



THE INFLUENCE OF INTERLEUKIN-10 (*IL-10*) GENE POLYMORPHISM ON THE SUSCEPTIBILITY AND PROTECTION AGAINST DENGUE IN SABAH POPULATION



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Background:

Dengue is a growing public health concern in tropical and subtropical countries of Southeast Asian countries including Malaysia. Genetic host variations are an important contributor to disease outcomes, and many case-control studies have associated it with single nucleotide polymorphisms (SNPs) such as *IL-10* in dengue pathogenesis by regulating immune activity, such as B-cell proliferation and cytokine production.

Aim: This study aims to identify the association of *IL-10* [-819 C/T (rs1800871)] gene polymorphism with dengue in Sabah population.

Methods:

Study design: This is a cross-sectional, case control study involving 156 subjects of main ethnic groups in Sabah: 84 dengue cases and 72 healthy controls of Kadazan-dusun and Bajau ethnic groups. Subjects were recruited either prospectively or retrospectively between January to June 2019. The study has been approved by Malaysia National Medical Research Registry; NMRR-18-1556-42651-IIR, Jabatan Kesihatan Negeri Sabah; (55) dlm.JKN(SB)100-13 and Universiti Malaysia Sabah ethics committee; JKEtika 3/18 (2). A written informed consent was obtained from subjects prior to their participations in the study.

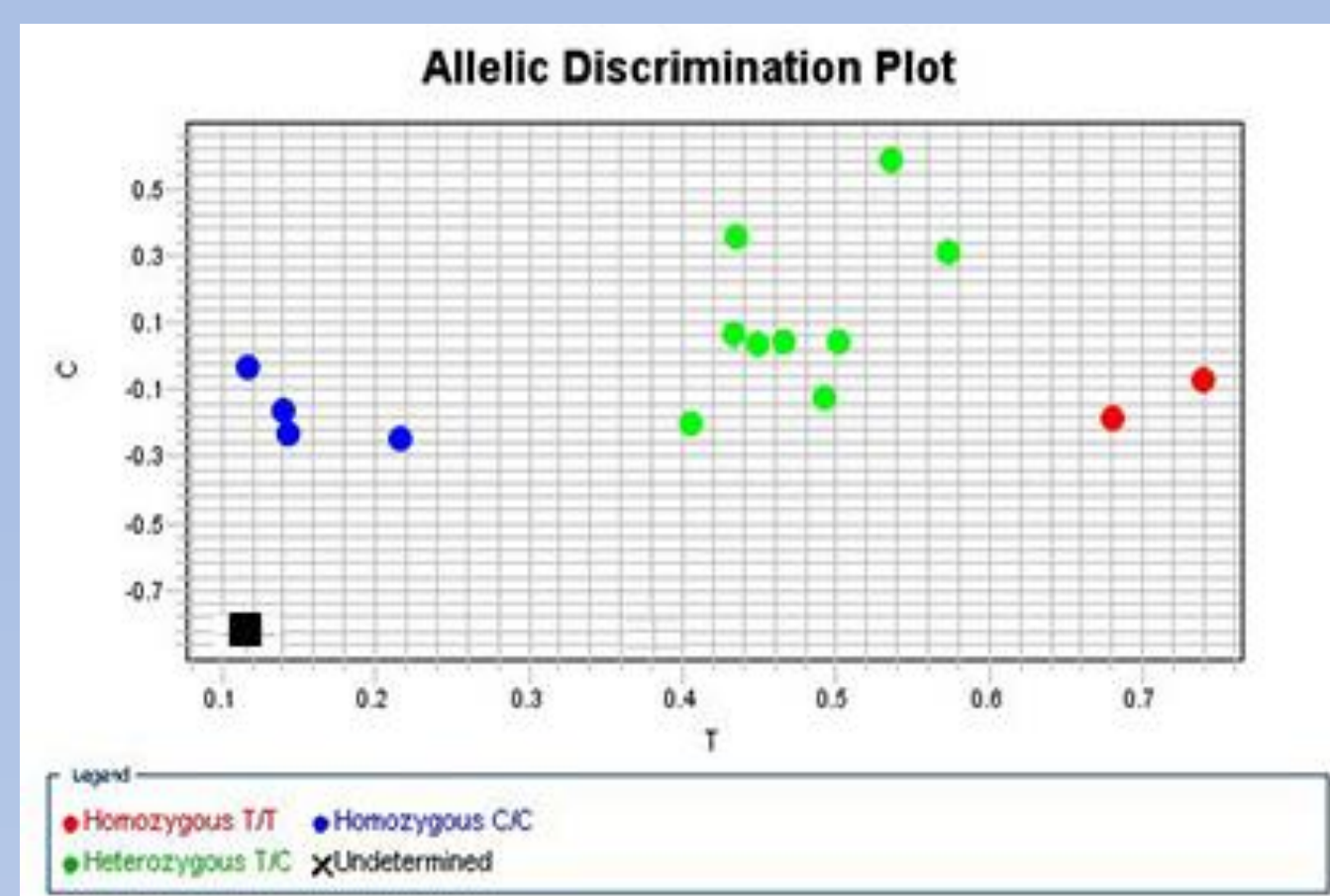
Subject Recruitment and Sample Collection: Cases group consist of patients with dengue fever while control group consist of healthy adult that matched with age, sex, ethnicity with the cases. Adults who are age above 18 years old, diagnosed with dengue fever or/and dengue hemorrhagic fever and consented to participate were be included in this study. Subjects with major medical illness, ethnicity mixed with non-indigenous Sabahan population and disagree with testing procedures were be excluded. 3mL of blood from the study subjects were collected in EDTA tube according to a standard method.

Diagnosis of dengue: Clinical classification of dengue according to WHO Guidelines for Dengue Prevention and Control. All cases of dengue infection as confirmed by rapid test (NS1 or IgM/IgG detection) using acute serum samples

Genotyping: Genotyping of the genetic polymorphism of the *IL-10* was performed by using Real-time polymerase chain reaction (qRT-PCR). The real-time polymerase chain reaction was carried out by ABI StepOne Plus RT-PCR Thermal Cycler (Applied Biosystems, USA), according to the manufacturer's protocol.

Statistical analysis: Intergroup statistical analyses were performed using Chi-square (X^2) test and the Fisher exact test, where necessary, to compare each ethnic group's cases with ethnically matched healthy controls for the frequencies of *IL-10* C/C homozygous, C/T heterozygous and T/T homozygous genotypes. For all analyses, $p < 0.05$ were considered statistically significant. SPSS version 23 was used for statistical analysis.

Result



Conclusion

Significant association between polymorphism of *IL-10* [-819 C/T (rs1800871)] genes with dengue in Kadazan-Dusun and Bajau ethnicities and male gender. This outcome provides evidence that genetic polymorphisms in the immune system affect susceptibility to clinical phenotypes of dengue, and it may be considered as useful prognostic markers in the future personalized medication for Sabah population.

Ethnicity	Participant	Genotype Frequency				Allelic Frequency			OR (CI 95%)
		C/C	C/T	T/T	<i>p</i>	C	T	<i>p</i>	
Kadazan-Dusun	Case	19 (0.296)	33 (0.516)	12 (0.188)	0.002*	71 (0.554)	57 (0.445)	0.156	1.505 (0.897 - 2.525)
	Control	3 (0.057)	42 (0.792)	8 (0.151)		48 (0.453)	58 (0.547)		
Bajau	Case	3 (0.150)	9 (0.450)	8 (0.400)	0.011*	15 (0.375)	25 (0.625)	0.675	0.741 (0.300 - 1.832)
	Control	0 (0.000)	17 (0.895)	2 (0.105)		17 (0.447)	21 (0.553)		
Total	Case	22 (0.262)	42 (0.500)	20 (0.238)	<0.001*	86 (0.512)	82 (0.488)	0.341	1.275 (0.816 - 1.992)
	Control	3 (0.042)	59 (0.820)	10 (0.139)		65 (0.451)	79 (0.549)		

IL-10 gene polymorphism showed a significant association with dengue in the Kadazan-Dusun and Bajau ethnics in terms of genotype ($p=0.002$ and $p=0.011$, respectively) but not for the

Gender	Participant	Genotype Frequency				Allelic Frequency			OR (CI 95%)
		C/C	C/T	T/T	<i>p</i>	C	T	<i>p</i>	
Male	Case	13 (0.302)	18 (0.419)	12 (0.279)	<0.001*	44 (0.512)	42 (0.488)	0.971	1.048 (0.534 - 2.054)
	Control	1 (0.036)	26 (0.929)	1 (0.036)		28 (0.5)	28 (0.500)		
Female	Case	9 (0.219)	24 (0.585)	8 (0.195)	0.147	42 (0.512)	40 (0.488)	0.283	1.575 (0.772 - 3.212)
	Control	2 (0.08)	16 (0.640)	7 (0.280)		20 (0.400)	30 (0.600)		
Total	Case	22 (0.262)	42 (0.500)	20 (0.238)	<0.001*	86 (0.512)	82 (0.488)	0.341	1.275 (0.816 - 1.992)
	Control	3 (0.042)	59 (0.820)	10 (0.139)		65 (0.451)	79 (0.549)		

IL-10 polymorphism significantly associated with dengue ($p < 0.001$) in the male subjects in terms of genotypic frequency between cases and controls

Genotype	Kadazan-Dusun		Bajau		Total	
	Odd Ratio (95% CI)	<i>p</i>	Odd Ratio (95% CI)	<i>p</i>	Odd Ratio (95% CI)	<i>p</i>
CC vs TT	4.222 (0.932 - 19.132)	0.112	NA	0.944	3.667 (0.882 - 15.250)	0.125
CC vs (CT + TT)	7.037 (1.952 - 25.374)	0.002*	NA	0.248	8.161 (2.329 - 28.603)	<0.001*
(CC + CT) vs TT	1.269 (0.476 - 3.384)	0.818	5.667 (1.018 - 31.542)	0.082	1.906 (0.826 - 4.399)	0.186

- Comparison between C/C genotype and C/T plus T/T genotypes revealed that the heterozygous C/T plus homozygous T/T genotype has a higher risk of getting dengue in the pooled subjects ($p < 0.001$, odds ratio: 8.161, 95% CI 2.329 – 28.603).
- Comparison between C/C genotype with C/T plus T/T genotypes in the Kadazan-Dusun ethnic group showed a significant difference in odds ratio ($p=0.002$, odds ratio: 7.037, 95% CI 1.952 – 25.374).

Genotype	Male		Female		Total	
	Odd Ratio (95% CI)	<i>p</i>	Odd Ratio (95% CI)	<i>p</i>	Odd Ratio (95% CI)	<i>p</i>
CC vs TT	2.167 (0.173 - 27.077)	1.000	4.500 (0.730 - 27.740)	0.202	3.667 (0.882 - 15.250)	0.125
CC vs (TC + TT)	16.033 (1.983 - 129.656)	0.003*	4.500 (0.901 - 22.482)	0.103	8.161 (2.329 - 28.603)	<0.001*
(CC + TC) vs TT	6.968 (1.447 - 33.563)	0.017*	0.758 (0.250 - 2.297)	0.836	1.906 (0.826 - 4.399)	0.186

- Comparison between C/C genotype and C/T plus T/T genotypes in the male subjects showed a significant difference in odds ratio ($p=0.003$, odds ratio: 16.033, 95% CI 1.983– 129.656).
- Comparison of between T/T genotype and C/C plus C/T genotype in male subject reveals that the genotype showed a significant difference in odds ratio ($p=0.017$, odds ratio: 6.968, 95% CI 1.447 – 33.563).

Disclosure statement

This study was funded by Universiti Malaysia Sabah (UMS): Skim Penyelidikan Pensyarah Lantikan Baru (SLB0181-2018) and Skim Penyelidikan Bidang Keutamaan (SBK0414-2018). The authors declare no relationships to disclose.