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ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2022;11(1):159-65

Investigation of the use of seratec pmb test on postmortem peripheral blood samples for forensic purposes

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Received 27 August 2021; Accepted 31 October 2021
Available online 20.01.2022 with doi: 10.5455/medscience.2021.08.274

Abstract

The identification of the origin of biological stains at the crime scene is one of the important components of forensic science. In this study, it was aimed to evaluate the specificity performance of Seratec-PMB Test, which is used to differentiate menstrual blood from peripheral blood, on blood samples collected from dead bodies. Moreover, our purpose is to investigate the relationship between D-dimer accumulation in postmortem blood and other factors using this method. In this study, Seratec-PMB test is applied to peripheral blood samples of 200 dead bodies and the test results were compared in terms of parameters including age, gender, time between being found dead and sample collection, the cause of death. In Seratec-PMB Test, 71% of postmortem blood samples were positive and 29% were negative for D-dimer presence. A significant relationship was reported in the distribution of test results by age. SERATEC®-PMB test afforded a high rate of positive results in postmortem peripheral blood. It should be remembered that the test may give false positive results for postmortem peripheral blood. Since the test is a semi-quantitative test, it was understood that it would not allow any interpretation about the cause of death.

Keywords: D-dimer, immunochromatographic assay, menstrual blood, peripheral blood

Introduction

Determining the origin and type of biological stains at the crime scene is one of the most important components in forensic practice. In cases where blood is present on any surface, the accurate differentiation between peripheral blood that refers to a traumatic cause and menstrual blood can potentially provide information about the subject of consent, e.g., in sexual assault allegations [1, 2]. Moreover, it can be a crucial factor to speed up the conclusion of the case, as well as to provide supportive results for the victim or findings in favor of the defendant. Thus, a method that can be used to reliably differentiate between menstrual blood and peripheral blood would provide a significant advance in the analysis and interpretation of the evidence. Recently, the use of immunochromatographic assays that detect fibrinolysis degradation products as innovative methods for identifying menstrual blood in forensic samples has been successfully implemented [3]. Fibrinolysis is an enzymatic process that prevents unnecessary accumulation of intravascular fibrin and enables thrombus removal [4]. Fibrinolysis is tightly controlled by a number of proteins and receptors, including various inhibitors and cofactors [5]. Plasmic degradation of non-crosslinked fibrin yields fibrin degradation products, the smallest of which is the D-dimer [6-10]. During menstruation, fibrinolysis is an important stage to prevent blood coagulation and allow the menstrual blood to easily pour out. D-Dimer, one of the fibrin degradation products, has an average concentration of 200 times higher in menstrual blood than peripheral blood [11]. Similarly, it is known that D-dimer is formed in postmortem blood [12, 13]. Certain studies state that postmortem peripheral blood stains may not be easily distinguishable from menstrual blood [14, 15]. SERATEC PMB test (SERATEC GmbH, Göttingen, Germany), a new immunochromatographic test introduced in recent years, is a method developed for detecting human hemoglobin and D-dimers. This method is the first D-dimer test developed to distinguish between human peripheral blood and human menstrual blood,

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particularly in forensic samples. The SERATEC PMB test is easy and fast to use directly at the crime scene or in the lab and does not require special training for analysts. [16]. The SERATEC PMB test, which is newer and limited in use compared to other rapid tests for forensic purposes, was validated on different samples by Holtkötter with two different studies. However, larger studies are required to evaluate the performance of the test on postmortem blood samples under different conditions. In this study, it was aimed to investigate the specificity performance of SERATEC PMB Test, which is used in the differentiation between menstrual and peripheral blood, in postmortem peripheral blood samples and its feasibility for forensic purposes. Moreover, it is considered to evaluate the relationship between D-dimer accumulation in postmortem blood and other factors such as age, sex, time between being found dead and sample collection, and cause of death.

Materials and Methods

Postmortem blood samples were planned to be collected from Department of Morgue Specialization, Forensic Medicine Institute. Research permission was obtained with the decision of the Forensic Medicine Institute Education and Scientific Research Commission dated 20.05.2019 and numbered 21589509/2019/404. Ethical approval was obtained with the decision of Cerrahpaşa Medical Faculty Clinical Research Ethics Committee dated 03.07.2019 and numbered 83045809-604.01.02. This study was supported by the decision of Scientific Research Projects Coordination Unit of Istanbul University-Cerrahpasa dated 27.11.2019 and numbered 2019/21 Meeting. The study started after the necessary approval and equipment were completed.

Sample Collection

The study was performed on postmortem peripheral blood samples of cases delivered to Istanbul Morgue Specialization Department of Council of Forensic Medicine between 01.01.2020 and 10.07.2020 for forensic autopsy. The sampling process was completed when 200 cases were reached, considering age, gender, and time between being found dead and sample collection, as well as the variety of cause or origin of death. Sampling was not performed if the cause of death could not be clearly evaluated, sufficient data could not be obtained, the deceased was severely decayed, and sufficient samples were reached for one cause of death. The information recorded during the autopsy was later confirmed from autopsy reports. Bloodstains were produced within a maximum of 1 h by allowing three drops of postmortem peripheral blood onto 100% cotton, white and clean swatch using a sterile disposable syringe. The blood-stained swatches were dried for 24 h at room temperature. Moreover, 10 menstrual blood samples was collected by vaginal swabs from 10 volunteers with informed consent and a stain was created on 100% cotton, white and clean swatches with this swabs. These menstrual blood samples were regarded as the positive control group for the rapid test kits used in the study. All menstrual bloodstain samples were dried at room temperature for 24 h. Each dried swatch was stored in a separate envelope. The samples were analyzed gradually three days after the stain was produced and dried without waiting for the collection of other samples. Moreover, the D-dimer level was measured with competitive ELISA-based kits in 20 randomly selected postmortem blood samples, and compared with the SERATEC PMB test results.

Test Preparation and Procedures

SERATEC® PMB Test (SERATEC GmbH, Göttingen, Germany) is a chromatographic immunoassay method that contains monoclonal antibodies as active compounds. The cassette of the assay contains one sample well and one result window with a control line (labeled “C” for control), a result line for human hemoglobin (labeled “P” for peripheral blood), and a result line for D-dimer (labeled “M” for menstrual blood). Moreover, the assay includes a buffer solution for liquid sample dilution or dried sample extraction. The optimized sensitivity of the test is stated to be 20 ng/mL for hemoglobin and 400 ng/mL for D-dimer [16]. If a high-dose hook effect was suspected in hemoglobin detection, a dilution of the original sample was performed, and an analysis of this diluted sample was done again [16]. The sample numbers were written on the 1.5 ml buffer solution bottle and cassette provided by the SERATEC® PMB test kit for each sample prior to the study. Moreover, a form in which the results are maintained was created and the sample numbers, the date of application of the test and the test results were recorded on the form.

Application of the test

An area of 1 cm x 1 cm from the dried bloodstains was measured with a ruler and cut with a sterile disposable scalpel tip. The ruler was sterilized every time before cutting the bloodstain. Each cutting was placed into 1.5 ml buffer solution provided by the SERATEC® PMB test kit for extraction. After 60 min of extraction, three drops (~120 µL) of the extracted solution were added into the sample well in the test cassette using the disposable plastic pipette provided by the kit. Test results were evaluated after 10 min of incubation at room temperature.

Evaluation of Test Results

In the evaluation of the test results of the D-dimer cassette, the interpretation guidelines used by Holtkötter et al. [3] were considered. Table 1 shows the interpretive rules applied in this study. Although a pink band was visible for the “C” and “P” test result lines in the cassette, the results were categorized as "+", "++" and "+++", depending on the intensity of the pink band seen in the “M” test result line “M”. The "+" category has been interpreted as an extra light pink band visible only with careful inspection and too faint to be captured by a camera under normal circumstances, indicating a weak positive result. The"++" category has been interpreted as a light pink band visible at first glance without the need for careful examination, indicating a positive result. The category "+++" has been interpreted as pink band that can be clearly visible at first glance, indicating a strong positive result. The category "-" represents a pink band visible in the “C” and “P” test result line, with no visible band in the “M” line, which indicates a negative test result for D-dimer. All evaluations were performed by the first author (ÖFG). Note that ~2 weeks after the first evaluation, 10% of all test cassettes randomly selected (20 samples) were re-evaluated for inter-observation agreement. To test the inter-observer agreement, an evaluation was made by the second author (BK) on the same cassettes.

Statistical Evaluation

The research data were evaluated using SPSS 22 statistical

program, descriptive statistics, Pearson chi-square test and Mann–Whitney U test for the purpose. Interobserver agreement was tested using Kappa repeatability analysis.

Results

Cohen's kappa for measuring intra observer agreement for evaluation stages was 0.93, indicating almost perfect agreement. Inter observer agreement (0.73) showed substantial in accordance.

Postmortem Blood Samples

Note that 70.5% (n=141) of a total of 200 cases were male and 29.5% (n=59) were female. The mean age was 45.86±19.04 ranging from 0 to 89. When cases were classified as per causes of death, 8.5% (n=17) of all cases (n=17) were cardiovascular disease (CVD), 8.5% (n=17) were shotgun injuries, 8.5% (n=17) firearm bullet injury, 8.5% (n=17) traffic accident (TA), 8.5% (n=17) hanging, 8.5% (n=17) falling from height, 8.5% (n=17) stab wounds (STAW), 8.5% (n=17) sedatives/narcotics abuse , 6.5% (n=13) CO poisoning, 6% (n=12) drowning in water, 4% (n=8) tamponade and 15.5% (n=31) other conditions. Other conditions included gastrointestinal system (GIS) bleeding (n=5), infection (n=5), pulmonary embolism (n=4), epilepsy (n=3), decay (n=3), ligature strangulation (n=2), buried in the wreckage (n=2), stillbirth (n=2), aspiration (n=2), lung cancer (n=1), fistula bleeding (n=1), and corrosive substance intoxication (n=1). The time between being reported dead and sample collection ranges from 3 to 166 h. The time intervals between being found dead and sample collection were grouped as0–9, 10–14, 15–19, and ≥20 h. All the postmortem bloodstain samples (n=200) positively reacted for the presence of hemoglobin. In 43 of the samples, the hemoglobin (P) line gave a very weak positive reaction, making visualization difficult. Dilution was separately performed for these samples with the suspicion of high-dose hook effect and the test was repeated. Upon retesting, all the diluted postmortem blood samples showed a stronger positive reaction with readily visible pink bands for the presence of hemoglobin. The positive reaction rate for the presence of hemoglobin in postmortem blood samples using SERATEC® PMB Test was reported to be 100%. In the result labeled "M" for D-dimer, 71% (n=142) of all postmortem blood samples (n=200) were positive and 29% (n=58) were negative. The band intensity of positive results in "M" labeled result line for D-dimer varied between samples. Figure 1 shows the distribution of the cases according to the test results. There was no statistically significant difference in the distribution of test results by gender. Figure 2 shows the distribution of test results by gender. When the test results are evaluated according to age groups; 69.2% (n=9) of cases in the first and second decades (n=13) were positive, 30.8% (n=4) were negative, 62.5% of the cases in the third decade (n = 15) were positive, 37.5% (n=9) were negative, 59.5% (n=25) of the cases in the fourth decade were positive, 40.5% (n=17) were negative 76.3% (n=29) of the cases in the fifth decade were positive, 23.7% (n=9) were negative, 71% (n=22) of the cases in the sixth decade were positive, 29% (n=9) were negative, 82.8% (n=24) of the cases in the seventh decade were positive, 17.2% (n=5) were negative, 78% of the cases in the eighth decade or later,(n = 18) were positive and 21.7% (n=5) were negative. A statistically significant relationship was reported in the distribution of test results by age (p=0.041). After the age of 60, the positivity rate of the test was reported to be higher than other ages. Figure 3 shows the distribution of test results by age. There was no statistically significant relationship between the cause of death and test results (p=0.514). Table 2 lists the distribution of test results according to the causes of death. When the test results are evaluated in terms of time between being reported dead and sample collection; 14 (66.7%) of 21 test results were positive, 7 (33.3%) were negative for 0-9 h, 54 (75.0%) of 72 test results were positive, 18 (25%) were negative for 10-14 h, 47 (73.4%) of 64 test results were positive, 17 (26.6%) were negative for 15-19 h, 27 of 43 test results (62.8%) were positive and 16 (37.2%) were negative for >20 h. No statistically significant correlation was reported between the time from being found dead to sample collection and the test results (p = 0.460).

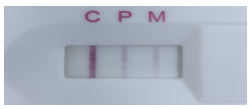
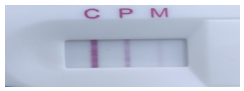
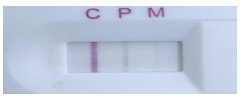
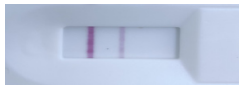
Postmortem Blood Samples with D-dimer detection

Note that 17 (85%) of 20 postmortem blood samples, which were randomly selected and for which D-dimer levels were measured by quantitative methods (Elisa), were positive and 3(15%) were negative. Biochemical D-dimer levels were reported to be more than 400 ng/mL for 17 postmortem blood samples that were positive in the test. Biochemical D-dimer levels of three samples that were negative in the test were reported to be equal to or less than 400 ng/mL. There was no statistically significant relationship between the D-dimer level and the band densities of test results (p=0.421). Furthermore, no statistically significant relationship was reported between age and D-dimer levels (p=0.487). Table 3 lists the cause of death, age, gender, time between being found dead and sample collection, D-dimer level and test results.

Menstrual Blood Samples

Of the menstrual bloodstain samples, 3(30%) were collected on day 1, 3(30%) on day 2, 2(20%) on day 3 and 2(20%) on day 4 of menstruation All the menstrual bloodstain samples (n=10) demonstrated a positive response for the presence of hemoglobin. The positive reaction rate for the presence of hemoglobin was 100% in the menstrual bloodstain samples with the SERATEC® PMB Test. A statistically significant relationship was reported between the days of menstruation on which the blood sample was collected and the test results (p<0.0001). After the onset of menstruation, the band intensity for test positivity decreased day by day.

Table 1. Guidelines for the interpretation of D-dimer results according to the intensity of the pink band seen in the "M" test result line in the cassette window [3]

Result	Description	Sample*
+++	Pink band that can be clearly visible at first glance, indicating a strong positive result	
++	Light pink band visible at first glance, indicating a positive result	
+	Extra light pink band visible only with careful inspection, indicating a weak positive result	
-	No visible band in the "M" test line, which indicates a negative result for D-dimer	

*High resolution formats of the images are added to the supplementary

Table 2. Distribution of test results according to causes of death

Cause/ Origin of Death	Negative		Positive		Total	
	n	%	n	%	n	%
Cardiovascular disease	3	17.6	14	82.4	17	8.5
Shotgun injury	6	35.3	11	64.7	17	8.5
Firearm bullet injury	3	17.6	14	82.4	17	8.5
Traffic accident	7	41.2	10	58.8	17	8.5
Hanging	2	11.8	15	88.2	17	8.5
Falling from height	7	41.2	10	58.8	17	8.5
Stab wounds	5	29.4	12	70.6	17	8.5
Sedatives/narcotics abuse	5	29.4	12	70.6	17	8.5
CO Poisoning	4	30.8	9	69.2	13	6.5
Drowning in water	4	33.3	8	66.7	12	6.0
Tamponade	1	12.5	7	87.5	8	4.0
Other Conditions	11	35.5	20	64.5	31	15.5
Total	58	29.0	142	71.0	200	100.0

Table 3. Cause of death, age, gender, time between being found dead and sample collection, D-dimer level and test results in postmortem blood samples with D-dimer detection

Cause/ Origin of Death	Age	Gender	Time (Hour)	D-Dimer (ng / mL)	Result
CO poisoning	62	Male	16	290	Negative
Traffic accident	51	Male	8	400	Negative
Firearm bullet injury	31	Female	15	370	Negative
Shotgun injury	55	Male	12	940	Weak Positive
Traffic accident	70	Male	10	1830	Positive
Shotgun injury	47	Male	16	560	Positive
CO poisoning	46	Male	10	1010	Positive
Shotgun injury	62	Male	15	2960	Positive
Sedatives/narcotics abuse	41	Male	20	2510	Positive
Hanging	38	Male	12	650	Positive
Drowning in water	26	Male	17	480	Positive
Tamponade	37	Male	6	3010	Strong Positive
Falling from height	54	Male	24	770	Strong Positive
GI bleeding	62	Female	13	910	Strong Positive
Infection	74	Female	13	80000	Strong Positive
Firearm bullet injury	19	Male	9	1830	Strong Positive
Hanging	53	Female	9	2010	Strong Positive
Firearm bullet injury	41	Male	11	1920	Strong Positive
Falling from height	59	Male	13	3770	Strong Positive
Hanging	49	Male	14	35100	Strong Positive

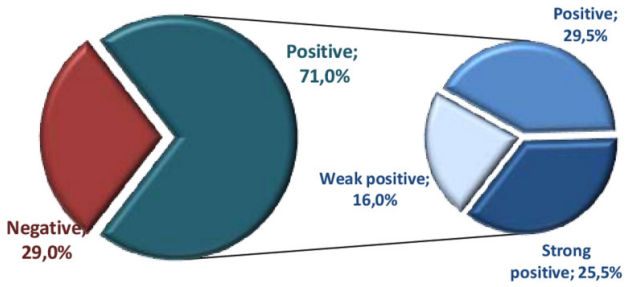


Figure 1. Distribution of the cases according to the test results

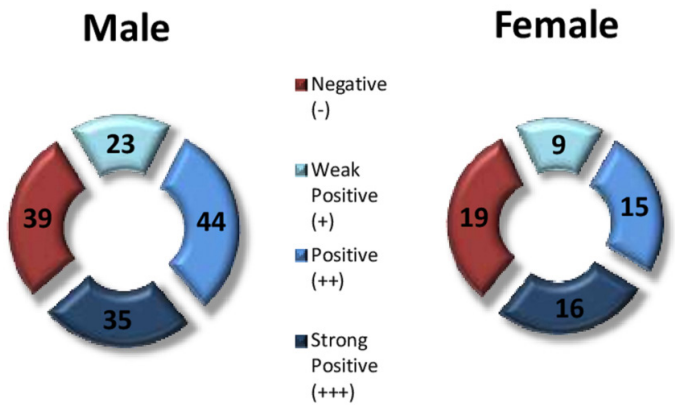


Figure 2. Distribution of test results by gender

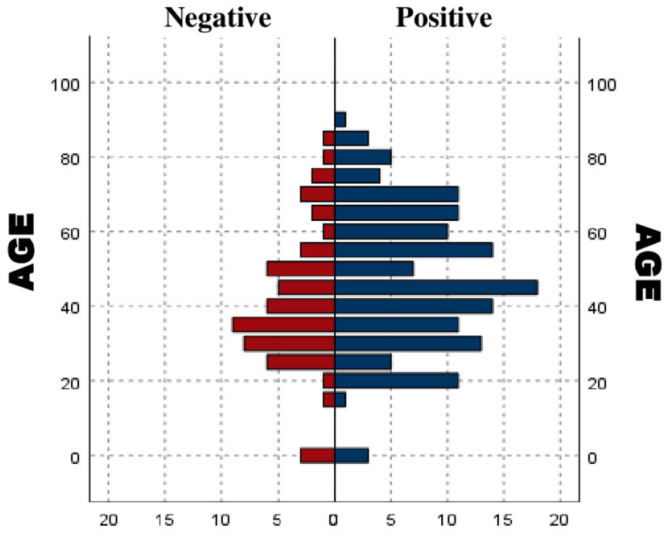


Figure 3. Distribution of test results by age

Discussion

Determining the origin and type of biological stains at the scene is one of the most important components in forensic practice. Blood is one of the most common bodily fluids at crime scenes. In cases where blood is present on any surface, the accurate differentiation of peripheral blood (expressing a traumatic cause) from menstrual blood may contribute to the correct conclusion of the case, especially in sexual assault claims [1,2]. All 10 postmortem and 48 menstrual blood samples in the study of Holtkötter et al. (2018), and 20 postmortem and 10 menstrual blood samples in the study by Wang were reported to have positive reactions for the presence of hemoglobin in the test. In this study, all 210 blood samples, including 200 postmortem and 10 menstrual blood, showed a positive reaction for the presence of hemoglobin in the test and were reported to be consistent with the literature [1,17]. In this study, positive results were obtained in 71% (142) and negative results in 29% (58) of 200 postmortem blood samples for the presence of D-dimer. In the study conducted by Holtkötter et al. (2015), 8 (89%) of the postmortem blood samples taken from nine individuals showed positive results with the immunochromatographic D-dimer test [3]. In another study conducted by Holtkötter et al. (2018), 7 out of 10 (70%) postmortem samples had a positive reaction for the presence of D-dimer by using SERATEC® PMB Test [1]. However, in another study conducted by Wang with a limited number of samples (20 postmortem blood samples), all samples showed positive D-dimer result in the SERATEC® PMB Test [17]. The results of test results in the present study were generally consistent with the studies of Holtkötter et al. The small sample size, lack of knowledge of the sample type and causes of death in the study conducted by Wang may have caused differences between studies. Our study, along with other studies, confirms that this test shows a high rate of false positivity on postmortem peripheral blood samples. The band intensities of D-dimer concentration varied between samples, and classification according to band density demonstrated that 25.5% (51) of all samples were strong positive, 29.5% (59) positive, 16% (32) weak positive. In the study conducted by Holtkötter et al. (2018), 3 of 10 postmortem samples gave a weak positive signal, while 4 gave a strong positive signal. Although there were certain differences in the interpretation of the test results according to the band density, the results were generally consistent. In the study conducted by Wang, it has been stated that 18 out of 20 postmortem blood samples gave strong positive results, while 2 of them showed positive results, which is inconsistent with this study. In the evaluation of the results of these two studies with our study, it seems probable that there may be differences because of the small number of cases, differences in case selection, causes of death, and interpretation of the band density of the test. Large-scale new studies with different samples will shed light on the interpretation of different findings on this topic. When test results are evaluated according to gender; 67.8% of all female were positive, 32.2% were negative, and 72.3% of all male were reported to be positive and 27.7% negative. In the study conducted by Wang, it has been stated that 7 out of 20 cases were female, 13 were male and there was no difference in test results according to gender. In the present study, there was no significant difference in the distribution of test results by gender, and it was compatible with Wang's study. A statistically significant relationship was reported between the test results and the distribution by age, and the test positivity after the age of 60 was higher than the other ages. In the study conducted by Wang, the age of the cases from which postmortem samples were obtained had no effect on the presence of D-dimer, and the results were inconsistent with our study. Wang's study was conducted with only 20 cases, and the causes of death of the cases were not specified. It was thought that there might be incompatibility between the results in the two studies due to differences in the number of cases and the causes of death. There was no other study investigating the relation of D-dimer level with age on postmortem blood samples. In the study of Tita-Nwa et al., 359 out of 776

followed-up individuals with known D-dimer levels were reported to have D-dimer levels of 200< (mean age: 75.2±0.59), 244 have 100-200 (mean age: 64.9±0.85) and 173 have <100 (mean age: 59.2±0.92) [18]. These results demonstrate that D-dimer levels are higher above a certain age, consistent with our study. However, since our method is a semi-quantitative method, it could not be determined to what extent the postmortem effect and antemortem effect contribute to the D-dimer level. It was seen that 17 (85%) of 20 postmortem blood samples, which were randomly selected and for which D-dimer levels were measured by quantitative methods (Elisa), were positive and 3 (15%) were negative. The test results were reported to correlate with the specified sensitivity level for D-dimer. It was thought that there is a requirement for new studies to be performed on more samples on this subject. The evaluation of 20 postmortem blood samples with D-dimer levels measured in biochemical analysis showed no consistent relationship between the D-dimer level and the band intensities of the test results. It was shown in this study that D-dimer was not the only determinant of the band intensity for SERATEC® PMB Test, and it was thought that the band intensity may differ depending on the differences in blood components affecting blood pH or blood viscosity and the factors affecting the extraction from the swatch. In the study conducted by Holtkötter et al. (2015), it has been reported that there were differences in the test results according to the causes of death in accordance with our study. Although there were differences in the test results according to the causes of death in the present study, a significant relationship could not be established between the causes of death and test results of 200 postmortem blood samples. In the control tests performed in our study, a significant relationship could not be established between the causes of death and D-dimer levels in 20 different post-mortem peripheral blood samples. Similarly, in the study conducted by Takeichi et al. with 76 patients who died of various reasons, no significant relationship could be established between causes of death and serum fibrin degradation products (D-dimer) values, consistently with our study. The evaluation of the test results per time between being found dead and sample collection showed 58 out of 196 sample taken within one day were negative, 32 were weak positive, 57 were positive and 49 were strong positive. In Wang's study, 10 out of 11 blood samples collected with one day interval between the date of death and the time of autopsy gave a strong intensity result for D-dimer detection, while 1 showed a moderate intensity and was found to be incompatible. It is thought that the findings in the two studies may be inconsistent due to the differences in the number of cases and the timing of sampling. In this study, time between being found dead and sample collection was taken as basis for the evaluation, while in Wang's study, the evaluation was made using the time between the date of death and the time of autopsy. In this study, 90% (n=10) of all menstrual blood samples collected from living donors for the validation of the test were positive and 10% negative for D-dimer. When classified according to band intensity, 30% (n=3) of all samples were found to be strong positive, 40% positive, 2% weak positive and 10% negative. In the study by Wang, 8 of 10 menstrual blood samples were positive, 2 were negative and when classified according to band intensity, 1 was reported as strong positive, 2 as positive, 5 as weak positive and 2 as negative. When the test results were evaluated as negative and positive, the two studies were compatible, but when evaluated according to band intensity, they were found to be inconsistent. It is thought that the test results according to the band intensity in the two studies may be inconsistent due to the differences between the case selection and the day of menstruation on which the sample was taken. Similarly, Bagwe's study showed positive D-dimer test result in all menstrual blood samples taken from 6 volunteers. Considering the number of cases and sampling days was on menstruation days 1 and 2, it was thought that this study was not correlated with our study [19]. In our study, it was observed that the band intensity decreases day by day for the test positivity after the onset of menstruation. In the study of Holtkötter et al. (2018), all 42 samples collected from 14 healthy volunteers on the 1st, 2nd and 3rd day of menstruation had a positive reaction for the presence of D-dimer, the majority gave a strong positive signal (52.4%), 12 of the 42 samples gave a positive reaction (28.6%) and 8 samples gave a weak positive signal (19%), indicating that the day the sample was collected did not affect the results of the test. When the test results were evaluated as only negative and positive, the two studies seem compatible, but when evaluated according to band intensity, there were inconsistency between two studies. It is thought that the test results according to the band intensity in the two studies may be inconsistent due to the differences in the case selection, volunteer characteristics and the menstruation day on which the sample was taken. It was thought that there is a need for new studies to be carried out on larger sample size. This special immunochromatographic test, which can be used in forensic medicine applications, might be considered as a presumptive test method for both menstrual blood and postmortem blood identification. Further examinations are required to distinguish whether the blood sample found at the crime scene with positive D-dimer result in the test is menstrual blood or postmortem blood containing D-dimer. The identification of epithelial cells by microscopic examination of the sample may be suggestive of menstrual blood [20]. Moreover, menstrual blood can be excluded with the Y chromosome detected in molecular genetic examinations. Moreover, possible limitations should be considered when analyzing with the SERATEC PMB test. D- dimer levels may rise and cause a false positive test result. In such uncertain cases, it would be more appropriate to reach a conclusion with further investigations [21-24].

Conclusion

In this study, all postmortem peripheral blood samples showed a positive reaction for the presence of hemoglobin as expected in the test. Although the test seems successful in this respect, only human blood was examined and different samples were not compared in our study. Thus, it is recommended to evaluate the performance of the test by conducting further studies with different body fluid samples and the blood specimens of various species. The increase in D-dimer levels in postmortem peripheral blood samples compared to antemortem peripheral blood samples was confirmed by the SERATEC® PMB test, which is a semi-quantitative rapid test, by obtaining a positive result with 71% of postmortem samples. Considering that SERATEC® PMB Test differentiates menstrual blood using D-dimer levels, it should be kept in mind that the test may give false positive results (71% according to this study) for postmortem peripheral blood. It was shown in this study that D-dimer was not the only determinant of the band intensity for SERATEC® PMB Test, and it was thought that the band density may differ depending on the differences in extraction from the swatch, and changes in blood components affecting pH

and blood viscosity. Although the observer and interobserver agreement was reported to be strong, the test is not quantitative. Therefore, although the test gives semi-quantitative results, it is thought that using a scoring system in case samples may lead to incorrect evaluations. It has been found that there is a requirement for more comprehensive studies by comparing the test results with biochemical D-dimer levels. There was no significant relationship between the time from being found dead to sample collection and the test results. This situation was reported to be inconsistent with similar studies in the literature. However, considering that similar studies in the literature have been performed on a small number of samples, the results of our study on this subject are more reliable. In this study, a significant relationship was reported between the days of menstruation on which the blood sample was collected and the test results, and it was observed that the band intensity for the test positivity decreased day by day after the onset of menstruation. In cases of sexual assault allegations, it may be useful to make an evaluation with a test for contradictory statements about the menstrual day. However, because our sample size for this part of our study is limited, our results require to be confirmed by a comprehensive study for such an evaluation. There were differences in the test results according to the causes of death in postmortem blood samples, and a significant relationship could not be established between the causes of death and test results. The SERATEC® PMB Test may be suitable as a pre-screening test to identify postmortem blood; however, it does not allow us to speculate on the cause of death.

Conflict of interests

The authors declare that they have no competing interests.

Financial Disclosure

This study was funded by Scientific Research Projects Coordination Unit of Istanbul University-Cerrahpasa. Project number: 34358.

Ethical approval

Ethical approval was obtained with the decision of Cerrahpaşa Medical Faculty Clinical Research Ethics Committee dated 03.07.2019 and numbered 83045809-604.01.02.

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