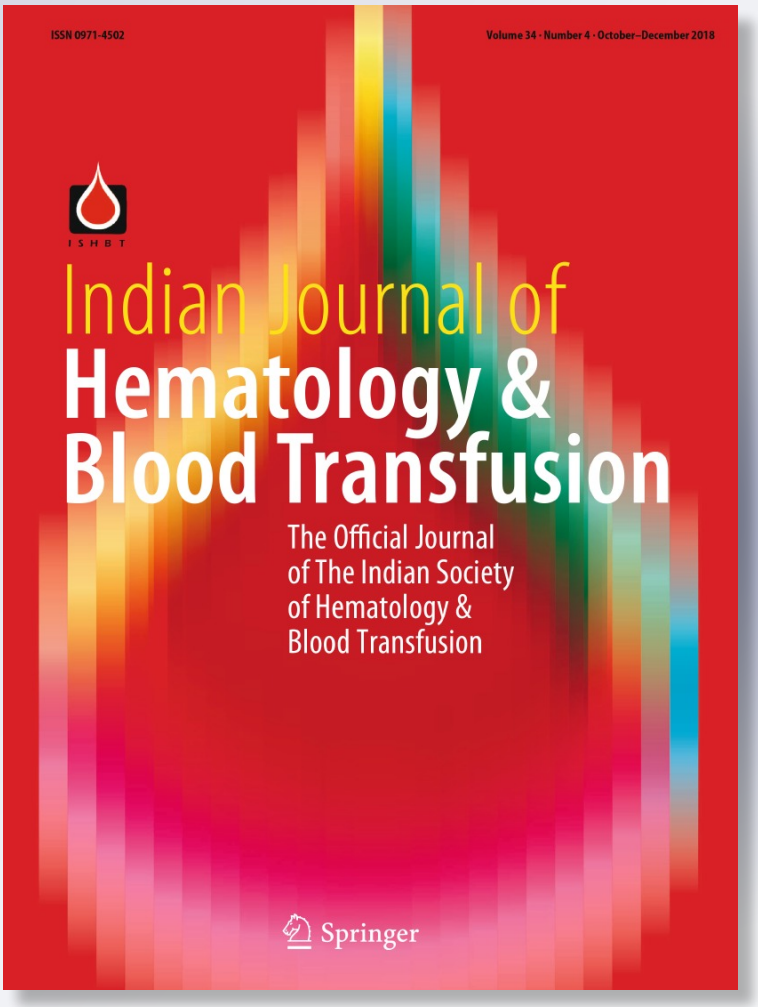
Relationship Between Haemostasis Parameters and Anxiety Under Examination Stress: A Pilot Study

Renad Ibrahimovich Zhdanov, Roman Vladimirovich Kupriyanov, Svetlana Igorevna Zhdanova & Vladimir Georgievich Dvoenosov

Indian Journal of Hematology and Blood Transfusion

ISSN 0971-4502
Volume 34
Number 4

Indian J Hematol Blood Transfus (2018)
34:727-730
DOI 10.1007/s12288-018-0980-7



Indian J Hematol Blood Transfus (Oct-Dec 2018) 34(4):727–730
https://doi.org/10.1007/s12288-018-0980-7

SHORT COMMUNICATION

Relationship Between Haemostasis Parameters and Anxiety Under Examination Stress: A Pilot Study

Renad Ibrahimovich Zhdanov[1,2] · Roman Vladimirovich Kupriyanov[2,3] · Svetlana Igorevna Zhdanova[4] · Vladimir Georgievich Dvoenosov[1]

Received: 19 December 2017 / Accepted: 3 July 2018 / Published online: 6 July 2018

Abstract This work aims to clarify how blood coagulation parameters reflect mild stress response in males and females. Healthy student volunteers of both sexes were used in this pilot study. A new global sensitive assay of haemostasis, spatial thrombodynamics, along with conventional coagulometry approach were used to evaluate of blood coagulation parameters. Psychodiagnostics scales (according to Spielberger and Taylor) are employed to evaluate anxiety as stress-induced response. We have selected exam stress, which despite being a mild stressor may nevertheless cause somatic disorders. We provide the first evidence of a statistically significant increase in initial clot growth velocity in women, but not men, in response to exam stress. The exam situation produces higher situational anxiety in female volunteers, and so they express remarkable stress-induced haemostatic responses, including plasma- and platelet-based changes. In contrast, male volunteers do not express pronounced stress-induced changes in haemostasis, and only display a decrease in plateletcrit value and an increase in prothrombin time. Mild form of stress (exam) induces changes in some blood coagulation parameters. A statistically significant remarkable increase in V_{init} value and some other plasma- and platelet-based parameters has been seen in female students (but not male ones) under exam stress.

Keywords Haemostasis · Blood coagulation · Anxiety · Gender · Stress · Thrombodynamics

✉ Renad Ibrahimovich Zhdanov
zrenad@gmail.com

Roman Vladimirovich Kupriyanov
kroman1@mail.ru

Svetlana Igorevna Zhdanova
votinia@mail.com

Vladimir Georgievich Dvoenosov
dvoenosovvg@yandex.ru

1 "Healthy Food" Open Laboratory, Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, 74, Karl Marx St., Kazan, Russian Federation 420008

2 Russin Institute for Advanced Study, Moscow State Pedagogical University, 1/1, Malaya Pirogovskaya St., Moscow, Russian Federation 119991

3 Chair of Social Work, Pedagogy and Psychology, Kazan National Research Technological University, 68, Karl Marx St., Kazan, Russian Federation 4200015

4 Chair of Pediatry, Kazan State Medical University, 49, Butlerov St., Kazan, Russian Federation 420012

Abbreviations

V_{init}	The initial velocity of induced thrombosis
V_{steady}	The steady velocity of induced thrombosis
d_{Speed}	The difference between the initial and steady velocity of induced thrombosis
PT	Prothrombin time
INR	International normalized ratio
APTT	Activated partial thromboplastin time
PLT	Absolute number of platelets
MPV	The average platelets' volume
PCT	Thrombocrit
STAI	State-trait anxiety inventory
TMAS	Teilor's Manifest Anxiety Scale

Introduction

Most articles studying stress and health are devoted to pathological processes as a result of distress [1, 2]. One of the major risk factors in the pathogenesis of cardiovascular diseases is acute mental stress [3]. Acute mental stress has been recognized as an important trigger of acute coronary syndrome and cardiovascular events [4–6]. We have selected to study a mild form of stress, student examination stress (exam stress) [7]. Mental stress-induced activation of haemostasis occurs through the stimulation of the sympathetic nervous system and the release of catecholamines [1, 2, 8]. Although a considerable amount of research has been conducted on the psychophysiological aspects of sex differences, little attention has been paid to how males and females react to environmental influences, and stress in particular [9]. To understand the mechanisms of stress response, it is important to evaluate its impact on healthy men and women. This work as a pilot study aims to clarify how blood coagulation parameters reflect exam stress response in men and women.

Methods

Healthy volunteers were used in this pilot study. Institutional and international regulations concerning the ethical use of human volunteers were followed. The study was approved by the Local Ethics Committee of the Kazan State Medical University (6.21.06.18). All volunteers were the University undergraduate natural sciences students (n = 81) of both sexes, aged 18–22 years. The control group consisted of 39 volunteers observed at the beginning of academic year just after summer vacations (September 15–30) (22 women and 17 men), and the clinical group under exam stress included 42 students observed at the day of their second examination of academic session just before exam and meal (19 women and 23 men). After obtaining informed consent from participants, a team of physicians conducted clinical observations and collected anamnesis data. This also allowed us to account for hereditary haemostatic diseases other acute or chronic diseases and an absent of heavy stressors for the last 6 months, in accordance with the protocol approved [10]. A new global assay of haemostasis, spatial thrombodynamics, was used to evaluate of thrombosis risk, employing the Thromboimager-2 device (HemaCore Ltd, Moscow, Russia) [11–13] along with standard coagulometry measurements [1, 10, 13]. For the thrombodynamics study, venous blood sampling from volunteers was carried out before meals between 8 and 9 a.m. out of exam session (control group) or just before the 2nd examination in session, with 3.2% of sodium citrate (VACUETTE Blood Collection Tubes, Greiner Bio-One, Austria). Blood samples were centrifuged for 20 min at $2500\times g$. Plasma was collected in 1.5 mL Eppendorf tubes, and was subsequently centrifuged for 5 min at $10{,}000\times g$ to obtain platelet-poor blood plasma (Eppendorf MiniSpin, USA). Prepared plasma samples were incubated with a coagulation contact pathway inhibitor and recalcination occurred at 37.0 °C (HemaCore, Moscow, Russia). This method and its corresponding device are based on a model of the spatial growth of fibrin clots [11–13]. The Spielberger State-Trait Anxiety Inventory (STAI) and Taylor Manifest Anxiety Scale (TMAS) were used to evaluate the degree of stress of the exam situation. Statistical analysis was performed using the Statistica-10 software package (StatSoft). Comparisons between men and women under exam stress compared to the control group were computed by means of the Student's t test. Significance levels were set at $p < 0.05$ with two tailed testing.

Results

A statistically significant decrease in the plateletcrit value (PCT) was observed for both women and men under exam stress compared to the control group (Table 1). A statistically significant increase in prothrombin time value (PT) was also seen in male volunteers under exam, compared to the control group. In women, there was a statistically significant reduction in platelet count (PLT) and an increase in mean platelet volume (MPV) under stress conditions compared to the control (Table 1), demonstrating that stress affects platelet haemostasis in women. The decrease in PCT value in volunteers of both sexes might indicate a stimulation of platelet formation and an increase in immature platelets in circulation, probably due to their increased consumption in thrombosis. This is consistent with published data [1, 2] showing that stress leads to increased platelet activation markers. This phenomenon could be considered adaptive from the perspective of stress-induced coagulation changes as a warning of possible bleeding [10, 14].

This is the first evidence of a statistically significant increase in the initial clot growth velocity (V_{init}), as well as a difference between initial and steady velocity of induced thrombosis (dSpeed), in female students between the stressed and control groups, determined via spatial thrombodynamics (Table 1) [11–13]. This phenomenon outlines the involvement of plasma haemostasis in females in response to exam stress. The V_{init} parameter is seemingly more sensitive to changes in hypercoagulability under stressful conditions. Other common coagulometric parameters, such as activated partial thromboplastin time

Table 1 Coagulometric, thrombodynamics and anxiety parameters of students under and out of examination stress

Category	Sex	Students out of exams (norm)	Students under examination stress	p
PT (s)	Males	14.7 ± 0.17	15.6 ± 0.56	< 0.05
	Females	14.9 ± 0.40	14.4 ± 0.22	–
INR	Males	1.051 ± 0.022	1.123 ± 0134	–
	Females	1.062 ± 0.065	1.032 ± 0.045	–
APTT (s)	Males	33.2 ± 2.6	32.9 ± 1.38	–
	Females	33.9 ± 2.0	33.4 ± 0.88	–
PLT (10^9/L)	Males	244 ± 24.6	223 ± 8.6	–
	Females	292 ± 40.9	219 ± 12.0	< 0.05
MPV (fL)	Males	8.77 ± 0.35	8.42 ± 0.20	–
	Females	7.0 ± 0.55	9.06 ± 0.12	< 0.05
PCT (%)	Males	0.212 ± 0.013	0.186 ± 0.006	< 0.05
	Females	0.228 ± 0.022	0.198 ± 0.009	< 0.05
Vinit (mcm/min)	Males	43.0 ± 1.61	45.1 ± 1.23	–
	Females	41.3 ± 1.67	48.7 ± 1.10	< 0.05
Vstead (mcm/min)	Males	21.7 ± 0.54	22.7 ± 0.51	–
	Females	23.3 ± 0.64	24.0 ± 0.33	–
dSpeed (mcm/min)	Males	21.3 ± 1.35	22.4 ± 0.91	–
	Females	18.0 ± 1.28	24.7 ± 1.03	< 0.05
State anxiety (STAI)	Males	22.0 ± 1.98	20.9 ± 2.30	–
	Females	23.8 ± 1.52	31.7 ± 2.43	< 0.05
Trait anxiety (STAI)	Males	39.6 ± 1.32	38.2 ± 2.66	–
	Females	42.3 ± 1.19	44.6 ± 1.39	–
TMAS	Males	15.0 ± 1.15	13.7 ± 2.16	–
	Females	14.7 ± 2.66	18.8 ± 1.55	–

$p < 0.05$—statistically significant differences of students' indicators under examination stress compared to control

(APTT), do not differ significantly between females and males. This finding is inconsistent too with other studies [3], due to the extreme sensitivity of the thrombodynamics methods, and the use of exams as a stressor, which does not represent as strong a stressor as natural disasters. Normally, changes in the haemostatic system due to stress represent a key mechanism in that following rupture of an atherosclerotic plaque, coronary thrombus growth depends upon coagulation activation initiated by tissue factor and platelets at the site of endothelial lesions [3].

Exam stress-induced alterations in the cardiovascular system are mainly started through activation of the sympathetic nervous system. Furthermore, we used psychodiagnostic tests to evaluate such a mild stressful situation. Table shows that there are statistically significant differences in STAI between the stressed and control groups, but only in females. This suggests that male students do not perceive exams as stressful situations, causing little response from the haemostatic system, with the exception of PT and PCT values. In contrast, females consider exams to be threatening, leading to increased situational anxiety and changes in the women's haemostatic biochemical parameters (Table 1). Furthermore, we have not found haemoconcentration phenomenon as it was reported earlier [15]. In the case, we talk about the impact of the exam situation on the human body, since other personal parameters (Trait Anxiety STAI and TMAS) do not express statistically significant differences due to examination stress in female and in male volunteers. Interestingly, correlation analysis revealed a statistically significant ($p < 0.05$) negative correlation (− 0.344958) between State Anxiety STAI and the lag time before clots begin to form in female participants, demonstrating that when situational anxiety increases, lag time decreases.

Conclusions

Mild form of stress (exam) induces changes in some blood coagulation parameters. This is the first time that a statistically significant increase in V_{init} has been seen in female students under exam stress. Exam situations produce higher situational anxiety in female volunteers compared to male volunteers, and this difference may be due to

psychoemotional or sociocultural factors. The female volunteers express remarkable stress-induced response, demonstrated by plasma- and platelet-based responses. In contrast, males do not express pronounced stress-induced changes in haemostasis, only exhibiting a decrease in PCT and an increase in PT values. These findings demonstrate the necessity of accounting for psychological factors in conducting comparative studies of female and male reactions to environmental influences.

Acknowledgements The authors are grateful to Professor Dr. Fazly Ataullakhanov (Lomonosov Moscow State University and "HemaCore" Ltd) for the support with thrombodynamics studies and fruitful discussions. The work was supported and 2016-2018 Grant of Russian Institute of Advanced Study at the Moscow State Pedagogical University (to R.V.K.).

Funding This work was funded by the subsidy allocated by the Russian Ministry of Education and Science, Grant # 19.9777.2017/8.9 to Kazan Federal University for the state assignment in the sphere of scientific activities.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards approved by the Local Ethics Committee of the Kazan State Medical University (6.21.06.18) and with the 1964 Helsinki declaration and its later amendments.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. von Känel R, Vökt F, Biasiutti FD, Stauber S, Wuillemin WA, Lukas PS (2012) Relation of psychological distress to the international normalized ratio in patients with venous thromboembolism with and without oral anticoagulant therapy. J Thromb Haemost 10(8):1547–1555. https://doi.org/10.1111/j.1538-7836.2012.04801.x
2. Stämpfli SF, Camici GG, Keller S, Rozenberg I, Arras M, Schuler B, Gassmann M, Garcia I, Lüscher TF, Tanner FC (2014) Restraint stress enhances arterial thrombosis in vivo–role of the sympathetic nervous system. Stress 17(1):126–132. https://doi.org/10.3109/10253890.2013.862616
3. von Känel R (2015) Acute mental stress and hemostasis: when physiology becomes vascular harm. Thromb Res 135(Suppl 1):S52–S55. https://doi.org/10.1016/S0049-3848(15)50444-1
4. Tofler GH, Stone PH, Maclure M, Edelman E, Davis VG, Robertson T et al (1990) Analysis of possible triggers of acute myocardial infarction (the MILIS study). Am J Cardiol 66:22–27. https://doi.org/10.1016/0002-9149(90)90729-K
5. Mostofsky E, Penner EA, Mittleman MA (2014) Outbursts of anger as a trigger of acute cardiovascular events: a systematic review and meta-analysis. Eur Heart J 35:1404–1410. https://doi.org/10.1093/eurheartj/ehu033
6. Steptoe A, Strike PC, Perkins-Porras L, McEwan JR, Whitehead DL (2006) Acute depressed mood as a trigger of acute coronary syndromes. Biol Psychiatry 60:837–842. https://doi.org/10.1016/j.biopsych.2006.03.041
7. Zunhammer M, Eberle H, Eichhammer P, Busch V (2013) Somatic symptoms evoked by exam stress in university students: the role of alexithymia, neuroticism, anxiety and depression. PLoS ONE 8(12):e84911. https://doi.org/10.1371/journal.pone.0084911
8. Weekes N, Lewis R, Patel F, Garrison-Jakel J, Berger DE, Lupien SJ (2006) Examination stress as an ecological inducer of cortisol and psychological responses to stress in undergraduate students. Stress 9(4):199–206. https://doi.org/10.1080/10253890601029751
9. Miller VM, Kaplan JR, Schork NJ, Ouyang P, Berga SL, Wenger NK, Shaw LJ, Webb RC, Mallampalli M, Steiner M, Taylor DA, Merz CN, Reckelhoff JF (2011) Strategies and methods to study sex differences in cardiovascular structure and function: a guide for basic scientists. Biol Sex Differ 2:14. https://doi.org/10.1186/2042-6410-2-14
10. Davydov DM, Zhdanov RI, Dvoenosov VG, Kravtsova OA, Voronina EN, Filipenko ML (2015) Resilience to orthostasis and haemorrhage: a pilot study of common genetic and conditioning mechanisms. Sci Rep 5:10703. https://doi.org/10.1038/srep10703
11. Tuktamyshov RN, Zhdanov RI (2015) The method of in vivo evaluation of haemostasis: spatial thrombodynamics. Hematology 20(10):584–586. https://doi.org/10.1179/1607845415Y.0000000022
12. Lipets E, Ataullakhanov F (2015) Global assays of hemostasis in the diagnostics of hypercoagulation and evaluation of thrombosis risk. Thromb J 13(4):2–15. https://doi.org/10.1186/s12959_015_0038_0
13. Sinauridze EI, Gorbatenko AS, Seregina EA, Lipets EN, Ataullakhanov FI (2017) Moderate plasma dilution using artificial plasma expanders shifts the haemostatic balance to hypercoagulation. Sci Rep 7(1):843. https://doi.org/10.1038/s41598-017-00927-w
14. Kupriyanov RV, Zhdanov RI (2014) Stress and allostasis: problems, outlooks and relationships. IP Pavlov J Higher Nerv Act (Zhurnal Vysshei Nervnoi Deyatelnosti imeni IP Pavlova) 64(1):21–31. https://doi.org/10.7868/S0044467714010080
15. Veldhuijzen van Zanten JJ, Ring C, Burns VE, Edwards KM, Drayson M, Carroll D (2004) Mental stress- induced hemoconcentration: sex differences and mechanisms. Psychophysiology 41(4):541–551. https://doi.org/10.1111/j.1469-8986.2004.00190.x