

## ORIGINAL ARTICLE

# MODERN DIAGNOSTIC CRITERION FOR ESTABLISHING THE REGIONAL ORIGIN OF BLOOD IN SEXUAL CRIMES

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**Olena P. Babkina<sup>1,2</sup>, Kateryna V. Varukha<sup>1</sup>, Iryna Y. Khmil<sup>1</sup>, Svitlana I. Danylchenko<sup>3</sup>, Iana A. Ushko<sup>1</sup>**<sup>1</sup> OO BOHOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE<sup>2</sup>KIEV CLINICAL BUREAU OF FORENSIC MEDICINE, KYIV, UKRAINE<sup>3</sup> PETRO MOHYLA BLACK SEA NATIONAL UNIVERSITY, MYKOLAIV, UKRAINE

## ABSTRACT

**The aim:** Determination of regional blood origin in cases of sexual violence, establishing the possibility of using prostaglandin F2alpha as a marker of blood of menstrual origin.**Materials and methods:** The material for the study were samples of vaginal fluid, menstrual blood and capillary blood from females, the age distribution of women was carried out according to the gynecological classification according to the age periods of women's lives depending on the functional state of their reproductive system: the first group – women at the age of 18-29, the second group – women at the age of 30-45.**Results:** Among objects of biological origin, fluids, especially blood, occupy an important place. The content of PGF2a has age fluctuations: its content is higher by 6-12% in women aged 30-45 than in women 18-29 years old. PGF2a levels above 13.1 ng / mg of dry tissue are a reliable sign of blood of menstrual origin, which is very important in determining the regional origin of blood in forensic examinations for sexual violence / sexual crimes.**Conclusions:** In cases of sexual violence against women, in addition to recording external harm, attention should also be paid to the examination of objects of biological origin, identification by species, sex, regional, organ or cell.**KEY WORDS:** sexual abuse, laboratory diagnostics, blood, forensics, COVID-19

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## INTRODUCTION

The current and most important problem today is the spread of a dangerous infection caused by a new virus that leads to the development of respiratory diseases in humans, namely acute respiratory disease COVID-19. Given the rapid spread of COVID-2019 worldwide, the severity of the disease in 20-30% and high mortality, governments in almost all countries have taken measures to implement global quarantine and declare a global emergency [1-5]. Under quarantine conditions due to the COVID-19 pandemic, the majority of the population is forced to stay at home, significantly reducing the number of contacts. At the same time, on a domestic basis quite often there are conflicts between family members, both psychological, physical and sexual, as a result of which women often suffer. Thus, the number of appeals to law enforcement agencies, hotlines of social services and charitable organizations about cases of domestic violence against women has increased sharply, sometimes to 1,500 per day, according to information sources. Therefore, it is very important in case of suspicion of domestic violence to respond quickly for law enforcement agencies, social services, medical workers in order to provide assistance to victims, timely detection and recording of signs of psychological, physical, sexual violence, impartial investigation. Timely appointment and forensic examination, including examination of physical

evidence, are required to detect the presence and record of physical and sexual violence against female victims. The most important task of forensic examination is to obtain from the minimum amount of biological material the data necessary for its comprehensive characterization. The study of micro-traces and micro-objects of biological origin, the identification of which by species, sex, regional, organ or cellular affiliation can provide significant assistance to justice in the investigation of crimes against human life and health [6, 7].

## THE AIM

Determination of regional blood origin in cases of sexual violence, establishing the possibility of using prostaglandin F2alpha as a marker of blood of menstrual origin.

## MATERIALS AND METHODS

The material for the study were samples of vaginal fluid, menstrual blood and capillary blood from females of reproductive age 18-45 years old, which were taken during their examination in the KNP "Center of Primary Health Care" №1 Shevchenkivskyi district of Kyiv, during 2015-2016. Sampling was performed with the informed consent of virtually healthy patients. The age distribution

of women was carried out according to the gynecological classification according to the age periods of women's lives depending on the functional state of their reproductive system: the first group – women at the age of 18-29 (n = 28), the second group – women at the age of 30-45 (n = 23). A sample of vaginal contents during menstruation on a tampon, a sample of vaginal contents in the postmenstrual period and a sample of capillary blood on gauze were removed for the study. The removed objects were dried and stored until examination. Prostaglandins in the samples were determined by the method of preparative isolation and systematic analysis of prostaglandins obtained by biosynthesis [2]. A standard solution of prostaglandin F<sub>2</sub>α, namely Enzaprost F (“CHINOIN” Pharmaceutical and Chemical Works Co.Ltd., Hungary) was used as a control. The evaluation of the quantitative content of PGF<sub>2</sub>α in the blood was performed directly on the chromatograms, taking into account the relationship between the size of the stain and the mass of the object, namely, a method was used that allows using software to automatically determine the size of the stain of the standard to accurately determine its quantitative content [8]. The digital data obtained in the studies was processed statistically according to the generally accepted methods of variation statistics, comparing the values of the content of PG F<sub>2</sub>α in different liquids. Differences between liquids were considered significant under the condition  $P < 0.001$  \* (\* P - achieved level of significance of PGF<sub>2</sub>α).

The work was carried out in accordance with the requirements of the «Instructions on the forensic medical examination» (Order of the Ministry of Health of Ukraine No. 6 of 01/17/1995), in accordance with the requirements and norms, a typical provision on ethics of the Ministry of Health of Ukraine No. 690 of 09/23/2009, «The procedure for the removal of biological objects from the dead, whose bodies are subject to forensic examination and pathological examination, for scientific purposes» (2018).

## RESULTS

In total, PGF<sub>2</sub>α content in the effluent was studied in 51 samples, along with 28 samples derived from virtually healthy women aged 18-29 and 23 samples of women aged 30-45. A similar number of samples were studied to determine the content of PGF<sub>2</sub>α in blood of menstrual origin and 32 samples with capillary blood. The total number of studied samples obtained from practically healthy women is 134.

Studies of the content of PGF<sub>2</sub>α in the vaginal fluid of almost healthy women showed that its content in the vaginal fluid in healthy women aged 18-29 is  $9.25 \pm 0.03$  ng / mg with individual fluctuations from 2.75 ng / mg to 16. As for almost healthy women aged 30-45, the PGF<sub>2</sub>α content is  $10.35 \pm 0.04$  ng / mg, with individual variations from 5.79 ng / mg to 16.05 ng / mg. According to the data in the table and figure, it is seen that the content of PGF<sub>2</sub>α in the vaginal fluid of almost healthy women 30-45 years old, almost 11% more than in almost healthy women 18-29

years old. Comparison of their content taking into account age showed the dependence of PGF<sub>2</sub>α content on age ( $p < 0,001$ ) (Table I).

Using the method of TLC, we studied the content of PGF<sub>2</sub>α in the blood of menstrual origin. Studies of the content of PGF<sub>2</sub>α in the menstrual blood of almost healthy women showed that its content in the menstrual blood of healthy women aged 18-29 is  $13.62 \pm 0.04$  ng / mg with individual fluctuations from 5.58 ng / mg to 21, 48 ng / mg. As for almost healthy women aged 30-45, the content of PGF<sub>2</sub>α is  $14.48 \pm 0.02$  ng / mg, with individual variations from 5.64 ng / mg to 20.95 ng / mg. According to the data in the table and figure, it is seen that the content of PGF<sub>2</sub>α in the blood of almost healthy women aged 30-45, almost 6% more than in almost healthy women 18-29 years old. Comparison of their content depending on age showed a significant difference ( $p < 0,001$ ). Thus, the content of PGF<sub>2</sub>α in the menstrual blood of almost healthy women varies depending on age (Table II).

A study of the PGF<sub>2</sub>α content in the capillary blood of almost healthy women, taking into account their age, showed that there is an effect of a woman's age on the content of PGF<sub>2</sub>α in the capillary blood. According to the data in the table and figure shows that women aged 18-29 it was  $5.07 \pm 0.06$  ng / mg, with individual variations from 2.75 ng / mg to 7.09 ng / mg, and at the age of 30-45 is  $5.44 \pm 0.06$  ng / mg, with individual variations from 2.54 ng / mg to 8.62 ng / mg (Table III). Thus, the content of PGF<sub>2</sub>α in vaginal fluid and capillary blood is age-dependent. ( $P < 0.001$ ).

The obtained indicators of PGF<sub>2</sub>α in fluids of different regional origin (vaginal contents, blood of menstrual origin, and capillary blood) of almost healthy women allowed to conduct a comparative analysis of indicators and find out that the marker of menstrual blood is PGF<sub>2</sub>α (Table IV).

To determine the diagnostic criteria, we measured the limits of fluctuations of the mean values (M ratio), using the method of two-sigma estimation -  $M \pm 2\sigma$ , where M is the arithmetic mean,  $\sigma$  is the standard deviation.

As a result of our research, we found the dependence of PGF<sub>2</sub>α content, firstly, on the age of women, and secondly, on the regional origin of the fluid. It should be noted that the content of PGF<sub>2</sub>α in vaginal fluid, menstrual blood and capillary blood in women of reproductive age, has an age feature, its content is higher by 6-12% for women aged 30-45. Thus, in women 30-45 years old the content of PGF<sub>2</sub>α is always, in all fluids, higher than in women 18-29 years old ( $p < 0.001$ ): in vaginal fluid - by 11%; in menstrual blood - by 6%; in capillary blood - by 3%. PGF<sub>2</sub>α is highest in menstrual blood compared to vaginal fluid and capillary blood. Moreover, this trend is true for women of both ages: in women aged 30-45, the content of PGF<sub>2</sub>α is 2.6 times higher in menstrual blood than in capillary, and 1.4 times more than in vaginal fluid; in women aged 18-29, the content of PGF<sub>2</sub>α is 2.7 times higher in menstrual blood than in capillary, and 1.5 times higher than in vaginal fluid.

**Table I.** The content of PGF2 $\alpha$  in the vaginal fluid of almost healthy women of reproductive age

Unit of measure	Vaginal fluid of almost healthy women					
	women 18-29			women 30-45		
	n	M $\pm$ m	$\sigma$	n	M $\pm$ m	$\sigma$
PG F2 $\alpha$ ng / mg dry tissue	28	9,25 $\pm$ 0,03	0,19	23	10,35 $\pm$ 0,04	0,21
P	P<0,001*					

Note: \* P– achieved level of significance of PGF2 $\alpha$

**Table II.** The content of PG F2 $\alpha$  in the blood of menstrual origin of almost healthy women of reproductive age

Unit of measure	Menstrual blood of almost healthy women					
	women 18-29			women 30-45		
	n	M $\pm$ m	$\Sigma$	n	M $\pm$ m	$\sigma$
PGF2 $\alpha$ ng / mg dry tissue	28	13,62 $\pm$ 0,04	0,25	23	14,48 $\pm$ 0,02	0,1
P	P<0,001*					

Note: \* P– achieved level of significance of PGF2 $\alpha$

**Table III.** The amount of PGF2 $\alpha$  in the capillary blood of almost healthy women of reproductive age

Unit of measure	Capillary blood of almost healthy women					
	women 18-29			women 30-45		
	n	M $\pm$ m	$\sigma$	n	M $\pm$ m	$\Sigma$
PGF2 $\alpha$ ng / mg dry tissue	16	5,07 $\pm$ 0,06	0,26	16	5,44 $\pm$ 0,06	0,26
P	P<0,001*					

Note: \* P– achieved level of significance of PGF2 $\alpha$

**Table IV.** The content of PGF2 $\alpha$  in fluids of different regional origin of almost healthy women of different reproductive ages

Age group of women	PG F2 $\alpha$ ng / mg dry tissue, M $\pm$ 2 $\sigma$		
	Vaginal fluid	Menstrual blood	Capillary blood
18-29 (n = 28)	9,25 $\pm$ 0,03 (DI 2,75-16,59)	13,62 $\pm$ 0,04 (DI 5,58-21,48)	5,07 $\pm$ 0,06 (DI 2,75-7,09)
30-45 (n = 23)	10,35 $\pm$ 0,04 (DI 5,79-16,05)	14,48 $\pm$ 0,02 (DI 5,64-20,95)	5,44 $\pm$ 0,06 (DI 2,54-8,62)
P	P<0,001*	P<0,001*	P<0,001*

## DISCUSSION

As a result of our analysis, it can be safely stated that Ukraine has come much closer to European standards in terms of preventing domestic violence and respecting women's rights, becoming the 17th state to accede to the Istanbul Convention on November 7, 2011 and supporting the main objectives of the Convention. namely: "...protection of women from all forms of violence and prevention, prosecution and eradication of violence against women and domestic violence...", and a number of legislative documents were adopted and existing changes were made [9-11]. Thus, it should be noted that the Law of Ukraine № 2229-VIII "On Prevention and Counteraction to Domestic Violence" (came into force on 01/07/2018) defines the organizational and legal framework for preventing and combating domestic violence, the main directions of state policy in the field of prevention and counteraction domestic violence, aimed at protecting the rights and inter-

ests of victims of such violence, including sexual violence. The law clearly states that sexual violence is "a form of domestic violence that includes any acts of a sexual nature committed against an adult without his or her consent or against a child regardless of his or her consent, or in the presence of a child, as well as other offenses against sexual freedom or sexual integrity of a person, including those committed against a child or in his or her presence (Article 54 of Section I of the Law) [7].

In view of the above, it should be noted that in cases of sexual violence against women, in addition to fixing external injuries, attention should also be paid to the study of microtrace and microobjects of biological origin, identification by species, sex, regional, organ or cell affiliation, which can provide significant assistance in the investigation of crimes. Among objects of biological origin, fluids, especially blood, occupy an important place as evidence of various crimes that are accompanied by

external bleeding. Traces of blood (in forensic medicine) is any amount of fresh or altered blood outside the living organism that does not have morphological characteristics [6]. At the same time, one of the issues addressed in the study of physical evidence of biological origin in forensic practice is to determine their regional origin, including the identification of menstrual blood, especially in cases of sexual violence. After studying the data of domestic and world literature, we came to the conclusion that from a forensic point of view, this problem is not fully developed, is fragmentary, known diagnostic criteria do not take into account inherent in menstrual blood components, which leads to further study of this area, so this study is of great importance for forensic examination. In search of ways to solve this problem, we paid attention primarily to biochemical studies, which are increasingly used in forensic practice, in particular the biochemical properties of menstrual blood [12-14]. It is well known that menstruation (from the Latin *mensis* - month) - part of the menstrual cycle of the female body during which there is a rejection of the functional layer of the endometrium (uterine mucosa), accompanied by bleeding. The main role at the beginning of menstruation is played by spasm of arterioles. It is known that vasoconstrictors, which are PG F<sub>2a</sub>, endothelin-1 and platelet-activating factor (TAF) are produced within the endometrium and are involved in the contraction of blood vessels. They also contribute to the onset of menstruation and subsequent control over it. These mediators are regulated by vasodilators such as PG E<sub>2</sub>, prostacyclin, nitric oxide, which are also produced by the endometrium. PG F<sub>2a</sub> has a pronounced vasoconstrictive effect, exacerbates arterial spasm and endometrial ischemia, causes contraction of the myometrium, which on the one hand, reduces blood flow, on the other - helps to remove the rejected endometrium. Menstrual blood does not clot and has a darker color than the blood circulating in the vessels, contains a number of enzymes. Also, menstrual blood, mixed with the contents of the vagina, contains components of the vaginal epithelium, the epithelium of the mucous membrane, as well as a large number of bacteria - cocci, bacilli, etc. But the same components are contained in the blood from the female genital tract (for example, in sexual crimes), but not of menstrual origin [15, 16]. Therefore, it is important to find a criterion that would be a reliable marker of menstrual blood. Such a marker may be the vasoconstrictor prostaglandin F<sub>2alpha</sub> (PGF<sub>2α</sub>), which is produced in the endometrium during menstruation.

Given the above, we studied the possibility of using PG F<sub>2α</sub> as a marker of menstrual blood [17, 18]. However, to verify this, it was first necessary to check whether the content of PG F<sub>2α</sub> in the menstrual blood of different age groups of women who are practically healthy. Prior to that, we determined the content of PGF<sub>2α</sub> in the blood of menstrual origin, and capillary blood in almost healthy women aged 18-45.

Thus, we obtained statistically significant indicators that indicate the possibility of establishing the menstrual origin of the blood by the quantitative content of PG F<sub>2α</sub>.

In our opinion, the content of PGF<sub>2α</sub> above 13.1 ng / mg of dry tissue is a reliable sign of menstrual blood, which is of great diagnostic value in the differential diagnosis of regional origin of objects of biological origin (blood) in cases of sexual violence / sexual crimes.

## CONCLUSIONS

1. Legal assistance in cases of domestic violence in Ukraine is provided at the legislative level, as evidenced by the constant development and improvement of measures to prevent and combat domestic violence. Due to the increase in cases of domestic violence, especially sexual violence, in emergencies, including quarantine due to the COVID-19 pandemic, the issue of preventing and combating domestic violence needs special attention and further solution.
2. Timely forensic examinations to identify injuries with subsequent evidence of domestic violence, especially sexual violence, help the pre-trial investigation / court party to take effective administrative, criminal or other measures against the perpetrator. In cases of sexual violence against women, in addition to recording external harm, attention should also be paid to the examination of objects of biological origin, identification by species, sex, regional, organ or cell, which can provide significant assistance to justice in the investigation of crimes. Among objects of biological origin, fluids, especially blood, occupy an important place.
3. The content of PGF<sub>2α</sub> is the highest in menstrual blood 1.5 times compared to vaginal fluid and 2.7 times compared to capillary blood. The content of PGF<sub>2α</sub> has age fluctuations: its content is higher by 6-12% in women aged 30-45 than in women 18-29 years old. PGF<sub>2α</sub> levels above 13.1 ng / mg of dry tissue are a reliable sign of blood of menstrual origin, which is very important in determining the regional origin of blood in forensic examinations for sexual violence / sexual crimes.

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**ORCID and contributionship:***Olena P. Babkina*: 0000-0001-8120-6526 <sup>A, D, F</sup>*Kateryna V. Varukha*: 0000-0001-5933-4124 <sup>B, C, E</sup>*Iryna Y. Khmil*: 0000-0002-0157-0289 <sup>C</sup>*Svitlana I. Danylchenko*: 0000-0001-5312-0231 <sup>E, F</sup>*Iana A. Ushko*: 0000-0003-3017-7766 <sup>C</sup>**Conflict of interest:***The Authors declare no conflict of interest.***CORRESPONDING AUTHOR****Olena P. Babkina**

00 Bohomolets National Medical University  
 13, T. Shevchenko Boulevard, 01601 Kyiv, Ukraine  
 tel: +38 050 530-63-64,  
 e-mail: hpbsme@gmail.com

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