

The Influence of *MTHFR* C677T Variants on Neuropathy Risk Among T2dm Patients Receiving Monotherapy Metformin

Michael Resta Surya Yanuar, Enade Perdana Istyastono and Dita Maria Virginia

Faculty of Pharmacy, Universitas Sanata Dharma, Maguwoharjo, Kec. Depok, Kabupaten Sleman, Daerah Istimewa Yogyakarta 55281

Article Info

Submitted: 12-08-2022

Revised: 05-05-2023

Accepted: 05-05-2023

*Corresponding author
Dita Maria Virginia

Email:
virginia@usd.ac.id

ABSTRACT

Neuropathy is the most prevalent microvascular complication in type 2 diabetes mellitus (T2DM). Metformin consumption is associated with higher neuropathy risk. The Methylene tetrahydrofolate reductase (*MTHFR*) enzyme has confirmed its role in neuropathy. Metformin and *MTHFR* may decrease folate and induce hyper-homocysteine. The *MTHFR* gene variant, C677T, commonly found in exons, was studied for its connection to neuropathy risk in newly diagnosed T2DM patients initiating metformin treatment. This cross-sectional study recruited 103 patients. The neuropathy risk was examined according to medical judgement through Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS) criteria. Genotyping C677T was performed using PCR-RFLP. This study revealed that only one patient had a homozygote mutant, with over 50% of patients were detected with allele mutants. There were no statistical differences in patient characteristics between CC and CT genotypes ($p > 0.05$). The association between C677T and neuropathy risk was not significant statistically, either in the genotype model ($p = 0.97$), allele model ($p = 0.82$), and dominant model ($p = 0.91$). Even after adjusting for several confounding factors, no significant association was observed. In conclusion, C677T in our population did not influence neuropathy risk. More specific criteria and laboratory parameters indicated neuropathy should be examined in future studies.

Keywords: *MTHFR*, neuropathy, metformin, and T2DM

INTRODUCTION

In 2019, the International Diabetes Federation revealed its staggering global statistics that 463 million people between the ages of 20-79 years old diagnosed with diabetes. Notably, Indonesia was ranked 7th marking the only country in Southeast Asia among 10 countries with the highest diabetics populations in 2019, tallying 10.7 million cases. Furthermore, a national health survey conducted in 2018 reported that the Special Region of Yogyakarta (DIY) ranked as the third province that has the highest prevalence of diabetes in Indonesia (International Diabetes Foundation, 2019; Kementerian Kesehatan Republik Indonesia, 2018). Intriguingly, neuropathy stands as one of the prevalent microvascular complications in T2DM patients (Amelia *et al.*, 2019), and the risk of neuropathy escalates with metformin consumption as its side effects (Miyani & Waris, 2020).

Metformin is well known as the first-line therapy for type 2 diabetes mellitus (T2DM), recommended by the American Diabetes Association and The European Association for The Study of Diabetes (American Diabetes Association, 2020; Cosentino *et al.*, 2020). However, metformin consumption is associated with the reduction of vitamin B12 levels and an increase in homocysteine and methylmalonic acid levels in the blood. Furthermore, metformin is posited to stimulate the expression of angiogenic factors in peripheral nerves. Consequently, patients who take metformin often reported neuropathy as a side effect of metformin (Hashem *et al.*, 2021; Kim *et al.*, 2019; Ni *et al.*, 2017).

Previous studies reported neuropathy associated with methylenetetrahydrofolate reductase (Jiménez-Ramírez *et al.*, 2017). The *MTHFR* enzyme plays a pivotal role in intracellular folate metabolism and homeostasis.

MTHFR catalyzes the irreversible conversion of 5,10-, methylenetetrahydrofolate to 5-methyltetrahydrofolate which serves as the primary methyl donor for the re-methylation of homocysteine to methionine. Notably, metformin consumption disrupts folate-like activity. Thus, MTHFR might take a role in those mechanisms (Owen *et al.*, 2021). Furthermore, pharmacogenomic as personalized medicine is now considered to prevent side effects and optimize therapeutic effectiveness (Schwartz *et al.*, 2016). The *MTHFR* gene is one of the genetic targets observed related to neuropathy (Dell'Edera *et al.*, 2018; Wu *et al.*, 2017).

The *MTHFR* gene encodes MTHFR, and variation in the C677T could reduce MTHFR activity by as much as 50%. Numerous studies have reported mutations in the *MTHFR* gene as a contributing factor in diabetic neuropathy (Kakavand Hamidi *et al.*, 2017; Mottaghi *et al.*, 2019; Russo *et al.*, 2016; Wu *et al.*, 2017). Sadewa *et al.* (2002) conducted a profiling genetic variation of MTHFR C677T in the Javanese population. Furthermore, Suryandari *et al.* (2012) also conducted genetic variations of MTHFR C677T among men in Indonesia, though their study primarily focused on its implication for fertility.

However, no prior study has specifically investigated the relationship between the genetic variation of MTHFR C677T in T2DM patients consuming metformin on neuropathy risk. The two aforementioned previous studies (Russo *et al.*, 2016; Yigit *et al.*, 2013) have not revealed the molecular impact when the mutation occurs. Therefore, there is critical need to evaluate the genetic variation of *MTHFR* C677T in relation to the risk of diabetic neuropathy among T2DM patients consuming metformin as a monotherapy in DI. Yogyakarta, especially in the Sleman District. This research could help fill an existing knowledge gap and provide valuable insights into the potential implications of this genetic variation on neuropathy in this specific patient population.

MATERIALS AND METHODS

Research design and sampling

This cross-sectional study was conducted with the necessary approval granted by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada - Dr. Sardjito General Hospital in Yogyakarta, Indonesia (for patients recruitment) and Research Ethics Committee of Faculty of Health Science at Universitas Respati

Yogyakarta (for SNP C677T detection). Prior to data collection, written informed consent was obtained from all participating patients. All procedures and data collection obeyed the Declaration of Helsinki.

We conducted this study at ten primary healthcare facilities in Sleman, Province of DI Yogyakarta, Indonesia. Patients were included if they had a type 2 diabetes mellitus diagnosis and had been on metformin treatment for a minimum of three consecutive months. Patients were individually diagnosed by licensed medical practitioners based on medical judgments and in accordance with the criteria stipulated by the American Diabetes Association (ADA). We used the standard Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS) criteria to diagnose diabetic neuropathy.

Patients were excluded if they had uncontrolled hypertension, severe complications related to poor glucose control (such as amputations, foot ulcers, dialysis, and blindness due to severe diabetic retinopathy), renal disease, impaired hepatic function, or pregnancy.

Genetic variant examination

DNA isolation from blood samples was performed using the Tissue Genomic DNA Extraction Mini Kit 100 Prep (Proteinase K) following the manufacturer's (Favorgen) procedure. Subsequently, the quality of DNA was checked by electrophoresis and detected on a UV transilluminator. The *MTHFR* C677T mutation was analysed with PCR-based restriction fragment length polymorphism (RFLP) assay using restriction enzyme *Nla*IV.

The *MTHFR* gene of 351 bp was amplified by using primers: 5'-GCGAACTCAGCACTCCACC-3' (forward) and 5'-TCATCCCTCGCCTTGAACAG-3' (reverse). Each PCR mixture (25 μ L) contained DNA isolate, Go Taq Green Master Mix, primers (12.5 pmoles of each). The mixture was heated at 95°C for 5 min and underwent 35 cycles of amplification: denaturation at 95°C for 20 s, annealing at 60°C for 30 s, extension at 72°C for 30 s. The final extension took 5 min at 72°C. The PCR reaction was performed in the Bio-Rad T100 Thermal Cycler. After amplification, the 351 bp PCR product was digested with *Nla*IV in a 30 μ L reaction solution containing 10 μ L of PCR product, 3 μ L of 10X NE buffer, 16 μ L nuclease-free water, and 2 units of *Nla*IV at 37°C for 2 h. The enzyme deactivated at 65°C for 20 min.

The PCR product was analysed on 2% agarose gels and was photographed on an ultraviolet transilluminator. There were two variations of interpretation. First, wild-type (CC) individuals were identified by only a 214 and 137 bp fragment; heterozygotes (CT) by the 214, 137 bp, and 351 bp; and homozygote variants (TT) by the 351 bp. Second, CC was identified by 137 bp, 125 bp, and 189 bp; CT by 263 bp, 127 bp, 125 bp, and 89 bp; and TT by 263 bp and 89 bp. The author designed the primer and restriction enzyme to detect 2 SNPs. Therefore, there were variations in interpretation. However, it has already been confirmed and validated by the repetition of RFLP on multiple samples (Figure 1).

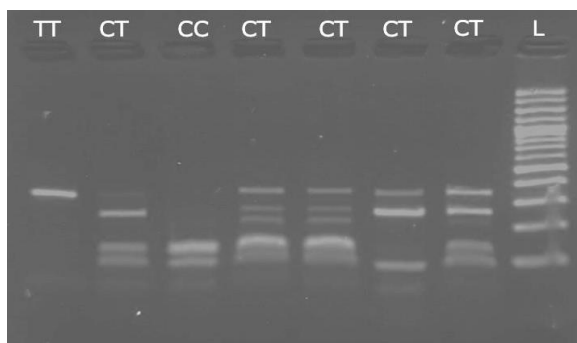


Figure 1. The pattern of wildtype homozygote, heterozygote, and mutant homozygote C677T

Statistical Analysis

The results are presented as the mean \pm standard deviation (SD) and frequency (percentage). To assess the distribution of genotypes among the patients and controls, the Hardy-Weinberg equilibrium was evaluated using the chi-square (χ^2) test. The relationships between the C677T mutation and diabetic neuropathy of the patients were analyzed using the χ^2 test. The χ^2 test was used to compare categorical variables appropriately, and odds ratios and 95% confidence intervals were used to assess the risk factors. Regression logistic was performed to evaluate the adjusted odd ratio (AOR) after adjusting for age, BMI, blood pressure, HbA1c level, smoking status, and T2DM family history. All p values were two-tailed, and p values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSIONS

Our study recruited 103 patients who had received a first-time diagnosis with T2DM at primary health care and were prescribed

metformin as monotherapy (Table I). The average age, HbA1c level, and fasting blood glucose (FBG) of the participants were 52.99 years, 10.47%, and 192.91 mg/dL, respectively. It was clear that our patients indicated an elderly group based on the mean of their age, but the mean of HbA1c and FBG were unexpectedly high for newly diagnosed T2DM patients. The majority of our respondents were female and tended to be obese; and blood pressure was controlled relatively. 45.6% of patients were diagnosed with neuropathy, but we did not definitively confirm whether it was caused by T2DM or metformin. Notably, our respondents were naïve metformin and newly diagnosed with T2DM. Therefore, we assumed that the neuropathy was induced by metformin consumption over the course of three months.

Only one patient exhibited the mutant TT genotype, while more than 50% of patients presented the heterozygote CT genotype (Table II). Assuredly, our study's SNP of C677T was inconsistent with Hardy-Weinberg Equilibrium ($p < 0.05$). Consequently, this present study only compared clinical characteristics among CC and CT genotypes. The clinical characteristics of the C677T genotype demonstrated no significant difference between wild-type homozygote and heterozygote ($p > 0.05$).

The crude odd ratio (COR), adjusted odd ratio (AOR), 95%CI, and p-value related to the association between C677T and neuropathy risk in the genotype, allele, and dominant models (Table III). We performed association statistics in the genotype model between CT and CC to neuropathy risk because only one patient has TT genotype. The odds ratio in the genotype model was different between COR and AOR. The CT had lower odds of neuropathy than CC in COR (COR = 0.98; 95%CI = 0.44 - 2.17), whereas its odds were higher in AOR (AOR = 1.15; 95%CI = 0.46 - 2.86), although it was not statistically significant in both ($p = 0.97$ and $p = 0.80$ in COR and AOR, respectively). We revealed that the T allele as a mutant had COR = 0.93 (95%CI = 0.51 - 1.61, p-value = 0.82) and AOR = 0.87 (95%CI = 0.46 - 1.63, p-value = 0.66) to neuropathy risk in the allele model. The recessive model could not be calculated due to the limited presence of the TT genotype. Moreover, the CT and TT genotype had COR = 0.95 (95%CI = 0.43 - 2.11, p-value = 0.91) and AOR = 0.83 (95%CI = 0.35 - 1.96, p-value = 0.67). Overall, the findings in the allele and dominant model were similar, indicating that the mutant had a lower risk of neuropathy than the wild type, either in COR or AOR.

Table I. Characteristic of T2DM patients consuming monotherapy metformin.

Characteristics	mean±SD / n(%)
Age (years)	52.99±9.52
Gender (male)	30 (29.1)
BMI (kg/m ²)	24.98±4.06
Systolic blood pressure (mmHg)	131.53±19.69
Diastolic blood pressure (mmHg)	81.50±9.40
HbA1c (%)	10.47±8.46
Fasting blood glucose (mg/dL)	192.91±75.67
Neuropathy (yes)	47 (45.6)
Family history of diabetes (yes)	23 (22.3)
Smoking status (active smoker)	15 (14.6)

Table II. The profile of subject's characteristic according to genotype C677T

Characteristics	Genotype			p-value*
	CC (n=41)	CT (n=61)	TT (n=1)	
Age (years)	51.80±10.80	53.75±8.63	55	0.32
Gender (male)	12 (29.3)	29 (47.5)	1	0.88
BMI (kg/m ²)	25.05±4.66	24.87±3.64	29	0.18
Systolic blood pressure (mmHg)	133.95±19.89	129.93±19.72	130	0.32
Diastolic blood pressure (mmHg)	82.07±10.07	81.15±9.06	80	0.63
HbA1c (%)	9.63±2.14	11.05±10.85	9.40	0.30
Fasting blood glucose (mg/dL)	184.59±62.08	198.23±84.17	210	0.38

*Compare between CC and CT genotype

Table III. Association between MTHFR C677T genetic variation with neuropathy risk

Genotype* model	Neuropathy (n(%))		COR 95% CI	p-value	AOR 95% CI	p-value
	Yes (n=47)	No (n=55)				
CT	28 (59.57)	33 (60.00)	0.98	0.97	1.15	0.80
CC	19 (40.43)	22 (40.00)	(0.44 - 2.17)		(0.46 - 2.86)	
Allele model						
T	28 (29.79)	35 (31.25)	0.93	0.82	0.87	0.66
C	66 (70.21)	77 (68.75)	(0.51 - 1.69)		(0.46 - 1.63)	
Dominant model						
CT+TT	28 (59.57)	34 (60.71)	0.95	0.91	0.83	0.67
CC	19 (40.43)	22 (39.29)	(0.43 - 2.11)		(0.35 - 1.96)	

*TT genotype was excluded because only one patient has TT

COR: crude odds ratio, AOR: adjusted odds ratio, data were adjusted for age, BMI, blood pressure, HbA1c level, smoking status, and T2DM family history

In brief, this present study could not find any significant association between MTHFR C677T and the risk of neuropathy, even after several confounding factors were added.

The genetic variation of C677T, recorded as rs1801133 in NCBI, is one of the prevalent MTHFR polymorphisms (Ni *et al.*, 2017). The SNP is a point mutation that occurs in exon 4 of the MTHFR gene, converting amino acid at codon 222, valine, to

alanine. The variation in the SNP C677T has been associated with a reduction in MTHFR activities (Liew & Gupta, 2015). The MTHFR enzyme has an important role in catalyzing homocysteine to methionine as a methyl donor. Therefore, activity induces hyper-homocysteine resulting in elevating peripheral neuropathy (Hsu *et al.*, 2020).

MTHFR genetic variation combined with hyperhomocysteinemia was found to be associated

with diabetic neuropathy (Mottaghi *et al.*, 2019; Hsu *et al.*, 2020). Previous studies confirmed that metformin consumption leads to peripheral neuropathy through B12 deficiency and high homocysteine (Elhadd *et al.*, 2018; Hashem *et al.*, 2021). Neuropathy impacts the quality of life and contributes to high morbidity and mortality rates among T2DM patients (Dornas *et al.*, 2021; Hicks *et al.*, 2021). Therefore, this current study is the first in the Indonesian population to examine the variation of C677T in the MTHFR gene related to metformin and T2DM to neuropathy risk.

A key highlighted finding of our study is the demonstration of no significant association between the C677T genetic variant and neuropathy among T2DM patients consuming monotherapy metformin, even after adjusting for several confounding factors. Several studies have mentioned that C677T increases the risk of diabetic neuropathy. Moreover, C677T was superior to A1298C as a risk factor for neuropathy (Hamidi *et al.*, 2017; Yigit *et al.*, 2013). Still, another study involving a different racial population from our population has similar results to our findings. Russo *et al.* (2015) could not discover any significant association between C677T and the risk of neuropathy among metformin users. Additionally, we found one cohort study that found C677T had a significant association with neuropathy, but interestingly the variant did not correlate with homocysteine (Jiménez-Ramírez *et al.*, 2017).

The discrepancy in these findings might be because of the unclear mechanism through which metformin impacts the MTHFR enzyme. Theoretically, metformin has the potential to reduce vitamin B12 and interfere with the methylation cycle, thus leading to hyperhomocysteinemia (Owen *et al.*, 2021). The collaboration of metformin and C677T in the MTHFR gene potentially induces neuropathy through hyperhomocysteinemia. Nevertheless, it was not clear whether the high homocysteine levels were induced by metformin or variant MTHFR.

While our study imposed certain restrictions by only recruiting patients with naive metformin who had received a first-time T2DM diagnosis, it is noteworthy that the mean HbA1c level was high. It is important to take into consideration that neuropathy is caused by uncontrolled diabetes (Amelia *et al.*, 2019; Nguyen *et al.*, 2019). Therefore, the high level of HbA1c could predispose our findings. Furthermore, other

factors that might affect our findings were lifestyle, including diet and physical activities. Those could influence neuropathy through improvement in controlling blood sugar and insulin sensitivity (Zilliox & Russell, 2019). Indeed, the medication factor is an important and critical point that affects disease progression (Al-Temimi *et al.*, 2021). Adherence to medication consumption might involve a confounding factor (da Silva *et al.*, 2019). Therefore, pharmacists should escalate their communication skills to counsel the patient regarding medication adherence (Selvadurai *et al.*, 2020).

This study does have several limitations. First, we did not control the baseline of HbA1c and blood glucose levels influencing neuropathy. Second, the homocysteine level was not measured in our study. If it was measured, it might explain the unclear path between metformin-MTHFR-homocysteine-neuropathy. Therefore, we recommend that future studies establish more rigorous baseline criteria and examine homocysteine levels.

CONCLUSION

The current study revealed that genetic variant C677T in the MTHFR gene had no significant association with neuropathy risk among newly diagnosed T2DM patients consuming monotherapy metformin. Due to no proven association, more rigorous criteria and laboratory parameters related to neuropathy should be examined in future research endeavors.

ACKNOWLEDGEMENT

This research was supported and funded by Ministry of Education, Culture, Research, and Technology (1989.9/LL5-INT/PG.02.00/2022, 028 Penel.LPPM-USD/V/2022) and Institute for Research and Community Services, Universitas Sanata Dharma (Research Grant Contract No. 007 Penel./LPPM-USD/II/2022).

AUTHORS CONTRIBUTIONS

M.R.S.Y contributed to data collection. E. P. I. contributed to the study conceptualization, edited, and reviewed the manuscript. D.M.V contributed to the study design and drafted a manuscript. All authors have approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

REFERENCES

- Al-Temimi, A. A., Krishnan, S., Sandhu, A. K., & Bangash, N. S. A. (2021). Medication Nonadherence: Implications for Patient Health Outcomes in Pharmacy Practice. *Indonesia Journal of Pharmacy*, 32(4): 416-428
- Amelia, R., Wahyuni, A. S., & Yunanda, Y. (2019). Diabetic Neuropathy among Type 2 Diabetes Mellitus Patients at Amplas Primary Health Care in Medan City. *Open Access Macedonian Journal of Medical Sciences*, 7(20): 3400-3403.
- American Diabetes Association. (2020). Standards of Medical Care in Diabetes. *The Journal of Clinical and Applied Research and Education*, 43(1): 1-212.
- Cosentino, F., Grant, P. J., Aboyans, V., Bailey, C. J., Ceriello, A., Delgado, V., Federici, M., Filippatos, G., Grobbee, D. E., Hansen, T. B., Huikuri, H. V., Johansson, I., Juni, P., Lettino, M., Marx, N., Mellbin, L. G., Ostgren, C. J., Rocca, B., Roffi, M., Chowdhury, T. A. (2020). 2019 ESC Guidelines on Diabetes, Pre-diabetes, and Cardiovascular Diseases Developed in Collaboration with the EASD. *European Heart Journal*, 41(2): 255-323.
- da Silva, W. H., Dantas, D. S., da Nóbrega, B. S., de Queiroz, M. D. S. R., & Alves, H. da S. (2019). Evaluation of Adherence to Pharmacological Treatment. *Brazilian Journal of Pharmaceutical Sciences*, 55: 18341.
- Dell'Edera, D., L'Episcopia, A., Simone, F., Lupo, M. G., Epifania, A. A., & Allegretti, A. (2018). Methylenetetrahydrofolate Reductase Gene C677T and A1298C Polymorphisms and Susceptibility to Recurrent Pregnancy Loss. *Biomedical Reports*, 8(2): 172-175.
- Dornas, R. M., Castelo, C., Pupe, B., José, O., & Nascimento, M. (2021). Evaluation of Quality of Life in Patients with Type 2 Diabetes Mellitus with Symptomatic Distal Symmetric Polyneuropathy / Avaliação da qualidade de vida em doentes com diabetes Mellitus tipo 2 com polyneuropathy simétrica distal sintomática. *Brazilian Journal of Health Review*, 4(3): 9936-9951.
- Elhadd, T., Ponirakis, G., Dabbous, Z., Siddique, M., Chinnaiyan, S., & Malik, R. A. (2018). Metformin Use is not Associated with B12 Deficiency or Neuropathy in Patients with Type 2 Diabetes Mellitus in Qatar. *Frontiers in Endocrinology*, 9(MAY): 248.
- Hashem, M. M., Esmael, A., Nassar, A. K., & El-Sherif, M. (2021). The Relationship between Exacerbated Diabetic Peripheral Neuropathy and Metformin Treatment in Type 2 Diabetes Mellitus. *Scientific Reports* 2021 11:1, 11(1): 1-9.
- Hicks, C. W., Wang, D., Matsushita, K., Gwen Windham, B., & Selvin, E. (2021). Peripheral Neuropathy and All-Cause and Cardiovascular Mortality in US Adults. *Annals of Internal Medicine*, 174(2): 167.
- International Diabetes Foundation. (2019). International Diabetes Federation - Facts & figures. <https://www.idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html>
- Jiménez-Ramírez, F. J., Castro, L. M., Ortiz, C., Concepción, J., Renta, J. Y., Morales-Borges, R. H., Miranda-Massari, J. R., & Duconge, J. (2017). Role of Treatment-modifying MTHFR677C>T and 1298A > C Polymorphisms in Metformin-treated Puerto Rican Patients with Type-2 Diabetes Mellitus and Peripheral Neuropathy. *Drug Metabolism and Personalized Therapy*, 32(1): 23-32.
- Hamidi, K. A., Radfar, M., & Amoli, M. M. (2017). Association between MTHFR Variant and Diabetic Neuropathy. *Pharmacological Reports* 2017 70:1, 70(1), 1-5.
- Hsu, T. R., Bumanlag, F., K. Mehta, A., Si, X., Yuan, Y., J. Dun, N., & Jun Luo, J. (2020). Homocysteine and Peripheral Neuropathy. *Journal of Neurology and Experimental Neuroscience*, 6(2): 58-61.
- Kementerian Kesehatan Republik Indonesia. (2018). Laporan Nasional Riset Kesehatan Dasar. In Kementerian Kesehatan RI.
- Kim, J., Ahn, C. W., Fang, S., Lee, H. S., & Park, J. S. (2019). Association between Metformin Dose and Vitamin B12 Deficiency in Patients with Type 2 Diabetes. *Medicine*, 98(46), e17918.
- Liew, S. C., & Gupta, E. Das. (2015). Methylenetetrahydrofolate Reductase (MTHFR) C677T Polymorphism: Epidemiology, Metabolism and the Associated Diseases. *European Journal of Medical Genetics*, 58(1), 1-10.
- Miyan, Z., & Waris, N. (2020). Association of Vitamin B12 Deficiency in People with Type 2 Diabetes on Metformin and without Metformin: a Multicenter Study, Karachi, Pakistan. *BMJ Open Diabetes Research and Care*, 8(1), e001151.
- Mottaghi, T., Khorvash, F., Kheirollahi, M., Maracy, M., & Askari, G. (2019). The MTHFR C677T Polymorphism Influences the Efficacy of Folic Acid Supplementation on the Nerve

- Conduction Studies in Patients with Diabetic Polyneuropathy; A Randomized, Double Blind, Placebo-controlled Study. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, 24(1): 36.
- Nguyen, K. T., Diep, B. T. T., Nguyen, V. D. K., Van Lam, H., Tran, K. Q., & Tran, N. Q. (2019). A Cross-sectional Study to Evaluate Diabetes Management, Control and Complications in 1631 Patients with Type 2 Diabetes Mellitus in Vietnam (DiabCare Asia). *International Journal of Diabetes in Developing Countries*, 40: 70-79.
- Ni, J., Zhang, L., Zhou, T., Xu, W. J., Xue, J. L., Cao, N., & Wang, X. (2017). Association between the MTHFR C677T Polymorphism, Blood Folate and Vitamin B12 Deficiency, and Elevated Serum Total Homocysteine in Healthy Individuals in Yunnan Province, China. *Journal of the Chinese Medical Association*, 80(3), 147-153.
- Owen, M. D., Baker, B. C., Scott, E. M., & Forbes, K. (2021). Interaction between Metformin, Folate and Vitamin B12 and the Potential Impact on Fetal Growth and Long-Term Metabolic Health in Diabetic Pregnancies. *International Journal of Molecular Sciences* 2021, 22(11): 5759.
- Russo, G. T., Giandalia, A., Romeo, E. L., Scarcella, C., Gambadoro, N., Zingale, R., Forte, F., Perdichizzi, G., Alibrandi, A., & Cucinotta, D. (2015). Diabetic Neuropathy is not Associated with Homocysteine, Folate, Vitamin B12 Levels, and MTHFR C677T Mutation in Type 2 Diabetic Outpatients Taking Metformin. *Journal of Endocrinological Investigation* 2015, 39(3): 305-314.
- Russo, G. T., Giandalia, A., Romeo, E. L., Scarcella, C., Gambadoro, N., Zingale, R., Forte, F., Perdichizzi, G., Alibrandi, A., & Cucinotta, D. (2016). Diabetic Neuropathy is not Associated with Homocysteine, Folate, Vitamin B12 Levels, and MTHFR C677T Mutation in Type 2 Diabetic Outpatients Taking Metformin. *Journal of Endocrinological Investigation*, 39(3): 305-314.
- Sadewa, A. H., Sunarti, Sutomo, R., Hayashi, C., Lee, M. J., Ayaki, H., Sofro, A. S. M., Matsuo, M., & Nishio, H. (2002). The C677T Mutation in the Methylenetetrahydrofolate Reductase Gene among the Indonesian Javanese Population. *Kobe Journal of Medical Sciences*, 48(5): 137-144.
- Schwartz, S. S., Epstein, S., Corkey, B. E., Grant, S. F. A., Gavin, J. R., & Aguilar, R. B. (2016). The Time is Right for a New Classification System for Diabetes: Rationale and Implications of the β -cell-centric Classification Schema. *Diabetes Care*, 39(2): 179-186.
- Suryandari, D. A., Yurnadi, Wiweko, B., & Yunaini, L. (2012). Genotype Distribution of Methylenetetrahydrofolate Reductase A1298C and C677T Gene in Indonesian Infertile Men. *Medical Journal of Indonesia*, 21(1): 23-27.
- Wu, S., Han, Y., Hu, Q., Zhang, X., Cui, G., Li, Z., & Guan, Y. (2017). Effects of Common Polymorphisms in the MTHFR and ACE Genes on Diabetic Peripheral Neuropathy Progression: a Meta-Analysis. *Molecular Neurobiology*, 54(4): 2435-2444.
- Yigit, S., Karakus, N., & Inanir, A. (2013). Association of MTHFR gene C677T Mutation with Diabetic Peripheral Neuropathy and Diabetic Retinopathy. *Molecular Vision*, 19: 1626-1630.
- Zilliox, L. A., & Russell, J. W. (2019). Physical Activity and Dietary Interventions in Diabetic Neuropathy: A Systematic Review. *Clinical Autonomic Research: Official Journal of the Clinical Autonomic Research Society*, 29(4): 443-455.