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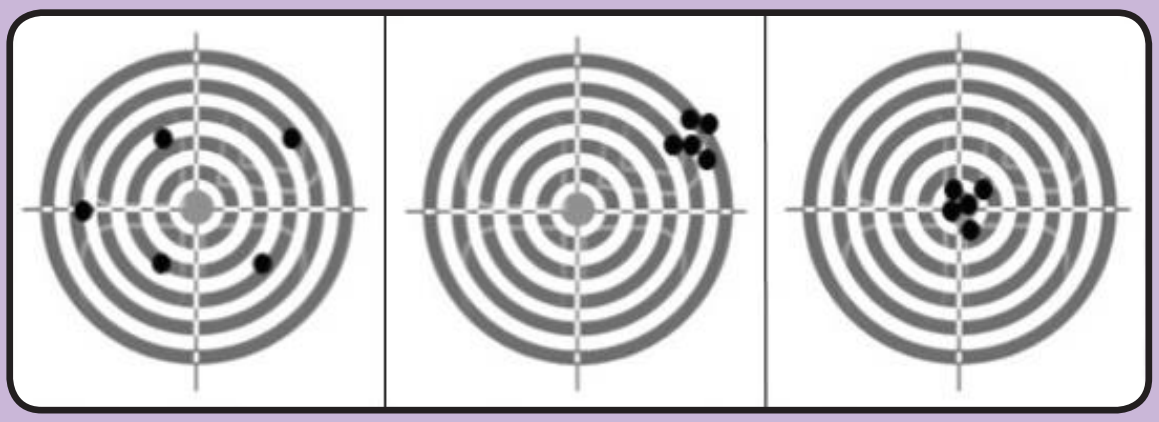

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We welcome journal submissions throughout the year but preferably by **March** and **September**. Articles submitted for publication are understood to be offered only to *JUMMEC* and which have not been sent to other journals for consideration.

Cover

Results of measurements of body weight using three different scales. Image courtesy of *Rafdzah Zaki.*

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The **Journal of Health and Translational Medicine (JUMMEC)** publishes both basic and applied science as well as clinical research studies on any area of medicine that is of interest and relevance to the medical community. This is a peer-reviewed journal that publishes Reviews Articles, Original Articles, Short Communications, Clinico-pathological Conference Abstracts, Case Reports, Letters to the Editor and Book Reviews.

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Foreword from the Editor

Dear Readers of JUMMEC,

Welcome to the first issue of *JUMMEC* for 2013. We aim to continue our efforts to develop this journal into the foremost choice for the publication of quality research in medical science. Therefore, we look forward to working productively with our authors and readers in this new year.

This issue presents four very interesting research articles from distinct fields. First, Lumbanraja describes a new prognostic system based on clinical characteristics for evaluating the severity of preeclampsia, which is a life-threatening disorder of widespread vascular endothelial malfunction and vasospasm in pregnant women. Preeclampsia typically develops after 20 weeks of gestation, but can also occur up to 4–6 weeks postpartum. Maternal age and gestational age appear to be strong predictors of poor clinical outcome in patients with preeclampsia.

Claustrophobic patients have a fear of confined spaces and often experience severe anxiety or panic attacks when exposed to small or crowded areas. A person suffering from a claustrophobic panic attack might find it difficult to breathe, sweat profusely, become nauseated, display heart palpitations, and/or become fearful that they will be unable to escape the situation. Rai *et al*. investigated the effect of Dexmedetomidine as a sedative agent to alleviate these symptoms for claustrophobic patients undergoing MRI.

Endometrial cancer (EC) is a type of uterine cancer that involves the lining of the uterus (the endometrium). Worldwide, EC ranked sixth among commonly diagnosed female cancers in 2008. Activation of oncogenic genes (e.g., *PIK3CA*) and inactivation of tumor suppressor genes (e.g., *PTEN*) are considered to be key genetic alterations involved in the development of EC. In this issue, Chung *et al.* report that *PTEN* and *PIK3CA* mutations are commonly identified in Malaysian women with EC.

Finally, Rafdzah *et al.* reviewed several routine statistical methods that are used to assess agreement and reliability for medical instruments measuring the same continuous outcomes. Indeed, accurate measurement of clinical values is fundamental, as incorrect measurements can result in inappropriate patient management that puts patients' lives at risk. The article discusses issues related to method comparison studies in medicine for the benefit of medical professionals and researchers.

The editorial board hopes that these contributions to *JUMMEC* arouse our readers' interest and boost their inspiration so much that the texts published here may instigate new research findings which will then be published in future issues of *JUMMEC*. By chance, are you interested in writing an article for the j*ournal*? Submission is open throughout the year. You can learn about the journal by visiting *JUMMEC* web site.

With best wishes,

Lau Yee Ling
Editor
The Journal of Health and Translational Medicine

CLAUSTROPHOBIA IN MRI: IS THERE A ROLE FOR DEXMEDETOMIDINE? AN OBSERVATIONAL CASE SERIES STUDY INVOLVING 11 PATIENTS

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ABSTRACT

MRI can be a distressing and traumatic experience in many patients, especially in those with underlying anxiety and/or claustrophobia. We conducted a study to determine if dexmedetomidine as a sedative agent can alleviate these symptoms. Dexmedetomidine is a potent and highly selective α-2 adrenergic receptor agonist which has sedative and analgesic properties. Eleven adult patients (n=11) with a histroy of anxiety and/or claustrophobia undergoing MRI who expressed their desire for sedation were recruited. Dexmedetomidine was infused at 0.5 to 1.0 µg/kg over 10 minutes prior to scanning. Eight patients (n=8) were able to complete the MRI scan comfortably. The findings suggest that dexmedetomidine provides adequate sedation that can allow patients with anxiety and/or claustrophobia to undergo MRI scanning succesfully in a large poproption of the population with anxiety. This result however is still preliminary and will need to be validated in a more robust clinical study.

Keywords: *Claustrophobia, dexmedetomidine, MRI, sedation*

Introduction

MRI is a special technique that produces detailed images of the body's internal environment in order to assist in the diagnosis and monitoring of many medical conditions. Patients encounter a noisy, enclosed and isolated environment because of the design and specific nature of MRI machine, even with the advances made in modern machines technologies.

Under such circumstances, a certain subset of patients would experience tremendous amount of distress, anxiety, agitation and claustrophobia. In extreme cases, these patients would not be able to complete the MRI examination despite the administration of sedatives such as benzodiazepines, opioids and hypnotics. These traditional pharmacological agents may not be very effective in producing adequate sedation that will ensure a motionless patient. It needs to be noted that at high doses these drugs possess possible undesirable adverse effects which may result in apnoea, airway obstruction and hypotension.

The ideal sedative drug that alleviate severe anxiety and/or claustrophobia in MRI has yet to be identified. Dexmedetomidine (Precedex® Hospira,Illinois,USA), a potent and highly selective α- 2 adrenergic receptor agonist, has sedative and analgesic properties with a distribution half-life of about 6 minutes and a terminal half-life of about 2 hours. However, there appears to be limited information being mentioned in literatures with regards of using this drug to manage patients with anxiety disorders who are undergoing MRI scanning. The purpose of this study was therefore to explore the potential value of dexmedetomidine, as an optional pharmocological agent, to enable anxious and/or claustrophobic patients to undergo MRI safetly and comfortably, whom would otherwise would not have been able to complete the scan.

Case series

The MRI scanner in our institution, University Malaya Medical Centre (UMMC), is a GE Signa® HDx (Wisconsin, USA) which has a high-field super-conducting magnet of 1.5 T and a tunnel length of 125cm with inner diameter of 70cm.

After obtaining ethics clearance, 11 adult patients (6 females and 5 males) who expressed their desire for sedation and consented to the use of dexmedetomidine for MRI examination, were recruited.

They were briefly counselled by one of the 3 authors on what to expect inside the scan tunnel and verbally supported positively to address their fears and anxieties. Over a period of 10 minutes, a dose of dexmedetomidine 0.5 mcg/kg to a maximum of 1.0 mcg/kg was infused when the patient was in the holding area of the MRI suite. This dose range was chosen based on manufacturer's recommendation (1). The Riker Sedation-Agitation Scale (2) was used to evaluate the patient's sedation/agitation after dexmedetomidine infusion and after MRI completion. Table 2 shows the ranking of sedation using Riker Sedation-Agitation Scale (SAS). A SAS 7 (dangerous agiation) was not included in the Table 2 because it would be extremely unlikely to achieve this score for this subset of patients.

Once a targeted SAS 3 to 4 was achieved with dexmedetomidine infusion, the patient was wheeled into the scan room. Patients had ear-muffs applied and were provided with a panic button. Standard monitoring consisted of non-invasive blood pressure, pulse oximetry (Sp02) and electrocardiogram. The monitoring of vital signs were continued during the imaging and recorded every 5 minutes. Oxygen supplementation with 3L /minute was given via nasal prongs. Side effects or complications including hypotension, bradycardia, restlessness, allergic reactions and desaturation were documented if were present during the procedure. After the procedure , the patient was monitored in the recovery bay and discharged home when fully conscious and hemodynamically stable. If the patient developed any serious side effect, the patient would be admitted for close observation and further management. In this case series,the duration of MRI examination ranged from 20 minutes to 50 minutes.

Table 1 summarises the SAS and outcome of MRI examinations. Eight patients completed the MRI and had stable respiratory and hemodynamic observations. They were satisfied with the sedative drug but patient No. 1 was noted to complaint of dry mouth. Pateint No. 5 had a SAS score of 2 after dexmedetomidine infusion but underwent MRI examination without any respiratory or hemodynamic issues.

Table 1: Patients' data on SAS and outcome of MRI scan

Patient No. (age)	Dose of dex infusion (μg/kg)	SAS after dex infusion	SAS after MRI scan	Previous MRI experience	Known claustrophobia	Outcome of MRI examination
1 (70)	0.5	4	4	4 MRI with sedation	Yes	Completed
2 (42)	0.7	4	4	2 MRI failed	Yes	Completed
3 (65)	0.7	4	4	2 MRI (1failed)	Unknown	Completed
4 (58)	0.7	4	4	2 MRI (1 failed)	Yes	Completed
5 (47)	0.9	2	2	1 MRI	Unknown	Completed
6 (49)	0.7	4	4	1 MRI	Yes	Completed
7 (54)	0.7	4	4	4 MRI with sedation	Unknown	Completed
8 (29)	0.7	4	4	1 MRI	Yes	Completed
9 (45)	0.7	4	Incomplete	1 MRI	Unknown	Abandoned
10 (37)	1	5	Incomplete	2 failed MRI (no sedation)	Yes	Abandoned
11 (65)	0.7	3	Incomplete	2 MRI (1 failed)	Yes	Abandoned

SAS =sedation-agitation scale

Three patients (n=3) failed to complete the MRI examination. Patient No. 10 was excessively anxious after the protocol limit infusion of dexmedetomidine 1 mcg/kg with a SAS score of 5 and refused to enter the scan room. Patient No. 11 had a history of cerebrovascular accident in the past. He was on 3 types of antihypertensive medications to manage his hypertension. The blood pressure at baseline was 130/80 mmHg and heart rate 60 beats/minute. He received dexmedetomidine at 0.7 mcg/kg to achieve a targeted SAS 3 before entering the scan room but 15 minutes later while the MRI was underway, his blood pressure dropped to 65/42 mmHg (2 readings) and heart rate to 50 beats/minute. He quickly responded to a fluid challenge and a bolus of vasopressor. He remained alert and responsive. However, the procedure was abandoned and later he was warded and observed, without further problems. Patient No. 9 rang the panic button after 30 minutes in the MRI and refused to complete the examination. He claimed he was "not fully asleep" and hence was agitated and anxious.

Discussion

MRI can meet the clinical needs quickly and accurately, producing a definitive diagnosis for patients to be manged appropirately and promptly by treating physicians. Although it is a non-invasive examination, there are several problems that patients face while MRI is in progress, which includes claustrophobia and noisy environment. Claustrophobia contributes heavily to the anxiety and agitation experienced by patients. Claustrophobia represents a big issue since patient's are unable to undergo MRI examination. This is inspite of the use of various sedative drugs. Claustrophobia may be more severe with the use of older MRI machines. The newer MRI machines are designed to reduce this by incorporating wider space for patients to lie. In fact, newer open scanners or scanners with upright systems have been designed to further help reduce claustrophobia in some patients (3).

If the patient is nervous and claustrophobic, there are several strategies that can help to reduce the anxiety level associated with MRI scan such as providing the patient with a panic button, setting the patient in a prone position for the scan (4), anxiolytics, airing music during scanning and systemic desensitization (5). If nothing helps, general anaesthesia may be the final option. Reports relating to the incidences of failed MRI examinations due to claustrophobia vary from country to country. Anxiety-related reactions have been reported to occur in approximately 4%-30% of patients undergoing MRI (6). In 1998, the Department of Biomedical Imaging, UMMC reported that the incidence of failed MRI examinations due to claustrophobia was 0.54% in 3324 patients (7). More recently, Enders et al quoted that 2.3% of all patients scheduled for MRI suffer from claustrophobia (3).

Dexmedetomidine is a centrally-acting dextro-enantiomer of medetomidine and binds to the α 2-adrenergic receptor and produces dose-dependent sedation. Dexmedetomidine is emerging as an effective therapeutic agent in the management of a wide range of clinical conditions with an efficacious and safe profile (8). In the United States, it is the only drug in its class (α- 2 agonist) approved for continuous intravenous sedation of intubated and mechanically ventilated patients in the intensive care setting for not longer than 24 hours. In 2008 the FDA approved new indication for dexmedetomidine, expanding its use for sedation in non-intubated patients in a monitored setting for surgery and other procedures.

Lubish and her co-workers noted that dexmedetomidine offers several advantages over other sedative drugs and the primary benefit of using it is in its minimal respiratory depression as a side effect (9). Results following the use of dexmedetomidine in children undergoing MRI have been published (10) but there are no reports on dexmedetomidine as a single bolus infusion in severely anxious adults for MRI examinations. This study revealed that 8 out of 11 patients with anxiety and/or claustrophobia benefited from the administration of dexmedetomidine and completed their MRI examination without any significant problems. In these 8 patients vital signs were stable throughout the procedure. The Riker Sedation-Agitation Scale (Table 2), a validated and reliable tool for sedation assessment, has established its value in intensive care setting. This was employed in the study because it is descriptive, flexible and most importantly easy to use.

Table 2: Riker Sedation-Agitation Scale (SAS)

6	Very agitated	Does not calm despite frequent verbal reminding of limits, requires physical restraint
5	Agitated	Anxious or mildly agitated, attempting to sit up, calms down to verbal instructions
4	Calm and cooperative	Calm, awakens easily, follows commands
3	Sedated	Difficult to arouse, awakens to verbal stimuli or gentle shaking but drifts off again, follows simple commands.
2	Very sedated	Arouses to physical stimuli but does not communicate or follow commands, may move spontaneously.
1	Unarousable	Minimal or no response to noxious stimuli, does not communicate or follow commands.

Of the 11 patients, 3 failed to complete the MRI examination. Explanations for the premature termination of MRI and suggestions for solutions are explored here. A single bolus of dexmedetomidine may be inadequate

for every patient and hence there is a need to adjust and individualize treatment. A higher bolus has to be considered with or without a maintenance infusion of dexmedetomidine. This would possibly benefit Patients No. 9 and 10. The addition of another sedative such as midazolam may even be necessary. Dexmedetomidine was probably not suitable for Patient No.11. He might have a poor left ventricular ejection fraction of the heart that was unknown to the authors. Co-morbidities of patients receiving this drug have to be carefully screened, particularly cardiac disease because dexmedetomidine causes a dose-dependent decrease in arterial blood pressure and a concomitant decrease in heart rate and cardiac output.

In conclusion, the present study demonstrates that a single bolus dose of dexmedetomidine at 0.5 – 1.0 mcg/kg without maintenance may have the potential as a sedative and anxiolytic for anxious and/or claustrophobic patients undergoing MRI. However, further studies are warranted before this drug can be recommended for routine use in clinical practise in view of the limitations of this study.

Acknowledgement

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DETERMINING THE MATERNAL CHARACTERISTICS THAT PREDICTS THE ADVERSE OUTCOMES FOR PATIENTS WITH PREECLAMPSIA

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ABSTRACT

BACKGROUND:
Preeclampsia is a major cause of maternal morbidity and mortality worldwide. Despite the advances made in the field of obstretics, the ability to predict maternal and neonatal outcome in pregnant women with pre-eclampsia remains under developed.

OBJECTIVE:
To determine the clinical characteristics that could be used as a prognostic tool that would aid in clinical assessments and interventions, which in turn will reduce the rate of mortality in pregnant women with preeclampsia.

METHODS:
This nested case control study enrolled 40 subjects diagnosed clinically with pre-eclampsia. Using logistic regression, we determined the cilinical characteristics that could be used as a prognostic tool.

RESULTS:
Maternal and gestational age were strong predictors that indicate poor prognosis in severe patients with preeclampsia at <37 weeks gestation. The scoring card models developed in this study had good calibration and discrimination value with a $p > 0.05$ and AUC 0.850 (95% CI 0.732 to 0.969). Subjects with total scores of 0, 1, and 2 had 3.1%, 27.6%, and 80.6% poor prognosis, respectively.

CONCLUSION:
Maternal age and gestational age are strong predictors for poor clinical outcomes in patients with preeclampsia.

Keywords**:** *severe preeclampsia, clinical predictor, poor prognosis*

Introduction

Preeclampsia is a pregnancy-specific disorder that occurs in 3 to 5% of all pregnancies (1). This condition remains a major cause of maternal and perinatal, morbidity and mortality and poses a threat to many developing countries worldwide (1). Although several criterias and guidelines for diagnosing and managing pre-eclampsia in pregnant women have been established in recent years, the overall morbidity and mortality have not dramatically changed (2-5). The cause for pre-ecplamsia has been attributed namely due to the cytokines or factors released by the placenta, thus the main strategy for treating this condition is to deliver the placenta from the mother as soon as possible (1). However, in doing so, there are consequential maternal and perinatal risks that need to be weighted. In many instances, while this results in good neonatal outcome, maternal health may still be affected and remains a risk (2,5).

Our ability to predict maternal and perinatal outcomes in pre-eaclampsia to date remains poor, even with advancing technologies that are reported every year (6). In many rural areas, general practitioners (GP) remain the main front-liners that manage pregnant women At times where doctors are not available midwives, with limited diagnostic facilities provide services to pregnant women. It is fortunate

that in many cases, pregnancy is usually uneventful and that most pregnant mothers complete their pregnancies with no complications. The main challenge however, arises when certain condition such as pre-eclampsia occurs. It now becomes necessary for healthcare providers to refer high-risk patients to an appropriate referral centre. In many cases, healthcare providers are pressed into making the correct decision in a short time, and in many instances a wrong call of judgement is inevitable since these are uncommon conditions to manage. This is compounded by the fact that clinical signs alone are not strong indicative and thus is not predictive of the patient outcome and prognosis (6,7). Whilst more sophisticated equipments and devices may be available for use in such instances, many underdeveloped countries and even rural areas may not be able to benefit from it due to the high cost required. Extensive training programs are an option, however, considering that number of healthcare providers are few, and that the cost to ensure exclusive training would be prohibitive for under-developed nations, this option is also unlikely to be possible in the near future. As such, the is an urgent need to develop an easy method for staff of lesser skills to be able to make an early diagnosis, and make predictive outcome and prognosis in order to weigh the consequences of delay referral. Thus the aim of the present study is to establish pre-eclampsia prognostic system based on clinical characteristics for evaluation of the severity and outcome of this condition thereby creating a system by which staff in many underprivellaged healthcare centres may benefit.

Methods

We conducted a prospective nested case control study from September 2011 to August 2012 involving patients at 28-36 weeks of singleton gestation who were diagnosed with severe pre-eclampsia with intrauterine pregnancy carrying a viable fetus. Patients with a history of diabetes mellitus and renal disorder were excluded from this study. Gestational age was determined by history taking (last menstrual period) and biparietal diameter/femur length based on ultrasonography (Mindray DP-1100 Plus). The subjects were said to have pre-eclampsia if blood pressure was ≥160 mm Hg systolic or 110 mm Hg diastolic on two occasions at least 6 h apart during bed rest and proteinuria was 3+ or greater. Obstetric status examination includes uterine fundal height, fetal position, fetal presentation, and estimated birth weight measured (Jhonson Tausak). Written informed consent to participate in the study was obtained from the subjects. The study was approved by the Local Ethical and Research Committees.

All patients (n=40) with pre-eclampsia were admitted, stabilized, evaluated, and planned to have expectant management. Twenty-eight patients (n=28) were found to be unstable in the first 24 hours and required immediate delivery. Corticosteroids were used in all patients before the pregnancies were terminated. Expectant management is defined as conservative management until any complications as the result of PE became apparent, warranting termination of the expectant management (n = 12). Expectant management consisted of bed rest and monitoring of maternal blood pressure every hour and urine output every 4 hour. The patients were questioned frequently about headache, visual disturbance, and right upper quadrant pain. Blood tests included hemoglobin, hemoatocrite, platelet count, serum liver enzymes, ureum, creatinine, uric acid, lactate dehydrogenase and coagulation profile. Oral antihypertensive medication (Nifedipine 30-120 mg per 24 h) was initiated with target <20% decreases in mean arterial pressure. Magnesium sulfate was given as antiseizure. Dexamethasone intramuscular was given for fetal lung maturation. Fetal assessment consisted of initial ultrasonography to estimate gestational age and amniotic fluid index. Fetal heart rate was reassured every 15 minutes. The patients were delivered if contraindication to expectant management developed or when pregnancy has reached 37 weeks. Indication in the foetus to terminate the preganancy ealy (fetal indidcation) was when any signs of fetal distress requiring was observed. The mode of delivery was determined by attending physician based on obstetric and fetal indications.

Data are presented as median or range, as where deemed appropriate. All variables was analyzed using chi square (CI 95%). If $p < 0.05$ in bivariate analysis, we continue to proceed for a multivariate analysis (backward and stepwise) and we choose a prognostic model based on the callibration and discrimination tests. A simulation to count probability and cut off, was performed in order to create a scoring system.

Results

Forty (N=40) subjects who fulfilled our inclusion criteria were recruited for this study. Among them, 28 patients (70%) had immediate delivery and the remaining 12 were managed expectantly. Prolongation time for gestation varied between 24 hours and 171 hours. One patient developed intra-uterine fetal death, i.e patient who underwent 171-hours of prolonged labour. Characteristics of subjects, i.e. maternal age, gestational age, gravida and history of preeclampsia are shown in the table 1.

Table 1. Characteristics of Subjects

Characteristics	**n**	**%**
Maternal age (Year)		
<20 and >35	14	35%
20 – 35	26	65%
Gestational age (weeks)		
28 – 33	23	57.5%
34-36	17	42.5%
Previous Preeclampsia		
(+)	6	15%
(-)	34	85%
Gravida		
Primigravida and Grandemultigravida	14	35%
Multigravida	26	65%

The age group between 20-35 years represented approximately 65% of the subject population. Primigravida and grand-multigravida, i.e. history of more than 4 pregnancies previously, represented 35% of the patients. Previous history of pre-eclampsia was found only in 15% of the recruited subjects.

Eligible variables for multivariate analysis (p<0.25 based on bivariate analysis) were maternal age, gestational age, and previous PE (table 2).

In order to develop a prognostic model using logistic regression (backward stepwise), we included all variables with p < 0.25. Among the four, only two variables were found to be significant (table 3).

Based on Hosmer Lemeshow test, this model was well calibrated with p value of > 0.05 (table 4).

This model was also well discriminated based on the area under the curve (table 5). Discrimination of scoring model was 0.835 (CO 95%; 0.709-0.961).

Table 2. The Result of Bivariate Analysis between All Variable with Prognosis from Severe Preeclampsia < 37 weeks

Variable	Prognosis		n	p Value	OR	CI 95%	
	Poor (<24 hour)	Good (≥ 24 hour)				Min	Max
Maternal age (year)							
20 - 35	9 (22.5%)	17 (42.5%)	26 (65%)	p=0.101	3.4	0.87– 13.239	
<20 & >35	9 (22.5%)	5 (12.5%)	14 (35%)				
Gestational Age							
28 - 33	16 (40%)	7 (17.5%)	23 (54.5%)	P<0.001	17.143	3.06 – 95.9	
34 - 36	2 (5%)	15 (37.5%)	17 (45.5%)				
Previous PE							
No	17 (42.5%)	17 (42.5%)	34 (85%)	p=0.197	0.2	0.21 – 1.897	
Yes	1 (2.5%)	5 (12.5%)	6 (15%)				
Gravida							
Primi& Grande	7 (17.5%)	7 (17.5%)	14 (35%)	p =0.744	1.364	0.370– 5.028	
Multi	11 (27.5%)	15 (37.5%)	26 (65%)				

Table 3. Logistic regression analysis (backward stepwise)

							95% C.I. for EXP(B)	
	B	S.E.	Wald	Df	Sig.	Exp(B)	Lower	Upper
Maternal age	2.092	.932	5.034	1	.025	8.102	1.303	50.386
Gestational age	3.165	1.224	6.692	1	.010	23.700	2.154	260.814
Constant	-3.833	1.257	9.296	1	.002	.022		

Table 3. Subject Probability had Poor Prognosis

Patient Score	Constanta	Coefisien	Y = -3.437 + 2.474 x total score	P= 1 / (1 + exp (-y))
0	-3.437	2.474	-3.437	0.031
1	-3.437	2.474	-0.963	0.276
2	-3.437	2.474	1.511	0.806

Table 4. Hosmer and Lemeshow Test

Step	Chi-square	Df	Sig.
1	2.022	5	.846
2	.428	2	.807

Table 5. Area under the curve

Area	Std. Error	Asymptotic Sig	Asymptotic 95% CI	
			Lower Bound	Upper Bound
.835	.064	.001	.709	.961

We the determined the subject probability of poor prognosis (table 6).

After calculating the probability of poor prognosis, we made a scoring card that could be used in everyday practice.

Based on the area under the curve of the total score desribed above, we were able to create a reference table

(table 7). From table 7, we determined the optimum cut off. At a score greater than 2, the sensitivity was 100% and specificity was 44.4%.

From the table above, we also made a scoring card that could be used daily by our healthcare provider.

Table 6. Subject Probability for Poor Prognosis

Score	Cons	Coeff.	Y = -3.437 + 2.474 x score	P
0	-3.437	2.474	-3.437	0.180
1	-3.437	2.474	-0.963	0.724
2	-3.437	2.474	1.511	0.968

PROBABILITY FOR POOR PROGNOSIS CARD

Scoring Card for Severe Preeclampsia with gestational age <37 weeks
Patient Name :
Fill some with complete data. Provide a cross in the column corresponding to the patient's condition.

No		**Yes**	**No**	**Patient Score**
1	How old are you <20 year old or > 35 year old ?	1	0	
2	Is your gestational age 28-33 weeks ?	1	0	
	Total Score			

Based on total score, whether subject probability had poor prognosis. Provide a cross in the column corresponding to the patient's condition.

Score	**Probability Poor Prognosis (%)**
0	3.1%
1	27.6%
2	80.6%
Day/Date Prognosis made :	
Doctor	
Signature	

Table 7. Intersection

No	(+) if Greater Than or Equal To	Sens.	Specificity
1	-1.0000	1.000	.000
2	.5000	1.000	.444
3	1.5000	.538	.926
4	3.0000	.000	1.000

SCORING CARD FOR POOR PROGNOSIS

Scoring Card for Severe Preeclampsia on Gestational Age <37 weeks
Patient Name :
Fill some with complete data. Provide a cross in the column corresponding to the patient's condition

No		**Yes**	**No**	**Patient Score**
1	How old are you <20 year old or > 35 year old	1	0	
2	Is your gestational age 28-33 weeks	1	0	
	Total Score			

Subject had poor prognosis if score 2
Subject had good prognosis if score 0 - 1
Based on total score whether subject had good or poor prognosis?

Day/Date Prognosis made :
Doctor
Signature

Discussion

The present study was able to demonstrate that using multi-variate analyses, we were able to develop a predictive table and a scoring card that would help make diagnosing and risk factor assesment of potential pregnant patients with impeding pre-eclampsia easier, namely for the less experience healthcare providers. The data presented here were based on the 40 subjects recruited for our study which included pregnant women with severe preeclampsia. What was interesting to note is that in our analyses shows that there are no significant association between maternal age with the prognosis associated with severe preeclampsia. It is also worth noting that extreme maternal age is closely linked to an increased risk of preeclampsia in some studies. Research on the risk of preeclampsia during antenatal follow up consisting of 52 cohort and case control in other studies demosntrates that pregnant women over age 40 years had twice the risk of preeclampsia as compared with younger age patients (6). In addition, previous studies have shown that the risk of occurrence of pre-eclampsia will increase by 30% for every age since the age of 34 years (9). Another point worth mentioning is that in our study, primigravida and grand-multigravida had poor prognosis as compared with multigravida. Our further analyses also indicates that there is no significant relationship between the increase in gravida and poor prognosis in pre-eclampsia, provided they are not of the grand-multiparagravidarum group. The reason for the increased risk in primagradvida remains unknown, and has been desribed previously (10).

In contrary to common belief, our study demonstrated no significant relationship between a previous history of pre-eclampsia and that of the increased progrnosis of developing severe pre-eclampsia in future pregnancies. Previous studies have shown that the risk of developing pre-eclampsia can increase from 2.5% in women who had a single birth to 3.4% of pregnancies in multigravida pregnancies (10). A history of previous preeclampsia is a risk factor for the occurrence of preeclampsia in subsequent pregnancies. In fact, it has been mentioned that the incidence of preeclampsia are likely to be repeated up to twenty-fold in the next pregnancy compared with women without a history of pre-eclampsia (13). Duckitt and Harrington reported that there is a likelihood of up to seven times the incidence of pre-eclampsia in women with no history of pre-eclampsia as compared to women with no history of pre-eclampsia (6). The incidence of recurrence of pre-eclampsia is also dependent on how previous events occur, for example how the outcome of treatment and how easy it was to manage the condition previously, although the exact relationship does not appear to be clearly demonstrated (12). If pre-eclampsia occurs in pregnancy of less than 28 weeks, the risk that pre-eclampsia can develop in subsequent pregnancies is 38.6%. At 29-32 weeks of gestation, the risk of recurrence of pre-eclampsia was reported to be 29.1%. For pregnancies of 33-36 weeks of gestation, the risk of recurrence was 21.9% and in cases of pregnancy ≥ 37 weeks, the risk of recurrence was 12.9% (14). It also said that women with recurrent preeclampsia is often associated with the incidence of more severe preeclampsia as compared with women who previously experienced pre-eclampsia. They are predisposed to a number of high risk conditions which includes preterm labor, placenta and fetal death solution (13).

When looking into the gestational age and prognosis, our study suggests that there is a significant association between these two factors. Based on gestational age, pre-eclampsia can be categorized as the early onset preeclampsia (before 34 weeks gestation) or the late onset (≥ 34 weeks) (14). Early onset preeclampsia is associated with abnormal placentation and can be diagnosed based on the abnormal uterine artery found from using the Doppler examination. Another feature that is consistent with this condition is the stunted fetal growth and deterioration in the mother's health condition. In contrast to early pre-eclampsia, late pre-eclampsia is the result of maternal factors and rarely, other than symptomatic features that can be observed as a late stage presentation, this condition has no specific signs that can be used as an indicator such as those in early pre-eclampsia.

There are several limitations that is worth menitioning in this paper. To achieve a good analyses a much larger sample would be needed, employing mutlicentre cooperations and longer term follow ups. Such results would provide better representation and thus more meaningful data that could be sufficiently robust for healthcare providers to use as a "pre-clampsia score card". It needs to be reminded that the present study does provide a certain platform and justification for such a large scale study to be conducted in the nead future, and thus is of value at the present time. Another limitation is that the recruitment of subjects were restricted to patients without any other complications, which may not be reflective of the conditions being presented by many pre-eclamptic patients at the time of presentation. The reason for this was for us to have a restrive data which will provide lesser number of variables that could lead to increased variations in our predictive modelling. However, in doing so, this has lead to the possible limitation to the scoring system we developed, that is unable to be adapted into real life situation. This limitation needs to be overcome in future studies.

In conclusion, the present study was able to develop a scoring system which could assist healtcare providers in making a prediction of the outcome of pre-eclamptic patients, but needs to be validated in a more robust study due the present limitations. Our analyses demonstrates that maternal age and gestational age could be used as a predictor for the occurrence of clinical deterioration of severe preeclampsia In pregnant women with severe preeclampsia <37 weeks and therefore should be taken into consideration when applying to future studies.

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PRELIMINARY STUDY ON THE OCCURRENCE OF PTEN AND PIK3CA GENE MUTATIONS IN ENDOMETRIAL CANCER AMONG MALAYSIAN WOMEN

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ABSTRACT

Genetic mutations in endometrial cancer (EC) have been extensively studied in the Western population but not much in Asian cohorts. This study has demonstrated that *PTEN* and *PIK3CA* mutations are commonly found in EC among Malaysian women. Following RNA extraction from 20 cancerous and 18 non-cancerous tissues, the presence of mutations in 9 exons of *PTEN* and 3 exons of *PIK3CA* genes were detected using real-time PCR, accompanied by High Resolution Melt (HRM) analysis. Sequencing confirmed specificity of each PCR product. The mutations for both genes were detected in the samples with varying frequencies. Notably, all samples expressed mutation of *PTEN* at exon 7 but none in exon 4. Further analysis demonstrated that strong concurrent mutations occurred between exons 7 of *PTEN* with exon 20 region 1 of *PIK3CA* gene (90%). Our data showed mutations are present in EC and not the non-cancerous tissues. Larger samples are being collected to validate this observation.

Keywords: *Uterine cancer, Malaysian, genetic abnormalities*

Introduction

Worldwide, endometrial cancer (EC) ranks sixth among commonly diagnosed female cancer with 288,000 new cases and mortality rate from 1.7 to 2.4% per 100,000 women in 2008 (1). It is also the top gynecological malignancy in the United States, making it the 8th leading cause of cancer death among women worldwide. American Cancer Society has estimated about 49,560 new cases of EC and 8,190 women succumbing to this disease in 2013 (2) . In Malaysia, National Cancer Incidence reported that EC contributed to 4.1% of total cancer cases involving women in 2007. This was a rise from 3.3% in 2003. There are 2 types of EC namely Type 1 and Type 2, with the former having better prognosis and survival rate of 83% compared to 25% for the latter (3). Several factors are thought to be linked to the rising trend of EC incidence worldwide, including the increase in obesity incidence, unopposed exposure to estrogen due to hormonal treatment after menopause and nullparity (4).

In addition to the environmental and hormonal factors, genetics may represent an important key regulator in EC cancer occurrence and progression. Oncogenes and tumor suppressor genes are the two gene classifications in which their mutations affect the development of cancer cells (5). Activation of oncogenic genes such as catalytic subunit α of phosphatidylinositol-4,5-bisphosphate 3-kinase (*PIK3CA*) gene, and inactivation of tumor suppressor genes such as Phosphatase and tensin homolog (*PTEN*) gene, are thought to be the key genetic changes involved in endometrial cancer development (6,7).

PTEN is responsible for controlling cell growth by regulating the cell cycle at G_1/S checkpoint, and loss of *PTEN* gene function was reported in 83% of EC cases (8). *PTEN* often acts with phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) gene to control the activity of AKT signaling, which is important in proliferating cancer cells. Up to 26% of tumors harboring *PTEN* mutation also have mutation in *PIK3CA*. Mutation in *PIK3CA* can lead to an additive effect on PI3K signaling activation. In fact, it was found that the mutation of *PIK3CA* were more common in tumor with *PTEN* mutations compared to those without (7). Additionally, contribution of *PIK3CA* and *PTEN* gene mutations are often implicated

with endometrial cancer, both individually as well as by co-existing together (7,9-14). Given the importance of these genes in EC progression, we aim to investigate in this preliminary study whether such mutations can be detected in a small cohort of patients with endometrial cancer admitted to University Malaya Medical Center (UMMC).

Methodology

Ethics statement

The study was approved by the Ethical Committee of University Malaya Medical Center (Ref No. 865.19). Written informed consent was obtained from all participants.

Tissue inclusion and exclusion criteria

All the cancer tissues used in this study were from patients with confirmed endometrial cancer, while the control (non-tumor) tissues were from patients with non-tumor conditions such as post-menopausal bleeding, and from dilation and curettage samples. All the cancer tissues were confirmed by pathologist to be type 1 endometrial cancer of endometriod adenocarcinoma. Patients who were pregnant, under-age and had been diagnosed for other types of cancer were excluded from this study.

Human endometrium tissue processing and RNA extraction

All 38 snap frozen tissue samples (20 cancer and 18 controls) were collected from the University of Malaya, Faculty of Medicine, Biobank Unit. These tissues were cut to 2 mm length and transferred to 1.5 ml microcentrifuge tubes containing 100 µl phosphate buffered saline (Life Technologies, NY, USA). Next, equivalent amount of stainless steel beads with diameter of 1.6 mm (Next Advance, New York, USA) were added into the tubes. The tissues were then homogenized by using a bullet blender (Next Advance, New York, USA). Total RNA were extracted from homogenized tissues using TRIsure (Bioline, London, UK) according to the manufacture's protocol, and the yield of the RNA was quantified using NanoDrop ND-2000 (Thermo Fisher Scientific Inc, *Massachusetts, USA). T*otal RNA was converted into cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc, *Massachusetts, USA).*

Real Time PCR and analysis of High Resolution Melt (HRM)

Real Time PCR was performed using ABI StepOne Plus (Applied Biosystem, California, USA) in 40 cycles. Each PCR reaction included5x HOT FIREPol EvaGreen qPCR Mix (Solis Biodyne, Tartu, Estonia), 10 pmol/µl forward and reverse primers, 10 ng/µl cDNA template and PCR grade H_2O prior to HRM analysis using High Resolution Melt Software v3.0.1 (Applied Biosystem, California, USA). Exons 1-9 of *PTEN* gene and exons 9 and 20 of *PIK3CA* gene were analyzed for mutation. All PCR reactions were performed in duplicates, and data shown were from at least 2 independent experiments. Primers used are shown in Table 1.

Table 1: List of primer sequences

Exon	Forward (3'→5')	Reverse (3'→5')
PTEN		
1	CAGAAGAAGCCCCGCCACCAG	AGAGGAGCAGCCGCAGAAATG
2	TTTCAGATATTTCTTTCCTTA	AACAAGAATATAAAACATCAA
3	TAATTTCAAATGTTAGCTCAT	AAGATATTTCAAGCATACAA
4	GTTTGTTAGTATTAGTACTTT	ACAACATAGTACAGTACATC
5 (1)	ACCTGTTAAGTTTGTATGCAAC	CTTTCCAGCTTTACAGTGAA
5 (2)	GCTAAGTGAAGATGACAATCA	TCCAGGAAGAGGAAAGGAAA
6	CATAGCAATTTAGTGAAATAACT	GATATGGTTAAGAAAACTGTTC
7	TGACAGTTTGACAGTTAAAGG	GGATATTTCTCCCAATGAAAG
8 (1)	TTAAATATGTCATTTCATTTCTTTT	TTGCTTTGTCAAGATCATT
8 (2)	GTGCAGATAATGACAAGGAATA	TCATGTTACTGCTACGTAAAC
9	TTCATTTTAAATTTTCTTTCT	TTTTCATGGTGTTTTATCCCTC
PIK3CA		
9	GATTGGTTCTTTCCTGTCTCTG	CCACAAATATCAATTTACAACCATTG
20 (1)	TGGGGTAAAGGGAATCAAAAG	CCTATGCAATCGGTCTTTGC
20 (2)	TTGCATACATTCGAAAGACC	GGGGATTTTTGTTTTGTTTTG

Sequencing of PCR Products

PCR products were purified using MSB Spin PCRapace kit (Stratec, Berlin, Germany) and the validity of each product is confirmed with sequencing analysis (AIT Biotech Pte Ltd, Singapore).

Results

Patient demographic distribution

Cancer tissues used in this study were all from type 1 endometrial cancer with varying stages and histograde. Stage 1A and Grade 2 were the predominant classifications, with 8 and 10 cases, respectively (Table 2a). Control tissues were collected from non-tumor conditions: post-menapausal bleeding (10 cases), endometrial hyperplasia (3 cases) and endometrial fibroid (5 cases) (Table 2b). The patient cohort in this study comprised of 3 ethnic groups (Malays, Chinese and Indians) and their age ranged from 30 to 79 years old. As shown in Table 3, the Malays represented the majority (50%) of the cancer patients compared to 22.2% in the control group. Approximately 30% and 20% of the remaining cancer cases were Chinese and Indian, compared to about 56% and 22% in control cases. Stratification analysis according to age showed that almost 80% of cancer patients were above 50 years old, consistent with the aetiology of this disease that mostly affecting post-menopausal women (Table 4).

Table 2(a): Staging and grading information for cancer tissues in this study

CANCER TISSUES		Histograde			
		1	2	3	**Total**
Tumor stages	1A	3	5	0	**8**
	1B	0	3	1	**4**
	2	1	2	0	**3**
	3C1	1	0	0	**1**
	4A	1	0	1	**2**
	4B	0	0	2	**2**
	Total	**6**	**10**	**4**	**20**

Table 2(b): Control tissues classifications for tissues used in this study

CONTROL TISSUES	
Conditions	**No.**
Post-menapausal bleeding	10
Hyperplasia	3
Fibroid	5
Total	**18**

Table 3: Patients demography according to race distribution among cases and controls

	Race			**Total**
	Malay	**Chinese**	**Indian**	
Cases	10 (50)	6 (30)	4 (20)	**20**
Control	4 (22.2)	10 (55.6)	4 (22.2)	**18**
Total	**14**	**16**	**8**	**38**

*Data are given as frequency (percentage)

Table 4: Patients demography according to age distribution among cases and controls

	Age group					**Total**
	30-39	**40-49**	**50-59**	**60-69**	**70-79**	
Cases	3 (15)	1 (5)	6 (30)	5 (25)	5 (25)	**20**
Control	5 (27.7)	7 (38.9)	5 (27.8)	1 (5.6)	0 (0)	**18**
Total	**8**	**8**	**11**	**6**	**5**	**38**

*Data are given as frequency (percentage)

Occurrence of tumor mutation

We screened for presence of mutations in the tissues as summarized in Table 5(a) and (b). Our findings were verified by capillary sequencing. Analysis of *PIK3CA* gene mutations showed highest occurrence in exon 20 region 1 (90%) followed by exon 9 (65%) and lastly in exon 20 region 2 (55%). Exons of *PTEN* gene exhibited different percentages of mutation occurrences with all samples being mutated in exon 7 (100%) compared to no samples being mutated in exon 4 (0%). None of these mutations were detected in the control tissues (0%).

Table 5(a): Frequency of mutations occurrence in cases and controls of PIK3CA gene

PIK3CA	Cases (n=20)	Controls (n=18)
	n (%)	**n (%)**
PIK3CA-9	13 (65)	0 (0)
PIK3CA 20-1	18 (90)	
PIK3CA 20-2	11 (55)	

*Data are given as frequency (percentage). RNA from cases and controls were extracted and subjected to real time PCR followed by HRM analysis. Frequency above represents total number of patients with mutation.

Table 5(b): Frequency of mutations occurrence in cases and controls of PTEN gene

PTEN	Cases (n=20)	Controls (n=18)
	n (%)	**n (%)**
PTEN 1	15 (75)	0 (0)
PTEN 2	2 (10)	
PTEN 3	2 (10)	
PTEN 4	0 (0)	
PTEN 5-1	16 (80)	
PTEN 5-2	14 (70)	
PTEN 6	13 (65)	
PTEN 7	20 (100)	
PTEN 8-1	5 (25)	
PTEN 8-2	18 (90)	
PTEN 9	6 (30)	

*Data are given as frequency (percentage). RNA from cases and controls were extracted and subjected to real time PCR followed by HRM analysis. Frequency above represents total number of patients with mutation.

Simultaneous PIK3CA and PTEN gene mutations

We further analyzed the trends of simultaneous mutations occurrence between exons of a gene as well as between genes (Table 6). For *PTEN*, the highest concurrent mutation was between exon 7 and exon 8 region 2 (18/20 cases), while for *PIK3CA*, frequent concurrent mutation were found between exon 9 and exon 20 region 1 (11 / 20 cases). Simultaneous mutations analysis was also performed between the two genes. Exon 7 of *PTEN* gene showed strong co-occurrence with exon 20 region 1 of *PIK3CA* gene (18/20 cases).

Table 6: Simultaneous occurrence of mutation between exons and genes

		PTEN										***PIK3CA***		
		1	**2**	**3**	**5 (1)**	**5 (2)**	**6**	**7**	**8 (1)**	**8 (2)**	**9**	**9**	**20 (1)**	**20 (2)**
PTEN	**1**		2	2	**12**	**11**	9	**15**	5	**13**	6	**10**	**14**	6
	2			1	2	2	2	2	2	2	2	1	2	1
	3				2	1	2	2	2	2	2	1	2	2
	5 (1)					**12**	**10**	**16**	5	**14**	5	9	**15**	7
	5 (2)						9	**14**	3	**12**	4	8	**13**	7
	6							**13**	5	**13**	4	**11**	12	9
	7								6	**18**	6	**13**	**18**	**11**
	8 (1)									5	3	4	5	2
	8 (2)										5	**13**	**16**	**11**
	9											4	5	4
PIK3CA	**9**												**11**	9
	20 (1)													9
	20 (2)													

*Data shown are frequency of concurrent occurrence; Bolded numbers represent more than 50% mutation co-occurrence

Discussion

In this study, we screened for various mutations of *PTEN* and *PIK3CA* genes in 20 endometrial cancer and 18 non-cancerous endometrial tissues collected from Malaysian women. In addition, we also analyzed the patterns of mutation co-occurrence between exons in these genes. While contribution of these genes in endometrial cancer has been studied in depth in western population (15), such information are not available for Malaysian women. Such information may provide a significant clinical implication, as status of mutations in tumors may predict resistance of tumor cells against selected drug therapy.

PIK3CA gene mutation was highly expressed in our study, where it demonstrated at least 55% frequency in all three different exons examined. *PIK3CA* is an important catalytic subunit of the phosphatidylinositol 3-kinases (PI3Ks) that regulates cell proliferation, adhesion and survival (10). Many investigative agents, such as rapamycin, RAD001 and evorolimus that primarily blocks mTOR (a downstream molecule of PI3K pathway) are also known to inhibit the action of *PIK3CA* (16,17). Determining the status of mutation in genes is crucial before making any clinical decision, considering the recent report that suggested mutation in another oncogenic gene, the K-*ras* gene may be the cause of cancer cells developing resistance towards mTOR inhibitors (16). This emphasizes the importance of mutation screening prior to decisions of therapeutic interventions. Interestingly, it was demonstrated that mutations in *PIK3CA* gene can lead to constant activation of PI3K pathway, and therefore blocking the effect of mTOR inhibitors (12,17). It was also shown recently, mutation in exon 20 of this gene is associated with high-grade endometrial cancer and that another mutation site in this gene, H1047R correlated with shorter survival (18).

Our analysis further demonstrated that this gene (*PIK3CA* exon 2 region 1) occurred in the presence of *PTEN* gene mutations (exon 7), consistent with previous findings (7,12). It is worthwhile to note that co-existence of these two gene mutations seems to be frequent in endometrial cancer but is quite rare in other cancers (13). It was also reported that *PTEN* mutations are observed specifically in endometrial cancer but not in other gynecological malignancies (14) and that it is frequently found in type 1 endometrial cancer (18). In contrast to cancer types, where *PTEN* mutation translates to increased metastatic potential, such mutation in endometrial cancer may be associated with a favorable survival (9).

Endometrial cancer is in part a genetic-driven process, and many clinical decisions are now being made based on the genetic profiling of the tumor. While the sample size in this study is relatively small, our data strongly suggests that mutations in *PTEN* and *PIK3CA* genes can be detected in the endometrial cancer samples presented by Malaysian women but not in controls, and this may imply that the etiology of this tumor in this region share similarities with those from the Western countries. A more comprehensive subsequent analysis is warranted to validate this finding in a larger sample cohort and to investigate the association of these mutations with the prognosis of these women. Determination of these mutations may allow for a more informed clinical diagnosis and to make a choice of the therapy required.

Conclusion

This preliminary study showed the presence of mutations in *PTEN* and *PIK3CA* among patients with endometrial cancer admitted to UMMC, with strong co-occurrence of

mutations between exon 7 of *PTEN* with exon 20 region 1 of *PIK3CA* gene.

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METHOD COMPARISON STUDIES IN MEDICINE

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ABSTRACT

INTRODUCTION AND OBJECTIVE:

Most of important variables measured in medicine are in numerical forms or continuous in nature. New instruments and tests are constantly being developed for the purpose of measuring various variables, with the aim of providing cheaper, non-invasive, more convenient and safe methods. When a new method of measurement or instrument is invented, the quality of the instrument has to be assessed. Agreement and reliability are both important parameters in determining the quality of an instrument. This article will discuss some issues related to methods comparison study in medicine for the benefit of medical professional and researcher.

METHOD:

This is a narrative review and this article review the most common statistical methods used to assess agreement and reliability of medical instruments that measure the same continuous outcome. The two methods discussed in detail were the Bland-Altman Limits of Agreement, and Intra-class Correlation Coefficient (ICC). This article also discussed some issues related to method comparison studies including the application of inappropriate statistical methods, multiple statistical methods, and the strengths and weaknesses of each method. The importance of appropriate statistical method in the analysis of agreement and reliability in medicine is also highlighted in this article.

CONCLUSION:

There is no single perfect method to assess agreement and reliability; however researchers should be aware of the inappropriate methods that they should avoid when analysing data in method comparison studies. Inappropriate analysis will lead to invalid conclusions and thus validated instrument might not be accurate or reliable. Consequently this will affect the quality of care given to a patient.

Keywords: *agreement, reliability, method comparison study, validation study*

Introduction

In medicine, accurate measurement of clinical values is vital. Most of important variables measured in medicine are in numerical forms or continuous in nature, such as blood pressure, body temperature, haemoglobin level, and many other clinical values. Inaccurate measurement of these variables will result in inappropriate management of the patient, thus putting the patient's life at risk.

There are numerous instruments or machines that have been invented for the purpose of measuring various variables. New instruments and tests are constantly being developed, with the aim of providing cheaper, non-invasive, more convenient and safe methods. When a new method of measurement or instrument is invented, the quality of the instrument has to be assessed. This is where a method comparison study or a validation study comes into medicine. This article will discuss some issues related to methods comparison study in medicine for the benefit of medical professional and researcher.

Agreement versus Reliability

Agreement and reliability are both important parameters in determining the quality of an instrument. To illustrate the

concept of agreement and reliability in a simple language, imagine if we have three target boards (see Figure 1) that show the results of five repeated measurements of body weight of the same person, using three different scales (A, B and C). Figure 1(a) shows that after taking five measurements using scale A, the results of the measurements are scattered all over the target board. This suggest that the measurements are not near each other (poor reliability), and are not near their intended target or true value (poor agreement).

Figure 1(b) shows that all five measurements from scale B appear in more or less the same location on the target board, but not in the centre of the target board. This suggests that five different measurements were almost the same (good reliability), but they did not hit the intended target (poor agreement). Figure 1(c) shows that all five measurements from scale C are close to each other (good reliability), and hit the centre of the target board (good agreement).

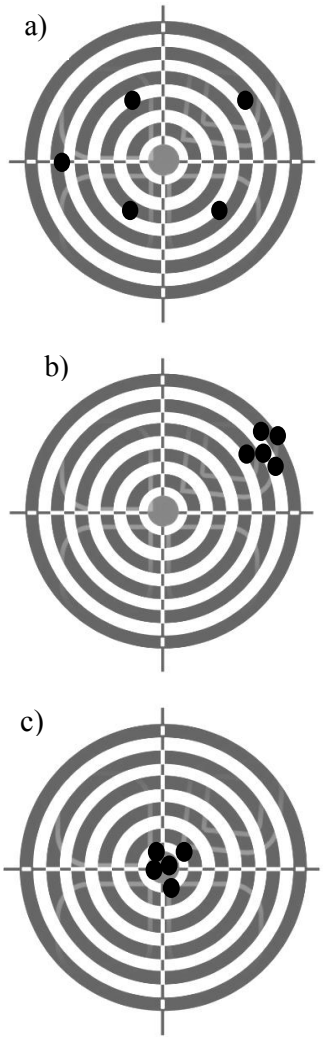

Figure 1: Results of measurements of body weight using three different scales A, B and C.

In most clinical situations, we use the same instrument to evaluate changes over time and also to differentiate values from the normal or abnormal cut-off point (which is usually derived from population-based studies). One of the examples of this situation is in the screening of hypertension cases, and the assessment of reduction of blood pressure post-treatment, in a clinic or health centre. Both blood pressure measurements are performed using the same blood pressure machine, or sphygmomanometer.

So, agreement and reliability parameters are equally important in determining the quality of instruments. In fact, it is difficult to be certain about the agreement of an instrument if the instrument is not reliable. Similarly, a precise instrument or instrument with good reliability will not necessarily measure the "true" value. Therefore, when comparing two instruments, or methods of measurement, we should consider assessing both agreement (accuracy) and reliability (precision).

An instrument with high agreement will not be useful if it is unreliable. Ideally, these parameters should be assessed together. However, we have conducted two systematic reviews (1, 2) and found that this is not commonly followed in practice, especially with respect to agreement studies. Most of the reliability studies (71%) also measured agreement at the same time (2). However, only 30% of agreement studies assessed reliability (1). Researchers tend to focus on one aspect of quality when validating instruments, although there is a possibility of agreement and reliability studies being conducted separately for the same instrument. Nonetheless, it is important to ensure the reliability of the instrument first, before testing for agreement, because it is impossible to assess the agreement of an unreliable instrument.

Statistical Methods of assessing Agreement and Reliability

There are several methods and approaches that have been used to measure agreement and reliability. The most common method to assess agreement found in the systematic review (1) is the Bland-Altman Limits of Agreement (LoA), followed by Correlation Coefficient (*r*), comparing means, comparing slope and intercept, and Intra-class Correlation Coefficient. According to the systematic review of reliability studies (2), various methods have also been used to estimate reliability, and among these popular methods include: Intra-class Correlation Coefficient, comparing means, Bland-Altman Limits of Agreement, and Correlation Coefficient (*r*). However, Correlation Coefficient (*r*), comparing means, and ICC have been shown to be inappropriate in assessing agreement. Whereas, in the analysis of reliability, Correlation Coefficient (*r*), Bland-Altman Limits of Agreement and comparing means were thought to be inappropriate.

Agreement Analysis

In 1983, Bland and Altman introduced Limits of Agreement (LoA) to quantify agreement (3). Bland and Altman (4), stated that it is very unlikely for two different methods or instruments to be exactly in agreement, or give identical results for all individuals. However, what is important is how close the values obtained by the new method (predicted values) are to the gold standard method (actual values). This is because a very small difference in the predicted and the actual value will not have an effect on decisions of patient management (4). So they started with an estimation of the difference between measurements by two methods or instruments (4). The formula for Limits of Agreement (LoA) is given as (4):

$$\text{LoA} = \text{mean difference} \pm 1.96 \times (\text{standard deviation of differences})$$

The 95% Limits of Agreement is dependent on the assumptions that the mean and standard deviation of the differences are constant throughout the range of measurement, and the distribution of these differences follow approximately a normal distribution (3). It is important to check for these assumptions (3). Altman and Bland (1983) proposed a scatter plot of the differences of two measurements against the average of the two measurements, and a histogram of the differences, to check for these assumptions (3). Initially, the scatter plot is only to check the assumption and not the analysis of agreement, but then it becomes a graphical presentation of agreement (see Figure 2).

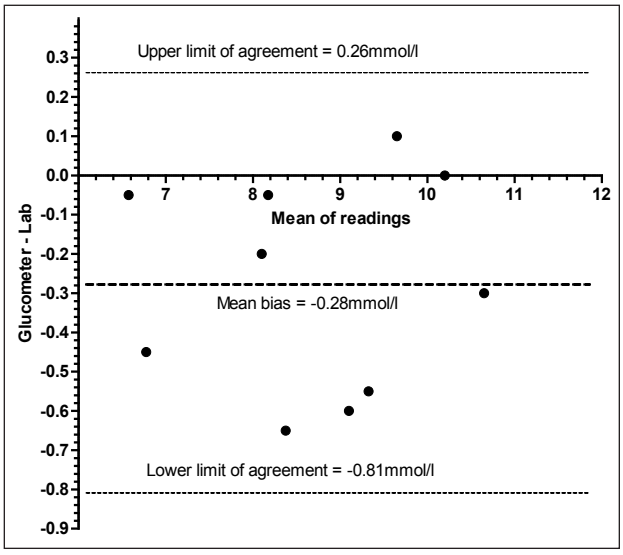

Figure 2: The Bland-Altman Plot

Reliability Analysis

The Intra-class Correlation Coefficient was originally proposed by Sir Ronald Aylmer Fisher (5, 6). He was a statistician from England, and Fisher's exact test was one of his well-known contributions to statistics (5, 7). The earliest ICCs were modifications of the Pearson Correlation Coefficient (8). However, the modern version of ICC is now calculated using variance estimates, obtained from the analysis of variance or ANOVA, through partitioning of the total variance between and within subject variance (9, 10).

The general formula for ICC is given as (8):

$$\text{ICC} = \frac{\text{Subject variability } (\delta_S^2)}{\text{Subject variability } (\delta_S^2) + \text{Measurement error } (\delta_E^2)}$$

Values obtained from ANOVA table:

Measurement error, (δ_E^2) Mean square of Error, MS_E

Subject variability, δ_S^2

$$= \frac{\text{Mean square of Subject, } MS_S - \text{ Mean square of Error, } MS_E}{\text{Number of observer}}$$

ICC is a ratio of variances derived from ANOVA, so it is unit-less. The closer this ratio is to 1.0, the higher the reliability (8). Chinn (1991) recommended that any measure should have an Intra-class Correlation Coefficient of at least 0.6 to be useful Chinn, 1991). Rosner (11) suggested the interpretation of ICC as shown in Table 1:

Table 1: Interpretation of ICC

ICC value	Interpretation
< 0.4	poor reliability
0.4 ≤ ICC < 0.75	fair to good reliability
≥ 0.75	excellent reliability

Is the most popular method the best?

Agreement Analysis

Although the Bland-Altman Limits of Agreement is the most popular method used to assess agreement, there are a few issues and limitation related to it of which medical researchers should be aware of.

Confidence Interval for Limits of Agreement

Limits of agreement is actually just an estimate of the values which apply to the whole population (4). So, whatever value of limits of agreement are obtained from a study, they only apply to that study population. If a similar study was repeated in a different study population, this second sample would give different limits of agreement. Therefore, to infer the limits of agreement to the whole population, a 95% confidence interval (CI) of the upper and lower limit of agreement should be calculated, as suggested by Bland and Altman (4). The 95% confidence intervals can be calculated by finding the appropriate point of the *t* distribution with *n* - 1 degrees of freedom and the standard deviation of the difference, SD (4):

$$\text{CI for upper limit of agreement} = \text{Mean Bias} + (1.96(\text{SD}) \pm t\sqrt{\frac{3\text{SD}^2}{\text{n}}});$$

$$\text{CI for lower limit of agreement} = \text{Mean Bias} - (1.96(\text{SD}) \pm t\sqrt{\frac{3\text{SD}^2}{\text{n}}});$$

However, this is rarely practised by researchers. Out of 178 papers reviewed earlier (1) that used the Bland-Altman method to assess agreement, only one paper considered the 95% confidence interval of limits of agreement. Bland and Altman are also aware of this problem and regret that these confidence intervals are seldom quoted (12). Theoretically, without reporting the confidence interval, their conclusion about the agreement of methods measured can only be applied to the measurement during the research, and cannot be inferred to clinical practice.

This issue has also been discussed in detail by Hamilton and Stamey (2007), who suggested that Limits of Agreement only provide a reference interval, and can be misleading if the Confidence Interval (CI) is not considered (13). They concluded that Limits of Agreement should never be used as the decisive factor in concluding agreement between two instruments (13).

Interpretation of Bland-Altman Limits of Agreement

One of the reasons why the Bland-Altman Method is so popular is its simplicity (14). Although the interpretation of limits of agreement seems to be simple and easy, medical researcher should be aware of the appropriate way of interpreting the Bland-Altman analysis. Mistakes or inappropriate interpretation of limits of agreement can occur as found in the following published article.

In 2005, a study tested the agreement of three peak flow meters (A, B and C) using three statistical methods (Pearson's Correlation Coefficient, t-test, and the Bland-Altman method) (15). For peak flow meters A and B, the limits of agreement were found to be 40 l/min to 60 l/min. The authors interpreted this as the differences between peak flow meter A and B to range from 40–60 l/min (15). They did not comment whether peak flow B would overestimate the value of peak flow A, which is the most important clinical finding desired. Furthermore, the overall conclusions on the agreement of the peak flow meters were made based on a paired t-test.

In fact Bland and Altman themselves made a mistake in the interpretation of the limits of agreement in one of their earlier publications (4), where they compared the readings between a large peak flow meter (PEFR) and mini peak flow meter. By plotting the difference (Large PEFR – mini PEFR) against the mean, the upper limit of agreement was 75.5 l/min and the lower limit of agreement was -79.7 l/min (4). Their interpretation was that the mini peak flow meter may be 80 l/min below or 76 l/min above the large peak flow meter. However, because the difference was calculated from Large PEFR – mini PEFR, the positive difference means that the mini PEFR underestimates the large PEFR, and the negative difference means that the mini PEFR overestimates the large PEFR. So, the appropriate interpretation should be that the mini PEFR may be 80 l/min above or 76 l/min below the large PEFR.

Thus, a mix of negative and positive values of limits of agreement might confuse some researchers. In addition, imagine if we apply the 95% confidence interval for the limits of agreement. This would create further confusion and make the Bland-Altman method appear to not be as straightforward as originally thought. Therefore, medical researcher should put an effort to really understand this method and interpret the result appropriately.

Proportional Bias

Hopkins (2004) demonstrated that the Bland-Altman plot indicates incorrectly that there is a systematic bias in the relationship between two measures (16). Using a fixedly generated data, Hopkins clearly showed the proportional bias produced in the Bland-Altman plot, but not in the regression (ordinary least squares method) analysis. If a slope of regression line fitted to the Bland-Altman plot differs significantly from zero, it is argued that proportional bias exists (17). Using randomly generated data, Hopkins showed that proportional bias was produced in the Bland-Altman plot, but not in the regression (ordinary least squares method) analysis, and concluded that the Bland-Altman plot should not be used to make conclusions about bias for any instrument (16). He added that bias in the Bland-Altman plots was not restricted to calibrated instruments, but could arise as an artefact of random error between measures that have not been calibrated (16). Commenting on Hopkins' article, Batterham (2004) favoured the ordinary least squares regression technique, rather than the Bland-Altman limits of agreement (18).

However, Ludbrook (2002) claimed that the presence of bias in the analysis was a result of some kind of statistical assumption (17). Ludbrook (2010) recommended that a linear regression line be fitted to the Bland–Altman plot to check for this bias (19). It was argued that, if the slope of the regression line fitted to the Bland–Altman plot is not significantly different from zero then the proportional bias is absent(19). Thus we should not be worried about any artifactual bias. However, recent study (20) showed that testing the slope of regression line of the Bland–Altman plot does not remove the artifactual bias in the prediction.

The main concern about the proportional bias is that this will result in artefactual bias in the prediction. The predicted bias will consist of artefact and real bias, which cannot be differentiated by the researcher (16). Therefore the Bland–Altman method should be used with caution and should be complemented by other methods.

Reliability Analysis

Intra-class Correlation Coefficient or ICC is the most popular method used to assess the reliability of medical instruments. There are a few concerns regarding the application of ICC in evaluating reliability:

Choosing appropriate type of ICC

There are different types of ICC, and confusion exists regarding which ICC to use (8). Muller and Buttner (2004) demonstrated that different types of ICC may result in quite different values for the same dataset, under the same sampling theory (21). So it is important to determine which type of ICC is suitable, depending on the purpose of the analysis. Weir (2005) suggested some issues that should be considered when choosing an ICC test:

(a) One- or two-way model:
- For the one-way model each subject is assumed to be assessed by different raters, and the raters are also assumed to be selected from the population. This model allows for situations where all subjects are not rated by all raters. In this model, all sources of error are lumped together. A one-way model should be considered when information on which raters rated the subject is not known (8).
- The two-way model assumes that each subject was assessed by the same raters, and requires raters to be crossed with subjects (i.e. each rater rates all subjects). The two-way model allows the error to be devised into random and fixed errors (8, 22).

(b) Random- or fixed-effect model
- In a fixed-effects model, the levels of variable are fixed or specified in advance (11). The fixed factor is considered when all levels of the factor of interest are included in the analysis. Raters are considered as fixed effects, but items/subjects are treated as random effects (no generalization beyond the sample). So, there is no attempt to generalise the result on reliability (8).
- Under a random-effects model, both factors (raters and items/subjects) are viewed as random effects (11). Random factor is considered when the analysis is to be generalised to other levels (8).

(c) Single or mean score (8):
- Single Measures ICC should be reported if only a single measure on a subject was taken.
- If two or more trials were measured on a subject, then Average Measures ICC should be reported. The Averaged Measures ICC will always be higher than the Single Measures ICC

Between-subjects variability

The ICC is influenced greatly by between-subjects variability. If the ICC is applied to data from a group of individuals with a wide range of the measured characteristics, the value of the ICC will indicate higher reliability, compared to the same analysis when applied to a group of data with a narrow range of the same characteristic (8). However, according to Weir (2005) this is an unfair criticism, because the ICC is not meant to provide an index of absolute measurement error (8). In general, the ICC is a ratio and does not quantify precision.

Single or Multiple methods?

According to both our systematic reviews published recently (1, 2), most reliability studies (86%) relied on a single statistical method to assess reliability, in contrast with agreement studies where most of the studies (65%) used a combination of statistical methods (see Table 2). A strong case for using multiple methods in assessing agreement and reliability is because each statistical method has its own strengths and weaknesses. The usage of multiple methods has the advantage of compensating for the limitations of any one single method. As long as the methods chosen are appropriate for it purposes. Luiz and Szklo (2005) suggested that more than one statistical method to assess agreement may be reported usefully, since no strategy seems to be fool proof (23). Similarly, in reliability studies, it was suggested that no single reliability estimate should be used for reliability studies, and a combination of methods was more likely to provide more information on the reliability of an instrument (9).

However, another possible reason for using multiple methods is the researcher's limited understanding of the statistical methods for agreement and reliability. This is probably the reason for the application of multiple inappropriate statistical methods in a single study; for example, the use of both correlation coefficient and significance test of the difference between means, to test for agreement and reliability. Both of these methods have been clearly shown to be inappropriate statistical methods to assess agreement and reliability (3, 24).

Table 2: Single versus multiple methods

	AGREEMENT (N=210)	RELIABILITY (N=42)
Overall:		
Multiple methods	137 (65%)	6 (14%)
Single method	73 (35%)	36 (86%)
p<0.0001		
According to year:		
2007		
Multiple methods	n=70	n=26
Single method	43 (61%)	6 (23%)
p=0.0002	27 (39%)	20(77%)
2008		
Multiple methods	n=70	n=7
Single method	46 (66%)	0
p=0.0009*	24 (34%)	7 (100%)
2009		
Multiple methods	n=70	n=9
Single method	48 (69%)	0
p<0.0001*	22 (31%)	9 (100%)
(*Fisher's exact)		

Application of Inappropriate Statistical Methods

The proportion of studies with inappropriate statistical methods, found in both earlier systematic reviews, will reflect the proportion of medical instruments that have been validated using inappropriate methods in current clinical practice. As found in the earlier systematic reviews, eight (19%) of reliability studies (2) and twenty (10%) of agreement studies (1) used inappropriate methods, which means that there is a distinct possibility that some medical instruments or equipment used currently were validated using inappropriate methods, with consequently erroneous conclusions being drawn from these methods. This equipment, therefore, may not be as precise or accurate as believed, which could, potentially, affect the management of patients, the quality of care given to patients and, worse, it could cost lives. Inappropriate application of statistical methods in method comparison studies also reflects the lack of knowledge in this area among medical researchers. This is alarming and it is important for clinicians or medical researchers to be aware of this.

The Importance of Appropriate Statistical Method in Medicine

Patient Care

In clinical situations, the duty of a doctor is to provide the best care or treatment for their patients. Most of the time, doctors have to decide what is the best available option for their patients. In some cases, this may involve life and death decisions; for example, deciding to thrombolyse patient with myocardial infarction in an Accident and Emergency department. Doctors have to assess a patient thoroughly and, assisted by information from some medical equipment such as electrocardiogram (ECG) and blood pressure machines, before the decision to thrombolyse the patient can be made.

In 2009, a study to assess the accuracy and precision of five currently available blood glucose meters in South Africa was conducted (25). The study compared five glucometers that utilise different analytical techniques (reflectometry or amperometry), and all the glucometers were calibrated (25). The authors found that although all the devices showed satisfactory precision, there was substantial discordance when their results were compared to a laboratory reference (25). Only three out of the five glucometers fulfilled the criteria suggested by the International Standardisation Organisation. All meters demonstrated significant deviation from the American Diabetes Association guidelines, as more than 60% of the measurements exceeded the recommended percentage of deviation (25).

It is well-known that both type 1 and type 2 diabetes show a direct relationship between the degree of glucose control and the risk of systemic complications (26). Many clinical organisations such as the American Diabetes Association promote the self-monitoring of blood glucose, because it allows diabetic patients to achieve and maintain specific glycaemic goals (26). The variability observed with the accuracy of glucometers can impact patient care in different settings, some of which include the diabetic patient on insulin in a home care or a clinical setting. Most of the time, glucose determinations and insulin adjustments are made according to glucometer readings. Inaccuracies can lead to misclassification of hypoglycaemic or hyperglycaemic episodes. It is, therefore, imperative that glucometer values are accurate and precise. Otherwise, a failure in this regard may lead to critical medical errors.

The variation amongst these glucometers found in the study (25) were probably a result of the improper evaluation of the glucometer in the validation study. This suggests that there is a necessity for proper evaluation, and it is important to be sure that appropriate statistical methods for the validation of the instrument has been used in any research or clinical situation.

Evidence-Based Medicine

The practice of Evidence-Based Medicine (EBM) has been promoted to ensure the best quality of care is given to the patient. One example is in the treatment of hypertension. According to the most recent National Institute of Clinical Excellence (NICE) *Clinical Guidelines on Hypertension* (27), antihypertensive drug treatment should be offered to people of any age with stage 2 hypertension. Stage 2 hypertension is defined as a patient with blood pressure of 160/100 mmHg or higher, and whose subsequent ambulatory blood pressure monitoring (ABPM), daytime average or home blood pressure monitoring (HBPM) average blood pressure, is 150/95 mmHg or higher (27).

The recommendation from the guidelines was derived from the views of experts, patients, carers and industry, and includes the best available evidence (from research) (27). Without doubt, researchers must have used some instrument to measure blood pressure in the process of producing evidence. However, which instrument was used in their studies: the automatic blood pressure machine or manual sphygmomanometer? Were these machines validated, and if the machines were validated, which statistical method was used? If the instruments used were not validated, or were validated using inappropriate statistical methods, we can actually question the quality of the evidence from such studies. A lack of precision and validity of an instrument in research may result in invalid evidence. The main goal of research, especially in epidemiological studies, is about applying the evidence to the population for practice. Appropriate statistical analysis is actually the “root” of Evidence-Based Medicine.

Conclusion

Although there is no single perfect method, researchers should be aware of the inappropriate methods that they

should avoid when analysing data in method comparison studies (i.e. to assess agreement and reliability). This is important because inappropriate analysis will lead to invalid conclusions and thus validated instrument might not be accurate or reliable. This will result in inaccuracy of prediction or diagnosis, and inappropriate management or treatment. Consequently this will affect the quality of care given to a patient and, most importantly, inappropriate treatment might put the patient's life at risk. Poor quality of care will also jeopardise the doctor-patient relationship. Inaccurate measurements cannot be used as an excuse for making any mistake in the management of patients. Therefore it is vital to ensure the validity of an instrument, and appropriate statistical methods should be applied in a validation study. In other words, appropriate statistical methods should be used when testing agreement and reliability of an instrument.

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