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RELATIONSHIP SPIROMETRY VALUES AND DIABETES CONTROL IN TYPE 2 DIABETES MELLITUS PATIENTS

Husdaryanti Sahabuddin [1], Irawaty Djaharuddin [2]*, Muhammad Ilyas [3], Bulkis Natsir [4], Harun Iskandar [5], Siti Nurisyah [6] and Edward Pandu Wiriansya [7]

[1] Student of Specialist Medical Education, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Hasanuddin University, Wahidin Sudirohusodo Hospital, Makassar, Indonesia.
[2,3,4,5,6,7] Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Hasanuddin University, Wahidin Sudirohusodo Hospital, Makassar, Indonesia.
*Corresponding Author

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Abstract

Background: Diabetes mellitus (DM) is a chronic disorder with persistently elevated blood glucose levels that leads to the development of microvascular and macrovascular complications over several years. The bidirectional relationship between DM and the lungs is clinically proven to have progressive pulmonary function abnormalities with decreased Forced Expiratory Volume in 1 second (FEV1) and Forced Vital Capacity (FVC) in individuals with poor DM control. Methods: This study was an observational analytic study using a Case Control approach method in patients with Diabetes Mellitus at Pangkajene Health Center. Each research subject underwent spirometry examination. Results: This study involved 133 research subjects, consisting of 3 groups, uncontrolled diabetes mellitus patients 38.2%, controlled 36.6%, and control group 25.2%. The mean age was 60.13 ± 9.82, 40 males and 91 females. The mean HbA1c value was 8.55 ± 1.69. Spirometric examinations were performed on all groups with parameters of Forced Vital Capacity (FVC), Forced Expiratory Volume in the first second (FEV1), and FEV1/FVC ratio. The mean Forced Vital Capacity (FVC) value in all samples was 73.65%, the mean Forced Expiratory Volume in the first second (FEV1) value was 60.47%, and the mean FEV1/FVC value was 86.25%. Conclusion: There was a significant weak negative correlation between HbA1c and FVC and FEV1 in uncontrolled DM patients. This indicates that the higher the HbA1c value, the lower the FVC and FEV1 values will be.

Keywords: Spirometry, HbA1c, Diabetes Mellitus.

1. INTRODUCTION

Diabetes mellitus (DM) is currently a global health threat. Various epidemiological studies show a trend of increasing incidence and prevalence of type 2 DM in various parts of the world. The WHO organization predicts an increase in the number of type 2 DM patients from 8.4 million in 2000 to around 21.3 million in 2030. International Diabetes Federation (IDF) predictions also show that in 2019-2030 there will be an increase in the number of DM patients from 10.7 million to 13.7 million in 2030.[1] Pulmonary function examination is an examination to determine whether lung function is normal or abnormal. Lung function examination is carried out based on certain indications. A sudden decrease in lung function can cause respiratory failure and can cause death in sufferers. Spirometry examination is not only used to determine the diagnosis but also to assess the severity of obstruction, restrictions, and the effects of treatment. There are some sufferers who do not show any complaints but the spirometry examination shows obstruction or restriction. This can be used as an early warning of lung function disorders that may occur so that we can determine preventive measures as soon as possible. [2] A spirometry examination is an examination to measure a person's static and dynamic lung volume using a spirometer. Simple spirometry usually provides sufficient information. A

number of inexpensive electronic spirometers can precisely measure certain parameters such as vital capacity, forced expiratory volume in the first second (VEP1) and peak expiratory flow. A spirometer cannot make a specific diagnosis but can determine the presence of obstructive and restrictive disorders and can provide an estimate of the degree of abnormality.[3]

Diabetes mellitus (DM) is a chronic disorder with continuously elevated blood glucose levels that over several years leads to the development of micro-vascular and macro-vascular complications. It is estimated that by 2035, one in ten adults will be affected by metabolic syndrome. Complications of DM include retinopathy, nephropathy, neuropathy, coronary artery disease, and peripheral vascular complications. The two-way effects that occur in DM and the lungs are clinically proven to have progressive lung function abnormalities with the results of Forced Expiratory Volume in 1 second (VEP1) and Reduction of Forced Vital Capacity (VAC) in individuals with poor DM control and long duration of DM. Suboptimal lung function has been observed in immune-mediated DM patients in relation to DM duration and control. [6]

This pathogenesis is thought to involve microangiopathic processes and non-enzymatic glycosylation of tissue proteins. This process results in disruption of collagen and elastin cross-linking with decreased strength and elasticity of the connective tissue. Due to the presence of abundant connective tissue and extensive microvascular circulation, it increases the possibility that the lung becomes a target organ in diabetes patients. It has been proven that pulmonary complications in diabetes are due to thickening of the walls of alveoli, alveolar capillaries and pulmonary arterioles and these changes lead to pulmonary dysfunction. Lung dysfunction may be one of the earliest measurable non-metabolic changes in diabetes.[7]

Zunairah et al (2022) explain the relationship between DM and restrictive lung disorders. Clinically, DM is associated with a progressive decrease in VEP1 which consequently causes bronchial hyperresponsiveness. The development of respiratory complications in DM is considered multifactorial. This can be caused by decreased elastic recoil, decreased lung volume, decreased respiratory muscle performance, decreased carbon monoxide diffusion capacity (DLCO), and autonomic neuropathy of the respiratory muscles. In addition, Weynand et al (1999) showed homogeneous thickening of the basal lamina of pulmonary capillaries throughout the lung parenchyma. All lung function parameters were lower in diabetics of both sexes compared to non-diabetic controls with a greater decline in men than in women and due to reduced lung elastic recoil.[2]

Research conducted by Zunaira et al (2022) on 100 patients with diabetes mellitus found 57 patients with normal spirometry results, while the rest had restricted results. The factors that have a significant influence are the HbA1c value and the duration of diabetes mellitus. The higher the HbA1c level, the lower the lung function.

A similar study was also conducted by Hawra et al (2021) on 101 diabetes mellitus patients whose lung function (KVP, KV, and KVP1) was checked with the results that patients with uncontrolled diabetes had lower spirometry results compared to the control group.[3]

2. METHOD

This research is an analytical observational study using a case control approach. The research was carried out at the Pangkajene Community Health Center, Sidrap Regency starting January 2024 until an appropriate sample size was obtained.

The population of this study were all patients diagnosed with diabetes mellitus. Patients with a registered diagnosis of diabetes mellitus who met the inclusion criteria. The estimated sample size in this study was calculated based on the sample size formula for unpaired numerical comparative analysis of 2 groups. This research uses consecutive sampling, which is a sample selection method that is carried out by selecting all individuals encountered who meet the selection criteria, until the desired sample size is met. In this study, the data collected is primary data which includes personal data and anamnesis and physical examination of the patient, namely name, age, gender, disease history, smoking history, use of diabetes medication and data from spirometry examination results, HbA1c laboratory results and photographs. thoracic.

Data collection was carried out by interviewing respondents who were indicated by their willingness to sign informed consent. Data obtained from medical records is entered into a master table/matrix using the Excel program, then processed using SPSS. Data analysis using statistical programs. The data has been collected and processed using SPSS (Statistical Package for Social Science) software version 26 (IBM Production).

Data was analyzed descriptively and analytically. The data analysis used is Univariate analysis carried out by means of the variables in this study arranged descriptively through a frequency table to determine the characteristics of the data distribution. This table contains the characteristics of the research subjects and frequency distribution and Bivariate analysis carried out on two or more related variables. Research data is presented in the form of narratives, tables and pictures/graphs. Presentation in narrative form is used to explain tables or figures. The data is interpreted descriptively to describe the variables that have been determined.

The data was compiled in the form of a research report which was presented before the staff of the Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Hasanuddin University. In carrying out this research, every action was carried out with the permission and knowledge of the patients who were used as research samples through an informed consent form and was declared to fulfill the ethical requirements for implementation from the Commission for Biomedical Research on Humans, Faculty of Medicine, Hasanuddin University with number: 947/ UN4.6.5.31/ PP36 / 2023 of prevaccinated sera absorbance value.

3. RESULT

This research consisted of 131 samples who met the inclusion criteria and participated as samples in this research. The research sample was divided into 3 groups, namely the controlled DM group, uncontrolled DM group, and the non-DM group as a control.

3.1 Basic characteristics of the research sample

In this research sample, there were 6 subjects aged 30-40 years, consisting of 1 man and 5 women, 17 people aged 41 - 50 years, consisting of 5 men and 12 women, aged 51 - 60 years old as many as 40 people , consisting of 9 men and 31 women, aged 61 – 70 years as many as 45 people, consisting of 15 men and 30 women, and aged 71-80 years as many as 23 people, consisting of 10 men and 13 women with a total of 40 men and 91 women (table 1).

Table 1: Age characteristics of all subjects

Age	Male	Female
30-40	1	5
41-50	5	12
51-60	9	31
61-70	15	30
71-80	10	13
Total	40	91

The research sample consisted of 30.5% men and 69.5% women respectively. As many as 20.6% of the entire sample had a history of smoking. In this study, the total sample size was 131 patients who were divided into three groups, namely 25.2% non-DM patients, 36.6% Diabetes Mellitus patients with a controlled glycemic index, and 38.2% Diabetes Mellitus patients with an uncontrolled glycemic index. controlled. Apart from that, 81.6% of diabetes mellitus patients use oral medication and 18.4% use insulin.

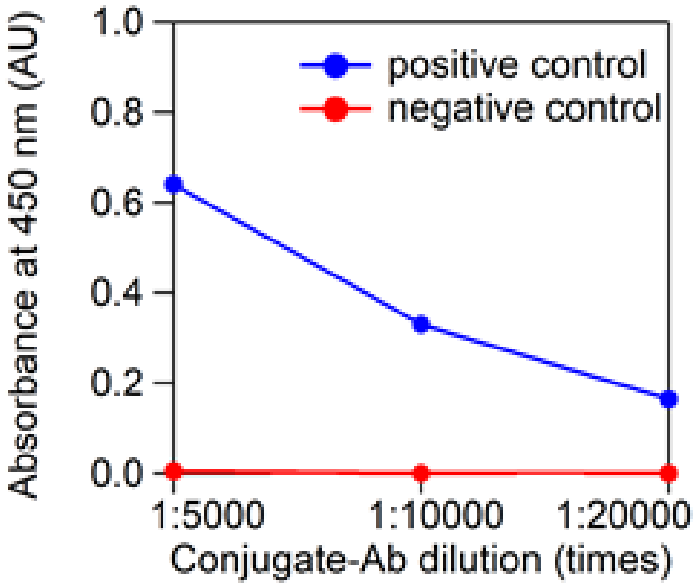

Figure 1: Optimization of antigen concentration and sample dilution. The sample used was seropositive DENV-3 post-vaccination in mice. Variation of antigen concentration are 25, 50, 100, 200 ng/well. Sera dilution performed was started from 1:200 to 1:3200

3.2 Optimization of conjugate antibody dilution

A serum dilution of 1:200 and an antigen concentration of 100 ng were used to determine the optimum conjugate dilution. It is known that TMB substrate has the best sensitivity by using a conjugate dilution of 1:5000 to 1:50.000. Conjugate-Ab dilution variations of 1:5000, 1:10000, and 1:20000 were used in this system optimization. The parameter to determine our optimal conditions is the highest OD450 ratio from positive samples to negative samples. Based on this, the conjugate dilution of 1:5000 was chosen as the optimum point (Figure 2).

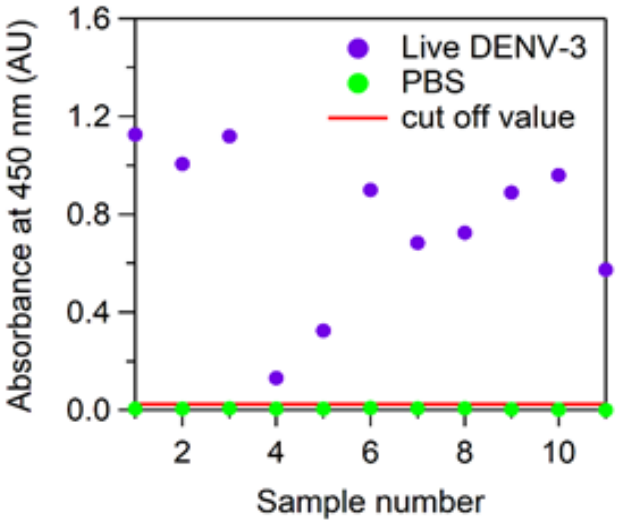

Figure 2: Optimization of conjugate-Ab dilution. The sample used was the seropositive DENV-3 post-vaccination in mice and negative controls (PBS treatment) with conjugate-Ab dilution started from 1:5000 to 1:20000

3.3 Detection of IgG antibody against prM/E DENV-3 and Live wild type virus DENV-3

Detection of IgG anti-dengue prM/E DENV-3 were carried out based on the ELISA optimization results using sample dilution 1:200, coating antigen concentration 100 ng, and conjugate-Ab dilution 1:5000. In the group of mice that received prM/E DENV-3 vaccination, all samples have detected IgG anti-dengue that determined by absorbance value above the cut off value with a mean absorbance value of 0.039. Meanwhile, in the group of negative control mice that were injected with PBS, all samples had absorbance values below the cut off value with a mean absorbance value of 0.0057. The data can be seen in Figure 3 below. These results indicate that the optimized in-house ELISA system can work well.

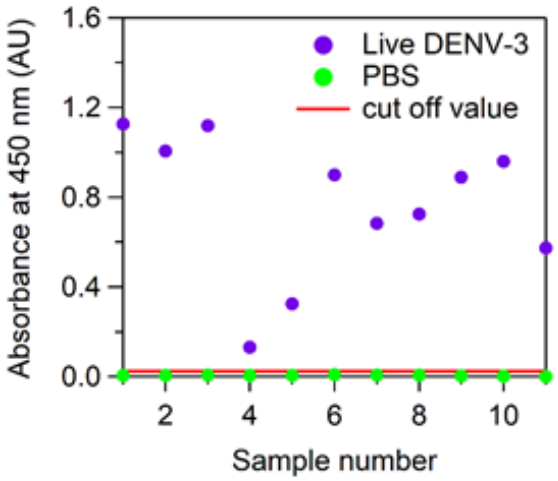

Figure 3: The results of IgG anti-dengue detection using the optimized in-house ELISA method on the mice seropositive that injected with prM/E DENV-3 recombinant protein (blue dots) and mice seronegative that injected with PBS (green dots). The cut off value is two times above the mean+2SD of prevaccinated sera absorbance value

Detection of IgG anti-dengue DENV-3 wt using the ELISA method based on optimization results. The results of this detection have a fairly high absorbance value with a mean of 0.777 with a fairly large data distribution (SD = 0.3056), but all of them were still above the cut off value, which indicates the optimized in-house ELISA system can work well. The data can be seen on Figure 4 below

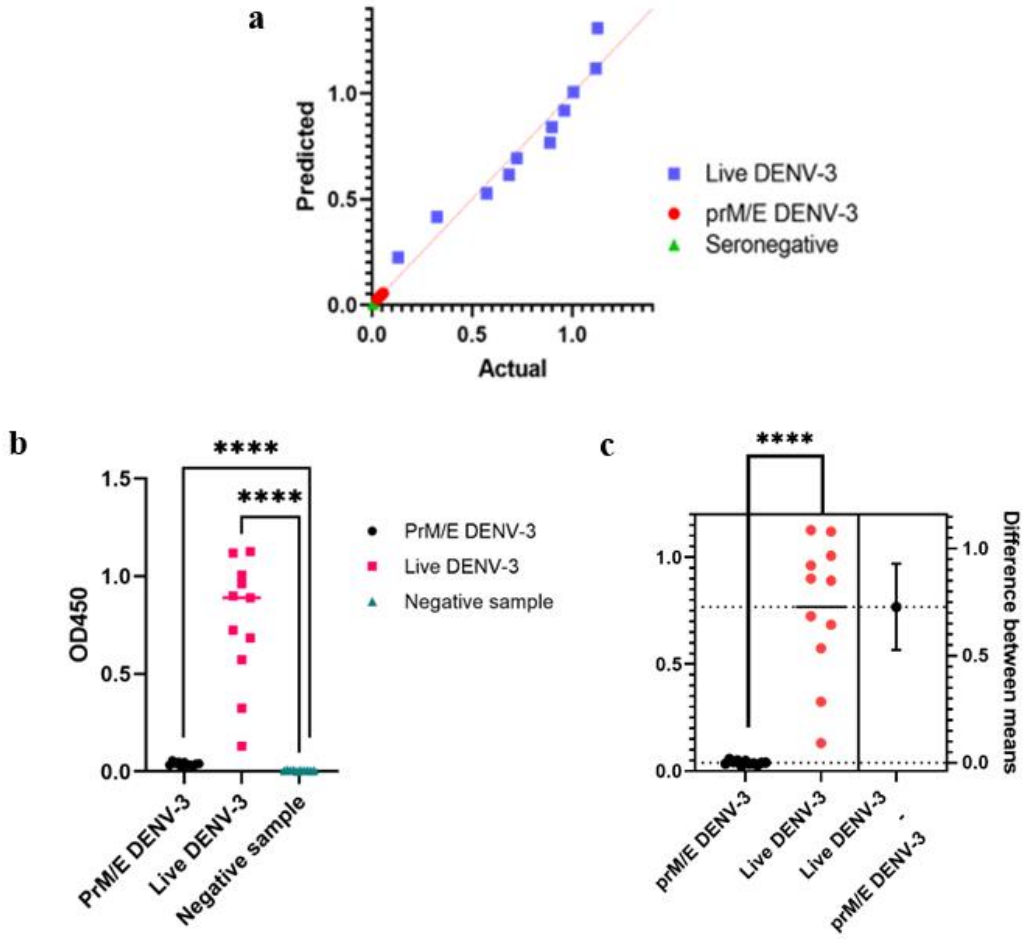

Figure 4: The results of IgG anti-dengue detection using the optimized in-house ELISA method on the mice seropositive that injected with live wild-type virus DENV-3 (purple dots) and mice seronegative that injected with PBS (green dots). The cut off value is two times above the mean+2SD of prevaccinated sera absorbance value

Data processing and analysis was carried out using the SPSS version 21.0 and visualize by GraphPad 9.1.0. The all data distributed normal and passed Kolmogorov-Smirnov test with p>0.1000 (Figure 5a). All data were analyzed by independent parametric t-test to show the significance between groups. We detected strongly significant differences (p<0.0001) between seropositive prM/E DENV-3 samples from 12 mice and seronegative PBS samples. The significant differences were also detected strongly between seropositive live wild-type virus DENV-3 injected samples obtained from 11 mice and seronegative PBS samples (p<0.0001). This result indicates that the optimization of in-house ELISA system can clearly determine the seropositive and seronegative samples (Figure 5b). The significant differences between IgG anti-dengue titer resulted in the prM/E DENV-3 treated group and the live wild-type virus DENV-3 treated group (p<0.0001) could also be detected clearly.

Together, these results showed that the optimization of in-house ELISA system could specifically determine the IgG titer against different antigens and antibody binding.

3.4 Discussion

Dengue fever caused by the dengue virus (DENV) is one of the acute infections whose expansion was still being reported worldwide till now. Even though the efforts of prevention and control of dengue virus infection had been processed in Indonesia by the Ministry of Health, the incidence rate was still considered high seasonally [27]. Since Dengvaxia®, the only available anti-dengue vaccine, showed contraindications on children under nine-year old, the novel anti-dengue vaccine became required [28]. Few of anti-dengue vaccine candidates could be viewed and read in some reviews article [29]. However, those candidates also are still in development stages and still understudied. Recently, QDENGA® (TAK-003) was approved for being used in Indonesia with tight monitoring process which could take time for resulting informative surveillance report from the whole nation. In addition, a prototype of halal anti-dengue vaccine candidates in the form of the prM/E DENV-1,-2,-3,-4 recombinant proteins had been well-developed, however still understudied [21], [22]. Therefore, we would like to detect the efficiency of these recombinant proteins. However, the specificity of commercial ELISA kit in detecting the anti-dengue vaccine candidate of ours was resulted in too high background and unspecific binding while being compared to the used controls. In order to face and well handle this matter and reach better quality of detection and giving the non-bias results, we tried to optimize the in-house ELISA method for the anti-dengue IgG titer in this study.

In some experimental cases, the optimization of one certain detection in order to increase the specificity and efficiency is indeed required. In recent previous studies, an in-house Dot-ELISA rapid test for detection of Toxoplasma gondii infection [30], recombinant nucleoprotein-based indirect ELISA for Crimean-Congo hemorrhagic fever virus-specific antibodies [31], and even helps to solve the problem faced clinically for detecting the antibody form patients receiving TNF-α inhibitors [32]. Besides, there were also changes and validations performed in some previous studies in order to improve the detection while still using the available commercial ELISA kit [33]. Until now, there is no available golden standard for detecting the anti-dengue antibody due to the recombinant protein antigen. Previous studies showed that ICT assay, a relative cost-effective assay, showed low sensitivity for anti-dengue IgM and IgG, while ELISA system definitely has limitations for rapid detection [34], [35]. Regarding this observation, a note to the clinical laboratories had been addressed by previous scientific communication letter [36].

In this study, we also considered the use of blocker and substrate as the important stage. There are several types of protein blockers and substrates for ELISA system available, where each blocker and substrate showed their own advantages and disadvantages. However, BSA was chosen to be used as the protein blocker in this study since BSA had been proven to be a well-working blocker which successfully blocked the non-specific protein-surface bindings [37]. In addition, BSA is relatively cost-effective and considered as hassle-free storage reagent. Being compared to the other type of protein blocker, such as the non-ionic detergent blocker which still facilitated the non-specific binding, BSA showed much higher sensitivity [38]. For the substrate, TMB was chosen as the substrate for this ELISA system optimization. TMB had been demonstrated to have far higher sensitivity, even for the detection of tiny

concentration in picogram (pg), and faster reaction rate than other types of ELISA system substrates, such as ABTS (2,2'-azino-di[3-ethylbenzthizoline]), ONGP (ortho-nitrophenyl-β-galactoside), and OPD (O-phenylenediamine dihydrochloride) in previous studies [39], [40]. In addition, TMB is also safe for being routinely used in laboratory, while OPD is cancerogenic [40].

In conclusion, based on our results, the in-house indirect ELISA system had been optimized from sample dilution, concentration of antigen, and conjugate-Ab dilution. The problem we had with commercial ELISA kit was solved with this optimization. This optimized in-house indirect ELISA system could be used as a guidance and solid protocol for the other three recombinant proteins, prM/E DENV-1,-2,-4, with the suitable antigen used based on the desirable antibody detected. However, we also encourage the researchers worldwide to perform more studies regarding anti-dengue candidate development, the optimization of dengue infection detections including the reliable biomarkers, and the golden standard of anti-dengue IgM and IgG detection.

4. AUTHOR CONTRIBUTION

CSWL and Sunarno conceived and designed the study. GN and Fithriani performed most of the experiments. CSWL, Sunarno, NSDP, HHI and GN analyzed the data. CSWL, HHI and NSDP wrote the first draft. Sunarno, MDH, DTS and SP gave required corrections and comments. CSWL, HHI and NSDP revised the manuscript. All authors read and approved the final version of manuscript.

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