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Comparison of C- Reactive Protein (CRP) Analysis Agglutination Method and Floresense Immunoassay in Smokers at PT. Anugerah Santosa Abadi Surabaya

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Abstract
Introduction: *Smoking has detrimental consequences on the development of diseases with inflammatory components, which cause tissue damage and cause the body to secrete more C-Reactive Protein (CRP). To ensure accurate diagnosis, a variety of techniques, including the latex agglutination method and the Fluorescence Immunoassay (FIA), are available for measuring CRP levels in human blood.* ***Objective:*** *This research is comparing the results of CRP testing performed on smokers at PT. ASA, Surabaya using the agglutination method and the FIA.* ***Method:*** *This research is analytic research with a cross sectional design. This research conduct in PT. ASA, Surabaya from May to June 2023.* ***Result and Discussion:*** *The results show that the CRP agglutination method shows negative results in 27 people (90%). Most of the CRP examination results using the FIA method show negative results in 27 people (90%).* ***Conclusion:*** *Diagnostic test for CRP agglutination using the FIA method obtain a sensitivity of 100% and a specificity of 100%. We can conclude that, there is no difference in CRP levels between the agglutination method and FIA*

Keywords: CRP; FIA; Immunoassay;

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Introduction

Smoking is still becoming a global problem because it effects on health and fatalities. According to WHO figures from 2022, smoking is responsible for 8 million annual deaths. Around 1.2 million deaths are attributed to exposure to secondhand smoke among passive smokers, of whom more than 7 million smoke cigarettes regularly. More than 80% of the 1.3 billion smokers worldwide come from countries with low and middle economies (Organization, 2023). The Central Bureau of Statistics (BPS) estimates that in Indonesia, the proportion of smokers over the age of 15 will be 28.69% in 2020, 28.96% in 2021, and 28.26% in 2022. East Java province saw a 28.51% increase of smokers between 2022 and 2023. To lessen its detrimental effects on public health, smoking is a serious issue that requires attention and effective action (Azizah, Fannya, & Mindhumalid, 2017)

Cigarettes contain 4.000 harmful substances, 200 are carcinogenic substances that cause diseases with inflammatory components, such as chronic obstructive pulmonary disease (COPD). Esophageal cancer, emphysema, laryngeal cancer, bronchitis, lung cancer, pharynxngeal cancer and cardiovascular disease. When tissue damage occurred, the CRP levels will increase with duration of smoking and the number of cigarettes smoked each day (Zulaikhah & Sampurna, 2022)

The CRP as an indicator of acute inflammation that produced by liver, and can be detected in various diseases (Bastian, Sari, & Pratama, 2022). Latex Agglutination and Fluorescence Immunoassay (FIA) commonly used to determine CRP in human blood (Eckschlager, Schwenoha, Roth, Bogner, & Oostingh, 2019)

Quantitatively, the method of latex agglutination is preferred due to its simplicity, affordability, and rapid application. However, this method has a lower sensitivity and can only detect CRP levels of >6 mg/dL (Pramonodjati, Prabandari, & Sudjono, 2019). Another method used to measure CRP is the Fluorescence Immuno Assay (FIA). The FIA method is a sensitive technique used to measure compounds, including drugs, hormones, and proteins, as well as to identify antibodies. The way it works is by adding fluorescence markers and molecules (Indriani, Amalia, & Levita, 2021)

The latex agglutination method has the benefit of low cost and can be widely implemented. Meanwhile, the FIA method offers high sensitivity and specificity, as well as fast analysis times. Because each method has its own advantages and limitations, the researchers were interested in comparing the results of CRP examination using latex agglutination and Fluorescence Immunoassay (FIA) methods among smokers in PT. Santosa Abadi Award. This research aims to determine the comparison of C-reactive protein (CRP) test results using latex agglutination method and Fluorescence Immuno Assay (FIA) among smokers at PT. Santosa Abadi Award. This study aims to compare the results of C-reactive protein (CRP) agglutination method and Fluorescence Immunoassay (FIA) examination in smokers at PT. ASA, Surabaya.

Method

This research is an analytical research with a cross-sectional design. This research was conducted in PT. ASA, Surabaya, from May to June 2023. The population of this study consists of active smokers in PT. ASA, Surabaya. The sample in this study consists of some active smokers in PT. ASA, Surabaya. The sampling technique is a purposive sampling method, with a total of 30 participants.

The independent variable in this research is CRP levels in active smokers using the latex agglutination method. The dependent variable in this study is CRP levels in active smokers using the Fluorescence Immunoassay (FIA) method. This research obtained ethical permission from the ethical commission of Airlangga University Surabaya with number 637/HRECC.FODM/V/2023.

Technique of collecting data

1. Blood sampling procedure

a. Prepare tools and materials
b. Label the vacutainer tube with its identity to prevent sample confusion.
c. Apply a torniquet 3 cm above the arm fold to do blood damming.
d. Use the index finger to palpate to locate the vein that needs to be punctured.
e. The area of the vein to be punctured must be noticed for inflammation, dermatitis, or scars.
f. Hold the syringe with your right hand, and cotton on your left, then wipe the area to be pierced with 70% cotton swab wait until dry.
g. Pierce the vein slowly with a needle in a position forming an angle of 150C. Push the needle slowly into the vein, if blood is seen entering the syringe, pull gently until a blood volume of 3 cc is obtained.
h. Remove the torniquet, give a dry cotton swab where the puncture marks.
i. Band-aid is used to plaster puncture wounds.
j. Place the blood in the vaunter tube and slowly homogenize it.

2. Agglutination method examination procedure

a. Qualitative Examination:
    a) Carefully homogenize the CRP latex reagent,
    b) pipette up the slide circle of serum sample by 1 drop (50μL), positive control (CP) and negative control (CN),
    c) Add 1 drop of latex reagent (CRP antigen) each to the circle.
    d) Homogenize by rotating on the rotator at a speed of 100 rpm for 2 minutes.
    e) Examine the results under bright light. Agglutination that occurs shows positive CRP (CRP in specimens ≥ 6 mg / L)

b. Semi Quantitative Examination:

Serum with a qualitatively positive method, followed by the determination of CRP in serum. That is by diluting the sample in series, by means of:

a) Pipette 50 µL NaCl 0.9% to the top of 6 circle slides.
b) After that, a pipette of 50 µL of serum to the top of circle I (dilution 2 times), homogenized.
c) Suspension pipette from circle I as much as 50 µL to the top of circle II (dilution 4 times), up to slide V (dilution 32 times).
d) If the result in circle V is still positive, pipette 50 L to circle VI (for stock).
e) Add to each circle of CRP latex reagent 1 drop.
f) Homogenize by rotating on the rotator at a speed of 100 rpm for 2 minutes. After that, the results are read under bright light. The highest dilution that is still positive (appears agglutination) multiplied by 6 mg/L indicates the CRP titer in the serum specimen examined

3. Fluorescence Immuno Assay (FIA) method examination procedure

a. Prior to testing, confirm that the Test Cartridge lot number corresponds to both the ID Chip and the Detection buffer.
b. Insert the ID Chip into the FIA Wondfo System.
c. 5 µL serum or plasma sampling with transfer pipette
d. Add it into the Detection Buffer tube.
e. Cover the detection buffer tube and thoroughly combine the sample mixture.
f. Beat approximately 10 times.
g. Pipette the sample mixture 75 µL and insert it into the test cartridge; a. Wait a few minutes according to the manufacturer's instructions, usually between 10-15 minutes. b. After the specified time, the test results will appear in the indicator area of the test kit

Data Analysis

After collecting the data, the Mann Whitney test is used to analyses it.

Research and Discussions

Result

All smokers in this research is men. The ages distribution of respondents is shown in Table 1 as follows:

Table 1

Age of respondents

Age	Total	Percentage
21-30 years	13	43,33
31-40 years	10	33.33
41-50 years	7	23.33
Total	**30**	**100**

According to Table 1 a total of 13 people (43.33%) who smoke are between the ages of 20 and 30. 10 people (33.33%) were between the ages of 31 and 40, while 7 people (23.33%) were between the ages of 41 and 50. The following is a depiction of the findings from a CRP test conducted on active smokers at PT. ASA, Surabaya, using the latex agglutination method:

Table 2
CRP Test Results using Latex Agglutination Method

Results of CRP latex agglutination method	Total	%
Negative	27	90
Positive	3	10
Total	**30**	**100**

According to Table 2, most of the CRP test results using the latex agglutination method show negative results, with 27 individuals (90%), while 3 individuals (10%) show positive results. The results of CRP examination using the *Fluorescence Immunoassay* (FIA) method in active smokers at PT. Anugerah Santosa Abadi, Surabaya, is displayed as follows:

Table 3
CRP Examination Results using the Fluorescence Immunoassay Method

FIA method CRP results	Total	%
Negative (< 5)	27	90
Positive (>5)	3	10
Total	**30**	**100**

According to Table 3, it is known that most of the results of CRP examination using the *Fluorescence Immunoassay* method show negative results, with 27 individuals (90%), while 3 individuals (10%) show positive results. The test results obtained are then tested with diagnostic tests to determine the sensitivity and specificity of the latex agglutination method compared to the *Fluorescence Immunoassay* (FIA) method.

Diagnostic test results show 100% sensitivity and 100% specificity for latex agglutination test with FIA. Furthermore, data analysis is carried out to determine the difference in CRP examination results between the latex agglutination method and the FIA method using the Mann-Whitney test. The results of the Mann-Whitney test show a significance value of 1.00 ($p > 0.05$), so there is no significant difference in CRP levels between the latex agglutination method and FIA (*Fluorescence Immunoassay*)

Discussion

The results show that most smokers are in the range age of 20-30 years, with 13 individuals (43.33%). This finding is different from the Central Statistics Agency data in 2022, which states that most smokers are in the range age of 35-39 years. Smoking habits are also influenced by age, with drastic increases during productive age due to misconceptions about smoking that begin as early as adolescence. Due to increased illness susceptibility and the need to stop smoking, smoking behaviors typically fall dramatically in the aged population (Lianzi & Pitaloka, 2014)

The results from latex agglutination method and FIA method showed that most smokers had negative CRP results (no increase in CRP levels), with 27 individuals (90%). In this study, most active smokers had negative CRP results (no increase in CRP levels), which may be due to the short duration of smoking among respondents and the type of cigarettes they consumed. Most responders are between the ages of 20 and 30. This suggests that smoking cigarettes for a short period of time does not result in inflammation. Additionally, the cigarettes which smoked are having filters, which lessen exposure to the poisons that are included in cigarettes, particularly nicotine, tar, and carbon monoxide (CO) gas. This condition shows that respondents have not experienced tissue or organ damage that will stimulate the secretion of inflammatory biomarkers, namely CRP (Pramonodjati et al., 2019)

CRP examination can be done using the latex agglutination method and the FIA (*Fluorescence Immunoassay*) method. The principle of the latex agglutination method for CRP examination is to coat antibodies on particles to determine the presence of antigens in serum specimens. In this test, a suspension of latex particles coated with human anti-CRP antibodies is added to the tested serum specimen. Visible agglutination signifies an increase in CRP levels to clinically significant levels (Kalma, K at (Pitaloka, 2021)

On the other hand, the FIA method works by adding fluorescence markers and light-emitting molecules in the detection process. The measured analyte can be either an antigen or an antibody, and a fluorescence label is affixed to one or both reactants (Indriani et al., 2021)

Analysis of the data shows no significant difference in CRP levels between the latex agglutination method and the FIA method. The diagnostic test results show 100% sensitivity and 100% specificity for the latex agglutination method compared to the FIA. CRP (C-reactive protein) levels can be measured by various methods, including latex agglutination method and FIA (Fluorescence Immunoassay) method. If no difference in results is detected between these two methods, some possible reasons are: Method Sensitivity: Both the latex agglutination method and the FIA method have high sensitivity in detecting CRP.

They are designed to detect very low levels of CRP in blood samples. If there is no difference in the results obtained from these two methods, it may be because they have similar detection limits. In this study, the sensitivity of the latex agglutination method to FIA for CRP was 100%. Proper Technique and Methodology: The correct technique and adherence to proper procedures can contribute to obtaining similar examination results. Technical factors such as dilution of the sample, use of appropriate reagents, and regular incubation time can affect the test results. If the methods and techniques used in these two methods have been optimized, the results will likely be consistent. Reagent Quality: The quality of the reagents used in both methods can affect the test results. If a high-quality reagent that meets the requirements set is used, it is likely that the results will be similar between the latex agglutination method and the FIA method (Guo et al., 2018)

Conclusion

Based on the results of this study, it can be concluded that most of the results of the latex agglutination method and the FIA (Fluorescence Immunoassay) method showed negative CRP results, with 27 individuals (90%) on both methods. Diagnostic tests of the latex agglutination method compared to FIA resulted in 100% sensitivity and 100% specificity. There was no significant difference in CRP levels between latex agglutination methods and FIA (Fluorescence Immunoassay).

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