ORIGINAL ARTICLE

Smart Medical Journal

Smart Medical Journal (SMedJour) December 2024, Vol. 7, No.3, pp :163 -168
DOI: https://doi.org/10.13057/smj.v7i3.91999
E-ISSN: 2621-0916 | P-ISSN: 2621-1408

Comparison of Anti-Dengue Antibody Diagnostic Tests Using Immunochromatography and Fluorescence Immunoassay Methods at dr. Ario Wirawan Salatiga Pulmonology Hospital

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Recived: 13/08/2024
Accepted: 07/03/2025
Published: 24/03/2025

ABSTRACT

Introduction: Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus, which is transmitted through the bite of the Aedes aegypti. The agent is the dengue virus, which belongs to the Flaviridae family and Flavivirus genus, consisting of 4 serotypes, namely Den-1, Den-2, Den-3, and Den-4. Antibodies that appear generally are IgG and IgM. The methods used in examining dengue antibodies include Immunochromatographic (ICT) and Fluorescent Immunoassay (FIA) methods. The ELFA method is used for diagnostic testing.
Objective: To determine whether there is a difference in anti-dengue antibody examination using ICT and FIA methods at the dr. Ario Wirawan Salatiga Pulmonology Hospital.
Method: The type of research used is descriptive comparative with a cross-sectional method. This research was conducted from October to November 2023. 44 samples used in this study were examined for diagnostic tests using ELFA method, and then anti-dengue IgG and IgM were examined using the ICT and the FIA methods
Results: The results of the research showed that the sensitivity of IgG method and IgG of ICT method were 86.21% and 75.86%, respectively, and the specificity of the IgG of the FIA method and the IgG of the ICT method was 80.00% and 93.30% respectively. Meanwhile, the sensitivity of IgM by FIA method and ICT method is 80.00% and 93.30%, respectively, while the specificity of IgM by FIA method and IgM by ICT method is 82.76%.
Conclusion: There is a significant difference between examining anti-dengue antibodies using Immunochromatography (ICT) and Fluorescence Immunoassay (FIA) methods.

Keywords: DHF; anti-dengue antibodies; ELFA; ICT; FIA

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus. Dengue virus is one of four serotypes of viruses from the genus flavivirus, Flaviridaefamily. This disease is transmitted through the bite of the Aedes aegypti mosquito. This disease can attack everyone, and if there is no appropriate treatment, it can cause death[1]. DHF is a disease found in most tropical and subtropical countries. The primary host of this disease is humans. Dengue virus belongs to the Flaviridae family, and the Flavivirus genus consists of 4 serotypes, namely Den-1, Den-2, Den-3, and Den-4[2].

In Indonesia, the first case occurred in Surabaya in 1968, with 58 people infected with DB, and 24 of them died. Every year, there are always outbreaks in several provinces. In 1998 and 2004,

79,480 people were infected, and more than 800 people died. In the following year, infected cases increased, but the number of deaths fell significantly compared to 1998 and 2004. In 2008, there were 137,469 people with 1,187 deaths with a Case Fatality Rate (CFR) of 0.86%, and in 2009, there were 154,855 people with 1,384 deaths or a CFR of 0.89[3].

The diagnosis of dengue fever is made clinically, usually characterized by fever without symptoms, petechiae rash with thrombocytopenia with a count of less than 100 x 109/L, and plasma leakage due to increased vascular permeability and relative leukopenia. Serological examination and PCR for diagnosis of dengue fever if clinically indicated[4].

After entering the body, the dengue virus will reproduce in reticuloendothelial cells. As a result of this infection, humoral and cellular immune responses arise, including anti-neutralization, anti-hemagglutinin, and anti-complement. IgM will be formed in primary infections, and IgG will be formed in secondary infections[5].

A Rapid Diagnostic Test (RDT) is a rapid test that uses the immunochromatographic (ICT) method to detect antibodies in patients. In this method, there are 3 C lines as controls, which must always appear during the examination. In contrast, the G and M lines are the lines for examining antibodies containing colloidal gold conjugated anti-IgG and IgM, which change to maroon or purple if the result is positive. The use of RDT speeds up the diagnosis of dengue infection and can also differentiate primary infection from secondary infection[6].

The Fluorescent Immunoassay (FIA) method is used to detect IgG and IgM antibodies using the lateral flow method. This examination has a way of working that is similar to the ICT method. Results are reported as Cut-Off Index (COI) values[7].

The gold standard examination of this research is the ELISA method. Over time, the ELFA method can also be used as a gold standard for examining IgG and IgM antibodies This study used the ELFA method as a reference standard for examining IgG and IgM antibodies[8].

Research conducted by Ruchusatsawat Kriangsak et al., 2022 stated that FIA has excellent sensitivity for detecting NS1 but is less sensitive for detecting dengue IgG and IgM antibodies. Meanwhile, ICT lacks sensitivity and specificity for the detection of anti-dengue IgG and IgM[7]. The study aim to insvestigate the comparation of anti-dengue antibody diagnostic test using immunochromatography and Fluorescent Immunoassay method and to decide the better method to examine antibody anti-dengue.

METHOD

This research uses a cross-sectional design (Cross-Sectional). This research compares one sample with another sample, both independent samples and paired samples. The population of this study was patients with symptoms of dengue fever from August to September 2023 at Dr. Ario Wirawan Salatiga Pulmonology Hospital. The amount of the population is 50 patient that meet the requirement of the inclusion and exclusion.

The samples included in the inclusion criteria were all patients with suspected dengue fever, while the exclusion criteria were patients with platelet disorders such as Idiopathic Thrombocytopenia Purpura (ITP). The sampling technique in this study used simple random sampling that taken from population who meet the requirement of the inclusion and exclusion. Determination of sample size was calculated using the Slovin formula. The data obtained then calculated for sensitivity and specificity against the reference standard. The sample for this study was 44 patients with symptoms of dengue hemorrhagic fever.

RESULT

The number of patients who has symptoms of dengue hemorrhagic fever from October to November 2023 at Dr. Ario Wirawan Salatiga Pulmonology Hospital was as many as 50 patients then 44 sample are taken by using slovin formula. Consisting of 21 male and 23 female patients with an age range of 2-93 years. All samples were then examined for dengue IgG and IgM using the ELFA method, after which IgG and IgM examinations were conducted using the ICT and FIA methods.

Table 1. Sample distribution

	Score	Average
Gender		
Male	21	47.72%
Female	23	52.28%
Age		
<18	15	34.10%
18-30	10	22.72%
>30	19	43.17%
Domicile		
Salatiga	25	56.81
Outside of Salatiga	19	43.19%

Analytical validity of examination methods

Table 2 results of anti-dengue antibody examination

	ELFA		ICT		FIA	
	IgG	IgM	IgG	IgM	IgG	IgM
Non-Reactive	15	29	14	25	12	22
Reactive	29	15	30	19	32	12
Total	44	44	44	44	44	44

The results of the IgG and IgM antibody examination using the ELFA method, FIA method, and ICT method in the table show the ELFA IgG examination. Fifteen patients had non-reactive results, and 29 patients had positive results. Using the ICT method, 14 patients were non-reactive, and 30 patients were reactive. Using the FIA method, 12 patients had non-reactive results, and 32 patients had reactive results.

In the IgM examination using the ELFA method, 29 patients had non-reactive results, and 15 patients had reactive results. In the ICT method, 25 patients had non-reactive results, and 19 patients had non-reactive results. In the FIA method, 22 patients had non-reactive results, and 12 patients had reactive results.

ICT examination, FIA compared with ELFA

Table 3. results of IgG examination compared with ELFA

		IgG_ELFA		
		Reactive	Non-Reactive	Total
IgG ICT	Reactive	22	1	23
	Non-Reactive	7	14	21

Table 3 shows that the proportion of reactive samples for IgG ICT and IgG ELFA was 22 patients, while the non-reactive results for IgG ICT and IgG ELFA were 14 patients. The sensitivity of IgG ICT compared to ELFA was for IgG ICT 75.86%. The specificity for IgG ICT compared with the ELFA method was 93.33 %.

Table 4. IgG FIA examination compared with ELFA

		IgG_ELFA		Total
		Reactive	Non-Reactive	
IgG FIA	Reactive	3	25	28
	Non-Reactive	12	4	16

Table 4 shows that the proportion of reactive samples for IgG FIA and IgG ELFA was 25 patients, and the non-reactive results for IgG FIA and IgG ELFA were 12 patients. The sensitivity of IgG FIA compared with ELFA was for IgG FIA 86.21%. The specificity for IgG FIA compared with the ELFA method is 80.00 %.

Table 5. IgM ICT examination compared with ELFA

		IgM_ELFA		Total
		Reactive	Non-Reactive	
IgM ICT	Reactive	14	5	19
	Non-Reactive	1	24	25

Table 5 shows that the proportion of reactive samples for IgM ICT and IgM ELFA is 14 samples, while the proportion for non-reactive IgM ICT and IgM ELFA results is 24. The sensitivity of the IgM ICT reagent compared to ELFA is 93.33%, while the specificity of the IgM ICT reagent compared to the ELFA method is 82.76%.

Table 6. IgM FIA examination compared with ELFA

		IgM_ELFA		Total
		Reactive	Non-Reactive	
IgM FIA	Reactive	12	5	17
	Non-Reactive	3	24	27

Table 6 shows that the proportion of reactive samples for IgM FIA and IgM ELFA is 12, and for the proportion of IgM FIA and IgM ELFA samples, it is 24. The sensitivity of the IgM FIA reagent compared with ELFA is 80.00%, while the specificity of the IgM ICT reagent compared with the ELFA method is 82.76%.

DISCUSSION

The ICT and FIA methods have their respective advantages and disadvantages. Based on several previous studies, the Immunochromatography (ICT) method has reasonable specificity, around 90%, but low sensitivity, ranging from 10 to 99% [9]. The FIA Fluorescent Immunoassay method uses a susceptible detection system and has a sensitivity of 70 - 100% and a specificity of 83.5 - 91.7% [10]. The ELFA method as a reference standard has a sensitivity and specificity of 91.91%. The results of this study show that anti-dengue IgG using the FIA method is more sensitive, with a sensitivity level of 86.21%, compared to the ICT method, with a sensitivity level of 75.86%. IgG examination using the ICT method is more specific, with a specificity level of 93.30%, compared to the FIA method, with a specificity level of 80.00%. The results of this study are slightly different from research conducted by Ruchusatwat Kriangsak et al, 2022 which stated that IgG FIA was more sensitive (43.04%). However, the specificity of IgG FIA was higher (89.84%) compared to IgG ICT (53.66%)[7].

Dengue IgM examination using the ICT method has a higher sensitivity level, 93.30%, than the FIA method, which is 80.00%. The specificity of dengue IgM examination using the FIA and ICT methods has the same specificity, namely 82.76%. It is slightly different from research conducted by

Ruchusatsawat Kriangsak et al, 2022, which compared the IgM FIA and IgG ICT methods with the ELISA method as the Gold Standard. It was found that the IgM antibody results of the ICT method were more sensitive (53.80%) compared to IgM ICT (40.51%) and IgM FIA also had higher specificity (89.84%) compared to IgM ICT (80.89%)[7].

Limitations in this study include the fact that the researchers did not differentiate between primary and secondary infections. The respondents studied had not been confirmed as suffering from dengue fever as evidenced by dengue virus culture and/or dengue virus PCR, and due to time constraints, the number of respondents studied was relatively small. Suggestions from this research are they can add viral culture examination and/or the gold standard, namely dengue PCR examination. Increasing the number of respondents, so that research can be maximized, for further research, it is possible to add several brands of anti-dengue IgG and IgM tests with various brands and with varying specificities and reagent specificities.

CONCLUSION

Based on the research that has been conducted, it can be concluded that anti-dengue IgG examination using the FIA and ICT methods is equally specific, but the sensitivity of the FIA method is superior to the ICT method. The anti-dengue IgM examination using the FIA method is more specific than using ICT but ICT is more sensitive than FIA.

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