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Original Article

Anemia, hematinic deficiencies, and hyperhomocysteinemia in male and female burning mouth syndrome patients

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Received 25 November 2021

Available online 9 December 2021

KEYWORDS

Burning mouth syndrome;
Hemoglobin;
Iron;
Vitamin B12;

Abstract *Background/purpose:* Our previous study found that 19.8%, 16.2%, 4.8%, 2.3%, 19.2%, and 12.3% of 884 burning mouth syndrome (BMS) patients have anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity, respectively. This study mainly evaluated the anemia, hematinic deficiencies, and hyperhomocysteinemia in 212 male and 672 female BMS patients. *Materials and methods:* The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid,

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Folic acid;
Homocysteine

homocysteine, GPCA levels in 212 male and 672 female BMS patients were measured and compared with the corresponding levels in 106 male and 336 female healthy control subjects, respectively.

Results: We found that 212 male BMS patients had significantly lower mean blood Hb, serum iron, vitamin B12, and folic acid levels and significantly higher mean serum homocysteine levels than 106 male healthy control subjects. Moreover, 672 female BMS patients had significantly lower mean blood Hb and serum iron levels and significantly higher mean serum homocysteine level than 336 female healthy control subjects. In addition, 212 male BMS patients had significantly higher mean blood Hb and serum homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of folic acid deficiency and hyperhomocysteinemia than 672 female BMS patients.

Conclusion: The male BMS patients do have significantly higher mean blood Hb and serum homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of folic acid deficiency and hyperhomocysteinemia than the female BMS patients.

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Introduction

Burning mouth syndrome (BMS) is characterized by the presence of burning sensation of the oral mucosa in the absence of clinically apparent mucosal alterations. It occurs more commonly in the middle-aged and elderly women and the prevalence of BMS increases with advancing age. BMS can be classified into two clinical forms: primary and secondary BMS. The primary BMS is essential or idiopathic, in which the organic local/systemic causes cannot be identified, and peripheral and central neuropathological pathways are involved. The secondary BMS is caused by local, systemic, and/or psychological factors.^{1,2}

In our oral mucosal disease clinic, BMS patients are relatively frequently encountered.^{1–11} Our previous study of 884 BMS patients found that all of them complained of burning sensation at some sites of oral mucosa, especially the tip and lateral borders of the tongue and anterior hard palate. Moreover, dry mouth, numbness of oral mucosa, and dysfunction of taste can be observed in 48.1%, 30.7% and 16.7% of 884 BMS patients.² We also discovered that 19.8%, 16.2%, 4.8%, 2.3%, 19.2%, and 12.3% of 884 BMS patients have anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity, respectively.² In another our previous study, 399 primary and secondary BMS patients without or with hematinic deficiencies were treated with vitamin BC capsules plus none, one, or two deficient hematinics depending on the corresponding deficiency statuses of the patients. Of the 399 BMS patients, 177 (44.4%) showed complete remission of all oral symptoms after treatment.¹¹ To the best of our knowledge, none of previous studies compared the complete blood count data, serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels between a large group of male and female BMS patients. Therefore, in this study, we divided the 884 BMS patients into 212 male and 672 female BMS patients.

We mainly evaluated whether 212 male BMS patients had significantly lower frequencies of blood hemoglobin (Hb) and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 672 female BMS patients. We also assessed whether there were significantly higher frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity in 212 male and 672 female BMS patients than in 106 male and 336 female healthy control subjects, respectively.

Materials and methods

Participants

This study included 212 male BMS patients (age range 18–88 years, mean 53.3 ± 16.9 years) and 672 female BMS patients (age range 18–90 years, mean 57.0 ± 13.5 years).² For two BMS patients, one age- (± 2 years of each patient's age) and sex-matched healthy control subject was selected. Thus, 106 male (age range 20–90 years, mean 54.5 ± 14.7 years) and 336 female (age range 18–88 years, mean 58.5 ± 13.0 years) age- and sex-matched healthy control subjects were selected and included in this study.² All the patients and control subjects were seen consecutively, diagnosed, and treated in the Department of Dentistry of National Taiwan University Hospital (NTUH) from July 2007 to July 2017. The detailed inclusion and exclusion criteria for 884 BMS patients and 442 healthy control subjects have been described previously.² In addition, none of the BMS patients had taken any prescription medication for BMS at least 3 months before entering the study.

The blood samples were drawn from 884 BMS patients and 442 healthy control subjects for the measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine concentrations, and the serum GPCA

positivity. All BMS patients and healthy control subjects signed the informed consent forms before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH (201212066RIND).

Determination of blood hemoglobin, iron, vitamin B12, folic acid, and homocysteine concentrations.

The complete blood count and serum iron, vitamin B12, folic acid, and homocysteine concentrations were determined by the routine tests performed in the Department of Laboratory Medicine, NTUH.^{1–11}

Determination of serum gastric parietal cell antibody level

The serum GPCA level was detected by the indirect immunofluorescence technique with rat stomach as a substrate as described previously.^{2–10} Sera were scored as positive when they produced fluorescence at a dilution of 10-fold or more.

Statistical analysis

Comparisons of the mean corpuscular volume (MCV) and mean blood Hb and serum iron, vitamin B12, folic acid, and homocysteine levels between 212 male or 672 female BMS patients and 106 male or 336 female healthy control subjects, respectively, as well as between 212 male and 672 female BMS patients were performed by Student's *t*-test. The differences in frequencies of microcytosis (MCV <80 fL),^{12,13} macrocytosis (MCV ≥100 fL),^{14–16} blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity between 212 male or 672 female BMS patients and 106 male or 336 female healthy control subjects, respectively, as well as between 212 male and 672 female BMS patients were compared by chi-square test. In addition, the differences in frequencies of 6 different types of anemia between 212 male and 672 female BMS patients were also compared by chi-square test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The MCV, mean blood Hb and serum iron, vitamin B12, folic acid, and homocysteine levels in 212 male and 672 female BMS patients and in 106 male and 336 female healthy control subjects are shown in Table 1. We found that 212 male BMS patients had significantly lower mean blood Hb, serum iron, vitamin B12, and folic acid levels and significantly higher mean serum homocysteine levels than 106 male healthy control subjects (all *P*-values < 0.05, Table 1). Moreover, 672 female BMS patients had significantly lower MCV and mean blood Hb and serum iron levels, and significantly higher mean serum homocysteine level than 336 female healthy control subjects (all *P*-values < 0.05, Table 1). In addition, 212 male BMS patients had significantly lower mean serum vitamin B12 and folic acid levels and significantly higher mean blood Hb and serum homocysteine levels than 672 female BMS patients (all *P*-values < 0.001,

Table 1). However, no significant difference in the MCV and mean serum iron level was found between 212 male and 672 female BMS patients (Table 1).

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV <80 fL,^{12,13} macrocytosis of erythrocyte was defined as having MCV ≥100 fL,^{14–16} and men with Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb deficiency or anemia.¹⁷ Furthermore, patients with the serum iron level <60 µg/dL,¹⁸ the serum vitamin B12 level <200 pg/mL,¹⁹ or the folic acid level <4 ng/mL²⁰ were defined as having serum iron, vitamin B12 or folic acid deficiency, respectively. In addition, patients with the blood homocysteine level >12.3 µM (which was the mean serum homocysteine level of healthy control subjects plus two standard deviations) were defined as having hyperhomocysteinemia.² By the above-mentioned definitions, 5.2%, 7.1%, 18.9%, 13.2%, 5.2%, 4.7%, 32.5%, and 12.3% of 212 male BMS patients and 8.5%, 4.6%, 20.1%, 17.1%, 4.6%, 1.5%, 15.0%, and 12.4% of 672 female BMS patients were diagnosed as having microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity, respectively (Table 2). Moreover, 212 male BMS patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 106 male healthy control subjects (all *P*-values < 0.05, Table 2). Furthermore, 672 female BMS patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 336 female healthy control subjects (all *P*-values < 0.001, Table 2). In addition, 212 male BMS patients had significantly higher frequencies of folic acid deficiency and hyperhomocysteinemia than 672 female BMS patients (both *P*-values < 0.05, Table 2).

Forty male and 135 female BMS patients had anemia (defined as having an Hb concentration <13 g/dL for men and <12 g/dL for women).¹⁷ Of the 40 anemic male BMS patients, 3 had pernicious anemia (PA, defined as having anemia, an MCV ≥100 fL, a serum vitamin B12 level <200 pg/mL, and the presence of serum GPCA positivity),^{14–16} 7 had macrocytic anemia (defined as having anemia and an MCV ≥100 fL) other than PA,^{14–16} 26 had normocytic anemia (defined as having anemia and an MCV between 80 fL and 99.9 fL),^{21–24} one had iron deficiency anemia (IDA, defined as having anemia, an MCV <80 fL, and a serum iron level <60 µg/dL),^{13,17,18} two had thalassemia trait-induced anemia (defined as having anemia, a red blood cell count >5.0 M/µL, an MCV <74 fL, and a Mentzer index (MCV/RBC) < 13),²⁵ and one had microcytic anemia (defined as having anemia and an MCV <80 fL)^{12,18} other than IDA and thalassemia trait-induced anemia. Thus, by strict WHO criteria the normocytic anemia (65.0%, 26/40) was the most common type of anemia in our 40 anemic male BMS patients (Table 3).

Of the 135 anemic female BMS patients, 12 had PA,^{14–16} 8 had macrocytic anemia other than PA,^{14–16} 69 had normocytic anemia,^{21–24} 20 had IDA,^{13,17,18} 25 had thalassemia trait-induced anemia,²⁵ and one had microcytic anemia^{12,18}

other than IDA and thalassemia trait-induced anemia. Therefore, by strict WHO criteria the normocytic anemia (51.1%, 69/135), thalassemia trait-induced anemia (18.5%, 25/135), and IDA (14.8%, 20/135) were the three most

common types of anemia in our 135 anemic female BMS patients (Table 3). In addition, 672 female BMS patients had significantly higher frequency of thalassemia trait-induced anemia than 212 male BMS patients ($P < 0.001$, Table 3).

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels between 212 male or 672 female BMS patients and 106 male or 336 female healthy control subjects, respectively, as well as between 212 male BMS patients and 672 female BMS patients.

Group	MCV (fL)	Hb (g/dL)	Iron ($\mu\text{g}/\text{dL}$)	Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
Male BMS patients (n = 212)	90.2 \pm 7.0	14.6 \pm 1.5	92.4 \pm 26.8	545.3 \pm 256.6	11.6 \pm 6.3	11.2 \pm 5.7
^a P-value	0.583	0.001	<0.001	0.002	0.027	0.003
^b P-value	0.165	<0.001	0.200	<0.001	<0.001	<0.001
Female BMS patients (n = 672)	89.4 \pm 7.4	13.1 \pm 1.2	89.3 \pm 31.8	669.3 \pm 264.9	15.2 \pm 7.5	8.7 \pm 3.5
^a P-value	0.035	<0.001	<0.001	0.343	0.830	<0.001
Male healthy control subjects (n = 106)	90.6 \pm 3.8	15.1 \pm 0.8	105.2 \pm 28.0	634.6 \pm 210.9	13.2 \pm 5.5	9.5 \pm 1.8
Female healthy control subjects (n = 336)	90.3 \pm 3.5	13.5 \pm 0.7	97.8 \pm 27.3	685.3 \pm 225.4	15.1 \pm 5.7	8.0 \pm 2.0

^a Comparisons of means of parameters between 212 male or 672 female BMS patients and 106 male or 336 female healthy control subjects by Student's *t*-test, respectively.

^b Comparisons of means of parameters between 212 male BMS patients and 672 female BMS patients by Student's *t*-test.

Table 2 Comparisons of frequencies of microcytosis (mean corpuscular volume or MCV <80 fL), macrocytosis (MCV \geq 100 fL), blood hemoglobin (Hb) and serum iron, vitamin B12, and folic acid deficiencies, and gastric parietal cell antibody (GPCA) positivity between 212 male or 672 female BMS patients and 106 male or 336 female healthy control subjects, respectively, as well as between 212 male BMS patients and 672 female BMS patients.

Group	Patient number (%)							
	Microcytosis (MCV <80 fL)	Macrocytosis (MCV \geq 100 fL)	Hb deficiency (Men <13 g/dL, women <12 g/dL)	Iron deficiency (<60 $\mu\text{g}/\text{dL}$)	Vitamin B12 deficiency (<200 pg/mL)	Folic acid deficiency (<4 ng/mL)	Hyperhomo -cysteinemia (>12.3 μM)	GPCA positivity
Male BMS patients (n = 212)	11 (5.2)	15 (7.1)	40 (18.9)	28 (13.2)	11 (5.2)	10 (4.7)	69 (32.5)	26 (12.3)
^a P-value	0.039	0.012	<0.001	<0.001	0.039	0.053	<0.001	0.004
^b P-value	0.155	0.219	0.772	0.215	0.874	0.013	<0.001	0.931
Female BMS patients (n = 672)	57 (8.5)	31 (4.6)	135 (20.1)	115 (17.1)	31 (4.6)	10 (1.5)	101 (15.0)	83 (12.4)
^a P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.056	<0.001	<0.001
Male healthy control subjects (n = 106)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.9)	2 (1.9)
Female healthy control subjects (n = 336)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (2.7)	6 (1.8)

^a Comparisons of frequencies of parameters between 212 male or 672 female BMS patients and 106 male or 336 female healthy control subjects by chi-square test, respectively.

^b Comparisons of frequencies of parameters between 212 male BMS patients and 672 female BMS patients by chi-square test.

Table 3 Comparison of frequencies of 6 different types of anemia between 212 male BMS patients and 672 female BMS patients.

Anemia type	Patient number (%) ^a		P-value
	Male BMS patients (n = 212)	Female BMS patients (n = 672)	
Pernicious anemia	3 (1.4)	12 (1.8)	0.953
Other macrocytic anemia	7 (3.3)	8 (1.2)	0.077
Normocytic anemia	26 (12.3)	69 (10.3)	0.489
Iron deficiency anemia	1 (0.5)	20 (3.0)	0.067
Thalassemia trait-induced anemia	2 (0.9)	25 (3.7)	<0.001
Other microcytic anemia	1 (0.5)	1 (0.1)	0.973
Total	40 (18.9)	135 (20.1)	0.772

^a Comparison of frequencies of 6 different types of anemia between 212 male BMS patients and 672 female BMS patients by chi-square test.

Discussion

The major findings of this study were that the male BMS patients had significantly higher mean blood Hb and serum homocysteine levels, significantly higher frequencies of folic acid deficiency and hyperhomocysteinemia, and significantly lower mean serum vitamin B12 and folic acid levels than the female BMS patients. The male BMS patients also had higher mean serum iron level, higher frequency of vitamin B12 deficiency, and lower frequencies of Hb and serum iron deficiencies than the female BMS patients, but the differences were not significant between the male and female BMS patients.

Sex hormones can have a significant influence on the red blood cell, blood Hb, and serum iron levels.^{26–32} Androgens can stimulate erythropoiesis and increase levels of red blood cells, hemoglobin, and hematocrit. Androgens stimulate hematopoietic system by various mechanisms, including stimulation of erythropoietin release, increase in bone marrow activity, and augmentation of iron incorporation into the red cells.^{26,27} Erythropoietin is a kidney-produced hormone that stimulates the production and maintenance of red blood cells.²⁸ However, in subjects with chronic mountain sickness (Monge's disease), estrogens have a striking negative effect on the erythropoietic response in a dose-dependent manner. Addition of estrogen at 5–10 nM (physiological estrogen levels: 0.1–10 nM) to erythroid cells isolated from females with chronic mountain sickness results in a significant decrease in red blood cell production, and the addition of estrogen at 50–100 nM leads to extremely low red blood cell production.²⁹ In menopause women, estrogen decreases because of the cessation of ovarian functions and iron increases as a result of decreasing menstrual periods. Nevertheless, estrogen deficiency up-regulates hepcidin, which inhibits intestinal iron absorption, leading to lower serum iron levels.³⁰ Therefore, the difference in the sex hormone levels between men and women can partially explain why men usually have higher blood Hb and serum iron levels.

In general, each healthy pregnancy depletes the mother of approximately 500 mg of iron. Menstrual blood losses are highly variable, ranging from 10 to 250 mL (4–100 mg of iron) per period. A man must absorb about 1 mg of iron to maintain equilibrium. During childbearing years, an adult female loses an average of 2 mg of iron daily and must absorb a similar quantity of iron in order to maintain equilibrium.³¹ However, in the postmenopausal women, iron deficiency is uncommon in the absence of menstrual bleeding. Furthermore, because women eat less food than men, they must be more than twice as efficient as men in the absorption of iron to avoid iron deficiency. Therefore, anemia is twice as prevalent in females as in males.³² This difference is significantly greater during the childbearing years due to pregnancies and menses.³² Because our male BMS patients had a mean age of 53.3 years and the female BMS patients had a mean age of 57.0 years that was beyond the childbearing years, this could explain why the male BMS patients had higher mean serum iron level and lower frequencies of Hb and serum iron deficiencies than the female BMS patients.

We further explained why male BMS patients had the lower mean serum vitamin B12 and folic acid levels and higher frequencies of vitamin B12 and folic acid deficiencies than female BMS patients. Previous studies discovered significantly lower mean folate levels in buccal mucosal cells and sera of 25 smokers than in those of 34 non-smokers.³³ Pivathilake et al.³⁴ also demonstrated lower buccal mucosal cell folate and vitamin B12 concentrations in 39 current smokers than in 60 noncurrent smokers.³⁴ Our previous study of serum vitamin B12 and folic acid levels in oral precancer patients also found significantly lower mean serum folic acid levels in 87 cigarette smokers than in 44 non-smokers and in 26 smokers consuming >20 cigarettes per day than in 61 smokers consuming ≤20 cigarettes per day.³⁵ The mean serum folic acid level was also lower in 52 betel quid chewers than in 79 non-chewers.³⁵ The findings of above-mentioned studies indicate the existence of vitamin B12 and folic acid deficiencies in the sera and oral mucosal cells of the smokers and betel quid chewers. We suggest that the mechanisms of vitamin B12 and folic acid deficiencies may arise from elevated vitamin B12 and folic acid consumption in response to rapid tissue proliferation or repair caused by the irritation or damage of oral mucosal cells by the carcinogens in tobacco or betel quid.^{36,37} The present study found that male BMS patients had significantly lower mean serum vitamin B12 and folic acid levels and significantly higher frequency of folic acid deficiency than female BMS patients. Although male BMS patients had higher frequency of vitamin B12 deficiency than female BMS patients, the difference was not significant. In this study, we did not assess the frequencies of cigarette smoking and betel quid chewing habits in our 212 male and 672 female BMS patients. However, in the Taiwan population, the males ≥18 years of age had a significantly higher prevalence of smoking habit (23.1% for men and 2.9% for women) or betel quid chewing habit (16.8% for men and 1.2% for women) than the females ≥18 years of age.³⁸ Because there is a significantly higher prevalence of smoking or betel quid chewing habit in men than in women in the Taiwan population, we strongly suggest that the smoking or betel quid chewing habit may be the major causes that

result in the lower mean serum vitamin B12 and folic acid levels and higher frequencies of vitamin B12 and folic acid deficiencies in male BMS patients than in female BMS patients.

In this study, the normocytic anemia (65.0%, 26/40) was the most common type of anemia in our 40 anemic male BMS patients, followed by the other macrocytic anemia (17.5%, 7/40) and PA (7.5%, 3/40) (Table 3). Furthermore, the normocytic anemia (51.1%, 69/135), thalassemia trait-induced anemia (18.5%, 25/135), and IDA (14.8%, 20/135) were the three most common types of anemia in our 135 anemic female BMS patients (Table 3). From the above-mentioned findings, we discovered that regardless of the normocytic anemia, the male BMS patients tended to have macrocytic anemia (PA and other macrocytic anemia) and the female BMS patients were prone to have microcytic anemia (thalassemia trait-induced anemia and IDA). As stated before, male BMS patients had the lower mean serum vitamin B12 and folic acid levels and higher frequencies of vitamin B12 and folic acid deficiencies than female BMS patients in the present study. Moreover, female BMS patients had the lower mean blood Hb and serum iron levels and higher frequencies of blood Hb and serum iron deficiencies than male BMS patients. These results could easily explain why the male BMS patients tended to have macrocytic anemia and the female BMS patients were prone to have microcytic anemia in the present study. In addition, this study also found that 672 female BMS patients had significantly higher frequency of thalassemia trait-induced anemia than 212 male BMS patients. The thalassemia trait-induced anemia is predominantly due to gene mutation resulting in insufficient production of either the α -globin or β -globin chains of the Hb molecule.²⁵ However, it needs further investigations to clarify the reasons why female BMS patients had significantly higher frequency of thalassemia trait-induced anemia than male BMS patients in the present study.

Homocysteine is formed during methionine metabolism.³⁹ Both vitamin B12 and folic acid function as coenzymes for the conversion of homocysteine to methionine.⁴⁰ Thus, patients with vitamin B12 and/or folic acid deficiencies may have hyperhomocysteinemia. A previous study has shown that a supplementation with folic acid and vitamins B12 and B6 can reduce blood homocysteine levels.⁴¹ Our previous studies also demonstrated that supplementations with vitamin B12 capsules plus corresponding deficient vitamin B12 and/or folic acid can reduce the abnormally high serum homocysteine level to significantly lower levels in patients with either atrophic glossitis or BMS.^{11,42} In this study, significantly lower mean serum vitamin B12 and folic acid levels and higher frequencies of serum vitamin B12 and folic acid deficiencies in male BMS patients than in female BMS patients could explain why our male BMS patients had significantly higher mean serum homocysteine level and high frequency of hyperhomocysteinemia than our female BMS patients.

The results of this study conclude that the male BMS patients do have significantly higher mean blood Hb and serum homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of folic acid deficiency and hyperhomocysteinemia than the female BMS patients.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This study was partially supported by the grants (No. 102-2314-B-002-125-MY3 and No. 105-2314-B-002-075-MY2) of Ministry of Science and Technology, Taiwan.

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