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Urinary chiro- and myo-inositol levels as a biological marker for type 2 diabetes mellitus

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Abstract. *Background:* The aim of this study was to investigate the role of the urinary chiro- and myo-inositol levels in predicting type 2 diabetes mellitus (T2DM).
Subjects and methods: A total of 212 normal controls and 101 type 2 diabetic patients were enrolled this study. The concentrations of urinary chiro- and myo-inositol were measured by high performance liquid chromatography/mass spectrometry.
Results: The concentration of urinary chiro-inositol was significantly higher in the diabetic subjects (2.24 ± 5.18 ng/L) than those in the control group (0.38 ± 0.62 ng/L; $p < 0.001$). The urinary myo-inositol level of the diabetic subjects (36.95 ± 37.77 ng/L) was also significantly higher than that of the controls (8.17 ± 13.29 ng/L; $p < 0.001$). The urinary chiro-inositol multiplied by myo-inositol level of the diabetic subjects (148.10 ± 544.91) was significantly higher than in the controls (5.12 ± 24.15; $p < 0.001$). The area under the receiver operating characteristic curve for the urinary chiro-inositol multiplied by myo-inositol level to predict T2DM was 0.840 (confidence interval 0.789–0.891, $p < 0.001$). The cut-off value for the urinary chiro-inositol multiplied by myo-inositol level to predict T2DM was 2.20 (sensitivity 81.3%, specificity 70.3%).
Conclusions: The urinary chiro- and myo-inositol concentrations were increased in the type 2 diabetic patients and the urinary chiro- times the myo-inositol was considered to be a sufficient marker in predicting T2DM.

Keywords: Chiro-inositol, myo-inositol, diabetes mellitus

1. Introduction

The prevalence of diabetes is rapidly increasing. The prevalence of diabetes for all age-groups, worldwide, was estimated to be 2.8% in 2000 and projected to be 4.4% by 2030 [1]. In Korea, 9.8% of all age groups and 22% of people > 60 years of age had diabetes, according to the 2009 Korea National Health and Nutrition Examination Survey (KNHANES IV-3). The rate of awareness of people > 30 years of ages increased to 70% in 2007. However, the awareness of age $30 \sim 39$ was only 30% [2]. Thus, it is important that diabetes mellitus must be detected, while it is in a mild stage and young age.

Many studies tried to estimate the risk for the development of type 2 diabetes. Most researchers have depended on a 2-hour oral glucose tolerance test (OGTT) for identifying impaired glucose tolerance. Previous studies have demonstrated that OGTT-derived measures of insulin resistance and impaired insulin secretion can predict future development of type 2 diabetes [3,4]. In contrast, some researchers questioned the reliability of OGTT. Abdul-Ghani et al. [5] reported that the insulin secretion/insulin resistance index was a useful predictor of future development of type 2 diabetes and considered to be more powerful than OGTT. Michael et al. [6] pronounced that persons at high risk for diabetes mellitus were better identified by using a simple prediction model than by relying exclusively on the results of a 2-hour glucose tolerance test. Further, Henry et al. [7] also reported that two-risk scoring systems, which use clinical data and fasting blood tests, were better in identifying the risk for type 2 diabetes. Furthermore, oral glucose test takes a long time, over

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2 hours, high cost, patient's fear or rejection of needle puncture and medical labor. It is required to find out convenient and easy-accessible method for screening.

In the pathogenesis of type 2 diabetes, it has become evident that hyperglycemia is associated with both insulin resistance and beta-cell dysfunction [8]. Some actions of insulin are performed by inositolphosphoglycan (IPG) mediators that are released by cells after stimulation of insulin [9,10]. IPG molecules are hydrolyzed from glycosylphosphatidyl-inositols (GPIs) in cell membranes, in response to insulin and are considered putative insulin mediators. IPGs exist as two forms: chiro-inositols and myo-inositols. Majority of IPGs exist as myo-inositols, and myo-inositols are converted to chiro-inositol by insulin.

Previous studies had reported the relationship of IPGs and diabetes. Ostlund et al. [11] found that both type 1 and type 2 diabetic patients had increased urinary D-chiro- and myo-inositol excretion compared with normal subjects. Kawa et al. [12] also found that urinary chiro- and myo-inositol excretion was elevated in diabetic db/db mouse and streptozotocin diabetic rat. Other study also reported that in women with polycystic ovarian syndrome, urinary chiro-inositol excretion was elevated and hyperinsulinemia with insulin resistance was detected [13]. Large urinary losses are entirely consistent with deficient chiro-inositol content, which was noted previously in the inositol phosphoglycan fractions purified human diabetic muscle [14]. These studies suggested that a deficiency in a chiro-inositol phophoglycan (DCI-IPG) might contribute to insulin resistance in individuals with impared glucose tolerance or type 2 diabetes [14,15]. Thus, a defect in an alternative insulin-signaling pathway, in which IPG acts as a mediator of insulin action, which contributes to the pathophysiology of the insulin resistance of type 2 diabetes.

Measuring urinary excretion of inositols is convenient. Larger urinary excretion of IPGs may be associated with larger defect of serum IPGs, putative insulin mediators, and contribute to development of type 2 diabetes. Thus, the aim of this study is to investigate the role of the urinary chiro- and myo-inositol levels in predicting type 2 diabetes mellitus.

2. Subjects and methods

2.1. Subjects

Total of 313 subjects, 160 males and 153 females were participated in this study, and they were divided into two groups, type 2 diabetes mellitus and non-diabetic control group. The diabetes group consisted of 101 patients with 53 males and 48 females, with the age ranging from 27 to 83 years old that visited the Chungnam National University Hospital from 2009 to 2010. They were all type 2 diabetes and had taken oral hypoglycemic agents or insulin injections. The control group consisted of 212 subjects, 107 males and 105 females with age ranging from 21 to 69 years old. They visited the hospital for medical examination. They had have no history of diabetes mellitus, nor any other accompanying medical diseases and their fasting blood glucose levels were below 100 mg/dL. We excluded subjects with renal dysfunction (serum creatinine $>$ 1.5 mg/dl or glomerular filtration rate $<$ 60 ml/min), clinically significant hepatic disease, abnormal liver enzyme (AST $>$ 100 IU/L or ALT $>$ 100 IU/L) and history of hospitalization for a major cardiovascular event in previous 3 months. Further, we also excluded subjects with infection and detected malignant patients.

We explained our study to all participants and received written informed consent from all participating subjects. The study protocol was reviewed and approved by the Chungnam National University Institutional Review Board.

2.2. Laboratory tests

We prepared blood samples in the morning, after overnight fasting (over 8hrs) and checked fasting plasma glucose, HDL, LDL, Triglyceride, total cholesterol, BUN and creatinine, using a blood chemistry analyzer (Hitachi 747, Tokyo, Japan). Glycosyalted hemoglobin was measured using a high-performance liquid chromatography (Biorad, Hercules, CA, USA). We prepared spot urine soon after blood sampling. The concentrations of urinary chiro- and myo-inositol were measured by high performance liquid chromatography/mass spectrometry (Applied Biosystems 4000 Q TRAP, Foster city, CA, USA).

2.3. Statistical analysis

Data was expressed as mean $\pm$ SD. Unpaired Student's t-test was used in comparison of baseline characteristics between the two groups. Pearson's correlation was used to assess associations between variables. Statistical significance was considered at the level of $p <$ 0.05. The comparison of prediction factor for development of type 2 diabetes was tested with the area under the receiver-operating characteristic (ROC) curve. Statistical analysis was performed with the SPSS (version 18.0 for Windows; SPSS Inc., Chicago, IL).

Table 1
Comparison of anthropometric and metabolic characteristics of the two study groups

	Normal ($n = 212$)	Type 2 DM ($n = 102$)	P value
Age (yr)	42.8 ± 10.1	55.9 ± 11.3	< 0.001
Sex (Male/Female)	107/105	53/48	
Body Mass Index (kg/m^2)	23.36 ± 2.74	25.69 ± 3.95	< 0.001
Fasting Plasma Glucose (mg/dl)	87.27 ± 6.75	140.21 ± 46.71	< 0.001
Triglyceride (mg/dl)	106.90 ± 61.22	159.76 ± 91.31	< 0.001
HDL-C (mg/dl)	56.94 ± 9.09	47.08 ± 11.91	< 0.001
LDL-C (mg/dl)	102.66 ± 26.39	95.13 ± 30.39	0.034
Total Cholesterol (mg/dl)	180.96 ± 30.52	173.70 ± 40.44	0.079
BUN (mg/dl)	11.47 ± 2.97	15.58 ± 4.69	< 0.001
Creatinine (mg/dl)	0.86 ± 0.15	0.97 ± 0.17	< 0.001
GFR (ml/min)	85.93 ± 16.99	70.04 ± 18.60	< 0.001
Urine chiro-inositol (ng/dl)	0.38 ± 0.62	2.24 ± 5.18	< 0.001
Urine myo-inositol (ng/dl)	8.17 ± 13.29	36.95 ± 37.77	< 0.001

Data are expressed as mean $\pm$ SD. P values were calculated using an unpaired T-test. HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; BUN, blood urea nitrogen; Cr, creatinine; GFR, glomerular filtration rate.

Table 2
Sensitivity and specificity of urinary chiro- and myo-inositol, chiro-inositol times myo-inositol as a marker for type 2 diabetes mellitus

	Cut-off value (ng/ml)	Sensitivity (%)	Specificity (%)	Areas under ROC (95% CI)
Urine chiro-inositol	0.31	70.8	69.8	0.791 (0.740–0.843)
Urine myo-inositol	7.62	79.2	70.3	0.828 (0.774–0.883)
Urine chiro-inositol $\times$ Urine myo-inositol	2.20	81.3	70.3	0.840 (0.789–0.891)

3. Results

3.1. Baseline characteristics of type 2 diabetic group and control group

Table 1 presented the anthropometric and metabolic characteristics of the two study groups. The diabetic group was older and had higher BMI with elevated triglyceride and LDL-cholesterol, with lower HDL-cholesterol level. Equivalently prepared urine samples from both groups revealed diabetic patients to have approximately 5 times as much urine chiro- and myo-inositol concentration. Urine chiro-inositol concentration in non-diabetic and diabetic patients was 0.38 ± 0.62 ng/ml and 2.24 ± 5.18 ng/ml, respectively. Urine myo-inositol concentration in non-diabetic and diabetic patients was 8.17 ± 13.29 ng/ml and 36.95 ± 37.77 ng/ml, respectively. Statistical significance was acceptable ($p < 0.001$ for chiro-inositol; $p < 0.001$ for myo-inositol).

3.2. Comparison of markers for type 2 diabetes prediction

The concentrations of urinary chiro-inositol, myo-inositol, and chiro-inositol multiplied by myo-inositol value were considered to be the markers for the prediction of type 2 diabetes. The urinary chiro-inositol multiplied by myo-inositol concentration in the diabetic subjects (148.10 ± 544.91 ng/ml) was significantly higher than in the controls (5.12 ± 24.15 ng/ml; $p < 0.001$). The cut-off value to predict type 2 diabetes was 0.31 ng/dl (sensitivity 70.8%; specificity 69.8%) for urinary chiro-inositol and 7.62 ng/dl (sensitivity 79.2%; specificity 70.3%) for urinary myo-inositol. The cut-off value for urine chiro-inositol multiplied by urine myo-inositol concentration was 2.20 (sensitivity 81.3%; specificity 70.3%), as presented in Table 2. The area under the ROC curve was presented in Fig. 1. The area under the ROC curve (95% confidence interval) to predict type 2 diabetes was 0.791 (0.740–0.843, $p < 0.001$) for urinary chiro-inositol and 0.828 (0.774–0.883, $p < 0.001$) for urinary myo-inositol. The value of urinary chiro-inositol multiplied by urinary myo-inositol concentration had the greatest area under the ROC curve (0.837, confidence interval 0.787–0.888, $p < 0.001$). As a cut-off value of 2.20 in urinary chiro-inositol times the urinary myo-inositol concentration, the sensitivity was 81.3% and the specificity was 70.3%.

3.3. Comparison of markers that might affect urinary excretion of chiro- and myo-inositol in type 2 diabetes

Table 3 presented the comparison of markers that might affect urinary chiro- and myo-inositol levels in type 2 diabetes. We checked the relation of urinary ex-

Table 3
The comparison of markers that might affect urinary chiro- and myo-inositol levels in type 2 diabetes

	Urinary chiro-inositol		Urinary myo-inositol	
	r	p	r	p
BMI (kg/m^2)	0.011	0.873	−0.056	0.421
FBS (mg/dl)	0.396	0.081	0.302	< 0.001
HbA1C (%)	0.286	0.125	0.324	< 0.001
Duration of DM (yr)	−0.070	0.508	0.130	0.208
GFR (ml/min)	−0.156	0.131	−0.144	0.159
Myo-/Chiro-inositol	0.267	0.010	0.886	< 0.001

P values were calculated using a Pearson's correlation. BMI, body mass index; FBS, fasting blood glucose; HbA1C, hemoglobin A1c; GFR, glomerular filtration rate.

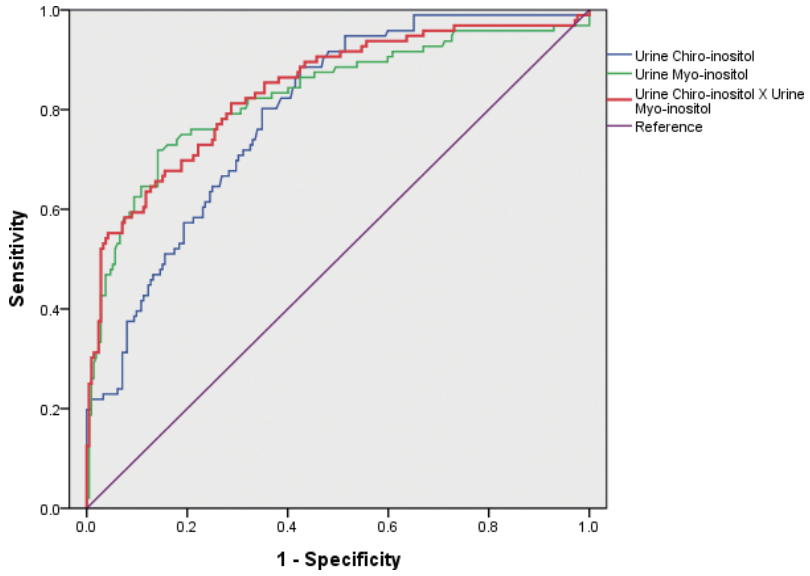

Fig. 1. ROC curves for the diagnostic accuracy of urine chiro-inositol, urine myo-inositol and urine chiro-inositol times urine myo-inositol. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-2012-0925)

cretion of chiro- and myo-inositols with obesity. Urinary excretion of chiro-inositol was not correlated with BMI (correlation efficient = 0.011, $p = 0.873$). Urinary excretion of myo-inositol was also not correlated with BMI (correlation efficient = −0.056, $p = 0.421$). Urinary excretion of myo-inositol showed positive correlation with fasting plasma glucose (correlation coefficient = 0.302, $p < 0.001$) and HbA1c (correlation coefficient = 0.324, $p < 0.001$). However, urinary excretion of chiro-inositol did not show any significant correlation with fasting plasma glucose (correlation coefficient = 0.396, $p = 0.081$) and HbA1c (correlation coefficient = 0.286, $p = 0.125$). The duration of type 2 DM showed no correlation with excretion of both chiro- (correlation coefficient = −0.070, $p = 0.508$) and myo-inositol (correlation coefficient = 0.130, $p = 0.208$).

Renal dysfunction might affect the excretion of inositol. In type 2 diabetes group, there were 28 patients with renal dysfunction (GFR < 60 ml/min). Urinary excretion of chiro-inositol was 4.72 ng/dl in renal dysfunction group and 1.85 ng/dl in normal renal function group ($p = 0.38$). Urinary excretion of myo-inositol was 44.31 ng/dl in renal dysfunction group and 33.72 ng/dl in normal renal function group ($p = 0.224$). There was no significant difference between urinary excretion of chiro- and myo-inositol and renal dysfunction.

In type 2 diabetes group, there were 28 patients with insulin injection and 73 patients with oral hypoglycemic agent. We divided the group into 2 subgroups by whether they took the insulin injection or not and compared FBS, HbA1c, duration of DM, serum insulin and urine excretions of chiro- and myo-inositol. Patients with insulin injection were more poorly controlled state (more elevated in FBS, HbA1c) and had longer duration of DM. Although the patients with in-

sulin injection excreted more urine chiro-inositol and urine myo-inositol, serum level of insulin was not significantly different ($p = 0.637$).

Urinary excretion of chiro-inositol showed positive correlation with urinary excretion of myo-inositol (correlation efficient $= 0.430$, $p = 0.01$). The ratio of urinary excretion of myo-inositol to chiro-inositol presented positive correlation with urinary excretion of myo-inositol (correlation coefficient $= 0.886$, $p < 0.001$) and urinary excretion of chiro-inositol (correlation coefficient $= 0.267$, $p = 0.01$).

4. Discussion

Many researchers presented biologic markers for prediction of type 2 diabetes. Kim et al. [16] suggested HbA1c over 5.95% for the prediction of type 2 diabetes mellitus with sensitivity 77% and specificity 89.4% in Koreans. Marguerite et al. [17] reported that 2-hour glucose by OGTT was useful for predicting diabetes risk, regardless of age. The reference value was 140 mg/dl with sensitivity 90%, specificity 68%, and area under ROC curve was 0.829 (0.782–0.876). Abdul-Ghani et al. [18] also used oral glucose tolerance test, but reported that subjects with 1-hour plasma glucose over 155 mg/dl present high risk for future diabetes. The 1-hour plasma glucose level had a greatest area under ROC curve (0.84) than that of fasting glucose level (0.75) and 2-hr plasma glucose level (0.79). In our study, the urinary chiro-inositol multiplied by urinary myo-inositol showed an area under ROC curve of 0.837 (0.787–0.888) and sensitivity 81.3%, specificity 70.3%. Thus, the value of the urinary chiro-inositol multiplied by urinary myo-inositol is suitable for the prediction of type 2 diabetes.

Compared with OGTT, urine analysis is less invasive, has lower cost, requires no medical labor and is convenient. The examination also takes less time, because we examined the concentration of urine chiro- and myo-inositol with spot urine, not 24-hrs collected urine. So it is easier to apply to the subjects for the purpose of screening type 2 diabetes.

In concordance with our results, previous studies also reported that urinary excretion of chiro-inositol was elevated in diabetic patients, diabetic db/db mouse, streptozotocin diabetic rat and polycystic ovary syndrome patients with increased insulin resistance [11–13]. These studies compared groups quantitatively, using 24 hrs urinary excretion of chiro- and myo-inositol. In contrast, we used spot urine to compare the concentration of urine chiro- and myo-inositol between the groups. Most of inositols can be obtained from dietary intake of inositol-rich food or synthesized in the body and exist as myo-inositols in the body. Then myo-inositols are converted to chiro-inositols by insulin, in the insulin sensitive tissues, such as muscle, liver, and adipose tissues [19,20]. Because the serum and urinary chiro-inositol levels change all day long, we obtained urine samples after overnight fasting. In our study, results using spot urine represented the same results, like using 24 hr-collected. Further, GFR of most of all the participants in this study was within the normal range (over 60 ml/min). So we suggest that the spot urine can replace the 24 hr-collected things.

In contrast to our results, some studies reported decreased urinary excretion of chiro-inositol in conditions that are associated with insulin resistance [14,15,21,22]. In these studies, urine myo-inositol excretion was increased and urinary chiro-inositol excretion was decreased in the type 2 diabetic subjects. Larner et al. [23] had shown that the increased excretion of urinary myo-inositol was due to a competition between glucose and myo-inositol in renal tubular transport. However, the reason of decreased urinary chiro-inositol has not been proven. The difference in the method of measuring the urinary chiro-inositol might be one reason. Some studies used gas chromatography/mass spectrometry inositol assay [21], other studies used quantitative recovery through several purification steps, without internal recovery standard [14,15]. In our study, we relied on high performance liquid chromatography/mass spectrometry with the internal standard. We guess that this difference of methodology may attribute to discrepancy of results.

Furthermore, we have performed further analysis to evaluate what factors affected increased urinary excretion of chiro- and myo-inositols in our study. By the Nestler et al., obese women with PCO showed increased clearance on chiro-inositol [24]. We checked the relation of urinary excretion of chiro- and myo-inositols with obesity. However, there was no statistically significant correlation between two parameters. In the study of Nestler et al., the BMI of obese group was 36.6 ± 2.3 kg/m^2 and BMI of non-obese group was 24.2 ± 2.3 kg/m^2. But in this study, BMI of obese group was only 25.69 ± 3.96 kg/m^2 and non-obese group 23.36 ± 2.74 kg/m^2. Renal dysfunction, fasting plasma glucose, HbA1c and duration of type 2 DM might affect the excretion of inositol. In our study, the parameters did not show any correlation with urinary excretion of chiro- and myo-inositol. In type 2 dia-

betic group, the patients with insulin injection excreted more urinary chiro- and myo-inositol than patients with oral hypoglycemic agent. However, there was no significant difference in serum insulin level. The effect of insulin injection for urinary excretion of chiro- and myo-inositol was needed more studies.

The ratio of urinary excretion of myo-inositol to chiro-inositol presented positive correlation with urinary excretion of myo-inositol and chiro-inositol in this study. We supposed that the overall myo-inositol level might increase in type 2 diabetes patients and the epimerization of myo-inositol to chiro-inositol also increased. And, fasting plasma glucose might significantly affect the urinary excretion of myo-inositol and the epimerization of myo-inositol to chiro-inositol.

Despite of variability in urinary chiro-inositol excretion, most of the studies have shown deficient state of chiro-inositol in human diabetic muscle tissues and of chiro-inositol mediator content and bioactivity [14, 15]. Therefore, we supposed that type 2 diabetes had altered renal metabolism and a large urinary loss of chiro-inositol, which is contributed to increased urinary excretion of chiro-inositol. But the exact mechanism is unknown, so further evaluation and examination are also required to explain such difference.

There was a limitation in our study. We did not control dietary intake. The intake of chiro-inositol mainly depends on diet [19]. Although we prepared the spot urine after overnight fasting, previous diet might affect the urinary excretion.

In conclusion, we presented the value of the urinary chiro-inositol concentration multiplied by urinary myo-inositol concentration was a suitable biological marker for the prediction of type 2 diabetes, with the cut-off value of 2.14 (sensitivity 81.3%, specificity 70.3%) in this study.

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