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The Roles of C-X-C Motif Chemokine Ligand 10 (CXCL10) in Dengue

Siti Noraihan Khamis[1], Mohd Amin Mohd Mokhtar[2], Seok Mui Wang[3-6] and Fadzilah Mohd Nor[3,4,7]*

[1]Microbiology Laboratory, Department of Pathology, Hospital Tuanku Fauziah, Pusat Bandar Kangar, 01000, Kangar, Perlis, Malaysia.
[2]Department of Emergency Medicine, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000, Sungai Buloh, Selangor, Malaysia.
[3]Department of Medical Microbiology & Parasitology, Level 5, Academic Building, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000, Sungai Buloh, Selangor, Malaysia.
[4]Institute for Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000, Sungai Buloh, Selangor, Malaysia.
[5]Institute of Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000, Sungai Buloh, Selangor, Malaysia.
[6]Non-Destructive Biomedical and Pharmaceutical Research Center, Smart Manufacturing Research Institute (SMRI), Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, 42300 Puncak Alam, Selangor, Malaysia.
[7]Integrative Pharmacogenomics Institute (i-PROMISE), Level 7, FF3 Building, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, 42300 Puncak Alam, Selangor, Malaysia.

***Correspondence:** fadzi456@uitm.edu.my

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Abstract

Early diagnosis of dengue is crucial to prevent the progression to severe dengue (SD) leading to mortality rate reduction. This study aimed to determine the role of the CXCL10 in dengue and its potential utilization as one of the biomarkers for the early diagnosis of dengue. A case-control study was conducted involving healthy subjects as control (n = 10) and 193 subjects as dengue cases. The cases were categorized into dengue without warning signs (DwoWS: n = 70; 34.5 %), dengue with warning signs (DWWS: n = 108; 23.2 %), and severe dengue (SD: n = 15; 7.4 %). The socio-demographic characteristics, clinical presentations, and laboratory parameters (platelet and hematocrit) were documented. Serum CXCL10 quantification was performed using an enzyme-linked immunosorbent assay (ELISA). The descriptive analysis and Pearson's correlation test were used to analyze demographic data and the correlation between CXCL10, hematocrit, and platelet respectively. The difference in age (p = 0.02) and ethnicity (p = 0.02) were significant between cases and control. Males more frequently had SD in contrast to females (4:1). The frequent warning signs were abdominal pain (42.0 %), severe vomiting (38.3 %), bleeding tendency (15.0 %), and fluid accumulation (7.2 %). The increase in hematocrit (p = 0.039) and platelet reduction (p = 0.0005) were significant in SD. The mean of CXCL10 in control (134.85 ± 48.52 rg/mL) was significantly lower than in cases (545.22 ± 76.33 rg/mL, p = 0.0005). The CXCL10 is evident to be a potential biomarker in the early diagnosis of dengue.

Keywords: CXCL10, Dengue, Early Diagnosis, Severe Dengue, Hematocrit, Biomarker

INTRODUCTION

Dengue has been classified into dengue and severe dengue (SD), with dengue further categorized into dengue without warning signs (DwoWS) and dengue with warning signs (DWWS) attributed to its severity .[1] Dengue virus (DENV) has three structural proteins which are core (C) nucleocapsid protein, membrane-associated protein (prM), envelope protein (E), and seven non-structural (NS) proteins including NS1 which is essential for viral replication in the acute phase.[2] The DENV-2 was reported to be more virulent and highly associated with dengue hemorrhagic fever (DHF) in contrast to other serotypes.[3,4] On a similar note, the introduction of a new genotype or lineage in the population could lead to SD, as DENV-3 was reported in India causing a DHF outbreak in 2001, replacing DENV-2.[5]

Immunopathogenesis of dengue incorporates antibody-dependent enhancement and activation of cross-reactive memory T cells causing cytokines overproduction leading to increased capillary permeability and plasma leakage.[6,7] The cytokine includes inflammatory cytokines, chemokines, adhesion molecules, and growth factors.[8,9] The CXCL10 is a chemokine whose production is induced by IFN-γ and is highly expressed on primary cells that have been infected by DENV.[10] The CXCL10 interacts with heparin and heparan sulphate, competing with the virus thus impede viral binding.[11] This serves as an innate immunity in the early course of dengue.

To date, the diagnosis of dengue commonly involves NS1 antigen detection whereby the highest percentage were identified on day one to three of illness.[2,12] The NS1 antigen is a highly conserved glycoprotein that appears to be crucial for virus viability. The sensitivity of NS1 antigen reduces from day four of illness onwards and becomes undetectable in the convalescence phase.[13] While IgM or IgG antibody detection is preferred once a patient comes at day five of illness or more. Meanwhile, a combination assay that detects both antigen and antibody is frequently used as a rapid test. This test has a longer detection window as it detects both virus and antibodies, thus lessening the false negative results.[14]

In providing better management of dengue, early diagnosis of dengue is crucial to prevent the progress towards severe disease. With the knowledge of CXCL10 increased in the early phase of dengue, hence, this study was conducted with the aim to determine the involvement of the CXCL10 in all dengue categories, which potentially contribute to the biomarker development for an early diagnosis of dengue and SD detection.

MATERIALS AND METHODS

Study Design, Specimen Collection, and Selection Criteria

A case-control study was conducted with the definition of the cases based on the World Health Organization (WHO) 2009 criteria including DwoWS, DWWS, and SD. The control is defined as a population who has not been exposed to dengue, and was recruited from healthy adults (n=10) with the age ranges from 25 to 39-year-old to obtain population-based normal ranges. While the case group involved 193 specimens collected from patients who were admitted to a tertiary hospital in Selangor, Malaysia. The demographic characteristics were gathered along with clinical presentations (e.g., pleural effusion, abdominal pain, severe vomiting, and significant bleeding) and laboratory parameters (platelet and hematocrit).

The laboratory diagnosis of dengue was conducted using NS1 antigen using Platelia Dengue NS1 Antigen Strip (Bio-Rad Laboratories, France) or DENV specific antibodies IgM or IgG using PanBio Dengue IgM and IgG Capture ELISA (PanBio, Brisbane, Australia). Clinically diagnosed dengue patients with positive NS1 antigen or serological tests for either IgM or IgG detection or both were included in this study. Patients who had negative NS1 antigen with non-detected IgM or IgG, or those infected with other etiology were excluded.

Serum CXCL10 Quantification

Sera from the case and control groups were subjected for CXCL10 measurement using quantitative ELISA which was performed in duplicate (R&D Systems, USA). All sera from the case group were collected within the first week of illness (day 3 to 7 of fever). The enzyme immunoassay technique involves the use of a monoclonal antibody specific for CXCL10 which has been pre-coated onto a microplate. The CXCL10 that is existing in the samples is bound to the immobilized antibody. The second detector antibody, an enzyme-linked polyclonal antibody specific for CXCL10 is added after the unbound substances are washed. Following that, a substrate solution is introduced and reacts with the enzyme-antibody-target complex. This reaction generates color changes that are equal to the amount of CXCL10 bound at the initial steps. The reaction is terminated, and the color intensity is measured.

A 75 µL of Assay Diluent RD 1-56, was initially added to each microtiter well followed by 75 µL of samples, controls, and standards. It was then incubated for two hours at room temperature. The microtiter plate was washed four times using 400 µL of wash buffer. The wells were then filled with 200 µL CXCL10 conjugate and incubated at room temperature for two hours. Similar wash steps were performed. A total of 200 µL substrate solution, later loaded to the wells and again incubated for 30 minutes at room temperature. The color changed from blue to yellow after 50 µL of stop solution was introduced. The optical densities were measured using a microplate reader at the wavelength of 450 nm and 570 nm. Subsequently, a standard curve was constructed by plotting the mean absorbance for each standard to acquire the value of CXCL10.

Statistical Analysis

The descriptive analysis was performed to analyze the demographic data. Meanwhile, the relationship between CXCL10 and the dengue severity was analyzed using ANOVA, followed by Tukey HSD post hoc tests. The probability value of $p < 0.05$ was considered significant. The correlation between CXCL10, hematocrit, and platelet level was analyzed using Pearson's correlation test.

RESULTS

Demographic Characteristics

There were 70 (34.5 %) subjects classified as DwoWS, 108 (53.2 %) categorized into DWWS, and 15 (7.4 %) subjects grouped as SD. These were further analyzed according to their age, gender, and ethnicity as shown in Table 1.

Age

The subjects were ranged from 6 to 79 years of age with a mean of 30.7 ± 12.9 years. The youngest age in DwoWS was 6 years and the oldest age was 79 with a mean of 32.3 ± 14.4 years. Those who had DWWS were between 7 to 76 years old with a mean of 28.5 ± 11.3 years. The age range for subjects with SD was 13 to 60 years and the

Table 1. The demographic analysis including age, gender, and ethnicity between control and cases

n (%)	Control 10 (4.9 %)	Total Cases 193 (95.1 %)	DwoWS 70 (34.5 %)	DWWS 108 (53.2 %)	SD 15 (7.4 %)
Age					
Mean ± SD*	30.0 ± 5.1	30.7 ± 12.9	32.3 ± 14.4	28.5 ± 11.3	38.5 ± 13.1
Range	25 – 39	6 – 79	6 – 79	7 – 76	13 – 60
Gender [n (%)]					
Male	0	118 (58.1 %)	47 (23.2 %)	59 (29.1 %)	12 (5.9 %)
Female	10 (4.9 %)	85 (41.9 %)	23 (11.3 %)	49 (24.1 %)	3 (1.5 %)
Ethnicity [n (%)]					
Malay	10 (4.9 %)	103 (50.7 %)	36 (17.7 %)	62 (30.5 %)	5 (2.5 %)
Chinese	0	21 (10.3 %)	7 (3.4 %)	10 (4.9 %)	4 (2.0 %)
Indian	0	15 (7.4 %)	11 (5.4 %)	2 (1.0 %)	2 (1.0 %)
Non-Malaysian	0	54 (26.6 %)	16 (7.9 %)	34 (16.7 %)	4 (2.0 %)

DwoWS: dengue without warning signs, DWWS: dengue with warning signs, SD: severe dengue, SD*: standard deviation.

Table 2. The distribution of warning signs within dengue cases

Clinical Symptoms	Total [n (%)]	DwoWS [n (%)]	DWWS [n (%)]	SD [n (%)]
Abdominal pain				
No	112 (58.0 %)	70 (100 %)	36 (33.3 %)	6 (40.0 %)
Yes	81 (42.0 %)	0	72 (66.7 %)	9 (60.0 %)
Severe vomiting				
No	119 (61.7 %)	70 (100 %)	41 (38.0 %)	8 (53.3 %)
Yes	74 (38.3 %)	0	67 (62.0 %)	7 (46.7 %)
Bleeding tendency				
No	164 (85.0 %)	70 (100 %)	80 (74.1 %)	14 (93.3 %)
Gum bleeding	26 (13.5 %)	0	25 (23.1 %)	
Epistaxis	2 (1.0 %)	0	2 (1.9 %)	1 (6.7 %)
Heavy menses	1 (0.5 %)	0	1 (0.9 %)	0
				0
Fluid accumulation				
No	179 (92.7 %)	70 (100 %)	108 (100 %)	1 (6.7 %)
Pleural effusion	13 (6.7 %)	0	0	13 (86.7 %)
Ascites	1 (0.5 %)	0	0	1 (6.7 %)

DwoWS: dengue without warning signs, DWWS: dengue with warning signs, SD: severe dengue.

mean was 38.5 ± 13.1 years, as shown in Table 1. There was a significant difference in the mean age between dengue cases and control (p = 0.02).

Gender

The majority of the subjects were male (n = 118, 58.1 %) while the remaining 85 (41.9 %) were female. Males predominated in all dengue cases including DwoWS (n= 47, 23.2 %), DWWS (n= 59, 29.1 %), and SD (n = 12, 5.9 %) respectively. Precisely, the female was having a higher percentage of DWWS (n = 49, 24.1 %) as compared to DwoWS (n = 23, 11.3 %) and SD (n = 3, 1.5 %), as shown in Table 1. The control consists of all females (n = 10, 4.9 %). The difference between male and female subjects in cases was significant (p = 0.0005).

Ethnicity

Of all dengue cases, most of the subjects were of Malay ethnicity, as shown in Table 1. The Malay subjects were categorized into DWWS (n = 62, 30.5 %) followed by DwoWS (n = 36, 17.7 %) and SD (n = 5, 2.5 %). Similarly, Chinese subjects

were having a greater percentage of DWWS (n = 10, 4.9 %) as compared to DwoWs (n = 7, 3.4 %) and SD (n = 4, 2.0 %). Meanwhile, Indian subjects had contracted more for DwoWS (n = 11, 5.4 %) in contrast to other categories. The percentage for non-Malaysian subjects was raised in DWWS (n = 34, 16.7 %) followed by DwoWS (n = 16, 7.9 %) and SD (n = 4, 2.0 %). The difference in ethnicity was significant between cases and control (p = 0.02).

Clinical Presentations

In DWWS, most of the subjects complained of abdominal pain (n = 72, 66.7 %) and severe vomiting (n= 67, 62.0 %). Twenty-five subjects (23.1 %) presented with gum bleeding, two subjects (1.9 %) had epistaxis, and one (0.9 %) with heavy menses. In SD, most subjects (60 %) experienced abdominal pain, and 46.7 % were having persistent vomiting with one subject having gum bleeding (6.7 %). Of 15 subjects with SD, 13 suffered from pleural effusion (86.7 %) and one had ascites (6.7 %), as shown in Table 2. The difference in all warning signs between DwoWS, DWWS, and SD was significant (p = 0.0005).

Laboratory Parameters

Hematocrit and Platelet

The mean for hematocrit was 42.88 ± 5.57% with a minimum level of 22.40 % and a maximum of 58.40 %. The mean hematocrit in SD was elevated (46.36 ± 5.68%) as compared to DwoWS (42.73 ± 5.43 %) and DWWS (42.49 ± 5.68 %). The lowest hematocrit level in SD was 35.10 %, while the highest was 58.40 %. Meanwhile, the mean platelet of all dengue cases was 49.79 ± 38.01 × 10^9/L with a range of 2 to 230 × 10^9/L. The mean platelet was reduced in SD (12.47 ± 12.65 × 10^9/L) with the lowest level was 2 × 10^9/L and the greatest level of 51 × 10^9/L. These findings revealed that subjects with SD had a significant increase in the mean of hematocrit (p = 0.039) and a decreased mean of platelet as compared to those with DwoWS and DWWS (p = 0.0005).

Association of CXCL10 with Control and Dengue Cases

The control group had CXCL10 level ranging from 90.44 to 253.51 pg/mL. The mean for CXCL10 was significantly lower in control group (134.85 ± 48.52 pg/mL) in comparison to dengue cases (545.22 ± 76.33 pg/mL), DwoWS (527.56 ± 91.17 pg/mL), DWWS (555.99 ± 66.81 pg/mL), and SD (550.46 ± 48.43 pg/mL), with p = 0.0005, as shown in Figure. However, there was no significant correlation for the mean of CXCL10 between DwoWS versus (vs) DWWS (p = 0.65), DwoWS vs SD (p = 0.7) and DWWS vs SD (p = 0.99).

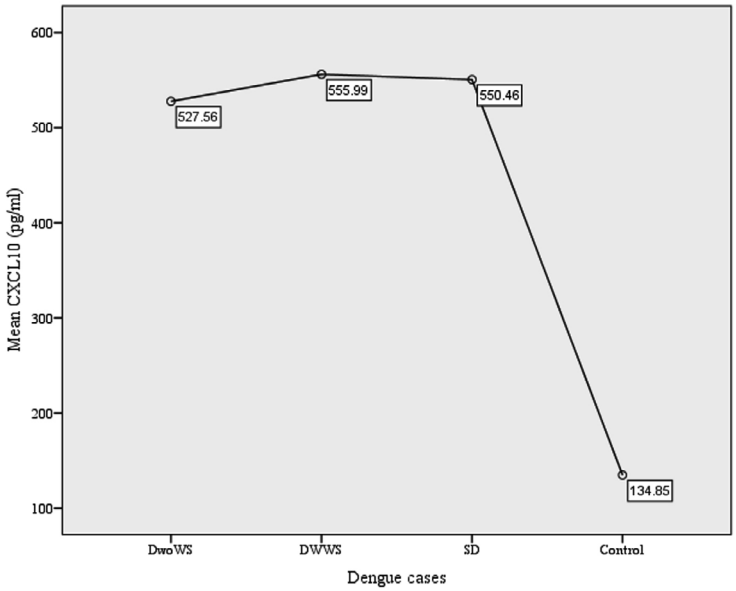

Figure. The mean of circulating serum CXCL10 level in control and dengue cases

Association of CXCL10 with Hematocrit and Platelet

There was a significant but weak positive correlation between the circulating level of CXCL10 and hematocrit (r = 0.2, p = 0.007). However, there was no significant correlation found between CXCL10 level and platelet count (r = -0.108, p = 0.14).

DISCUSSION

Dengue ranks as the most important mosquito-borne viral disease in the world and remains endemic in Malaysia, despite the current COVID-19 pandemic. The WHO reported that the largest number of dengue cases occurred in 2019, and continues affecting more than 15 countries worldwide, which made the combined impact of COVID-19 and dengue epidemics potentially result in devastating consequences for the populations at risk. Almost 3 billion people live in areas with a risk of dengue and each year up to 400 million people get infected with dengue with 22,000 died from severe dengue.[15] Although SD was seen in a small proportion of dengue, it does contribute to significant mortality. Five percent of hospitalized dengue patients in Vietnam progressed to SD,[16] while 6.6% of SD was documented by a former study performed in Vitoria, Brazil.[17] In Malaysia, Ahmad et al.[18] reported that SD contributed up to 4.9% of all other categories of dengue, with two other studies showing the percentage of SD cases were 4.6%.[18-20] In the present study, the prevalence of SD was 7.4% which was slightly higher in contrast to previous reports. This finding suggests a true reflection of Malaysia as one of the endemic countries for dengue.

Dengue affects all ages and is commonly seen in adults. A systematic literature review conducted from the year 2000 to 2012 found that the number of dengue cases was stable in adults as compared to children.[20,21] This was concurred by Ahmad et al.[18] that revealed 75% of dengue cases were frequently occurring in subjects of more than 15 years old. The prevalence of dengue was found to be higher in the age group of 20 to 29 years.[21] Similarly, the mean age for current dengue cases in our study was 30.7 years, which categorized the obtained mean age as a young adult. This is most probably linked to a productive age group and increasing outdoor activities leading to a high incidence of dengue exposure. Furthermore, the relationship of age with dengue cases was significant in the present study, as SD had a higher mean age (38.5) as compared to DwoWS (32.3) and DWWS (28.5). These findings corresponded with the previous reported study.[22] Our findings indicate that older subjects were at high risk for SD as increased age is associated with the presence of comorbidities and altered level of immunity.[23,24]

Males were reported to contract dengue more, in contrast to females. The percentage of males infected with dengue was higher in several studies ranging from 60.0% to 63.0%.[19-21] Based on Malaysia's surveillance data, adult males had a 4.17 times greater risk of contracting dengue in contrast to females.[24,25] Our analysis revealed that the male and female ratio in dengue cases was 1.57:1, while in SD the ratio was increased to 4:1. The difference in gender was significant (p = 0.0005) and these were in agreement with other former studies.[21,24]

Racial allocation and density are possible contributions to the ethnicity aspect of dengue distribution in the country. It was previously reported that Malay was more susceptible to dengue in contrast to other ethnicities.[26] Our findings revealed that 103 (50.7 %) subjects were Malay followed by non-Malaysian (n = 54, 26.6 %), Chinese (n = 21, 10.3 %), and Indian (n = 15, 7.4 %), with Malay dominating all the dengue cases. These findings were in parallel to previous studies.[20,21]

A small number of dengue cases may progress to SD despite most cases being self-limiting, thus attention should be given to dengue patients with warning signs as they would be susceptible to SD. The presence of warning signs (abdominal pain, severe vomiting, bleeding tendency, and clinical fluid accumulation) in this study was significantly associated with dengue cases (p = 0.0005) which corresponded to findings reported by previous studies.[18,27] The frequent warning signs from our analysis were abdominal pain (42.0 %), followed by severe vomiting (38.3 %), bleeding tendency (15.0 %), and fluid accumulation (7.2 %). These were concurrent with a study conducted in Brazil,[22] whereby 59.8 % of the subjects were having abdominal pain, 44.3 % vomiting, 29.9 % mucosal bleeding, and 28.9 % with pleural effusion.

Increased vascular permeability leads to plasma leakage causing hemoconcentration and manifests as a high hematocrit level. The level of hematocrit is associated with volume loss and severity of the disease.[18,27] Our findings demonstrated a significant elevation of mean hematocrit (46.36% ± 5.68) in SD compared to DwoWS (42.73 % ± 5.43) and DWWS (42.49 % ± 5.52) (p = 0.039). This is consistent with other studies that had raised hematocrit levels in DHF/DSS as compared to dengue fever.[28,29] Our analysis proved that high hematocrit is linked to SD, and its measurement has been the most common method used to identify hemoconcentration.

Thrombocytopenia in dengue occurs due to complex formation, following secretion of P-selectin which enables platelet binding to monocytes.[30] The present study demonstrated a significant (p = 0.0005) reduction of platelet count in SD (12.47 ± 12.65 × 10^9/L) vs DwoWS (54.56 ± 44.09 × 10^9/L) and DWWS (51.88 ± 33.21 × 10^9/L). The results were in agreement with Chen et al.[29] and Md-Sani et al.[23] who reported that thrombocytopenia was associated with plasma leakage and mortality in SD respectively.

The mean of CXCL10 for the control group in the present study was significantly lower (p = 0.0005) (134.85 ± 48.52 pg/mL) than all dengue cases (545.22 ± 76.33 pg/mL), and these were in parallel to the previous studies that had similar findings.[31,32] This can be explained as the production of CXCL10 was augmented in the presence of DENV, causing raised levels of CXCL10 in all dengue cases in contrast to healthy control.[31,32] Furthermore, CXCL10 competed with DENV in the early course of the disease, thus reducing viral uptake and replication.[11]

There were studies reported CXCL10 level was significantly elevated in DHF in contrast to dengue fever which implied that CXCL10 has the ability to serve as one of the biomarkers for dengue severity.[31,32] In the present study, CXCL10 demonstrated a significant relationship with hematocrit level, however, there was a weak positive correlation between CXCL10 and hematocrit level (r = 0.2, p = 0.007). Our finding suggests that CXCL10 is an essential factor (chemokine) causing raised hematocrit levels leading to vascular permeability despite the presence of weak correlation. This implied that CXCL10 is evident to be one of the crucial biomarkers that can be employed in diagnosing dengue in the early stage, as it has the ability to distinguish between dengue and non-dengue cases. However, our finding was inadequate to discriminate between DwoWS, DWWS, and SD. This was probably due to a limited number of sample sizes for SD with an absence of sequential blood collected for remaining days of fever in all dengue cases, which restricted us in reporting the effects of cytokine.

CONCLUSION

The CXCL10 involvement in all dengue categories with its association with demographic characteristics and selected laboratory parameters were ascertained in the present study. A crucial finding in the study substantiated the essential role of CXCL10 in the early diagnosis of dengue, whereby CXCL10 has the capacity to differentiate dengue from non-dengue cases, thus potentiated to be utilized as one of the supplementary tests in the current diagnosis of dengue. The other roles of CXCL10 discerning dengue versus severe dengue particularly as a marker for dengue severity remain to be explored.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All the authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Universiti Teknologi MARA (UiTM) (RAGS/1/2014/SKK04/UITM/4) and the Medical Research & Ethics Committee (MREC), Ministry of Health, Malaysia, NMRR-17-417-34493.

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