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Effect of valproic acid on overall survival in patients with high-grade gliomas undergoing temozolomide

A nationwide population-based cohort study in Taiwan

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Abstract

High-grade gliomas (HGGs) are a rapidly progressive and highly recurrent group of primary brain tumors. Despite aggressive surgical resection with chemoradiotherapy, prognoses remained poor. Valproic acid (VPA), a histone deacetylase inhibitor has shown the potential to inhibit glioma cell growth in vitro through several diverse mechanisms. However clinical studies regarding the effect of VPA on HGGs are limited. This study aimed to investigate whether using VPA in patients with HGGs under temozolomide (TMZ) would lead to a better overall survival (OS).

We used the Taiwan National Health Insurance Research database to conduct this population-based cohort study. A total of 2379 patients with HGGs under TMZ treatment were included and were further classified into VPA (n = 1212, VPA ≥ 84 defined daily dose [DDD]) and non-VPA (n = 1167, VPA < 84 DDD) groups. Each patient was followed from 1998 to 2013 or until death. A Cox proportional hazard regression was performed to evaluate the effect of VPA and OS.

The VPA group had a longer mean OS time compared with the non-VPA group (OS: 50.3 ± 41.0 vs 42.0 ± 37.2 months, $P < .001$). In patients between 18 and 40 years old, the difference is most significant (OS: 70.5 ± 48.7 vs 55.1 ± 46.0, $P = .001$). The adjusted hazard ratio is 0.81 (95% confidence interval, 0.72–0.91) for the VPA group relative to the non-VPA group.

VPA at over 84 DDD improved OS in HGGs TMZ treatment.

Abbreviations: AED = anti-epileptic drug, CAD = coronary artery disease, CKD = chronic kidney disease, DDD = defined daily dose, GBM = glioblastoma multiforme, HDAC = histone deacetylase, HGGs = high-grade gliomas, HTN = hypertension, OS = overall survival, TMZ = temozolomide, VPA = valproic acid.

Keywords: high-grade gliomas, survival, temozolomide, valproic acid

1. Introduction

High-grade gliomas (HGGs), such as anaplastic astrocytoma, anaplastic oligodendroglioma, and especially glioblastoma multiforme (GBM), are a rapidly progressive and highly recurrent group of primary brain tumors. Standard multimodal treatments presently used include aggressive resection with chemotherapy and radiotherapy[1]; however, prognoses remain discouraging. The median survival expectancy for GBM is only 14.6 months. 40% to 50% of grade III and 30% to 40% of grade IV glioma patients present with seizure and are often treated clinically with anti-epileptic drugs (AEDs).[2,3]

The poor outcomes have spurred the development of new and emerging adjuvant therapies in recent years. Leading among these

new chemotherapies, including immunotherapy and targeted therapy, investigated in clinical trials in recent years, are bevacizumab with temozolomide (TMZ),[4,5] cilengitide with TMZ,[6,7] rindopepimut with TMZ,[8] and other adjuvant therapies including alternating electric field therapy and carmustine wafer. Nevertheless, combination therapies have not shown survival benefits and are expensive.

Valproic acid (VPA), a drug with broad antiepileptic spectrum used for various kinds of seizures, was first approved in France in 1967. Subsequently, other indications for VPA included manic episodes in bipolar disorder and migraine prophylaxis. Since the discovery of its function as an inhibitor of class I and IIa histone deacetylases (HDACs), VPA was used in cancer therapy because of its mechanism of action, low cost, favorable side effects, and ease in crossing the blood-brain barrier. In recent years, several studies have shown increased cell apoptosis mediated by VPA-sensitized GBM cells to chemotherapy and radiotherapy.[9–13] Clinical trials demonstrated clinical benefits of VPA through the enhanced survival of GBM patients when combined with TMZ.[14–17]

While most studies investigated the effects of VPA on GBM, Watanabe et al[18] conducted a study stating that VPA also improved survival in patients with HGGs. However, the sample size is small, limiting the study power; therefore, we conducted this study using the population-based database to observe if the effect of VPA in HGGs still persisted through a larger sample size.

2. Material and methods

2.1. Data source

We conducted a nationwide population-based cohort study using the data from the Taiwan National Health Insurance Research Database that was established in 1995 by the government of Taiwan and covered 99.6% of Taiwan's population.[19,20] Medical claims data of all beneficiaries included disease diagnoses (according to International Classification of Diseases, Ninth Revision [ICD-9] codes) during hospitalization and clinic visits, prescription drugs, doses and durations, examinations, surgeries, and procedures generated during reimbursement for insurance. Obtaining informed consent from participants was exempted in this study because all data were de-identified such that personal health information of the insures was unidentifiable. Thus, this study did not violate participants' rights or adversely affect their welfare. The Chang Gung Medical Foundation Institutional Review Board reviewed and approved all procedures of this study (IRB No.: 201901156B1).

2.2. Study population

This is a retrospective nationwide population-based cohort study conducted from January 1, 1998, to December 31, 2012, with patients diagnosed with primary malignant brain tumors (ICD-9 code 191.0–191.9) under TMZ treatment. To increase the diagnostic accuracy of malignant brain tumors, we included patients with catastrophic illness registry identification only. TMZ is an oral chemotherapeutic drug that is only prescribed to patients diagnosed with HGGs (anaplastic astrocytoma, anaplastic oligodendroglioma, and GBM) in primary malignant brain tumors according to the Pharmaceutical Benefit and Reimbursement Scheme of the Bureau of National Health Insurance in Taiwan. Patients with age under 18 years or with a follow-up duration of less than 3 months were excluded from this
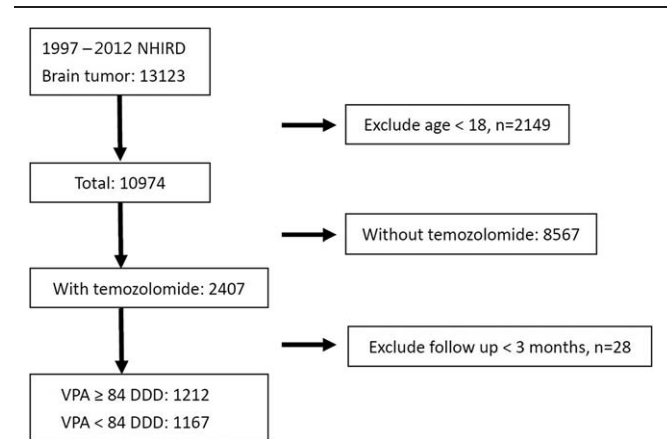
Figure 1. Enrollment schema of the study. DDD = defined daily dose, NHIRD = National Health Insurance Research Database, VPA = valproic acid.

study. A total of 2379 patients with HGGs who underwent TMZ treatment were eligible for inclusion in the analysis (Fig. 1).

2.3. VPA exposure

We identified patients who received VPA prescriptions after the diagnosis of HGGs in the inpatient or outpatient department from January 1, 1998, till death or the end of follow-up. The defined daily dose (DDD) recommended by the World Health Organization is the assumed average maintenance dose per day of a drug consumed for its main indication in adults. We categorized the patients into 2 groups: patients who used VPA ≥ 84 DDD as the VPA group and those who used VPA < 84 DDD as the non-VPA group.

2.4. Primary outcome

The study outcome was overall survival (OS). The date of death was defined in the Catastrophic Illnesses Patient Database. All patients were followed up until the end of this study (December 31, 2013) or occurrence of death, whichever came first.

2.5. Comorbidities

Comorbidities were defined by using ICD-9-CM codes recorded in the claims data: hypertension (HTN; ICD-9 code 401), diabetes mellitus (DM; ICD-9 code 250), chronic kidney disease (CKD; ICD-9 code 585), seizure (ICD-9 code 345), and coronary artery disease (CAD; ICD-9 code 414).

2.6. Statistical analysis

Baseline characteristics were compared between the VPA and non-VPA groups using Student t test. Kaplan–Meier analysis was used to evaluate the effect of VPA on the probability of survival, and the log-rank test was performed to examine the difference of OS. Cox proportional hazards ratio models were used to calculate the hazard ratios (HRs) after adjustment for VPA, gender, age, surgical excision, radiation therapy and the use of bevacizumab. For all analyses, a two-tailed P-value < .05 was considered statistically significant. All analyses were conducted using the SAS statistical software (Version 9.4; SAS Institute, Cary, NC).

2.7. Sensitivity analyses and subgroup analysis

Many comorbidities may affect the OS of HGGs. To examine the potential effect modifiers, we conducted sensitivity analyses by adding additional confounding factors including diabetes mellitus, HTN, CAD, CKD, seizure, and AEDs to the main model. We also examined the outcome stratified by groups according to gender and age and with or without seizures, surgical excisions, radiation therapy, and the use of bevacizumab. These sensitivity analyses and subgroup analysis were applied to evaluate the difference and consistency between OS and VPA use.

3. Results

3.1. Demographics

Patient demographics are summarized in Table 1. There were 1003 female and 1376 male patients. The VPA group showed a slightly younger age distribution, but it has similar gender and comorbidities compared with the non-VPA group. In the VPA group, the incidence rate of CAD was lower, but the seizure occurrence rate was higher compared with the non-VPA group (51.5% vs 32.2%, $P < .001$). In addition, of the 2098 (88.2%) patients who underwent surgical excision, more patients belonged to the VPA group than the non-VPA group (92.8% vs 83.4%, $P < .001$).

3.2. Overall survival and comorbidities

The mean OS time of the VPA group was longer (50.3 ± 41.0 months) compared with the non-VPA group (42.0 ± 37.2 months) with a P-value of $< .001$ (Table 2). The highest impact of VPA on OS (70.5 ± 48.7 months) was felt in the 18 to 40 year-old age group compared with the non-VPA group (55.1 ± 46.0 months), but there was no statistical significance in the > 65 year-old age group. Improvements in OS mediated by VPA were observed in patients irrespective of their diabetes, HTN, or surgical resection status and those receiving radiotherapy, without CKD, CAD or the use of bevacizumab. Patients with seizures had longer mean OS time in the VPA group compared

Table 1

Patient demographics and clinical characteristics of the valproic acid and non-valproic acid groups.

	VPA group	Non-VPA group	P-value
Total patients	1212	1167	
Age, yr (mean ± SD)	51.2 (15.0)	54.1 (15.8)	<.001
Gender			
Male	51.3 (15.0)	54.9 (16.1)	<.001
Female	51.1 (14.9)	53.1 (15.3)	.038
Age (yr)			<.001
18–40	315 (26.0%)	247 (21.2%)	
41–64	654 (54.0%)	582 (49.9%)	
≥65	243 (20.1%)	338 (29.0%)	
Gender			.41
Male	711 (58.7%)	665 (57.0%)	
Female	501 (41.3%)	502 (43.0%)	
CCI			.39
<4	1011 (83.42)	958 (82.09)	
≥4	201 (16.58)	209 (17.91)	
Comorbidity			
DM			
With	243 (20.1%)	244 (20.9%)	.60
Without	969 (80.0%)	923 (79.1%)	
HTN			
With	469 (38.7%)	469 (40.2%)	.46
Without	743 (61.3%)	698 (59.8%)	
CKD			
With	34 (2.81%)	38 (3.26%)	.52
Without	1178 (97.2%)	1129 (96.7%)	
Seizure			
With	624 (51.5%)	376 (32.2%)	<.001
Without	588 (48.5%)	791 (67.8%)	
CAD			
With	186 (15.4%)	221 (18.9%)	.02
Without	1026 (84.7%)	946 (81.1%)	
Surgery			<.001
Yes	1125 (92.8)	973 (83.4)	
No	87 (7.18)	194 (16.6)	
Radiation therapy			.41
Yes	1078 (88.9%)	1050 (90.0%)	
No	134 (11.1%)	117 (10.0%)	
Bevacizumab			.063
Yes	122 (10.1%)	89 (7.63%)	
No	1,090 (89.9%)	1,078 (92.4%)	

CAD = coronary artery disease, CCI = Charlson comorbidity index, CKD = chronic kidney disease, DM = diabetes mellitus, HTN = hypertension, SD = standard deviation, VPA = valproic acid.

Table 2

Overall survival of the valproic acid group and non-valproic acid group (months).

	VPA group	Non-VPA group	P-value[*]
Follow-up month			
Mean ± SD	50.3 ± 41.0	42.0 ± 37.2	<.001
Age (yr)			
18–40	70.5 ± 48.7	55.1 ± 46.0	.001
41–64	47.3 ± 37.2	42.2 ± 35.6	.014
≥65	31.9 ± 25.9	32.3 ± 28.9	.87
Gender			
Male	50.3 ± 41.6	39.7 ± 35.7	<.001
Female	50.1 ± 40.1	45.2 ± 38.8	.048
Comorbidity			
DM			
With	42.7 ± 35.6	35.3 ± 30.1	.013
Without	52.1 ± 42.0	43.8 ± 38.6	<.001
HTN			
With	43.5 ± 38.0	37.3 ± 32.7	.008
Without	54.5 ± 42.2	45.2 ± 39.6	<.001
CKD			
With	38.8 ± 30.1	36.3 ± 34.5	.74
Without	50.6 ± 41.2	42.2 ± 37.2	<.001
CAD			
With	43.0 ± 38.4	39.2 ± 34.7	0.30
Without	51.6 ± 41.3	42.7 ± 37.7	<0.001
Seizure			
With	62.7 ± 46.1	51.7 ± 43.5	.001
Without	37.0 ± 29.4	37.5 ± 32.8	.79
Surgery excision			
Yes	50.4 ± 40.6	43.0 ± 37.6	<.001
No	48.9 ± 45.4	37.3 ± 34.6	.035
Radiation therapy			
Yes	50.7 ± 40.5	42.2 ± 36.4	<.001
No	46.8 ± 44.4	40.3 ± 43.4	.24
Bevacizumab			
Yes	59.1 ± 45.2	49.5 ± 44.3	.13
No	49.3 ± 40.4	41.4 ± 36.5	<.001

[*] t-test.

CAD = coronary artery disease, CKD = chronic kidney disease, DM = diabetes mellitus, HTN = hypertension, VPA = valproic acid.
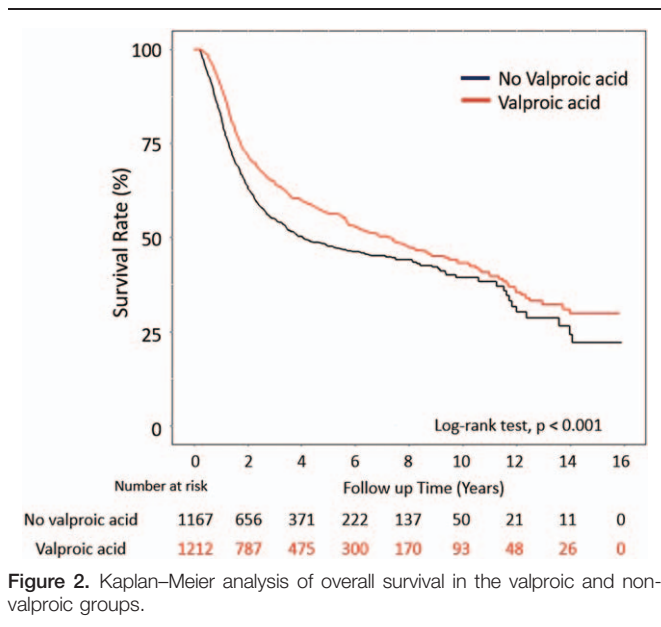
Figure 2. Kaplan–Meier analysis of overall survival in the valproic and non-valproic groups.

with those in the non-VPA group (62.7 vs 51.7 months). Figure 2 illustrates the results of the Kaplan–Meier method for the OS rate. The log-rank test revealed a significant difference over the entire Kaplan–Meier curve.

Table 3 showed that the sensitivity analysis adjustments exhibited a little effect on the OS between the VPA and non-VPA groups according to different models. Although the hazard ratios in the subgroup of age ≥65 years old, female patients, use of bevacizumab, patients without seizure, radiotherapy or did not receive surgical excision in subgroup analysis were not statistically significant, but the trend is still observed.

4. Discussion

This is the first population-based cohort study to demonstrate the impact of VPA on OS in patients with HGGs under standard treatment. Our study has several strengths. First, our database included a large number of HGG patients representing a nationwide population and a 16-year observation period and, therefore, has implications for health care in Taiwan, and beyond. Second, this study cohort is collected from a computerized database that included all HGG patients in Taiwan; we can then abolish the possibility of selection bias. The data on VPA and other medications were derived from a database that collects all available prescription details during this study period; we can eliminate the probability of recall bias.

Watanabe et al[18] demonstrated that VPA will delay hair loss and improve OS in HGG patients under radiotherapy. However, there are some limitations to that study. First, there is only a median VPA dose (800 mg/d), but the duration of the prescription was not shown. Second, only 24 patients (21.4%) were categorized in the VPA group, resulting in limited power to demonstrate the result. Third, the study did not include other possible drugs or comorbidities that might also affect the OS.

Previous studies have shown that VPA itself suppress glioma progression through anti-proliferative,[21] anti-angiogenic,[22] and apoptotic[23] effects in various glioma models. VPA was found as an HDAC inhibitor in 2001[24] and gained much interest in treating tumors. A combination of VPA with classic anticancer drugs leads to synergistic effects, which can have a promising therapeutic or adjuvant application in the treatment of cancer. Our previous study showed that the use of a combination of clinical concentrations of TMZ and VPA synergistically sensitized glioma cells to induce apoptosis possibly through the redox regulation mechanism.[25]

VPA has been found to mediate the radiosensitization of glioblastoma cells both in vitro and in preclinical in vivo studies[9,26–30] through its effects on HDAC. VPA also down-regulates the expression of O6-methylguanine-DNA methyltransferase in glioma cells[31] and to sensitize glioblastoma cells to TMZ in vitro[30–32] by protecting healthy neurons and sensitizing malignant glioma cells[27,33] to enhance radiation therapy. Besides radiosensitization and TMZ-sensitization, VPA-mediated histone hyperacetylation, and DNA demethylation are associated with changes in cell morphology, decreased cell viability, and increased apoptosis rates.[29,34–36] In addition, VPA in combination with TMZ and radiotherapy improved OS or progression-free survival in patients with GBM.[14,16,17,37]

Consistent with previous studies, our demographic is male dominant,[14,15] and the seizures rate is 41% in all HGG patients, which was comparable with that reported in GBM patients (30%–50%).[38] OS was highly relevant to age as previous studies[1,39] since VPA showed no benefits in OS in patients aged over 65 years.

In patients with CKD, VPA showed no benefit in OS compared with the non-VPA group. The incidence and treatment of seizures are complicated by chronic kidney failure causing higher seizure rates leading to challenges in the loading, titration, and maintenance of AEDs. Although VPA requires no dose adjustment in CKD patients since the drug is mostly metabolized by the liver, VPA can still cause tubulointerstitial nephritis, Fanconi syndrome, and hypernatremia,[40] further complicating the underlying CKD. We found no evidence that CKD could affect the OS of HGGs, but VPA did not prolong OS, warranting an investigation into the action of VPA in HGGs with CKD.

Epileptic patients receiving long-term VPA monotherapy exhibited altered circulatory markers of vascular risk that may contribute to the acceleration of the atherosclerotic process and were significantly associated with the duration of VPA exposure.[41] Another study showed that VPA induces higher serum C-reactive protein level, homocysteinemia, uric acid, oxidative stress markers, and increased carotid intima-media thickness, which enhanced cardiovascular risks.[42] It is plausible that the lack of improvement in OS in patients with CAD with VPA therapy may be due to the underlying CAD, which further increased mortality or morbidity.

In our study, 48.5% of patients were taking VPA in the absence of seizure, although the current guideline does not suggest prophylactic use of AEDs in brain tumors. However, the prophylactic use of antiepileptic agents is a relatively common practice previously in Taiwan. In recent years, the indications for prophylactic AEDs are stricter and prefer newer AEDs other than VPA. According to Lote et al,[37] seizure is associated with prolonged survival in patient with HGGs. Seizure may occur before any other symptoms leading to earlier diagnosis and treatment when tumor is small. Previous studies also showed that seizure is a marker for less aggressive tumor biology and more benign clinical behavior.[43,44] However, in patients without a seizure, VPA had no effect in improving OS, and this may support the long-term use of prophylactic AEDs that are not recommended in patients with HGGs.

Table 3

Adjusted hazard ratios of overall survival associated with valproic acid use.

	No valproic acid	Valproic acid		
	HR	HR	95%CI	*P*-value
Main model[*]	Reference	0.81	0.72–0.91	<.001
Full model1[†]	Reference	0.81	0.72–0.91	<.001
Full model2[‡]	Reference	0.82	0.72–0.92	<.001
Additional covariate[§]				
Main model+DM	Reference	0.81	0.72–0.91	<.001
Main model+HTN	Reference	0.81	0.72–0.91	<.001
Main model+CKD	Reference	0.81	0.72–0.91	<.001
Main model+CAD	Reference	0.81	0.72–0.91	<.001
Main model+seizure	Reference	0.85	0.75–0.96	<.001
Main model+gabapentin	Reference	0.82	0.73–0.92	<.001
Main model+carbamazepine	Reference	0.81	0.72–0.92	<.001
Main model+phenytoin	Reference	0.79	0.70–0.89	<.001
Main model+levetiracetam	Reference	0.83	0.73–0.93	.002
Main model+radiation Therapy	Reference	0.81	0.72–0.91	<.001
Main model+chemotherapy	Reference	0.81	0.72–0.91	<.001
Age (yr)				
18–40	Reference	0.73	0.57–0.93	.012
41–64	Reference	0.79	0.67–0.94	.006
≥65	Reference	0.88	0.70–1.11	.29
Gender				
Male	Reference	0.75	0.64–0.87	<.001
Female	Reference	0.91	0.76–1.10	.32
Seizure				
With	Reference	0.76	0.63–0.92	.005
Without	Reference	0.91	0.78–1.07	.25
Surgical excision				
No	Reference	0.87	0.60–1.25	.45
Yes	Reference	0.80	0.71–0.91	<.001
Radiation therapy				
With	Reference	0.80	0.71–0.91	.002
Without	Reference	0.79	0.56–1.12	.18
Bevacizumab				
With	Reference	0.86	0.58–1.28	.45
Without	Reference	0.80	0.71–0.91	<.001

[*] Main model adjusted valproic acid, gender, age, and surgical excision.
[†] Full model 1 adjusted valproic acid, gender, age, DM, HTN, CKD, CAD, and surgical excision.
[‡] Full model 2 adjusted valproic acid, gender, age, surgical excision, DM, HTN, CKD, CAD, gabapentin, carbamazepine, phenytoin, and levetiracetam.
[§] The models were adjusted for covariates in the main model as well as each additional listed covariate.
CAD=coronary artery disease, CKD=chronic kidney disease, DM=diabetes mellitus, HR = hazard ratio, HTN=hypertension.

VPA is mostly prescribe for seizure treatment and prevention especially postoperatively in patients with HGGs. Before the approval of levetiracetam intravenous form in 2012 in Taiwan, VPA is one of the most used AEDs for postoperative seizure prevention and treatment after brain tumor surgery, leading to the higher rate of VPA use in surgical excision group. The sensitivity analysis adjustment for surgical excision exhibited little effect on the association between VPA and OS. After subgroup analysis, VPA still showed positive effect on OS in patients receiving surgical excision.

VPA showed no improve on OS in patients who did not receive radiotherapy. We assume that these patients were unable to tolerate radiotherapy due to old age, poor performance status or multiple comorbidities. Bevacizumab is the second line chemotherapy in Taiwan for patients with HGGs failing response to TMZ. Previous study demonstrated that although the addition of bevacizumab to standard treatment regimens did not improve OS in newly diagnosed glioblastoma, but was associated with higher rate of response and 6-month progression-free survival in recurrent glioblastoma.[45] Fountzilas et al reported that bevacizumab in combination with VPA induced a prolonged partial response in a patient with recurrent glioblastoma.[46] Our study showed VPA has no survival benefit in patient with bevacizumab, however the dose and duration of bevacizumab were not recorded, thus further detailed studies are warranted.

There are several limitations to this study. First, we did not include patients with HGGs that are too ill to receive TMZ therapy which may affect the OS. However, our focus is on patients under TMZ treatment; thus, this will not affect our results. Second, we lack the precise pathological report including the molecular genetic studies, for example, isocitrate dehydrogenase mutation, O6-methylguanine-DNA methyltransferase promoter methylation, and 1p-19q co-deletions, which may affect the OS. Third, we lack the precise surgical procedure, including gross total resection, subtotal resection, or biopsy only.

5. Conclusion

VPA at over 84 DDD improved OS in HGGs. Further studies are required for a detailed analysis of the effect of VPA in different types of gliomas.

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Author contributions

Yu-Jen Kuo, Jen-Tsung Yang and Ming-Hsueh Lee conceived of the presented idea. Yao-Hsu Yang, I-Yun Lee, and Pau-Chung Chen performed the data retrieval and statistical analysis. Ting-Chung Wang, Martin Hsiu-Chu Lin, Wei-Hsun Yang, Chun-Yu Cheng, Kuo-Tai Chen, and Wei-Chao Huang contributed to the interpretation of the results. Yu-Jen Kuo took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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