Original Article

Impact of SLC47A1 (rs2289669 G>A) Variant on Metformin Associated Lactic Acidosis Patients

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Objective: To address the important role of SLC47A1 G>A (rs2289669) variants in type 2 diabetic Thai patients. The present study was undertaken to investigate the association between disease severity and incidence of those type 2 diabetic patients harboring this genetic abnormality.

Materials and Methods: Between January 2014 and December 2015, all patients diagnosed with metformin associated lactic acidosis [MALA] in Burapha University Hospital were enrolled. Full medical record and treatment outcome were collected. RT-PCR were performed in blood samples to identify the SLC47A1 G>A (rs2289669) variations genotyping.

Results: There were 10 patients (age 69.4±12.6 years, duration of diabetes 12.1±3.51 years, metformin dose 2,000±471.4 mg/day). The incidence of AA-alleles in MALA patients was 50%, which was significantly higher than the general type 2 diabetic patients (p<0.0001, 95% CI 3.09 to 8.01). Serum creatinine and anion gap of AA patients were significantly higher than non-AA alleles, (7.79+1.80 vs. 3.80+1.77 mg/dL, p = 0.032), (34.2+4.66 vs. 22.0+8.25 mEq/L, p = 0.045), respectively.

Conclusion: The present study has been demonstrated the incidence of homozygous MATE/SLC47 gene polymorphism in MALA patients was higher than general type 2 diabetic patient. MALA patients who carry this genetic mutation have more severe disease than those who do not carry this mutation.

Keywords: MATE1, SLC47A1, Metformin associate lactic acidosis, DM type 2

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Metformin is recommended to be the first line treatment of type 2 diabetes from various international guideline as a result of providing many proven clinical benefits including high efficacy, low cost, low risk of hypoglycemia without significant weight increase. On the contrary, metformin associated lactic acidosis [MALA] is a rare but fatal adverse effect related to treatment with metformin. Recent published data have suggested that some variants of SLC47A1 gene (rs2289669 G>A), which is encoding the multidrug and toxin extrusion 1 [MATE1] protein, can improve the glucose-lowering effect of metformin by slowing rate of elimination in type 2 diabetic population. However, there is a considerable ambiguity about the degree of variance in patients with MALA. To evaluate this problem, the present study had been conducted to establish relationship between the degree of severity in patients diagnosed with MALA and their genetic polymorphism of MATE/SLC47A1.

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Materials and Methods

All the participants provided written informed consent. Data of all MALA patients admitted at intensive care unit of Burapha University Hospital were record retrospectively between January 2009 and December 2013 and prospectively during January 2014 to December 2015. The study protocol was approved by the Ethical Committee, Burapha University on 11 June 2014, registration No36/2557.

Ten MALA patients were identified, nine of which were performed subsequent genetic testing. Relevant data comprised of baseline characteristics, daily dose of metformin, laboratory result, final diagnosis at discharge, risk factors for lactic acidosis, treatment, and discharge outcome were recorded. MALA was defined as an arterial pH <7.35 and plasma lactate concentration >5.0 mmol/L[(8)].

Continuous data were described as mean ± SD. Categorical variable were described as frequencies and percentages. Comparison used the single sample t-test for the incidence of SLC47A1 A-alleles, and Welch and Wilcoxon rank sum test with continuity correlation continuous variables between groups.

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Categorical variables were compared using Chi-square test. Statistical analyses were conducted using program R version 3.01, and a significance level of $p<0.05$ was applied.

DNA isolation

Genomic DNA was extracted from blood leucocytes with a commercial kit (FlexiGene, QIAGEN, USA). The DNA was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). All samples had 260/230 ratios greater than 2.0 and 260/280 ratios greater than 1.80.

Polymerase chain reaction

For genotyping of SLC47A1 c.922-158G>A (rs2289669) variations, the polymerase chain reaction [PCR] amplification contained 200 nM each primer (forward primer, 5'-CTG GTG AGT CAG TCC ATC CC-3'; reverse primer, 5'-CTG GTG GGA AAA CTT GGT CC-3') and 2X GoTaq® Green Master Mix (Promega, USA), for a final volume of 25 µL. The PCR program of Eppendorf ProS thermal cycler (Eppendorf ProS, Germany) was as followed: 95°C, 5 minutes followed by 10 cycles of 95°C, 30 seconds; annealing temperature step downs every 1 cycle of 1°C (from 65°C to 55°C); 72°C, 30 seconds. The annealing temperature for the final 20 cycles was 55°C with denaturation and extension phases as above and a final extension of 10 minutes at 72°C. For sequence analysis, 20 ng of purified PCR products were sequenced by direct cycle sequencing using fluorescent-labelled dideoxy terminators (Big Dye Terminator Cycle Sequencing Ready Reaction Kit; Applied peBiosystems, Foster City, CA), according to the manufacturer's protocol and ran on ABI Prism® 3500 automated DNA sequencer (Applied Biosystems, Foster City, CA).

Results

All ten patients diagnosed with MALA during the study period were treated with metformin. Of those nine were female and one male. The mean age of the participants was 69.4 years (ranging from 50 to 84 years) with five of them aged of more than 70. The mean duration of diabetes was 12.1 years, (ranging from 9 to 20 years). The mean daily dose of metformin was 2,000±471.4 mg, (range 1,000 to 3,000 mg). The mean arterial pH was 7.06±0.26, mean serum lactate was 9.86±7.19 mmol/L. The maximum of creatinine level was 9.7 mg/dL, while maximum anion gap was 42. One of ten died from severe sepsis. There were four patients with CKD stage 5 (40%), two with diabetic retinopathy (20%), and three with diabetic neuropathy (30%).

Chronic renal failure was a salient risk factors identified in the present study from which four patients (40%) were suffered. Six patients were dehydrate due to acute diarrhea. However, there were some others relevant aggravating factors including: dehydration from vomiting or GI bleeding, sepsis or NSAIDs used, as shown in Table 1.

Half of those renal failure patients with deleterious features including a higher creatinine level (6.77 and 4.81 mg/dL) significant low serum bicarbonate level ($p = 0.0598$) and significant hyperkalemia ($p = 0.0564$) were treated with acute hemodialysis, whilst best supportive care was offered in the rest half whom age was slightly older (74.6 and 64.2 years). In addition,

Table 1. Baseline characteristic

Case No.	Sex	Age (year)	Daily metformin dose (mg)	Risk factors	Creatinine		Precipitating cause	Admission			
					Baseline	At follow-up		Creatinine	AG	HCO_3	K
1	F	60	2,000	CKD	1.33	5.58	ARF, Hypovolemia	7.19	42	5	9.3
2	F	50	3,000	None	1.07	1.05	ARF	9.70	32	5	6.8
3	F	56	2,000	None	NA	1.09	ARF, Hypovolemia, NSAID	9.19	31	4.8	4.8
4	F	84	2,000	CKD	1.41	NA	Acute pyelonephritis, Septic shock	5.16	35	5.4	5.4
5	F	79	2,000	None	1.16	0.67	Diarrhea	7.73	31	4	6.8
6	M	83	2,000	CKD	1.2	1.12	GI bleed, Diarrhea, Hypovolemia	3.99	23	7.3	7.3
7	F	73	2,000	CKD	2.2	NA	Acute pyelonephritis, Diarrhea	5.79	32	2	6.2
8	F	59	2,000	None	NA	1.06	Acute pyelonephritis, Diarrhea	3.47	23	10	6.3
9	F	83	1,000	None	NA	0.62	Acute pulmonary embolism	1.05	9	4.8	4.8
10	F	67	2,000	None	NA	0.9	Diarrhea, Sepsis	4.70	23	4.7	4.7

F = female; M = male; CKD = chronic kidney disease; NA = not available; ARF = acute renal failure; NSAID = nonsteroidal anti-inflammatory drug; GI = gastrointestinal; AG = anion gap

Table 2. Demonstrate the different baseline characteristics between AA and non-AA group

	AA	non-AA	*p*-value
n*	4	5	-
M:F	0:4	1:4	-
pH	7.00	7.11	0.5697
Creatinine	7.79	3.80	0.0075
Anion gap	34.2	22.0	0.0264
K (potassium)	6.62	5.86	0.4365
HCO_3	6.4	11.6	0.2092
Lactate	11.38	8.35	0.5460

* Nine of ten MALA case were performed subsequent genetic testing

the median daily dose of metformin was numerically higher in those receiving dialysis (2,000 mg vs. 1,800 mg).

A highly significant incidence of Homozygous A-alleles in MALA patients was found around 50 percent (p<0.0001, 95% CI 3.09 to 8.01) comparing with 20.75 percent in simply type 2 diabetes[20], level of serum creatinine (7.79±1.80 mg/dL vs. 3.80±1.77 mg/dL, p = 0.032) and anion gap (34.2±4.66 vs. 22.0±8.25 mEq/L, p = 0.045) in AA patients were statistically higher significant than wild-type, as shown in Table 2. However, there was no meaningful difference in serum bicarbonate, potassium level and lactate level among these groups.

Discussion

Despite recent advance in oral hypoglycaemic drugs is developed, the prevalence and incidence of diabetes mellitus have been progressing worldwide especially in the developing countries. One epidemiologic study estimated that the prevalence of newly diagnosed type 2 diabetes in 2030 in the third world will be raised up to 69 percent from 2010, whilst only 20 percent expansion in developed countries[1]. Long standing hyperglycemia and peripheral insulin desensitization are two main deteriorating factors fostering both micro and macrovascular complication ultimately leading to increase morbidity and mortality.

Metformin has been widely accepted to be first line treatment for type 2 diabetes across international guidelines and health authorities for many years[2]. Besides glucose lowering effect, proven benefits of metformin have been firmly illustrated including: weight reduction; fewer untoward hypoglycaemic episodes. Moreover, the data from the UK Prospective Diabetes Study [UKPDS] have demonstrated that those newly diagnosed patients treated with metformin alone could significantly reduce diabetes-related mortality, total mortality, and delay the microvascular complication comparing with those who received insulin, sulfonylurea therapy, or only diet control[3].

The molecular mechanism determining pharmacological property of metformin is complicated and remain yet to be elucidated, however, it's widely established that metformin lowering hepatic gluconeogenesis and lipogenesis via activating AMP-activated protein kinase [AMPK] in liver. Phosphorylated AMPK may also increase level of glucose uptake in skeletal muscle by upregulating the expression of GLUT4 (encoded by gene SLC2A4) leading the overall systemic increase insulin sensitivity[4]. Stimulating AMPK by α-subunit phosphorylation may also inhibit aerobic mitochondrial respiratory chain that will subsequently converse aerobic to anaerobic metabolism[5-7], causing the accumulation of lactate as a by-product from this metabolic pathway.

A constellation of gastrointestinal symptoms like diarrhoea, nausea, vomiting, abdominal discomfort, and anorexia is commonly found as the adverse event-related to metformin use.

MALA is the most serious side effect, due to the high mortality rate at approximately 50%. Moreover, this mortal side effect is without any definite prognostic factors and the definitive cause of MALA is not known.

MALA is characterised by elevated blood lactate concentration (exceeding 45 mg/dl or 5.0 mEq/L), decreased blood pH (less than 7.35), and electrolyte disturbances with an increased anion gap in metformin treated patients[8].

The incidence of MALA have been reported around six to ten per 100,000 patient-years of exposure with the mortality rate of 30 to 50 percent. A systemic review from 591 cases reported showed the similar number of mortality rate ranged from three to 61 percent.

Confirmed predisposing factors for MALA may be comprised of concurrent liver disease, alcoholism, renal insufficiency, and poor tissue perfusion from any causes.

According to previous report by Kim et al[9] investigated the clinical profile and risk factors in 7 case of MALA. The study showed most patients had renal impairment on admission with or without other precipitating cause[9]. Recent study by Kajbaf et al demonstrated 56 cases of severe MALA, blood pH and lactate did not have prognostic value[10]. However, early recognition of MALA and initiation of treatment especially renal replacement therapy can improve

outcome.

In the present study, nine out of ten patients were suffering from acute renal failure. Of those, four patients had baseline chronic renal insufficiency. Aggravating factor for acute renal failure was urinary tract infection induced septicemia accounting for 50 percent and volume contraction from diarrhea around 30 percent. Those treated with acute hemodialysis were whose laboratory results revealed significant metabolic acidosis and severe hyperkalemia which was similar to previous study.

Clinical pharmacokinetic studies revealed that renal tubular secretion and glomerular filtration are the major route of metformin elimination. Therefore, this drug may accumulate in patients with kidney failure[11]. In addition, membrane transporters located in the renal and biliary duct was found to be responsible for this eliminating process.

The animal experimental model has been demonstrated that there is a substantial impairment of metformin uptake by hepatocyte in the OCT1-knockout mice. This abolish glucose-lowering effect of metformin was reassembly found in those patients habouring the polymorphisms of gene (SLC22A1)[12].

Apart from the organic cation transporter 2 (OCT2/SLC22A2)-, MATE1, and MATE2-K were considered to be a polyspecific antiporter, responsible for the final step of metformin excretion through bile and urine. Organic cation transporters, OCT1 (SLC22A1) and OCT2 (SLC22A2), which located in the basolateral membranes, are responsible for metformin uptake into liver and kidney cell[13,14]. The efflux of metformin out of the cell depends on the function of MATE1/SLC47A1 which is expressed in the luminal membranes of renal proximal tubules or bile canalicular membranes of hepatocytes[15,16]. Currently, SLC47A1/MATE1 gene was studied in pharmacogenomics of metformin. In vitro study suggested that the polymorphisms in SLC47A1 gene can affect the expression level and transport function of MATE1 in human kidney, eventually resulting in variation in the disposition and response to metformin[17].

The study about the effect of MATE/SLC47A1 gene variant on pharmacokinetics of metformin by Tsuda et al that compared pharmacokinetic characteristics of metformin between wild type [Mate1 (+/+)] and Mate1 knockout [Mate1 (/)] mice after a single intravenous administration of metformin. The result showed a 2-fold increase the area under the blood concentration-time curve of metformin and urinary excretion was significantly decreased in Mate1 (/) than Mate1 (+/+) mice(18).

Some studies have also demonstrated significant medication-gene interactions for glycemic outcomes. A result of study by Becker et al concluded that polymorphism of SLC47A1 rs2289669 was associated with the difference in HbA1c reduction in Caucasian patients[19]. The similar conclusion by He et al was also drawn in the study of the Chinese population that patients carrying rs2289669 homozygous A/A had a better glucose-lowering effect[20]. These results suggested that SLC47A1 rs2289669 which effect on MATE1 function may have an important role in the pharmacokinetics and pharmacodynamics of metformin. On the other hand, this variants may contain some detrimental effects on the safety profile. Metformin pharmacogenomics is required for its safe use in clinical situations. Furthermore, the study was reported by Toyama et al also highlighted the gene polymorphism of MATE/SLC47 to clarify the association between MATE dysfunction and metformin-induced lactic acidosis. In a mouse model, the result observed in Mate1-/- mice, but not in Mate1+/- mice at day 7 after drinking metformin-containing water, showed significantly higher blood lactate, lower pH and HCO_3 levels[21]. It would suggest that the deficiency of MATE activities may induce lactic acidosis[4,5]. Nevertheless, there has not been demonstrated this association of MALA and gene polymorphism in humans.

The present study which investigated MATE1 gene polymorphism in MALA patients reflected that the enrolled patients with MALA with AA genotype was 44.4% with $p<0.0001$ by single sample test which reflected a two fold increase on the results in the study of Chinese[20]. Moreover, these patients demonstrated a more advanced electrolyte imbalance than patients not included in the AA group. Consequently, there is a strong possibility that when combined with other precipitating causes, MATE1 gene polymorphism results in MALA. We propose that, genetic screening in the type 2 diabetes complicating with renal impairment before initiation the treatment with metformin should be further investigated. Relationship between laboratory results such as serum lactate level and anion gap related to treatment with metformin in Homozygous MATE should be evaluated in larger clinical trial.

However, there might be a selective bias in the present study, such as a small sample size, single center study, and incomplete some biochemistry data. Further study should be conducted to collect more sample size and improved the data collection protocol.

Conclusion

MALA traditionally occurs in diabetic patients with other comorbidities, especially renal failure when treated with metformin. Early recognition of this condition and initiation of renal replacement therapy can significantly improve outcomes-even in severe cases. Recent research has highlighted the part played by genetics in the development of MALA in mice. However, the present study has identified the high frequency of homozygous A-allele in patients with MALA. The present study concluded that SLC47A1rs2289669 G>A variants may be one of the patient variability factors of MALA. In addition, the present study also broadened conventional wisdom by making the connection of MATE1 gene frequency in patients diagnosed with MALA.

What is already known on this topic?

Previous studies demonstrated the association between gene polymorphism of MATE/SLC47 induced MATE dysfunction and metformin-induced lactic acidosis in mouse model. The study of the Chinese population demonstrated that the frequencies of patients carrying homozygous A/A was 20.75% and also had a better glucose-lowering effect.

What this study adds?

This study is the first to demonstrat MATE1 (rs2289669) gene distribution in MALA patients.

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Potential conflicts of interest

The authors declare no conflict of interest.

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