RESEARCH ARTICLE

MCBS
Mol Cell Biomed Sci. 2024; 8(2): 96-104
DOI: 10.21705/mcbs.v8i2.426

ACE I/D and *A2350G* Polymorphisms are Correlated with Body Mass Index, but Not with Body Weight and Essential Hypertension: Study in Javanese Postmenopausal Women

Sri Lestari Utami[1], Dorta Simamora[1], Ira Idawati[2], Jimmy Hadi Widjaja[3]

[1]*Biomedic Department, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia*
[2]*Histology Department, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia*
[3]*Anatomical Pathology Department, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia*

Background: Genetics was one of the risk factors for essential hypertension (EH). Research on *ACE* I/D and *A2350G* polymorphisms associated with risk factors for hypertension in Indonesia has never been done. Therefore, this study was conducted to analyze the relationship between the genotype and alleles of this gene with EH, body weight, and body mass index (BMI) in Javanese postmenopausal women.

Materials and methods: This cross-sectional study involved 69 postmenopausal Javanese women according to several criteria related with hypertension risk factors. The data were obtained from the measurement and questionnaire results, along with Towards Health Card Records. The polymerase chain reaction (PCR) genotyping method used was the restriction fragment length polymorphism and allele-specific.

Results: The prevalence of hypertension, prehypertension, and normotension in Javanese postmenopausal women were 0.246, 0.13, and 0.623, respectively. The frequency of BMI classification as underweight, normal, overweight, or obese were 0.029, 0.42, 0.261, and 0.29, respectively. The *ACE* I/D and *A2350G* polymorphism variant genotypes and frequencies found were II (0.464), ID (0.522), DD (0.014), and AA (1). Meanwhile, the alleles and their frequencies at *ACE* I/D gene polymorphism were I (0.725) and D (0.275). The II and ID genotype was mostly found in normotension subjects. The DD genotype was only available in hypertension subjects. There was no association between genotypes and alleles of *ACE* I/D, hypertension, body weight, and BMI classification ($p>0.05$). There was an association between these genotypes, alleles, and BMI ($p<0.05$).

Conclusion: *ACE* I/D polymorphism is susceptible for BMI in Javanese postmenopausal women.

Keywords: *Javanese postmenopausal, essential hypertension, ACE I/D, ACE A2350G*

Submission: January 21, 2024
Last Revision: April 17, 2024
Accepted for Publication: May 2, 2024

Corresponding Author:
Sri Lestari Utami
Biomedic Department, Faculty of Medicine
Universitas Wijaya Kusuma Surabaya
Jl. Dukuh Kupang XXV No.54, Surabaya 60225, Indonesia
e-mail: sri.lestari@uwks.ac.id

Cell and Biopharmaceutical Institute

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v8i2.426

Introduction

Essential hypertension (EH) or primary hypertension is defined as unclear-cause hypertension, as opposed to secondary hypertension. More than 90% of hypertensive patients are classified as EH patients. High blood pressure in hypertension can increase the risk of kidney disease, heart disease, and stroke because it is the main cause of premature death worldwide. According to Indonesia's Basic Health Research in 2018, people in DKI Jakarta Province with hypertension are 3.14 times more likely to suffer from chronic kidney disease than those without hypertension. Approximately 1.28 billion adults aged 30-78 years worldwide suffer from hypertension, of which 2/3 live in low-and middle-income countries, including Indonesia. The prevalence of hypertension in Indonesia was 34.11% based on blood pressure measurements in the population aged 18 years and over. The controlled and uncontrolled hypertension prevalence in postmenopausal women was 17.9% and 48.4%, respectively.[1–5] According to the WHO, among the risk factors for hypertension are unhealthy diets, physical inactivity, consumption of alcohol and tobacco, excessive body weight or obesity, family history of hypertension, age over 65 years, and the presence of comorbidities such as diabetes mellitus and kidney disease. These risk factors is also found in Indonesia with additional gender and employment status. The prevalence of women with hypertension is twice as high as that of men in the study with the Indonesian Family Life Survey (IFLS).[2,6]

A family history of hypertension as a risk factor shows that genetic inheritance plays a role in human health, including polymorphism. An allele variant in polymorphism is a condition of gene susceptibility to disease. A person with an allele of a gene that is considered defective will be more likely to develop the disease than one who does not have it. However, the environment will contribute to individuals being affected by diseases other than genetics. The frequency of genetic polymorphism is more than 1% and is generally present in the human population.[7] The *ACE* I/D (rs4340) and *A2350G* (rs4343) genes were associated with hypertension involving disorders of the renal-angiotensin-aldosterone system (RAAS). Angiotensin I Converting Enzyme (ACE) plays an important role in regulating blood pressure and salt and fluid homeostasis in the RAAS. Polymorphisms and mutations in the *ACE* have been implicated in many diseases, including cardiovascular pathophysiology, psoriasis, kidney disease, stroke, and Alzheimer's disease.[8,9]

Hypertension, body weight, and body mass index (BMI) are assumed to be related to the *ACE* I/D and *A2350G* polymorphisms as obesity risk factors. Therefore, it is necessary to examine these relationships in Javanese postmenopausal women, since the Javanese are the largest tribe in Indonesia (around 40%).[10] This study was conducted on postmenopausal women from the Javanese tribe, where two *ACE* polymorphisms were analyzed, along with their association with hypertension, including body weight and BMI risk factors.

Materials and methods

Study Design and Subject Recruitment

This was a cross-sectional study conducted in the Elderly Integrated Service Post (*Pos Pelayanan Terpadu/ Posyandu*). The Elderly Integrated Service Post provided services for the elderly, including health services such as measurement of blood pressure, weight, height, BMI, blood sugar, cholesterol, uric acid, and others.[11] Sixty-nine postmenopausal women were recruited from the research site as study subjects. Informed consents were obtained from each subject, and the protocol of this study was approved by the Committee of Ethics from the Faculty of Medicine, Universitas Brawijaya (No. 247/EC/KEPK/S3/06/2016).

The diagnosis of the subjects' hypertension and diabetes mellitus conditions was made based on the results of blood plasma glucose level and blood pressure measurements, the questionnaire, and the data on the Towards Health Card (*Kartu Menuju Sehat*/ KMS). The subjects were adjusted for other risk factors of hypertension, namely absence of diabetes mellitus (defined as blood plasma glucose level ≥199 mg/dL, no history of the disease, and absence of medication for diabetes mellitus), regular exercise (defined as engaging in light exercise activities such as walking and cycling for at least 30 minutes per day, 3-4 days per week), abstention from alcohol consumption, and non-smoking.

Determination of Hypertension and Diabetes Mellitus

Determination of hypertension and its classification was according to the reference from the International Society of Hypertension (ISH), where there were normal, prehypertension, and hypertension.[4] Determination of BMI was obtained from body weight (kg) divided by the square of height (m).[12] The BMI classification corresponded to the Ministry of Health of the Republic of Indonesia as extremely

underweight (<17), underweight (17-<18.5), normal (18.5-25), overweight (>25-27), and obesity (>27).[13]

DNA Isolation and Genotyping Polymorphism of ACE Genes

DNA isolation was performed on whole blood with the QIAamp DNA Blood Mini Kit (Cat No. 51104, Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) kit used was GoTaq® Green Master Mix (Cat No. M7122, Promega, Madison, WI, USA). The restriction enzyme in the restriction fragment length polymorphism (RFLP)-PCR used was BstUI (Cat No. R0518S, New England Biolabs, Ipswich, MA, USA). The total volume of the PCR mixture was 25 µL. The primers used to detect the *ACE* I/D and *A2350G* polymorphism genotype and the PCR programming followed the designs in previous studies.[14,15] The annealing temperature in the *ACE* I/D was 55°C. The number of PCR cycles performed was 30 and 35 times, respectively.

The components used in the RFLP process for a total volume of 15 µL were 1.5 µL 10X Buffer Restriction Enzyme, 0.5 µL Restriction Enzyme, 3 µL PCR Product, and 10 µL Water RNase-Free. The incubation with restriction enzyme was carried out at 60°C for 2 hours. The allelic-specific and RFLP PCR products obtained were visualized using a 2% gel electrophoresis and UV illuminator. These resulted in a strand with insertions (I) and deletions (D) that were 490 bp and 190 bp long, respectively. Genotype visualization showed homozygous II and DD and heterozygous ID. Each of these genotypes had a strand length of 490 bp for II, 190 bp for DD, and two bands of 490 bp and 190 bp for ID. The allele of *ACE A2350G* polymorphism was 122 bp band length A allele, and fragment length of 100 bp G allele.

Statistical Analysis

Normality and homogeneity tests of the data were carried out before determining further statistical analysis. The chi-square test was used to analyze the association between genotypes and alleles with hypertension and BMI classification. The association between genotypes and alleles with weight or BMI values used the one-way ANOVA, Kruskall-Wallis or Mann-Whitney tests, and Independent Samples T-test based on homogeneity results. Analysis of the relationship was significant if the p-value<0.05. Data were analyzed with SPSS version 22 (IBM Corporation, Armonk, NY, USA).

Results

Visualization of the genotype on the *ACE* I/D and *A2350G* polymorphisms and their lengths was shown in Figure 1 and Figure 2. The results showed that there were three genotypes of *ACE* I/D polymorphisms, namely II, ID, and DD, whose lengths were 490 bp, 190 bp+490 bp, and 190 bp, respectively. Meanwhile, the genotype of the *ACE A2350G* polymorphism and the length were AA (122 bp). Univariate and bivariate analyses of these genotypes as independent variables with weight, BMI, and hypertension as the dependent variables were presented in Table 1 and Table 2. The minimum value of BMI in this research is categorized in the underweight group, and the maximum value was classified as obese. The mean BMI values with genotypes II, ID, and DD were classified as normal, obese, and overweight, respectively. There was a relationship between BMI value with hypertension but not with BMI categories (p=0.016 and p=0.0916, respectively).

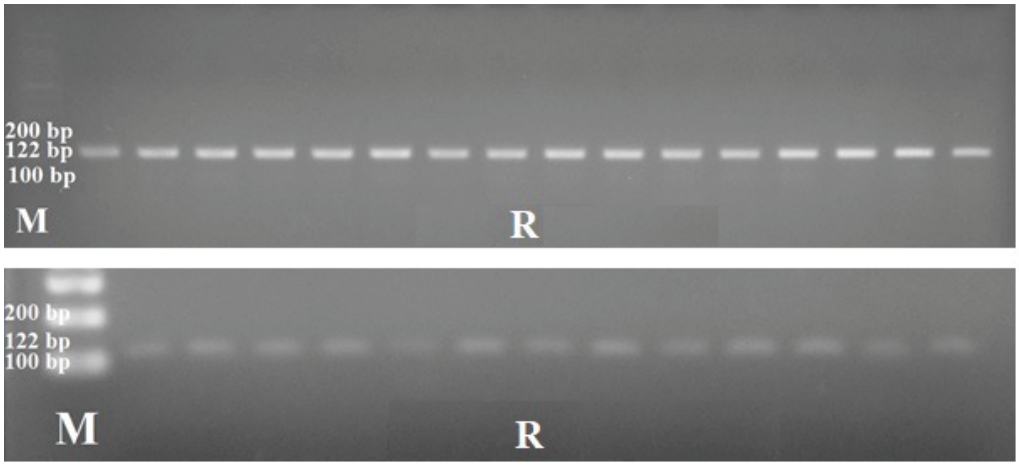

Figure 1. PCR and RFLP product visualization (above and below, respectively) shows genotypes AA, which are found in all respondents. The length of these genotype was 122 bp. The restriction enzyme was used BstUI. (M: Marker and R: Respondent).

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v8i2.426

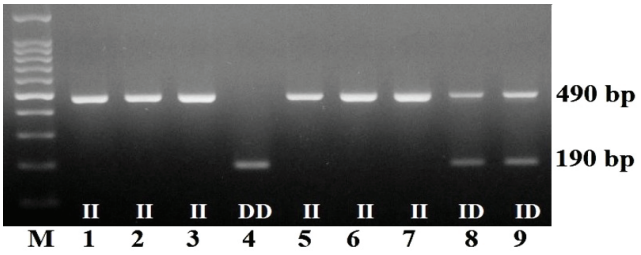

Figure 2. PCR product visualization shows genotypes II, ID, and DD, which are found in respondents. The lengths are 490 bp (lane 1-3 and 5-7), 490 bp and 190 bp (lane 8-9) and 190 bp (lane 4), respectively. M: Marker.

The genotype II, ID, and DD frequencies were 0.464, 0.522, and 0.014, respectively. The genotype DD was only found in hypertension. The II and ID genotypes were mostly obtained in respondents without hypertension, with a frequency of 0.623. The results showed that there was a relationship between the genotype polymorphism of the *ACE* I/D and BMI (p=0.016), but not with body weight, hypertension, and BMI categories.

The results showed that I and D alleles from the polymorphism *ACE* I/D, which was analyzed with other dependent variables (Table 3). The I allele frequency was the highest (0.449) in the normal respondents. The alleles in *ACE* I/D polymorphisms had a significant relationship with BMI (p=0.03), but not with body weight, hypertension, and BMI categories.

Discussion

The *ACE* codes for enzymes that control blood pressure and electrolyte balance.[9] The prevalence of hypertension in postmenopausal women at the Elderly Integrated Service Post who do not suffer from diabetes mellitus, do not smoke, do not consume alcohol, and exercise regularly is 0.2456. This number is higher than prevalence of hypertension based on a doctor's diagnosis or taking hypertension medication in individuals aged >18 years, both women and men, namely 0.1095 and 0.0574 respectively. Research in Brazil with older individuals in the community shows a prevalence of hypertension of 0.593, which is associated with diabetes mellitus and obesity.[2,16] This is caused by the presence of risk factors for hypertension in respondents, namely old age and the sex of postmenopausal women.[1,6] According to numerous studies, hypertension is a multifactorial disease caused by a variety of risk factors, including smoking, alcohol, obesity, diabetes, and genetic variation. Environmental, genetic, and lifestyle factors all interact to cause pathological conditions in hypertension disease.[8]

The percentages of genotypes and alleles II, ID, DD, I, and D in the *ACE* I/D were 46.4, 52.2, 1.4, 72.5, and 27.5, respectively. The proportion of genotypes shows that ID has the most value, while DD has the least. The results of this study are similar to the prevalence of the *ACE* genotype in the Saudi Arabian populations in both cases and controls.[17] The proportion of these genotypes differed slightly from a two-study in Yogyakarta (Indonesia), where genotype II had the greatest frequency, while DD was the lowest in both respondents with hypertension or normotension, even though the subjects at one research study had a worse functional outcome of ischemic stroke.[18,19]. However, the distribution of these genotypes is different for each ethnic population; in the South Indian population, the highest percentage is in the DD genotype in EH patients and II in

Table 1. Univariate and bivariate analysis of body weight, BMI, and genotypes I, ID, and DD.

General Data and Variables	Descriptive Value		Mean±SD			*p*-value (Genotypes *vs.* Variables)
	Mean±SD	Min-Max	Genotypes II	Genotypes ID	Genotypes DD	
Age (years old)	60.87±5.06	50.00-72.00				
Height (m)	150.47±0.21	142.00-160.00				
Weight value (kg)	58.62±10.48	37.00-85.00	54.90±7.85	62.06±11.54	52.00	0.063[1]
BMI value (kg/m²)	25.84±4.12	17.60-35.10	24.36±3.49	27.18±4.29	25.08	0.016*,[2]
Hypertension						0.421[3]

*p-value<0.05. [1]II, ID, and DD genotypes vs. weight value with Kruskall-Wallis test (the data was not normal and homogenous). [2,3]II, ID, and DD genotypes vs. BMI value or hypertension with one-way ANOVA test (the data was normal and homogenous).

Table 2. Univariate and bivariate analysis of diagnosis and genotypes I, ID, and DD on *ACE* I/D and *A2350G* polymorphisms.

Diagnosis Variables and Genotypes	n (%)	n (%)			*p*-value (Genotypes vs. Variables)
		Genotypes II	Genotypes ID	Genotypes DD	
Hypertension and BMI Classification					
Hypertension Classification					
Normal	43 (62.30)	19 (59.40)	24 (66.70)	0	
Prehypertension	9 (13.00)	3 (9.40)	6 (16.70)	0	0.249[1]
Hypertension	17 (24.60)	10 (31.30)	6 (16.70)	1 (100)	
BMI Classification					
Underweight	2 (2.90)	1 (3.10)	1 (2.80)	0	
Normal	29 (42.00)	17 (53.10)	11 (30.60)	1 (100)	0.437[1]
Overweight	18 (26.10)	8 (25.00)	10 (27.80)	0	0.916[2]
Obesity	20 (29.00)	6 (18.80)	14 (38.90)	0	
Genotypes and Alleles					
ACE I/D					
II	32 (46.40)				
ID	36 (52.20)				
DD	1 (1.40)				
ACE 2350G					
AA	69 (100)				

[1]II, ID, and DD genotypes vs. hypertension and BMI classification with chi-square test. [2]BMI *vs.* hypertension classification.

controls. While the frequency of allele D is greater than allele I.[20] Whereas in the Bania population in India, it was shown that the DD genotype had the greatest percentage in both cases and controls.[21] Different results were shown in studies in the north Indian population, where the results in the cases were the same as those in the south Indian population but the results in the controls were the same as those in the Bania population.[22,23]

The results showed that there was no relationship between genotype and allele frequencies in *ACE* I/D polymorphisms and hypertension. The results of this study are the same as those found in the Tunisian population,[24] as well as in other studies on both genotype and allele frequencies between case and control populations.[25] Research on Brazilian elderly also showed the same result. [16] There are also research results that show different results because there are significant differences in genotype and allele frequencies between the control and hypertension groups. The previous study was conducted in Bangladeshi and Indian (Odisha, South India, North India, Bania) populations.[20–22,26–28] The DD genotype was associated with the highest average systolic blood pressure (SBP) and diastolic blood pressure (DBP) in males and the lowest in genotype II. DD and ID genotypes were strongly associated with hypertension in men. The population of this study is from Bangladesh and India.[26,28] However, the DD genotype is significantly high in women with hypertension from the Odisha population.[27] These findings shed light on the potential importance of a gender-dependent association between genetic background and expression of the hypertension phenotype.[22,26–28] Another study in a North Indian population showed the ID genotype was significantly higher in hypertensive subjects, while the DD genotype was higher in control subjects. This genetic variation of the *ACE* is associated with several determinants

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v8i2.426

Table 3. Univariate and bivariate analysis of alleles I and D in *ACE* I/D polymorphisms.

Variables	Allele Frequency (n=138)		*p* -value (Alleles *vs.* Variables)
	I	D	
Alleles	100 (72.50)	38 (27.50)	
Hypertension Classification			
Normal, n (%)	62 (44.93)	24 (17.39)	
Prehypertension, n (%)	12 (8.70)	6 (4.35)	0.747[1]
Hypertension, n (%)	26 (18.84)	8 (5.80)	
BMI Classification			
Underweight, n (%)	3 (3.00)	1 (2.60)	
Normal, n (%)	45 (45.00)	13 (34.20)	0.595[1]
Overweight, n (%)	26 (26.00)	10 (26.30)	
Obesity, n (%)	26 (26.00)	14 (36.80)	
Weight BodyValue			
Minimum	37.00	37.00	
Maximum	85.00	85.00	0.155[2]
Mean	57.52	61.53	
SD	9.87	11.46	
BMI Value			
Minimum	17.60	17.60	
Maximum	35.06	35.06	0.03*,3
Mean	25.37	27.07	
SD	3.99	4.20	

*p-value< 0.05. [1]I and D alleles vs. hypertension and BMI classification with chi-square test. [2]I and D alleles vs. weight body value with Mann-Whitney tests (the data was not normal and homogeneous). [3]I and D alleles vs. BMI value with Independent Samples t-test (based on the number of two independent samples and also data that was normal and homogeneous).

of blood pressure in the Bania population, namely BMI, SBP, DBP, and waist-hip ratio (WHR). Other research states that there is a significant relationship between ID+DD and genotype II and hypertension.[18] while another is between *ACE* I/D and primary hypertension susceptibility through meta-analysis studies.[29] The same was found in the Saudi Arabian population, including those at risk for T2DM and hypertension developing from it.[17]

The results also showed that the genotype and polymorphism alleles of the *ACE* I/D had significant differences in average BMI, with values of 0.016 and 0.03, respectively. This result is the opposite when compared with the BMI category (p=0.437 and p=0.595, respectively). These results are different from the case-control study in Northwest Ethiopia.[30] Anthropometric parameters (age, SBP, DBP, Fasting Blood Glucose (FBG), weight, height,

BMI, waist circumference, WHR, duration hypertension dan diabetic nephropathy) can be taken as the risk indicator factors for hypertension and diabetes. These parameters were not associated with genotype variants.[31] Similar results were also found in Chinese patients with T2DM, if related to the BMI category, as well as in adult Koreans. In addition, allele I was more common in the obese group. The DD genotype and D allele are associated with obesity, hypertension, and pre-hypertension in Egyptian children. These frequencies were higher than the II genotype and I allele in obese children and adolescents. In addition, the genotype frequency is significantly different between hypertension and prehypertension *vs.* normotension but not between hypertension *vs.* prehypertension. These were contrasts with young Spanish adults both in body mass (kg) and BMI.[32–35] No association between *ACE* I/D polymorphism and hypertension, obesity, abnormal FBG, triglyceride, and high-density lipoprotein levels has also been found in Indian young adults. Meanwhile, in the Brazilian elderly with hypertension, the BMI value of this gene-genotype variation is also not related. The same result was also found in hypertensive patients with ischemic heart disease and ischemic stroke complications among the Ethiopian population. There is no relationship between *ACE* polymorphism, hypertension, and BMI in Tunisian people.[36–39]

The results of the study showed the AA genotype in the *ACE A2350G* polymorphism in Javanese postmenopausal women. A study in a Turkish population comparing the polymorphism of this gene between EH patients and elite athletes showed that the prevalence of the *ACE* rs4343 GG/GA/AA genotype frequency was 0.0741, 0.7778, 0.1481 in hypertensive patients; 0.0, 0.7778, 0.2222 in elite athletes and 0.04, 0.84, 0.12 in healthy controls.[15] Meanwhile, research in the northernmost province of China, focusing on the Han tribe, showed that the *ACE A2350G* was associated with an increased risk of suffering from EH in individuals from this region. G allele carriers were found to have a higher risk of EH.[40]

The relatively small sample size may limit the generalizability of the findings to the broader population. Future studies with larger sample sizes are recommended to validate these results. Including a larger and more diverse sample, possibly from other ethnic groups within Indonesia, would enhance the robustness and applicability of the findings. The sample is small due to the exclusion of several bias factors from hypertension risk factors, namely, respondents actively exercising, not consuming alcohol or smoking, and the absence of comorbid diabetes mellitus factors. The other not traced risk factors are unhealthy diets, family history of hypertension, and employment status.

The research on Javanese susceptibility hypertension and polymorphism gene analysis was required for the development of markers for the early detection of this disease. This biomarker will be used as a preventative tool for hypertension in people who are at high risk due to genetic factors by adjusting their lifestyle, such as diet, so they are not overweight or obese. This is following the priorities of health services that must be carried out at the Public Health Center, namely promotive and preventive. Public Health Center is a first-level health service facility.

Conclusion

Genotypic variation of the *ACE* I/D polymorphism was obtained with the fewest DD genotypes so that the II+ID genotypes dominated. Hypertension is still found even though risk factors for diabetes mellitus and exercise are applied to the criteria for selecting research subjects. Susceptibility can be proven in BMI but has not been proven in hypertension and body weight. Javanese postmenopausal women with a larger population genotype are needed for further verification processes with respondents who can be categorized as risk factors.

Acknowledgements

This research is funded by the Research Institutions and Community Service (LPPM), Universitas Wijaya Kusuma Surabaya. The authors are also grateful to Nur Syamsiatul Fajar from the Institute of Tropical Disease (Universitas Airlangga) for her assistance in the laboratory.

Authors Contribution

SLU and DS were involved in the research concept and planning. SLU, JHW, and II performed the data acquisition and collection, calculated the experimental data, and performed the analysis, along with assisting in interpreting the results. SLU and DS drafted the manuscript and designed the figures. All authors contributed to the manuscript's critical revision.

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v8i2.426

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