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Changes in D-dimer, Ferritin, and Fibrinogen in Healthy Smokers and Nonsmokers during the Covid-19 outbreak

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Abstract

Objective: The Covid-19 outbreak has altered our perspective on evaluating test markers in inflammatory diseases. Furthermore, natural alterations and baseline inflammatory responses, such as those caused by smoking, need a more exact interpretation of these clinical features. In this context, the aim of the study is to determine the changes in smokers and non-smokers in the specified parameters and to have a more detailed idea about the relevant parameters of Covid-19 disease.

Material and Methods: Blood samples were taken from 30 healthy volunteers on the first, third, and fifth days, 15 of whom were smokers, and 15 of whom were nonsmokers. Multiple D-dimer, ferritin, and fibrinogen tests were performed with repeated readings. After ensuring normality and homogeneity of the data and removing outliers, differences among groups were examined using the Student's t-test. A one-way ANOVA test was used to examine fluctuations over time.

Results: Smokers had significantly higher levels of d-dimer on the first day and fibrinogen on the third and fifth days than nonsmokers ($p=0.02$, $p=0.047$, and $p=0.039$, respectively). The ANOVA test discovered statistically significant distinctions in all metrics between participants and dates ($p<0.001$).

Conclusions: As a result of smoking, unexpected changes were detected in d-dimer, ferritin, and fibrinogen compared to non-smokers. According to these results, it was determined that smoking has a negative effect on inflammatory markers. When interpreting laboratory results in smokers with Covid-19 disease and similar diseases, it should be kept in mind that the specified parameters may vary depending on factors other than the disease.

Keywords: Covid-19; Biological variation; SARS-CoV-2; Smoking; Inflammation

Introduction

Coronavirus disease-2019 (Covid-19) has created a global pandemic with a significant fatality rate worldwide with severe or critical illness. Furthermore, it has been associated with SARS-CoV-2 infection-induced hyperinflammation of the innate and adaptive immune systems and the subsequent cytokine storm [1]. As a result, d-dimer, fibrinogen, and ferritin have influenced clinical decisions [2,3[. Tobacco smoke contains a complex mixture of chemicals, including reactive oxygen and nitrogen species, which can damage lipids, proteins, and nucleic acids, among other cellular and subcellular targets. Accumulating evidence shows that smoking-induced

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reactive oxygen species (ROS) and the consequent oxidative stress play a crucial role in inflammation and carcinogenesis [4]. Genetic support for a causal relationship between smoking behaviors and the Covid-19 phenotypes studied was found through Mendelian randomization analyses, which showed that genetic variants predicting differences in smoking initiation and heaviness were linked to an increased risk of SARS-CoV-2 infection and severe Covid-19-related outcomes [5]. Monitoring biochemical markers help understand a patient's clinical course in the context of other exams and clinical findings; however, other factors, such as smoking status, must be considered when concluding a patient's outcomes [6].

When performing diagnosis and treatment in patients or healthy individuals, it should be known that results within the population-based reference range may be pathological. In contrast, results outside it may be physiological [7]. One of the two main reasons for these differences is analytical variation, and the other is biological variation. Laboratory professionals regularly strive to minimize analytical variation. However, natural changes cannot be intervened due to their nature and are often overlooked. Therefore, it is essential to know the variations in diseased and healthy individuals to provide the most clinical benefit, which may affect the clinical picture. In order to clarify this point, we examined the interday changes of d-dimer, ferritin, and fibrinogen, which are important laboratory parameters for Covid-19 and various other inflammatory diseases in healthy individuals. In addition, increases in these parameters have been reported in healthy smokers [8,9]. These increases can be confusing for smokers in the case of Covid-19 or the like. Therefore, incorrect treatment may be applied by interpreting the clinical course of the patients differently. To prevent this situation, it is essential to have information about the changes in inflammatory parameters related to smoking. This study aims to investigate the effect of smoking on inflammatory laboratory parameters, which are essential in the follow-up of Covid-19 patients. Another aim is to provide a different perspective on the parameters specified in Covid-19 patients who smoke by detecting the changes in D-dimer, ferritin, and fibrinogen levels resulting from smoking.

Materials and Methods

Subjects and study design

Thirty healthy participants participated in the study of which 15 were smokers, and 15 were non-smokers and did not have tobacco exposure. Volunteers were chosen from the laboratory staff. The smokers smoked one pack (20 cigarettes) daily for at least ten years. As a result of smoking's prothrombotic, inflammatory, and endothelial effects, the groups were divided. On the first, third, and fifth days, subjects provided samples on three occasions. Pregnant women and current Covid-19 patients were excluded from the study.

Ethical approval

The subjects were given a thorough description of the study. Individuals provided informed consent. The study with the 2021-012 number was approved by the Amasya University non-invasive studies ethics committee.

Samples

On the first, third, and fifth day, the participants' fasting blood was taken in a yellow-capped tube with no gel addition and a blue-capped citrate tube. The same skilled phlebotomist collected blood samples between 8 and 10 a.m. after an 8-hour overnight fast. After drawing blood for 20 minutes, plasma and serum samples were collected by centrifuging the blood at 1500 G for 15 minutes. Samples were stored at -80 degrees Celsius until the day of the investigation. On the day of analysis, after waiting for the samples to dissolve at room temperature, they were centrifuged at 1500 G for 15 minutes. All tests were conducted in duplicate to rule out analytical variation. On a Siemens Advia Centaur XPT immunoassay autoanalyzer (Siemens Healthineers AG, Erlangen, Germany) with a reference range of 10-322 ng/mL, 2.1-3.0 within run % CV, and 2.7-5.4 between run % CV, serum ferritin measurements were done. Fibrinogen levels were detected using the Claus clotting technique and a Stago Compact Max 3 automated coagulation analyzer (Diagnostica Stago, Inc., Parsippany, NJ, USA), with a reference interval of 200-400 mg/dL, 1.60-1.72 within-run %CV, and 3.77-4.77 between-run %CV. Plasma d-dimer levels were analyzed with an Erba XL 1000 clinical chemistry analyzer using a turbidimetric method and Archem brand reagent (Archem Health Ind. Inc. Başakşehir, Istanbul, Turkey). The 99th percentile limit is 0.5 g FEU/mL, within the run % CV range of 1.3-3.7 and between the run % CV range of 3.3-4.5. All analyses were conducted on the same day using calibrators, controls, and reagents from the same lot. On the dates of the tests, one normal and one pathological quality control sample were assessed for every analyte to ensure they were both appropriate for analysis. All volunteers' assays were conducted using the same calibrator curve to reduce analytical variation.

Statistics

Between replicates, within people, and between individual values were evaluated, and the Shapiro-Wilk test was used to determine distribution. The Dixon-Reed criterion was applied to find outliers in the mean between-subject values for analytes. Furthermore, Cochran's C test was used to exclude outliers from within-subject results, such as repeated measurements. After processing the extreme values' findings, the variances' homogeneity was studied by applying the Bartlett test to all data, including repeated measurements. Due to the parametric nature of the data, Student's t-test was

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employed to evaluate day and daytime smoking. The one-way ANOVA test determined the parameters' changes over time and between individuals. A p-value less than 0.05 was deemed significant. Excel 2019 (Microsoft Corporation, Redmond, Washington, United States) and Minitab 19 were utilized to conduct statistical analyses (Minitab Ltd., Coventry CV3 2TE, UK).

Results

Thirteen women with a mean age of 42.58.4 and 17 men with a mean age of 44.19.5 were included in our study. Non-smokers were younger and comprised more women than smokers. There is no discernible difference in sex between smokers and non-smokers. The levels of d-dimer on the first day and fibrinogen on the third and fifth days were substantially higher in smokers than in non-smokers (Table 1). When all participants were evaluated, d-dimer levels differed considerably on days one and five. In the same examination, the mean values for d-dimer on the third and fifth days and ferritin on the first and third days differed significantly (Table 2). ANOVA revealed significant differences between days and between individuals. Table 3 displays the variations in parameters over time and across individuals.

Table 1: Descriptive statistics according to smoking.

		Mean	Std. Deviation	Minimum	Maximum	p-value
Age	Non- Smoker	39.933	8.163	28	52	0.03
	Smoker	46.933	8.639	34	60	
D-dimer 1st (FEU/mL)	Non- Smoker	0.062	0.044	0.015	0.14	0.02
	Smoker	0.102	0.045	0.025	0.175	
D-dimer 3rd (FEU/mL)	Non- Smoker	0.074	0.034	0.025	0.15	0.808
	Smoker	0.077	0.033	0.03	0.14	
D-dimer 5th (FEU/mL)	Non- Smoker	0.062	0.034	0.025	0.14	0.941
	Smoker	0.063	0.04	0.01	0.15	
Fibrinogen 1st (mg/dL)	Non- Smoker	249.967	46.241	186.5	341	0.207
	Smoker	277.5	68.302	192	416.5	
Fibrinogen 3rd (mg/dL)	Non- Smoker	259.1	49.299	173.5	349	0.047
	Smoker	287.833	83.558	155.5	410.5	
Fibrinogen 5th (mg/dL)	Non- Smoker	256.567	47.683	161.5	359.5	0.039
	Smoker	263.8	92.759	72	430.5	
Ferritin 1st (ng/mL)	Non- Smoker	43.707	67.447	2.85	219.65	0.861
	Smoker	47.533	49.836	4.7	203.65	
Ferritin 3rd (ng/mL)	Non- Smoker	46.22	67.893	3.05	213.5	0.604
	Smoker	59.31	68.895	5.245	274.6	
Ferritin 5th (ng/mL)	Non- Smoker	44.233	64.404	4.65	218.4	0.562
	Smoker	58.973	72.747	7.4	294.15	

Table 2: Day-to-day changes of parameters.

Measure 1		Measure 2	p
D-dimer 1st	-	D-dimer 3rd	0.374
D-dimer 1st	-	D-dimer 5th	0.032
D-dimer 3rd	-	D-dimer 5th	0.006
Fibrinogen 1st	-	Fibrinogen 3rd	0.199
Fibrinogen 1st	-	Fibrinogen 5th	0.817
Fibrinogen 3rd	-	Fibrinogen 5th	0.406
Ferritin 1st	-	Ferritin 3rd	0.025
Ferritin 1st	-	Ferritin 5th	0.145
Ferritin 3rd	-	Ferritin 5th	0.491

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Table 3: Variations in parameters over time and between individuals were shown in three groups.

		D-dimer	Ferritin	Fibrinogen
All individuals	Day-to-day	<0.001	<0.001	<0.001
	Between Subjects	<0.001	<0.001	<0.001
Smokers	Day-to-day	<0.001	<0.001	<0.001
	Between Subjects	<0.001	<0.001	<0.001
Non-smokers	Day-to-day	<0.001	<0.001	<0.001
	Between Subjects	<0.001	<0.001	<0.001

Discussion

Life is a continually evolving biological process. Laboratory parameters vary according to an individual's genetic traits, environmental variables, and smoking habits. Consequently, population-based reference ranges, commonly employed in medical laboratories, may not be adequate for the clinical evaluation of individuals. Additionally, distinct individual responses to infection may occur, particularly in pandemics such as COVID-19, for which the complete picture is not yet known.

We found significant increases in first-day d-dimer and third-fifth days fibrinogen values of smokers. A study on nearly three thousand elderly individuals determined that smoking increases c-reactive protein, d-dimer, and fibrinogen [10]. In another study on Sudanese smokers, d-dimer levels were higher than in healthy individuals [11]. A study on healthy individuals showed that smokers had increased fibrinogen levels [12]. In a study conducted with young women, it was stated that high fibrinogen levels were determined in smokers and that these individuals should be followed up with fibrinogen levels [13]. In a Korean study of 50405 participants, it was found that serum ferritin values were higher in former or current smokers and with the smoking amount in all subgroups of participants [14]. With the studies we mentioned, our data is compatible with the contribution to the literature except for ferritin. However, we could not detect any difference between smokers and non-smokers in terms of d-dimer levels on the third and fifth days and fibrinogen levels on the first day. In addition, when ferritin values were considered, there was a difference between groups. At this point, intra-individual and inter-individual biological variation came to the fore. In addition, smoking, a determining factor in inflammation, did not have a similar effect among smokers. One way Anova analysis revealed significant differences in the repeated measurements of both groups and individuals on different days.

The European Federation of Laboratory Medicine (EFLM) has created a database that reveals the effect of biological variables on laboratory results [15]. At this point, the aim is to consider the impacts of natural changes when interpreting laboratory results. For example, a study dealing with thromboembolic events in pregnant women argued that a biological variation-based approach would be helpful when interpreting d-dimer levels [16]. A different study revealed that biological variation was detected in fibrinogen, albeit at a low level [17]. In a survey conducted with a small number of individuals, researchers found the rate of biological variation in ferritin to be over 21 percent [18].

The inflammatory effects of cigarette smoking will differ according to individuals with different physical structures. Although we could not find any research on this subject in the literature, it is natural for smokers to have biological variations in laboratory parameters like non-smokers. This approach is essential for individuals who smoke and have inflammatory diseases such as Covid-19. According to the World Health Organization, smokers are more likely than never smokers to have more severe COVID-19 outcomes, such as admission to intensive care units and death. Furthermore, severe COVID-19 or fatalities from COVID-19 are more common in patients with tobacco-related comorbidities, such as COPD, lung cancer, and cardiovascular disease [19]. Therefore, when making clinical decisions in treating these patients, changes due to Covid-19 inflammation and those due to smoking should be considered in addition to biological variation. While the patient is recovering from Covid-19, the expected changes in parameters may not occur due to long-term smoking, and malpractice may be applicable. Alternatively, the source of the increase in inflammatory parameters may not be found. In the approach to different inflammatory diseases, knowing the basal inflammatory events such as smoking and interpreting the laboratory results with this knowledge will provide more patient benefits.

Conclusions

Variations in inflammatory laboratory parameters can be detected in healthy people based on their biological characteristics, and the population-based reference period may be inadequate for clinical evaluation. Therefore, every person should be examined internally. Clinicians will employ the same methodology when treating smoking and other comparable inflammatory diseases. From this perspective, it is beneficial to examine the factors contributing to the inflammatory response, particularly in diagnosing and treating Covid-19 and other systemic inflammatory illnesses.

Limitations

First, this study was done with healthy individuals and did not represent the clinical and biological features of patients with Covid-19 or other related diseases. In addition, the research is not an average biological variation analysis prepared according to the protocols outlined in the EFLM standards; rather, it is meant to track daily change. Moreover, since the amount and rate tobacco smokers consume are not precisely established, no interpretation was made. Lastly, the fact that we could not examine the effect of smoking on inflammatory parameters in individuals who had Covid-19 since the Covid-19 history of the individuals we selected is unknown was another factor that limited our study.

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Declaration of interest

All authors agree that there is no conflict of interest.

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Authors' contributions

All authors contributed equally to preparing the manuscript.

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