%

Panel No	TP	Alb	CRP	IgG	IgA	IgM
358	0.7%	0.0%	17.3%	3.5%	1.7%	8.3%
380	0.7%	1.1%	7.2%	0.5%	1.1%	2.1%
378	0.7%	1.7%	12.0%	1.8%	2.4%	3.9%
372	1.2%	0.0%	7.4%	1.6%	0.6%	1.3%
371	0.6%	0.0%	1.7%	3.6%	1.2%	3.0%
354	0.8%	1.0%	1.9%	1.2%	2.2%	2.9%

Panel No	C3	TTR	RBP	cysC	Pep I	Pep II	Type IV Collagen
358	1.5%	1.4%	2.3%	1.3%	10.4%	3.0%	1.9%
380	1.9%	1.4%	0.0%	0.9%	13.0%	4.2%	5.0%
378	1.3%	1.3%	2.4%	0.7%	3.3%	3.2%	2.0%
372	3.3%	3.1%	1.5%	1.6%	4.5%	11.2%	3.9%
371	0.9%	3.7%	1.2%	1.0%	1.2%	8.2%	n.m.
354	1.4%	2.3%	0.0%	0.0%	0.4%	0.8%	n.m.

Reference intervals in major serum proteins

Item	Age	M+F n	M+F Median	M+F LL	M+F UL	Male n	Male Median	Male LL	Male UL	Female n	Female Median	Female LL	Female UL
Unit	y.o	n	Median	LL	UL	n	Median	LL	UL	n	Median	LL	UL
BMI kg/m²	65>	563	21.8	17.4	29.8	272	23.1	18.2	30.1	489	21	17.3	29.1
	65≤	[illegible]	22.1	16.6	28.4	[illegible]	22.8	[illegible]	28.4	499	21.6	16.2	28.4
BP systolic mmHg	65>	[illegible]	114	90	142	[illegible]	118	94	148	[illegible]	112	89	141
	65≤	[illegible]	123	92	154	[illegible]	123	[illegible]	[illegible]	[illegible]	124	92	154
BP diastolic mmHg	65>	[illegible]	72	54	95	[illegible]	77	58	99	[illegible]	70	53	91
	65≤	[illegible]	73	53	93	[illegible]	74	[illegible]	[illegible]	[illegible]	73	53	92
TP g/dL	65>	[illegible]	7.2	6.5	8	[illegible]	7.3	6.5	8.1	[illegible]	7.2	6.5	8.0
	65≤	[illegible]	7.2	6.4	8.1	[illegible]	7.3	[illegible]	[illegible]	[illegible]	7.2	6.4	8.0
Alb g/dL	65>	[illegible]	4.4	3.8	4.8	[illegible]	4.4	3.9	4.8	[illegible]	4.3	3.8	4.8
	65≤	[illegible]	4.2	3.7	4.7	[illegible]	4.2	3.6	4.7	[illegible]	4.2	3.7	4.7
CRP mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IgG mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IgA mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IgM mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C3 mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TTR mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
RBP mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
cysC mg/L	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Pep I mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Pep II mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
PepI/PepII	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Type IV Collagen mg/dL	65>	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	65≤	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

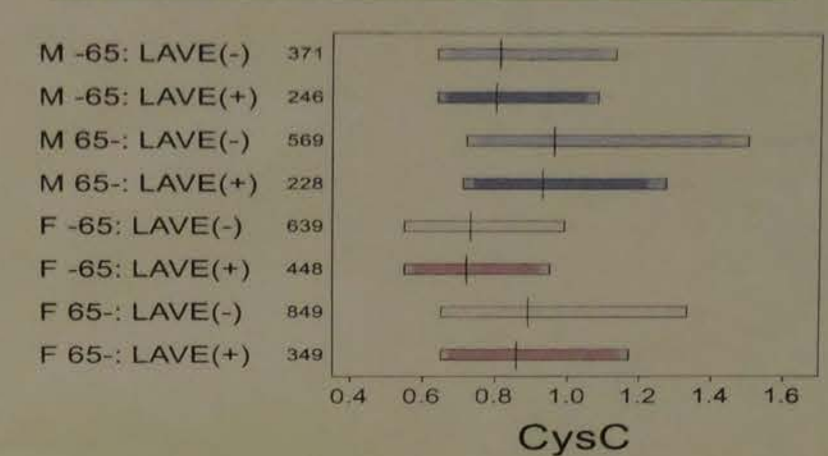
Performance of assays was reliable with high precision and accuracy.

The paper does not represent any conflict of interest by any of the authors.
The present work was approved by an Ethic Committee of Eiju General Hospital.
It was supported by a Grant-in-Aid from Life Extension Research Institute, Tokyo.

Multi-regression analysis in relation to age, sex, smoking, and BMI

Item	Male n	Male R	Male Age	Male Drkl.vl	Male Smkl.vl	Male BMI	Female n	Female R	Female Age	Female Drkl.vl	Female Smkl.vl	Female BMI
TP	891	0.19	-0.06	-0.01	-0.11	0.14	1436	0.17	-0.01	-0.04	-0.09	0.13
Alb	817	0.35	-0.34	0.02	-0.05	0.06	1290	0.22	-0.22	0.04	-0.04	-0.01
CRP	890	0.28	0.16	-0.02	0.15	0.21	1435	0.34	0.18	0.03	0.07	0.29
IgG	891	0.23	0.16	-0.05	-0.11	0.10	1436	0.23	0.08	-0.09	-0.12	0.09
IgA	891	0.21	0.18	0.03	-0.05	0.08	1436	0.19	0.15	0.01	-0.04	0.10
IgM	891	0.14	-0.13	-0.04	0.03	0.02	1436	0.23	-0.24	-0.05	-0.01	0.01
C3	891	0.40	-0.07	-0.11	0.08	0.37	1436	0.47	0.11	-0.06	0.00	0.44
TTR	891	0.44	-0.32	0.27	-0.05	0.09	1436	0.29	-0.09	0.22	0.01	0.13
RBP	891	0.31	-0.06	0.29	-0.05	0.08	1436	0.29	0.16	0.25	0.03	0.12
cys C	891	0.57	0.55	-0.12	0.05	0.14	1436	0.66	0.61	-0.09	0.08	0.19
Pep. I	891	0.21	-0.16	0.01	0.08	0.08	1436	0.08	0.01	-0.04	0.05	-0.07
Pep. II	891	0.12	0.11	0.04	0.04	0.04	1436	0.35	0.34	-0.01	0.00	-0.08
Pep I/Pep II	891	0.27	-0.23	-0.05	0.04	0.11	1436	0.28	-0.28	-0.05	0.05	0.04
Type IV Collagen	456	0.30	0.29	-0.06	0.00	0.00	727	0.23	0.23	0.02	0.08	-0.03

Age-dependent increase of cystatin C reflects decreased GFR



Age-dependent increase of cystatin C reflects decreased GFR.
Depriving increased Cr, and UN by modified LAVE made original cystatin C concentration lowered.

Analysis using "supernormal individuals"

Selection Criteria for Reference Intervals

Item	Unit	Criterion	Note
BMI	kg/m²	18.5-24.9	
BP	mm Hg	Systolic <140, and or diastolic <80	Aged <65 years old
		Systolic <150, and or diastolic <90	Aged <65 years old
Smoking		Non-smoker	
Alchohol		<80 g/week, drunk intermittently	
WBC	/µl	<8,000	
Hb	g/dL	>12.0	Male
		>11.0	Female
Albumin	g/dL	>3.2	
UA	g/dL	<7.0	Male
	g/dL	<8.0	Female
Cr	mg/dL		No cut-off specified
Glucose			No cut-off specified
HbA1c	%	<6.0	
TG	mg/dL	<150	
LDL-C	mg/dL	<140	
AST	U/L	<35	
ALT	U/L	<40	
GGT	U/L	<70	Male
		<30	Female
CRP	mg/dL	<0.3	
IgG	mg/dL	600-1800	
IgA	mg/dL	100-400	
IgM	mg/dL	50-400	
Cystatin C	mg/L	<1.10	
C3, RBP, TTR, Pep I, II			No cut-off specified

Male

Age		Stature	BW	BMI	BP systolic	BP diastolic	TP	Alb	CRP	IgG
	Unit	cm	Kg	Kg/m²	mm Hg	mm Hg	g/dL	g/dL	mg/dL	mg/dL
<65	Number	42	42	42	42	42	42	29	41	42
	mean	168.8	61.8	21.7	110.9	70.4	7.3	4.4	0.044	1239
	SD	5.6	5.7	1.6	11.4	8.0	0.4	0.2	0.051	197
	Medain	169.4	61.1	21.7	110.0	71.5	7.2	4.4	0.026	1221
	Max.	177.2	73.6	24.3	136	88	8.4	4.8	0.292	1747
	Min	156.1	49.6	18.7	90	50	6.3	4.1	0.007	892
≥65	Number	39	39	39	39	39	39	36	38	38
	mean	163.0	58.2	21.9	121.7	74.1	7.2	4.3	0.062	1271
	SD	4.9	5.5	1.6	11.0	6.3	0.6	0.3	0.062	215
	Medain	163.8	59.0	22.3	124.0	75.0	7.2	4.3	0.040	1260
	Max.	171.3	70.3	24.8	139	89	8.1	4.8	0.218	1647
	Min	148.8	48.5	19.2	96	59	5	3.1	0.004	690
p Value		2.85E-06	0.006	0.520	4.46E-05	0.021	0.653	0.080	0.166	0.498

Age	IgA	IgM	C3	TTR	RBP	cysC	Pep. I	Pep. II	Pep. I/Pep. II	Type IV col
	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/L	mg/dL	mg/dL	Ratio	mg/dL
<65	42	42	42	42	42	42	42	42	42	17
	240	79	104.3	26.9	2.9	0.8	61.3	13.9	5.0	121.8
	73	33	17	3.7	0.6	0.1	26.2	8.7	1.7	21.6
	233	69	104.0	26.3	2.9	0.8	54.5	11.1	5.2	117.5
	430	170	142	35.3	4.6	1.02	175	40.3	11.4	168.2
	132	26	74	18	1.8	0.65	32.9	4.9	1.8	[illegible]
≥65	38	38	38	38	38	38	38	38	38	18
	258	85	105.0	26.5	3.2	[illegible]	49.1	14.9	3.6	133.2
	91	38	19	4.7	0.7	[illegible]	32.4	9.2	2.1	24.7
	245	54	98.5	26.0	3.0	0.9	42.4	12.0	3.6	128.7
	494	189	149	39.6	3.7	1.09	137.5	46.9	8.7	173.4
	126	20	74	17.3	1.9	0.7	4.4	3	0.9	94.4
p Value	0.156	0.079	0.883	0.656	0.098	[illegible]	0.069	0.614	0.002	0.155

Female

Age		Stature	BW	BMI	BP systolic	BP diastolic	TP	Alb	CRP	IgG
	Unit	cm	Kg	Kg/m²	mm Hg	mm Hg	g/dL	g/dL	mg/dL	mg/dL
<65	Number	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Medain	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Max.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Min	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
≥65	Number	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Medain	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Max.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Min	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
p Value		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Age	IgA	IgM	C3	TTR	RBP	cysC	Pep. I	Pep. II	Pep. I/Pep. II	Type IV col
	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/L	mg/dL	mg/dL	Ratio	mg/dL
<65	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
≥65	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
p Value	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Conclusion

We initially set RI of major serum proteins in elderly population. It is a treasure box for physiologic and pathologic insights by a given protein, or proteins profiles. Cystatin C is a marker for aging on clinical setting.

Reference

[illegible]

Clinical Chemistry

PD-50

The extent of hemoglobin interference during serum total protein measurements is affected by the biuret reagent used

Ken-ichi Nagai

Department of Clinical Laboratory Saiseikai kawaguchi General Hospital

Introduction

The biuret method is widely used as a reference procedure for determining serum total protein concentrations in clinical laboratories because equal amounts of color are produced per gram of protein regardless of the type of protein. However, the reagents and analysis conditions employed can differ markedly between the various commercially available biuret assays.

Method

In the present study, we compared the extent of hemoglobin interference between eleven types of commercially available biuret assays (Fig. 1), A (SHINO-TEST, a 1-reagent system), B (SEKISUI MEDICAL, a 1-reagent system), C (Wako Pure Chemical, a 1-reagent system), D (SHINO-TEST, a 2-reagent system), E (KAINOS, a 2-reagent system), F (Wako Pure Chemical, a 2-reagent system), G (KANTO Chemistry, a 2-reagent system), H (SEROTEC, a 2-reagent system), I (NITTO-BO, a 2-reagent system) , J (SEKISUI MEDICAL, a 2-reagent system), and K (Wako Pure Chemical, a 2-reagent system). All analyses were performed using the FURUNO CA-270 plus autoanalyzer, but assays A, B, and C were carried out using the one point/dual wavelength method, whereas assays D, E,F,G,H,I,J and K were performed using the two points/dual wavelength method.

Results

The total protein levels of serum samples that had been spiked with 1.0 g/dL of hemoglobin were compared with those of Salines. In assays A, B, C, D, E,F,G,H,I,J and K 1.0 g/dL of hemoglobin was equivalent to total protein levels of 2.32, 2.27, 2.38, 1.14, 1.35, 0.14, 0.45, 0.93, 0.35, 0.32, and 0.05 g/dL, respectively (Fig. 2, 3).

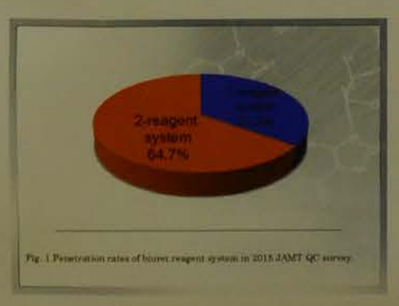


Fig. 1 Penetration rates of biuret reagent system in 2015 JAMT QC survey

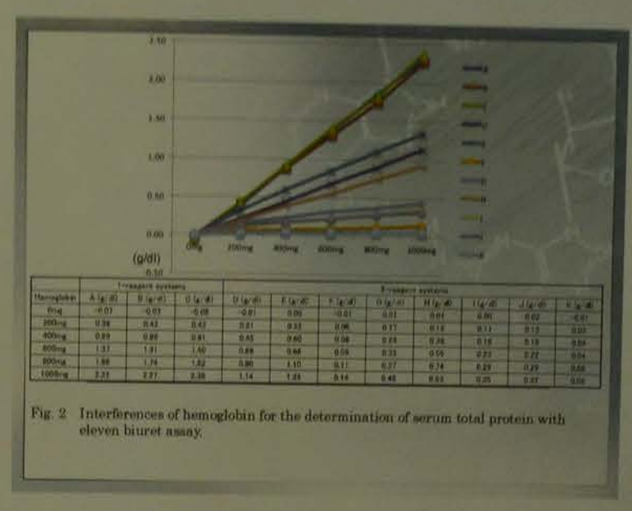


Fig. 2 Interferences of hemoglobin for the determination of serum total protein with eleven biuret asasy.

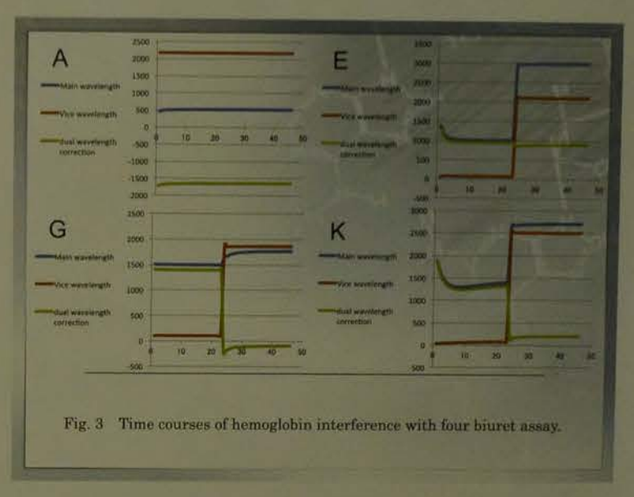


Fig. 3 Time courses of hemoglobin interference with four biuret assay.

Conclusions

In the 1-reagent systems, (assays A, B, and C), the effect of hemoglobin interference on serum total protein measurements was equivalent to the sum of the error due to the absorption of hemoglobin and the error caused by hemoglobin participating in the biuret reaction. In contrast, in assays D, E and H (2-reagent systems), the effect of hemoglobin interference was dependent on the hemoglobin concentration. Furthermore, in assay F, and K (a 2-reagent system) the two different hemoglobin interference mechanisms were considered to have canceled each other out. By contrast, in assay G, I, and J (a 2-reagent system) the was interference of half of them.

C-3 C-3

Clinical Chemistry

PD-51

Detection and Regulation of Apolipoprotein M in the Central Nervous System

Naoko Hisasue[1,3], Makoto Kurano[1], Yuki Morimoto[2], Koichi Tsuneyama[2], Tetsuo Kubota[3], Yutaka Yatomi[1]

1: Department of Clinical Laboratory, The University of Tokyo Hospital
2: Department of Pathology and Laboratory Medicine, Institute of Biomedical Sciences, Tokushima University Graduate School
3: Department of Microbiology and Immunology, Tokyo Medical and Dental University, Graduate School of Health Care Sciences

Background

It is well known that HDL-like lipoproteins exist in the **central nervous system (CNS)** and play important roles in the pathogenesis of several diseases. However, pleiotropic effects of those are yet to be known.

Apolipoprotein M (apoM) is a minor apolipoprotein riding on HDL. Recently apoM is elucidated to be a carrier of sphingosine 1-phosphate (S1P), a bioactive lipid mediator, which contributes to several pleiotropic effects of HDL, including anti-apoptosis and vaso-relaxation in the circulatory system.

In this study, we aimed to **examine the existence and regulation of apoM in CNS.**

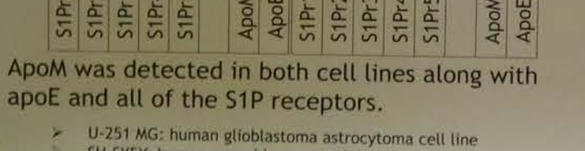
Methods & Results

Experiment 1

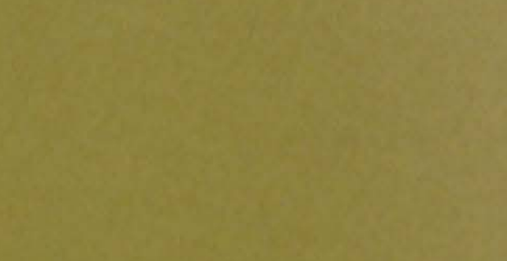
Detection of ApoM and S1P in Plasma and CSF

	Plasma	CSF
ApoM	10 - 60 ng/mL	0.1 - 1.0 ng/mL
S1P	300 - 800 nM	1 - 20 nM

ApoM and S1P were detected in CSF at 1 - 10 % and 0.3 - 6 % of plasma, respectively.

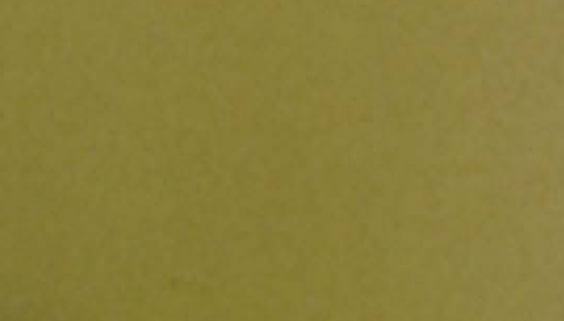


Immunostaining of the Human Brain for ApoM



Immunostaining with purified anti-apoM antibody revealed that apoM was expressed mainly in glial cells of the human brain.

Detection of ApoM, ApoE, and S1P Receptors by Reverse Transcription PCR from Cell Lines

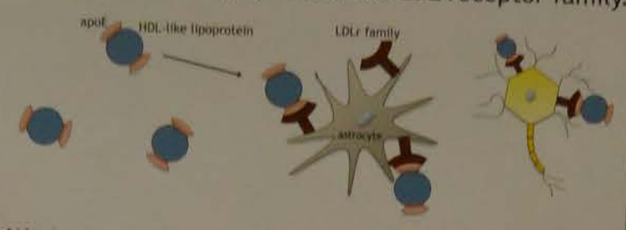


ApoM was detected in both cell lines along with apoE and all of the S1P receptors.

- U-251 MG: human glioblastoma astrocytoma cell line
- SH-SY5Y: human neuroblastoma cell line

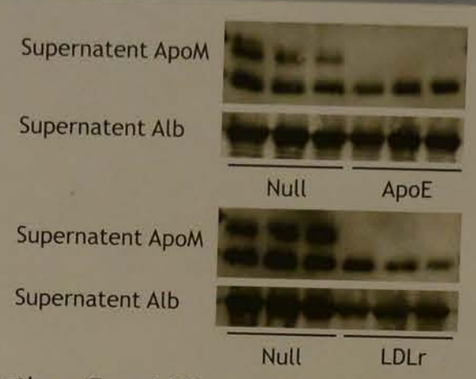
Experiment 2

Homeostasis of HDL-like lipoproteins in the CNS is largely regulated by apoE and the LDL receptor family.



We investigated the modulation of apoM in CNS by apolipoprotein E (apoE) and the LDL receptor, which are known to be involved in the homeostasis of apoM in the circulatory system.

Detection of ApoM in Adenoviral ApoE- and LDL Receptor-Overexpressed U-251 MG Cells



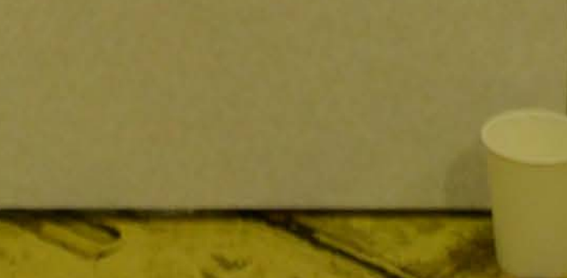
In both apoE- and LDL receptor-overexpressed U251 MG cells, supernatent apoM levels were decreased.

Conclusion

The present findings indicate:

① ApoM exists in CNS.
② ApoM may contribute to the pleiotropic properties of HDL-like lipoproteins in the CNS.
③ ApoE and LDL receptor are involved in the homeostasis of apoM in the CNS as well.

Considering apoE and LDL receptor have important roles in the pathogenesis of neurological diseases, such as Alzheimer's disease, **apoM in CNS** might possibly guide a way for **the development of novel laboratory testing and therapy for neurological diseases.**



ical
istry
-53

Urinary VEGF-A_{165}b and estimated Glome
- Does urinary VEGF-A_{165}b estimate kid

○ Ryosuke Kikuchi[1],
Yasuda Yoshinori[2], Hiroyuki Matsumoto[1], Toyoaki Muro
[1]Department of Medical Technique, Nagoya University
[2] Department of CKD Initiatives/nephrology, Nagoya University Gradu
[3] Department of Cardiology, Nagoya University Graduate Sch
[4]Department of Clinical Laboratory Medicine / Transfusion Medicine, Na

nd – What are CKD, VEGF-A and VEGF-A_{165}b ? -

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and Methods

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chemical Staining (IHC) : The successive sections were stained IHC in the

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sorbent assay (ELISA) according to the manufacturer's instructions (Human

alysis : Statistical analyses were completed with GraphPad Prism6.

Figure1. VEGF-A_{165}b
Human Kidney Tissu
To determine the expression of
tissues, IHC analyses were per
human kidney tissues revealed
podocyte and glomerular endot
kidney is the source of VEGF-A

	eGFR ≧ 60	60>eGFR ≧ 45	45>eGFR ≧ 30	30
Age, years	55 ± 12	58 ± 14	61 ± 14	
Male sex, %	63	84	58	
Weight (kg)	70 ± 16	62 ± 13	63 ± 10	
BMI (kg/m²)	25.8 ± 4.2	23.2 ± 4.2	23.5 ± 3.4	
Diabetes mellitus, %	14.8	16	36.8	
Hypertension, %	77.8	72	94.7	
Hypercholesterolemia, %	62.8	72	57.9	
Serum Creatinine (mg/dL)	1.19 ± 0.99	1.24 ± 0.79	1.22 ± 0.89	1.19
Serum Cystatin C (mg/dL)	1.07 ± 0.26	1.28 ± 0.27 *	1.59 ± 0.26 ***	2.35
eGFR cys (ml / min / 1.73 m²)	72.4 ± 18.5	57.6 ± 11.2 *	42.4 ± 6.9 ***	25.1
Inulin clearance (C$_{in}$, ml / min / 1.73 m²)	88.8 ± 16.8	48.8 ± 12.5 ***	41.9 ± 11.3 ***	19.7
Serum total VEGF-A (pg / mL)	247.6 ± 127.8	396.1 ± 334.3	370.8 ± 295.8	370.8
Serum VEGF-A_{165}b (pg / mL)	143.3 ± 85.8	241.4 ± 253.3	213.9 ± 152.8	200.2
Urinary total protein / Creatinine (g / gCr)	0.83 ± 1.16	1.07 ± 1.62	1.24 ± 1.34	3.79
Urinary micro albumin / Creatinine (mg / gCr)	344.5 ± 926.0	503.1 ± 933.8	915.1 ± 971.2	881.8
Urinary beta 2 microglobulin / Creatinine (ug / gCr)	617.2 ± 2981.7	950.8 ± 991.1	1308.7 ± 4120.7	9663.7
Urinary total VEGF-A / Creatine (ng / g Cr)	371.8 ± 287.2	253.1 ± 284.3 **	201.8 ± 113.7 **	241.8
Urinary VEGF-A_{165}b / Creatine (ng / g Cr)	180.9 ± 80.3	136.3 ± 45.2 *	118.4 ± 43.3 **	116.8

Correlation Between VEGF-A_{165}b and Estimated Glomerular Filtratio
d the relationship between urinary levels of VEGF-A_{165}b and eGFR in all subjects.
haracteristics table. **B**: Urinary VEGF-A_{165}b levels were correlated positively with eGFR. **C**: Urinary VEG
antly decreased compared to eGFR≧60 subjects.

lon

dicate the close association of low urinary VEGF-A_{165}b levels with kidney functio
EGF-A_{165}b represents a novel biomarker for kidney dysfunction.

Clinical Chemistry

PD-52

Role of adiponectin in chronic kidney disease.

Masashi Miyoshi Takayuki Nakao Toshio Doi

Division of Medical Technology, Tokushima University Hospital

Introduction

Adiponectin is one of the bioactive substances secreted by adipocytes. It may play an important role in the progression of chronic kidney disease (CKD).
However, information regarding the underlying protective mechanism of adiponectin limited.

Aim

To clarify adiponectin involvement in CKD.

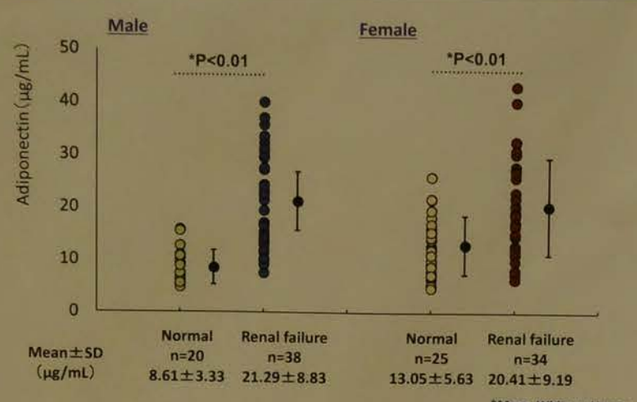
Method

We measured the level of adiponectin in patients with CKD and correlated the data with their functional index and risk stage. In addition, we examined the relationship between serum adiponectin levels and renal dysfunction.

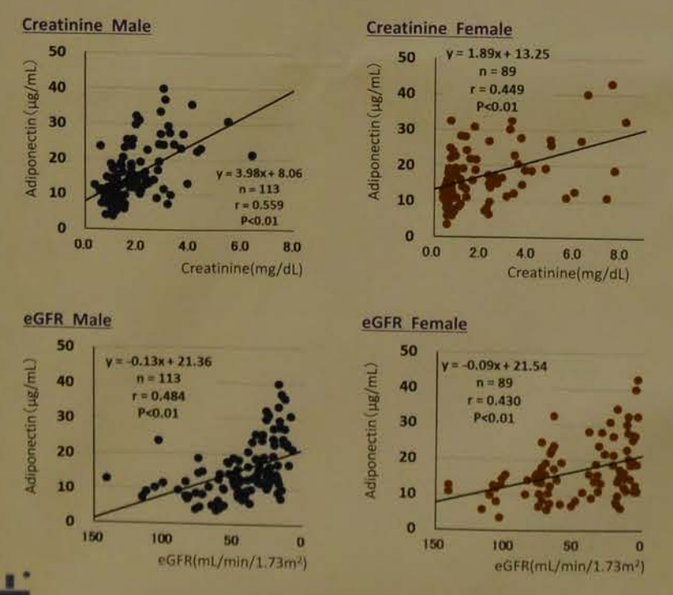
Study population

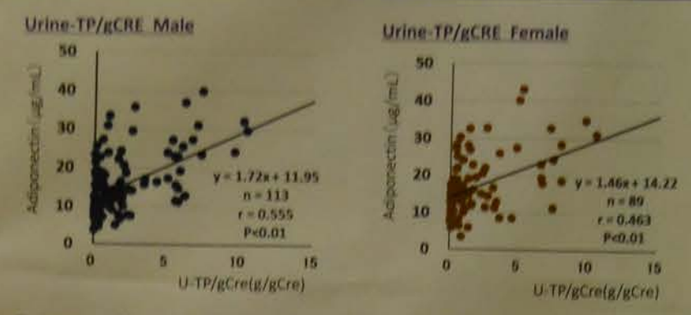
In this study, we included patients examined at the Tokushima University Hospital with CKD (n=228) .
In addition, healthy participants were enrolled as controls (n=45). We analyzed the patients according to the patient's sex.

Results



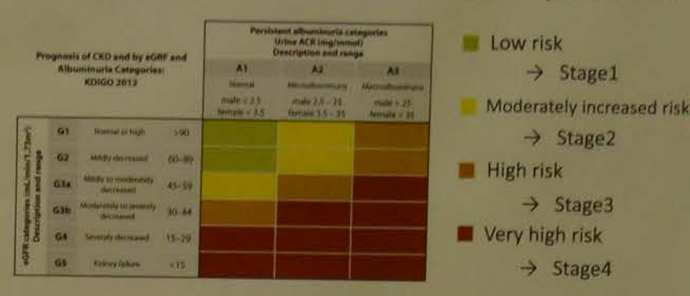
The patients with renal failure had elevated serum levels of adiponectin.

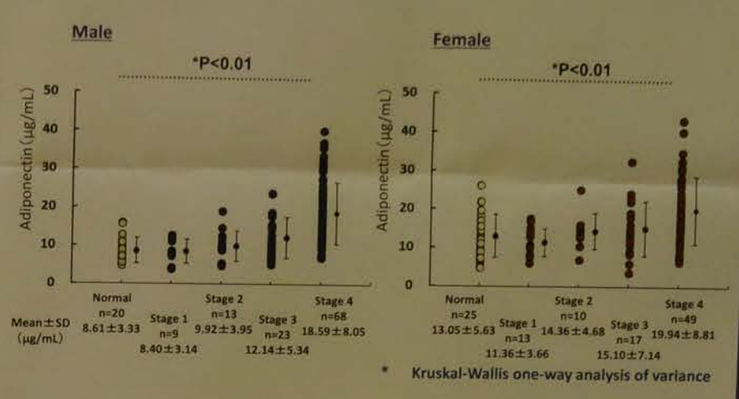




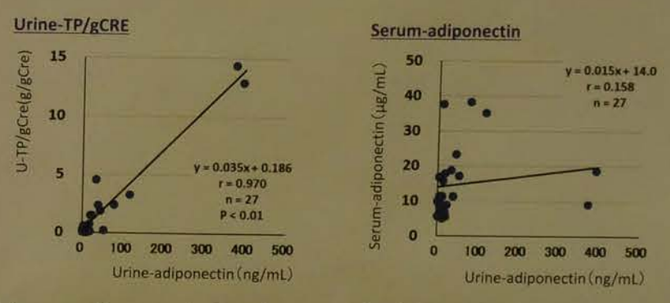
Serum adiponectin levels were significantly correlated with creatinine level, eGFR, and urinary protein levels.

We categorized the patients with CKD into 4 stages based on the risk of mortality or the stage of renal dysfunction.





Serum adiponectin levels increased with advanced risk stages.



Urinary adiponectin levels were significantly correlated with urinary protein levels, but not with serum adiponectin levels.

Discussion

In conclusion, serum adiponectin levels had increased in patients with progressive renal dysfunction. This finding is in contrast with the protective role of adiponectin.

Urinary adiponectin levels were not correlated with serum adiponectin levels, we found that high serum levels in patients with renal failure were not associated with an increase in urinary levels.

Summary

Serum adiponectin levels were associated with renal dysfunction.

Adiponectin plays a protective roles in the kidney.

Tokushima University Hospital

C-3 C-3

Clinical Chemistry

PD-53

PD-53

Urinary VEGF-A_{165}b and estimated Glomerular Filtration Rate
- Does urinary VEGF-A_{165}b estimate kidney function ? -

○ **Ryosuke Kikuchi**[1],

Yasuda Yoshinori[2], Hiroyuki Matsumoto[1], Toyoaki Murohara[3], Tadashi Matsushita[4]

[1]Department of Medical Technique, Nagoya University Hospital, JAPAN.
[2] Department of CKD Initiatives/nephrology, Nagoya University Graduate School of Medicine, JAPAN.
[3] Department of Cardiology, Nagoya University Graduate School of Medicine, JAPAN.
[4]Department of Clinical Laboratory Medicine / Transfusion Medicine, Nagoya University Hospital, JAPAN.

Background – What are CKD, VEGF-A and VEGF-A_{165}b ? -

Chronic kidney disease (CKD) is a public health concern in the worldwide. Vascular endothelial growth factor A (VEGF-A) is constitutively expressed by epithelial cells of nephron from embryonic to adult kidneys. CKD is associated with reduced VEGF-A expression. To date, essentially nothing is known about the role of VEGF-A splice isoforms, so called VEGF-A_{165}b, play in kidney physiology and pathology is still unclear.

Aim

This study aimed to investigate whether urinary VEGF-A_{165}b levels are able to estimate kidney function.

Materials and Methods

Clinical Samples : The kidney tissue was carefully excised from patient who had died and underwent autopsy. Total number of 157 patients were enrolled from *in* and *out* patient clinics at Nagoya University Hospital.

Immunohistochemical Staining (IHC) : The successive sections were stained IHC in the order of VEGF-A and VEGF-A_{165}b.

Measurement of Serum & Urinary VEGF-A_{165}b : As a pre-treatment, serum samples were treated with DTT (final concentration is 5 mM) for 60 min at 37 °C. Serum and urinary VEGF-A_{165}b levels was determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Human VEGF-A_{165}b ELISA Kit, MyBiosource).

Statistical Analysis : Statistical analyses were completed with GraphPad Prism6.

Results

Figure 1.

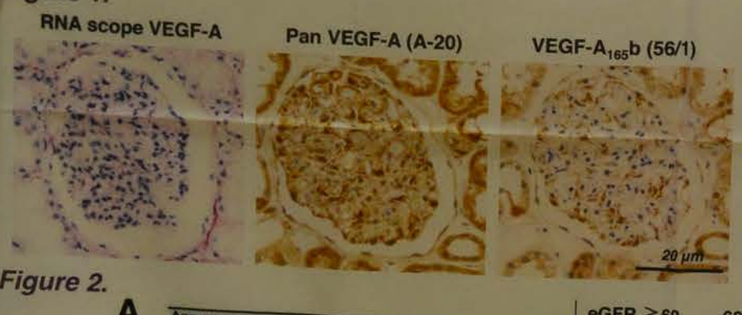


Figure1. VEGF-A_{165}b Expression in Human Kidney Tissue.
To determine the expression of VEGF-A_{165}b in human kidney tissues, IHC analyses were performed. IHC analyses of the human kidney tissues revealed VEGF-A_{165}b localization with podocyte and glomerular endothelial cells, indicating that kidney is the source of VEGF-A_{165}b.

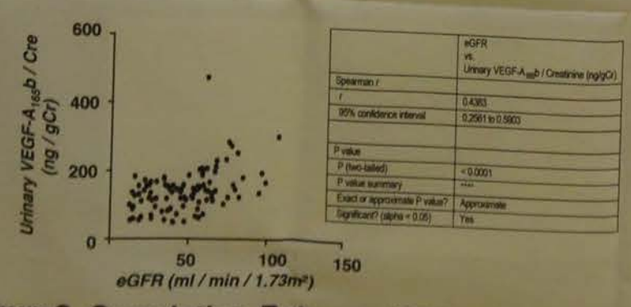
Figure 2.

A

Age, years	eGFR ≧60	60>eGFR≧45	45>eGFR≧30	30>eGFR
Male sex,%	55 ±12	58 ±14	61 ±14	64 ±11*
Weight (kg)	63	64	58	58
BMI (kg/m²)	70 ±16	62 ±15	63 ±10	61 ±12
Diabetes mellitus, %	25.6 ±4.2	23.2 ±4.2	23.5 ±2.4	23.8 ±4.1
Hypertension, %	14.8	16	36.8	23.1
Hypercholesterolemia, %	77.8	72	94.7	92.3
Serum Creatinine (mg/dL)	62.9	72	57.9	42.3
Serum Cystatin C (mg/dL)	1.10 ±0.99	1.24 ±0.79	1.22 ±0.59	1.10 ±0.58
eGFR cys (ml / min / 1.73 m²)	1.07 ±0.26	1.26 ±0.27 *	1.59 ±0.26 ***	2.56 ±0.51***
Inulin crearance (C_{in}, ml / min / 1.73 m²)	72.4 ±19.5	57.6 ±11.2 *	42.6 ±8.8 ***	23.1 ±6.3 ***
Serum total VEGF-A (pg / mL)	68.8 ±16.8	48.8 ±12.3 ***	41.5 ±11.3 ***	19.7 ±8.7 ***
Serum VEGF-A_{165}b (pg / mL)	247.6 ±127.8	366.1 ±334.3	370.9 ±255.6	376.0 ±256.1 *
Urinary total protein / Crestinine (g / gCr)	143.3 ±95.8	241.4 ±253.3	213.9 ±152.9	200.8 ±129.9 *
Urinary micro alubumin / Creatinine (mg / gCr)	0.83 ±1.16	1.07 ±1.92	1.04 ±1.54	1.74 ±1.19 **
Urinary beta 2 microglobulin / Creatinine (μg / gCr)	544.5 ±929.0	503.1 ±633.8	610.1 ±971.3	881.8 ±552.5 **
Urinary total VEGF-A / Creatine (ng / g Cr)	817.2 ±2881.7	563.8 ±991.1	1398.7 ±4122.3	6683.2 ±6971.2 ***
Urinary VEGF-A_{165}b / Creatine (ng / g Cr)	371.6 ±267.2	253.1 ±284. 2 **	201.8 ±113.7 **	241.6 ±191.6 **
	190.9 ±80.3	136.3 ±45.2 *	119.4 ±43.5 **	116.9 ±40.2 ***

Data expressed as Mean ± SD.
*P<0.05, **P<0.01, ***P<0.001, compared with GFR ≧60 subjects

B



	eGFR vs. Urinary VEGF-A_{165}b / Creatinine (ng/gCr)
Spearman r	
r	0.4083
95% confidence interval	0.2561 to 0.5503
P value	
P (two-tailed)	< 0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes

C

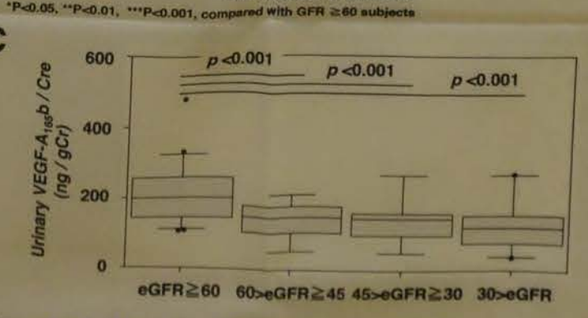


Figure2. Correlation Between VEGF-A_{165}b and Estimated Glomerular Filtration Rate.
We assessed the relationship between urinary levels of VEGF-A_{165}b and eGFR in all subjects.
A: Patient characteristics table. **B**: Urinary VEGF-A_{165}b levels were correlated positively with eGFR. **C**: Urinary VEGF-A_{165}b concentrations were significantly decreased compared to eGFR≧60 subjects.

Conclusion

Our data indicate the close association of low urinary VEGF-A_{165}b levels with kidney function, suggesting that *urinary VEGF-A_{165}b represents a novel biomarker for kidney dysfunction.*

A-3 A

f adiponectin in chronic kidney dise

Masashi Miyoshi Takayuki Nakao Tos

Division of Medical Technology, Tokushima University

ances secreted by
in the progression

erlying protective

ment in CKD.

patients with CKD
onal index and risk
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on

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rolled as controls
g to the patient's sex.

*P<0.01

Normal Renal failure

evated serum levels

Female

Urine-TP/gCRE Male

Urine-TP/gCRE Fem

Adiponectin (μg/mL)

U-TP/gCre(g/gCre)

Serum adiponectin levels were significantly
with creatinine level, eGFR, and urinary prot

We categorized the patients with CKD into 4
on the risk of mortality or the stage of renal d

Low ri
Moder
High r
Very h

Male

Female

*P<0.01

Normal Stage 1 Stage 2 Stage 3 Stage 4

Kruskal-Wallis one-way

Serum adiponectin levels increased with advance

Urine-TP/gCRE

Serum-adiponectin

Urine-adiponectin (ng/mL)

Urinary adiponectin levels were significantly cor
urinary protein levels, but not with serum adipon

Discussion

In conclusion, serum adiponectin levels had incr
patients with progressive renal dysfunction. This
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Urinary adiponectin levels were not correlated w
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patients with renal failure were not associated w
increase in urinary levels.

Summary

Serum adiponectin levels were associate
dysfunction.

Adiponectin plays a protective roles in th

ospital

Clinical Chemistry

PD-54

WAN-LING CHIU[1, 2], YU-HSUAN SHAO[1]
PEI-WEN LEE[2], CHIA-LING CHEN[3]

[1] Graduate Institute of Biomedical Informatics, Taipei Medical University, Taiwan
[2] Department of Clinical Laboratory, Taipei City Hospital Yang-Ming Branch, Taiwan
[3] Nursing Department, Far Eastern Memorial Hospital, Taiwan

臺北醫學大學 TAIPEI MEDICAL UNIVERSITY

臺北市立聯合醫院 TAIPEI CITY HOSPITAL

Maintain the Accuracy of point-of-care testing POCT for Glucometer

Introduction

NCCLS 1995 published AST2-P documents; bedside in vitro diagnostic test called POCT, it appears that the traditionally specialized inspectors work done more to non-professionals and individuals themselves to complete. POCT method is to use a small, portable instrument can be measured in minutes.

ISO 15197: 2013 new standard

1. When blood glucose results <100 mg / dl (or less), the error should be ±15 mg/dl.
2. When blood glucose results >100 mg / dl (or more), the error should be ±15%.
3. In addition to the results meet the acceptance criteria should be greater than 95%, the results should fall within 99% of the A and B zones of error grid.

Result

The intraclass correlation coefficient inferential statistic that can be used when quantitative measurements are made on units that are organized into groups. This result shows that the two blood glucometers and biochemical machine alignment are qualified standard. Among them, Roche performance was excellent, but no statistically significant difference in terms.

Interclass Correlation Coefficients (ICC)
BECKMEN & Roche

	ICC	95% (confidence interval)
Individual	0.985	0.968-0.992
Average	0.993	0.984-0.996

Interclass Correlation Coefficients (ICC)
BECKMEN & FEGO

	ICC	95% (confidence interval)
Individual	0.978	0.957-0.989
Average	0.989	0.978-0.994

Author Profile

邱琬玲 Wan-Ling Chiu
Medical Technologists of Taipei City Hospital
Taipei Association of Medical Technologists Supervisors
E-mail：charles230132@gmail.com

WHO Global report on diabetes

Methods

Taipei City Hospital Yang-Ming Branch

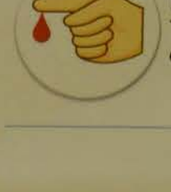
OPD
40 people

Gold stander

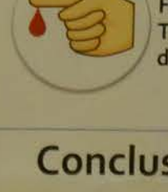
FEGO Glucometer Glucose Oxidase

Roche Accu-Check Active Glucose Dehydrogenase

Biochemical Beckman DxC 600 hexokinase method



Finger stick The second drop of blood



Finger stick The second drop of blood

Venous blood

Conclusion

Glucometer plays an important roles in the diabetics, Ease of operation is very easy to overlook usage restrictions. The use of chemical and biological stability of the machine and the use of quality control that is normally subject detection features, To help users understand the results of blood sugar detects whether maintenance and operation precautions in the acceptable range, and further consultation glucometer.

PD-55

Background

Methods

Results

Conclusion

Putative mechanisms for pita
changes of lipid-associat

statin

Clearance of LDL

HMGCoA

LDLR

SREBP-2

Free cholesterol

Oxy-cholesterol

LXR-α

CETP

SREBP-1c

Serum CETP

C-3 C-3

Clinical Chemistry

PD-55

Serum CETP status is independently associated with reduction rates in LDL-C in pitavastatin-treated diabetic patients

— possible involvement of LXR in its association —

Akihiro Shimada[1], Koji Oida[2], Hideo Kanehara[3], Yasuki Ito[4], Masayuki Iwano[5], Tsutomu Hirano[6] and Haruyoshi Yoshida[1,7], Hideki Kimura[1]

[1]Department of Clinical Laboratory and Nephrology, University of Fukui Hospital, [2]Division of Internal Medicine, Fukui Chuo Clinic, [3]Division of Endocrinology, Fukui-ken Saiseikai Hospital, [4]Research and Development Department, Denka Seiken Co. Ltd, [5]Division of Nephrology, Department of Medicine, School of Medicine, Faculty of Medical Sciences, University of Fukui, [6]Department of Medicine, Division of Diabetes, Metabolism, and Endocrinology, Showa University School of Medicine, [7]Division of Nephrology, Obama Municipal Hospital

Background

Statins decrease cholesteryl ester transfer protein (CETP) levels, which have been positively associated with hepatic lipid content as well as serum low density lipoproteins-cholesterol (LDL-C) levels. However, the relationship between the CETP status and statin-induced reductions in LDL-C levels has not yet been elucidated in detail. We herein examined the influence of the CETP status on the lipid-reducing effects of pitavastatin in hypercholesterolemic patients with type 2 diabetes mellitus as well as the molecular mechanism underlying pitavastatin-induced modifications in CETP levels.

Methods

Fifty-three patients were treated with 2 mg of pitavastatin for 3 months. Serum levels of LDL-C, small dense (sd) LDL-C, and CETP were measured before and after the pitavastatin treatment. The effects of pitavastatin, T0901317, a specific agonist for liver X receptor (LXR) that reflects hepatic cholesterol contents, and LXR silencing on CETP mRNA expression in HepG2 cells were also examined by a real-time PCR assay.

Results

The results are shown in Table and Figure.

Table1 The background of the enrolled patients

Characteristics	N=53
Age (years)	61.1±9.8[a]
Male, n (%)	27 (50.9)[b]
Body mass index (kg/m^2) n=52	24.8±4.5
Abdominal girth (cm) n=48	88.7±9.4
Systolic blood pressure (mmHg) n=52	127.9±12.0
Diastolic blood pressure (mmHg) n=52	73.3±9.2
HbA1c (%)	7.1±0.8
Complications	
Type 2 diabetes, n (%)	53(100.0)[b]
diabetic neuropathy, n (%)	10(18.9)
diabetic nephropathy, n (%)	5(9.4)
diabetic retinopathy, n (%)	4(7.5)
Hypertension, n (%)	23(43.4)
Coronary artery disease, n (%)	4(7.5)
Cerebral artery disease, n (%)	1(1.9)
Peripheral artery disease, n (%)	2(3.8)

[a]Data present mean±SD. [b]Data present the number and its percentage (%).

Table2 Concurrent medications

Drugs	n=53
Anti-diabetic agents	
sulfonyl urea	28 (52.8%)
biguanide	25 (47.2%)
α-glucosidase inhibitor	23 (43.4%)
pioglitazone	10 (18.9%)
Insulin	5 (9.4%)
Anti-hypertensive agents	
angiotensin II type 1 receptor blockers (ARBs)	18 (34.0%)
calcium channel blockers	13 (24.5%)
diuretics	7 (13.2%)
angiotensin-converting enzyme inhibitors	5 (9.4%)
Anti-lipidemic agents	
fenofibrate	2 (3.8%)

Data present the number and its percentage (%).

Table3 Clinical data profile of pre- and post-pitavastatin treatment

Variables	n	baseline	n	Post-treatment	Change(%)	*P*value
Body weight (kg)	53	64.1±15.4	52	64.4±16.2	0.7±2.4	0.052
HbA1c (%)	53	7.1±0.80	53	7.2±0.84	1.6±8.0	0.13
eGFR(ml/mim/m^2)	52	75.4±16.9	51	76.8±16.5	4.2±17.2	0.18
BUN (mg/dL)	52	14.7±4.2	50	14.8±3.9	−2.3±23.8	0.49
Uric acid (mg/dL)	52	5.2±1.4	51	4.9±1.2	−4.7±14.1	0.02
TC (mg/dL)	53	234.3±28.9	48	173.0±25.8	−25.7±9.9	<0.01
TG (mg/dL)	53	146.7±74.1	48	134.2±87.0	−4.1±55.3	0.61
HDL-C (mg/dL)	53	58.8±14.8	49	58.7±15.5	−0.6±11.4	0.70
LDL-C (mg/dL)	53	152.1±29.2	49	91.2±25.4	−39.4±13.6	<0.01
sd LDL-C (mg/dL)	53	52.0±18.1	49	28.7±10.4	−41.7±18.0	<0.01
sdLDL-C/LDL-C (%)	53	33.9±8.4	49	31.6±7.3	−4.5±15.6	0.047
ApoA-I (mg/dL)	53	142.9±26.6	48	148.5±26.7	3.5±8.4	<0.01
CETP (pg/mL)	48	2.54±0.60	46	1.98±0.73	−22.9±19.5	<0.01

Table4 Comparison of lipid data between patients with below- and abovemedian CETP levels

	CETP <2.6μg/mL (n = 21)	CETP ≧2.6pg/mL (n = 23)	p
Baseline CETP (pg/mL)	2.12 ± 0.3	3.01 ± 0.44	<0.01
Post-treatment CETP (pg/mL)	1.48 ± 0.36	2.44 ± 0.71	<0.01
Change in CETP (%)	−28.9 ± 20.3	−19.2 ± 17.0	0.09
Baseline LDL-C (mg/dL)	143.5 ± 234.1	157.0 ± 34.9	0.14
Post-treatment LDL-C (mg/dL)	75.1 ± 15.2	103.6 ± 26.3	<0.01
Change in LDL-C (%)	−46.4 ± 11.2	−33.1 ± 13.1	<0.01
Baseline sd LDL-C (mg/dL)	51.8 ± 16.3	51.7 ± 20.6	0.98
Post-treatment sd LDL-C (mg/dL)	23.9 ± 6.5	32.7 ± 12.1	<0.01
Change in LDL-C (%)	−49.6 ± 13.7	−33.5 ± 19.2	<0.01

Table 5 Linear regression analyses of baseline values and percent changes in clinical factors affecting the percent change in LDL-C levels

	Univariate			Multivariate		
	n	β[a]	*p*	n	β[b]	*p*
BMI (kg/m^2)	46	0.17	0.27[c]			
Gender (Male = 1, Female = 2)	47	−0.13	0.039			
Age (year)	47	−0.24	<0.1	44	−0.07	0.7
Body weight (kg)	47	0.3	0.04	44	0.07	0.68
Abdominal girth (cm)	48	0.11	0.47			
HbA1c (%)	47	0.08	0.57			
Uric acid (mg/dL)	47	0.05	0.74			
TC (mg/dL)	47	−0.37	0.011	44	−0.39	0.009
LDL-C (mg/dL)	47	−0.21	0.15[d]			
TG (mg/dL)	47	−0.1	0.5			
HDL-C (mg/dL)	47	−0.32	0.03	44	−0.16	0.29
sd LDL-C (mg/dL)	47	−0.29	0.04[d]			
ApoA-I (mg/dL)	47	−0.28	0.05[e]			
CETP (μg/mL)	44	0.38	0.01	44	0.41	0.006
Pioglitazone use	47	-0.02	0.9			
Change in Body weight (%)	47	0.19	0.2	43	0.36	0.01
Change in HbA1c (%)	47	−0.009	0.95			
Change in Uric acid (%)	47	0.14	0.33			
Change in TG (%)	46	0.22	0.15	43	0.17	0.2
Change in HDL-C (%)	47	−0.07	0.64			
Change in ApoA-I (%)	46	0.06	0.71			
Change in CETP (%)	44	0.44	0.003	43	0.57	0.0001

[a]standard regression coefficient [b]standard partial regression coefficient
[c]BMI was excluded from multivariate analysis, since colinearity between BMI and body weight was found
[d]LDL-C and sd LDL-C were excluded from multivariate analysis, since colinearity among TC, LDL-C and sd LDL-C was found
[e]ApoA-I was excluded from multivariate analysis, since colinearity between HDL-C and ApoA-I was found

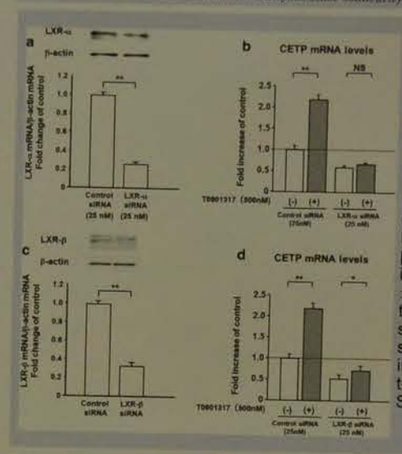


Figure 1 LXR silencing inhibited T0901317-stimulated CETP expression in HepG2 cells.
HepG2 cells (30–40 % confluence) were transfected with negative control siRNA or siRNA against LXR-α (a and b) or LXR-β (c and d) (25nM each). After a 48-h incubation, HepG2 cells that had been refreshed with DMEM were treated with or without T0901317 (500 nM) for an additional 24 h (b and d). Protein amounts of LXR-α (a), LXR-β (c), and β-actin in the cell lysates were determined by immunoblot analyses. Results were the mean ± SD of an independent experiment in triplicate or quadruplicate (n = 3-4). NS, not significant; *P < 0.05 and **P < 0.01 significantly different from cells under the indicated conditions, according to an unpaired t-test for Fig. 1a and c and ANOVA with Scheffé's post hoc test for Fig. 1b and d

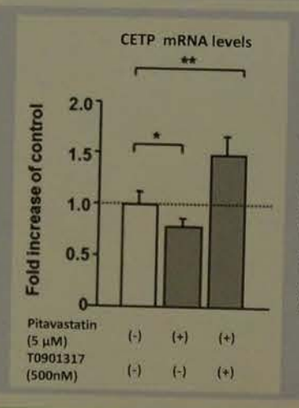


Figure 2 Pitavastatin decreased CETP mRNA levels and T0901317 abolished the decreasing effects.
HepG2 cells grown to semiconfluence were treated with DMEM containing no pitavastatin or T0901317 (control), 5 μM pitavastatin alone, or 5 μM pitavastatin plus T0901317 (500 nM) for 24 h. Results were the mean ± SD of three independent experiments in duplicate or triplicate (n = 8-9).
*P < 0.05 and **P < 0.01 significantly different from cells under the indicated conditions, according to ANOVA with Scheffé's post hoc test.

Putative mechanisms for pitavastatin-induced changes of lipid-assosiated proteins

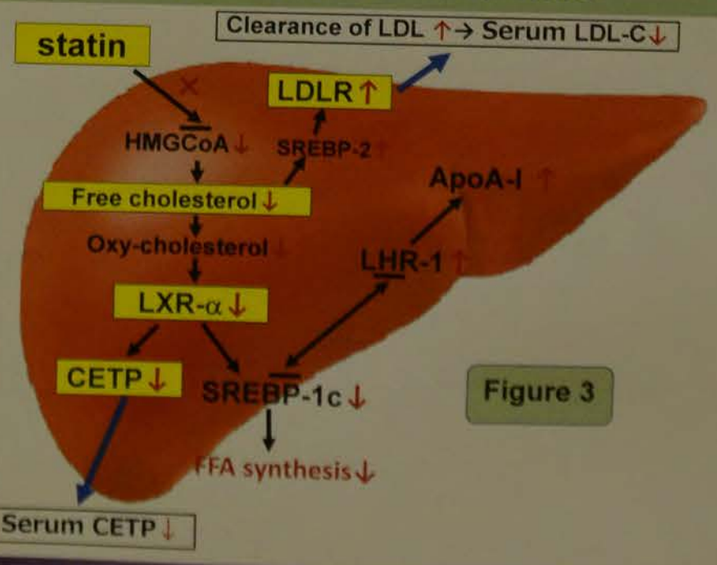


Figure 3

Conclusion

The baseline CETP level, a possible surrogate for hepatic cholesterol accumulation, was an independent positive determinant of pitavastatin-induced changes in LDL-C and sd LDL-C levels in hypercholesterolemic patients with type 2 diabetes mellitus. Concurrent and comparable reductions in serum CETP and LDL-C levels suggest that pitavastatin may decrease LXR activity as well as cholesterol synthesis in the liver.

Clinical Chemistry

PD-56

Evaluation of RG-II POCT Devices for Glucose Testing

Hye Jung Kim, Hye Lim Kim and Yong Lim*

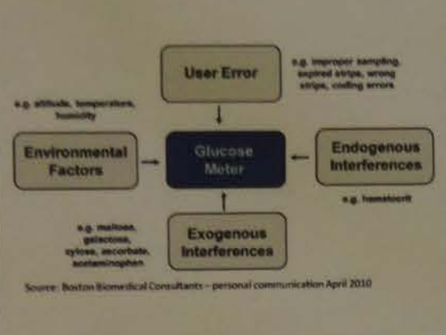
Bongseng Memorial Hospital, Busan, Korea

**Department of Clinical Laboratory Science, Dong-eui University, Busan, Korea*

Abstract

Point-of-care testing (POCT) is an increasingly popular means of providing laboratory testing at or near to the site of patient care. POCT provides rapid results and has the potential to improve patient outcome from earlier treatment. However, a faster result is not necessarily an equivalent result to traditional, core laboratory testing. Glucose meters are used routinely in hospital wards to manage blood glucose levels in patients requiring frequent monitoring of blood glucose. The primary objective of our study was to investigate whether all these RG II glucose meters (Sejong Biotechnology, Suwon, South Korea) results in hospitalized patients during routine clinical care jointly satisfy the specified quality specifications, as defined by Clinical and Laboratory Standards Institute (CLSI) guideline POCT12-A3. The records of hospitalized patients who underwent simultaneous measures of glucose levels with both glucose meters and a central laboratory analyser were retrospectively analysed. We also performed a prospective evaluation of the accuracy of the RG II glucose Strip. Glucose concentrations measured in 80 patients ranged from 2.01 to 32.17 mmol/L. The Bland-Altman difference plot between the auto analyser and RG II glucose meters revealed a mean bias of -3.1%, with analytical biases for the two methods varying from -38.6% to 32.4%. The glucose meter's values were within ± 14.3% for values 6.28 mmol/L of the comparative laboratory glucose values and 89% of the results were within 24% of the reference for glucose > 4.5 mmol/L and 65% of the results were within 1.3 mmol/L for glucose < 4.5 mmol/L. RG II glucose meter readings in hospital settings, especially in hypoglycaemic patients, should be confirmed by central laboratory analysers whenever possible.

Introduction



POCT12-A3

POCT12-A3 | *Point-of-Care Blood Glucose Testing in Acute and Chronic Care Facilities, 3rd Edition*
Preview Sample Pages

This document contains guidelines for performance of point-of-care blood glucose meter systems that stress quality control, training, and administrative responsibility.

Chairholder: David B. Sacks, MD
Organization: National Institutes of Health
Date of Publication: 1/30/2013
ISBN Number: 1-56238-868-1
Format: Approved Guideline
Edition: Third Edition
Pages: 64

Results

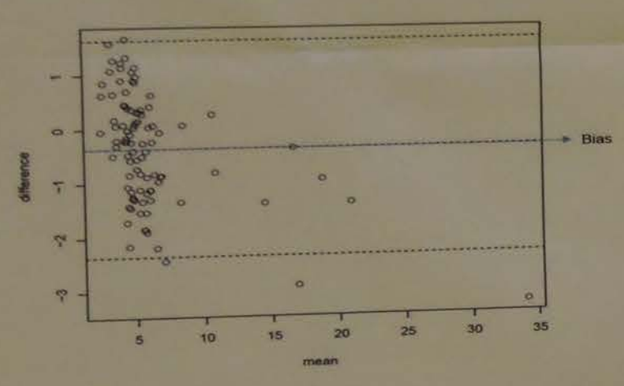


Fig. 1. Bland–Altman plot of the correlation between glucose meters and central laboratory analyser measurements of glucose concentrations in all patients. (n=840).

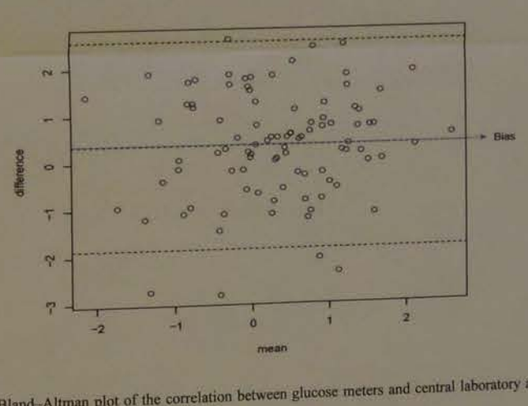


Fig. 2. Bland–Altman plot of the correlation between glucose meters and central laboratory analyser measurements of glucose concentrations above 5.54 mmol/L range. (n=450)

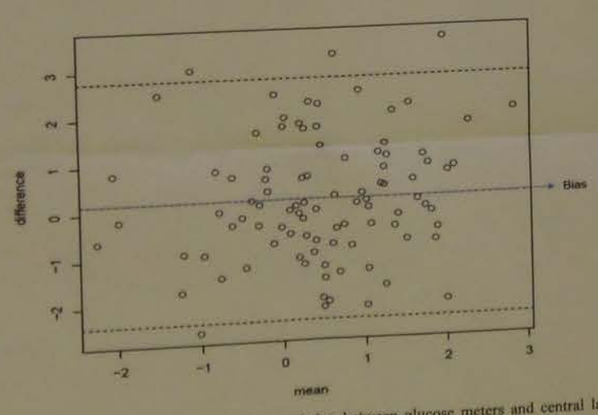


Fig. 3. Bland–Altman plot of the correlation between glucose meters and central laboratory analyser measurements of glucose concentrations below 5.54 mmol/L range. (n=390)

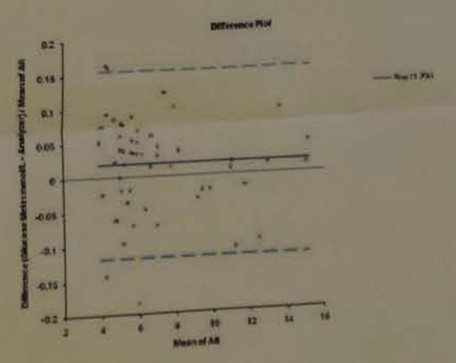


Fig. 4. Bland–Altman plot of the correlation between one glucose meter and central laboratory analyser measurements of glucose concentrations in sixty patients.

Conclusion

Erroneous results are not infrequent when glucose concentrations are measured with POCT glucose meters. Caution is required in interpreting POCT glucose meter results measured, as there were large, unpredictable errors in both directions from the reference BG value. Patients found to be hypoglycaemic or hyperglycaemic should be retested with a laboratory analyser to minimize misdiagnoses. This study demonstrated that RG II is not sufficiently accurate by health care professionals in all nursing units including the intensive care unit.

Clinical Chemistry

PD-57

Development of (long-wavelength biochemical analyzer

Yuka Sato[1], Masanori Seimiya[2], Toshi
Kazuyuki Matsushita[1]
1) Division of Laboratory Medicine, Chiba
2) International University of Health and We

【Background】

Indocyanine Green plasma disappearance rate (ICG-PDR) is important for understanding liver functions. However, the conventional manual method to determine the ICG-PDR has two disadvantages ;

The first issue is that the conventional manual method requires venipuncture two times because blood samples are needed before and after ICG loading in order to calculate the ICG-PDR.

The second issue is that the conventional manual method involves manual sampling and data input, which is inefficiently and may lead to careless mistakes. Therefore, an autoanalyzer method and auto data input system is desired.

【Objectives】

The aim of the present study was to development the **new autoanalyzer methods** (long-wavelength control method) and to evaluate the application for ICG-PDR.

【Materials and methods】

Serum samples were collected from 150 patients undergoing the ICG-PDR in our hospital. Written informed consent was obtained from all participants prior to blood sampling, and the study protocol was approved by the Ethics Committee of Chiba University Graduate School of Medicine.

ICG measurement methods:

1) Manual-conventional method (reference method); The absorbance of serum samples before and after ICG loading was measured at 805 nm using a spectrophotometer.

2) Manual-ICG decolorization method; The absorbance of an ICG-loaded sample was measured, a 6% solution of hypochlorous acid was added, and absorbance was measured for use as a blank.

3) Autoanalyzer conventional method; ICG was measured according to a reference method.

4) Autoanalyzer-ICG decolorization method; Sodium periodate solution (final concentration: 0.75%) was used for decolorization, and the absorbance was measured for use as a blank.

5) The new autoanalyzer methods is based on the fact that ICG has little absorption at 885 nm. Absorbance of serum at 805 nm without ICG loading is estimated from that at 885 nm with ICG loading. Subsequently, the estimate is subtracted from the absorbance of serum after ICG loading. And we performed a basic study.

【Result (

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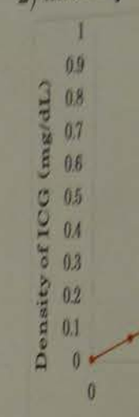

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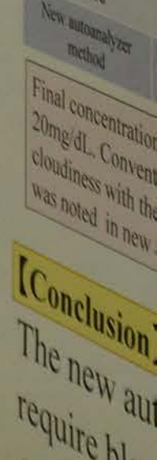

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Clinical Chemistry

PD-57

Development of ICG measurement (long-wavelength control) using an automatic biochemical analyzer

Yuka Sato[1], Masanori Seimiya[2], Toshihiko Yoshida[1], Yuji Sawabe[1], Kazuyuki Matsushita[1]

1) Division of Laboratory Medicine, Chiba University Hospital

2) International University of Health and Welfare

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【Result (performance of new autoanalyzer method)】

1) Reproducibility

Within-run reproducibility (n=20)

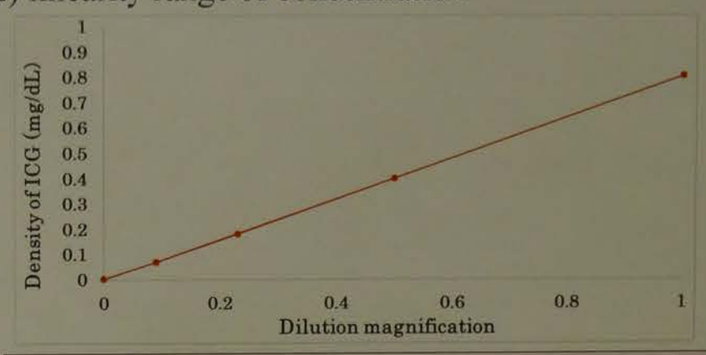
ICG value	CV (%)
0.15mg/dL (ICG 15%)	0.5%
0.32mg/dL (ICG 32%)	0.4%

Between-run reproducibility (n=24)

ICG value	CV (%)
0.13mg/dL (ICG 13%)	0.4%
0.27mg/dL (ICG 27%)	1.5%

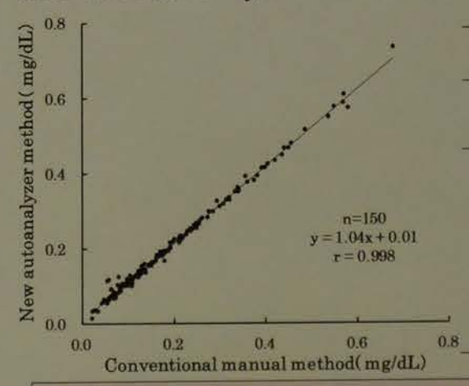
The reagent was stable for three weeks or longer after they were set in an automatic analyzer.

2) linearity range of concentration



The linearity range of concentration was 0-1mg/dL ICG.

3) Correlation with manual conventional method and various assays



Assay		r	Equation of regression
Decolorization manual method		0.998	y=0.98x+0.01
Autoanalyzer	Conventional manual method	0.998	y=1.05x+0.03
	Decolorization method	0.997	y=1.05x+0.01
	New autoanalyzer method	0.998	y=1.04x+0.01

A favorable correlation was confirmed; however, patients whose serum samples before ICG loading were turbid showed some deviation. The new autoanalyzer method may provide more accurate results for turbid samples.

4) Interference study

(mg/dL)

Assay	0.10 mg/dL (ICG 10%)				0.30 mg/dL (ICG 30%)			
	Hb	Chyle	BilC	BilF	Hb	Chyle	BilC	BilF
Manual Conventional method	0.13	0.79	0.10	0.11	0.34	0.94	0.30	0.31
New autoanalyzer method	0.08	0.14	0.10	0.11	0.28	0.34	0.31	0.31

Final concentration, Hb 500mg/dL; Chyle 1400FTU; BilC 21mg/dL; Bil-F 20mg/dL.. Conventional manual method came under a big influence in cloudiness with the chyle. No significant interference by these substances was noted in new autoanalyzer method.

【Conclusion】

The new autoanalyzer methods does not require blood sampling before ICG injection, and enables the automatic calculation of the retention rate, making it practicable.