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Authors

Protic, Dragana
Salcedo-Arellano, Maria Jimena
Stojkovic, Maja
et al.

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ORIGINAL CONTRIBUTION

Raising Knowledge and Awareness of Fragile X Syndrome in Serbia, Georgia, and Colombia: A Model for Other Developing Countries?

Dragana Protic^a, Maria Jimena Salcedo-Arellano^{b,c,d}, Maja Stojkovic^a, Wilmar Saldarriaga^{e,f}, Laura Alejandra Ávila Vidal^g, Robert M. Miller^h, Nazi Tabatadzeⁱ, Marina Peric^j, Randi Hagerman^{b,c}, and Dejan B. Budimirovic^{k,l,*}

^aDepartment of Pharmacology, Clinical Pharmacology and Toxicology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia; ^bDepartment of Pediatrics, University of California Davis School of Medicine, Sacramento, CA, USA; ^cMedical Investigation of Neurodevelopmental Disorders (MIND) Institute UCDHS, University of California Davis, Sacramento, CA, USA; ^dDepartment of Pathology and Laboratory Medicine, University of California Davis School of Medicine, Sacramento, CA, USA; ^eDepartment of Morphology, Universidad del Valle School of Medicine, Cali, Colombia; ^fDepartment of Obstetrics and Gynecology, Hospital Universitario del Valle, Evaristo Garcia, Cali, Colombia; ^gMedicine and Surgery, Universidad del Valle School of Medicine, Cali, Colombia; ^hNational Fragile X Foundation, McLean, VA, USA; ⁱFragile X Center, MediClub Georgia Medical Center, Tbilisi, Georgia; ^jFragile X Society-Balkans, Belgrade, Serbia; ^kDepartments of Psychiatry, Fragile X Clinic, Kennedy Krieger Institute, Baltimore, MD, USA; ^lDepartment of Psychiatry & Behavioral Sciences-Child Psychiatry, Johns Hopkins School of Medicine, Baltimore, MD, USA

Fragile X syndrome is the most common monogenetic cause of inherited intellectual disability and syndromic autism spectrum disorder. Fragile X syndrome is caused by an expansion (full mutation ≥ 200 CGGs repeats, normal 10-45 CGGs) of the fragile X mental retardation 1 (*FMR1*) gene, epigenetic silencing of the gene, which leads to reduction or lack of the gene's product: the fragile X mental retardation protein. In this cross-sectional study, we assessed general and pharmacotherapy knowledge (GK and PTK) of fragile X syndrome and satisfaction with education in neurodevelopmental disorders (NDDs) among senior medical students in Serbia (N=348), Georgia (N=112), and Colombia (N=58). A self-administered 18-item questionnaire included GK (8/18) and PTK (7/18) components and self-assessment of the participants education in NDDs (3/18). Roughly 1 in 5 respondents had correct answers on half or more facts about fragile X syndrome (GK>PTK), which ranged similarly 5-7 in Serbia, 6-8 in Georgia, and 5-8 in Colombia, respectively. No cohort had an average value greater than 9 (60%) that would represent passing score "cut-off." None of the participants answered all the questions correctly. More than two-thirds of the participants concluded that they gained inadequate knowledge of NDDs during their studies, and that their education in this field should be more intense. In conclusion, there is a major gap in knowledge regarding fragile X syndrome among senior medical students in these three developing countries. The finding could at least in part be generalized to other developing countries aimed toward increasing knowledge and awareness of NDDs and fostering an institutional collaboration between developed and developing countries.

*To whom all correspondence should be addressed: Dejan Budimirovic, MD, Department of Psychiatry, Psychiatry Mental Health Program, Outpatient Services, Kennedy Krieger Institute, Baltimore, MD; Email: budimirovic@kennedykrieger.org.

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; *FMR1*, fragile X mental retardation 1; FMRP, fragile X mental retardation protein; FXAD, fragile X-associated disorders; FXPOI, fragile X-associated primary ovarian insufficiency; FXS, fragile X syndrome; FXTAS, fragile X-associated tremor/ataxia syndrome; ID, intellectual disability; IRB, Institutional Review Board; KAP, knowledge, attitude, and practice; K-W, Kruskal-Wallis test; M-W, Mann-Whitney *U* test; NDDs, neurodevelopmental disorders; PCR, polymerase chain reaction; PM, premutation; SB, Southern blot; SD, standard deviation; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

Keywords: Fragile X syndrome, developing countries, knowledge and awareness, medical collaboration

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INTRODUCTION

Fragile X Syndrome (FXS) is a global neurodevelopmental disorder (NDD) caused by the full mutation (FM, ≥ 200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene and epigenetic silencing of the gene, which results in a deficiency or absence of fragile X mental retardation protein (FMRP) [1-4]. With an estimated prevalence of 1:4000 in males and 1:6000 in females, FXS is the most known single gene cause of inherited intellectual disability (ID) and autism spectrum disorder (ASD), which accounts for up to 5% of all ASD [3-7]. Reduced levels of FMRP are not only a basis for FXS since it leads to ID but also a contributor to the ASD phenotype [5,8-13]. Indeed, FMRP expression in the brain is the ultimate factor determining the severity of the neurobehavioral phenotype [11] and males with severe ID or severe ASD have the lowest FMRP values [10]. This is not surprising since FMRP is a RNA binding protein involved in the synaptic and dendritic maturity as well as synaptic plasticity [14]. Individuals with FXS present with a broad range of physical and neurobehavioral abnormalities including prominent ears, long face, hyperextensible finger joints, macroorchidism with puberty, stereotypies, aggression, poor eye contact, excessive shyness, tactile defensiveness, and hyperarousal. Common comorbid psychiatric conditions include attention deficit hyperactivity disorder (ADHD), social anxiety, and ASD [5,10,15-17].

The field of FXS leads the way as the most common monogenic form of ASD and the most translated among NDDs into clinical trials [18,19]. Yet, questions remain as to whether these trials were conducted with the optimal outcome endpoints or in the most appropriate age group [19-21]. While there remains a great need for safe and effective treatments for FXS, particularly for targeted treatments that surpass the symptom-based management in FXS, no medication is approved by the US regulatory agency for the treatment of FXS [22-24].

Since the discovery of the gene in 1991, many studies have focused on the molecular diagnoses of FXS and other fragile X-associated disorders (FXAD), including the fragile X-associated tremor ataxia syndrome (FXTAS) and the fragile X-associated primary ovarian insufficiency (FXPOI) experienced by premutation (PM; 55 to 200 CGG repeats) carriers. The genetic/medical diagnosis of FXS is determined by polymerase chain reaction (PCR) and Southern blotting. Furthermore, the next generation *FMR1* gene-specific PCR technology (Amplidex) detects the full range of fragile X expanded alleles and minimizes the need for Southern blot (SB) analysis [25-27]. The availability of the sensitive and precise assays, which includes quantification of FMRP, allowed more accurate *FMR1*-FMRP correlations; thus, detecting other phenotypical correlates of FMRP deficiency not reported

in previous relevant studies [8,10,13,28]. There are three general directions in which fragile X testing should be recommended: (i) clinical symptoms that suggest FXAD, (ii) family history of FXAD, intellectual or learning disabilities, ASD, or infertility and (iii) family or personal history of a fragile X genetics and inheritance (ie, carrier) [29]. A current recommendation of the American Academy of Pediatrics is to test individuals with ID, global developmental delay, ASD, and/or family history of the *FMR1* FM or PM [15,30]. Thus, the fragile X testing provides important information for the diagnosis, treatment and prevention of FXAD [24] to allow an early diagnosis of FXS with or without ASD [31]. Therapeutic development has been on a rapid pace since the early 2000s and experts in this field believe that treatment needs to be implemented very early (for example, within the first years of life) for the most effectiveness in improving long-term outcomes for individuals with FXS [32]. While the genetic testing has been widely used in developed countries such as the US, such testing is infrequently used in developing countries, due to high costs and the lack of trained local genetic laboratories conducting PCR and SB analysis. Consequently, prevalence of FXAD and conditions associated with them in the later countries remains unknown. For example, according to results from previous studies, medical professionals in Serbia were barely familiar with disorders associated with the PM of the *FMR1* gene (FXTAS and FXPOI) [33]. Nevertheless, their knowledge of the FM of the *FMR1* gene remains unknown [33,34]. Together, similar studies of knowledge and practices related to FXAD should be carried out in other developing countries.

Here, we aim to assess: (i) general knowledge of FXS, (ii) knowledge of pharmacotherapy of FXS, and (iii) satisfaction with education in NDDs among senior medical students in developing countries such as Serbia, Georgia, and Colombia. NDDs include a broad spectrum of disorders that disrupt the normal brain development. Thus, we also aim to initiate a universal and widely used action plan in these countries that may support a pathway towards raising knowledge and awareness of NDDs, focusing here on FXS. The research is applicable to all developing countries.

MATERIALS AND METHODS

Sample

This was a cross-sectional study conducted among senior medical students by investigators at the Faculty of Medicine at the University of Belgrade in Serbia, the Faculty of Medicine at the Tbilisi State Medical University in Georgia, and at the Universidad del Valle, School of Medicine in Cali, Colombia. Participation response rate

ranged from 16.52% in Georgia, 31.87% in Colombia, to 99.43% in Serbia. The sample distribution of the study participants was as follows (mean age in years): (i) 348 in Serbia (24.44 ± 1.18 , 227 females); (ii) 112 in Georgia (24.55 ± 0.90 , 77 females), and (iii) 58 in Colombia (24.57 ± 2.74 , 33 females). Anonymity of data was carried out for all participants. It was emphasized that the collected data would serve exclusively for statistical analysis, and it would be published only in a summary form as a group to establish a baseline of their knowledge related to FXS.

Measurement Tool

The instrument used for this study was a self-administered 18-item questionnaire survey. Design of the questionnaire was based on an extensive database search that included MEDLINE/PubMed, Web of Science, PsycINFO and Embase. A combination of the following keywords was used during the search: “fragile X syndrome,” “fragile X related disorders,” “drug development,” “clinical studies,” “preclinical studies,” and “pharmacological treatment.” If the data were limited or not available, an additional search included other fields of relevance (eg, “neurodevelopmental disorders,” “psychopharmacology,” etc.). In addition to the aforementioned systematic searches and the authors’ clinical experiences in the disorders related to fragile X, the questionnaire was developed by consulting a range of relevant literature involving FXS [5,9,33,35,36]. Testing of the questionnaire’s content validity was performed by a panel of three experts, who validated all items before the final version of the survey was distributed. The survey also included basic socio-demographic items (age, gender, and academic year of medical study). The complete questionnaire survey is available as supplementary material (Appendix A), which was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [37].

The survey consisted of 18 total questions, which were divided into three sections:

1. *General knowledge of FXS.* This section had eight items that aimed to assess general knowledge of: (i) FXS, including its frequency and symptoms; (ii) the availability of pharmacotherapy and non-pharmacological treatments in FXS; and (iii) drug development, ie, preclinical and clinical studies in FXS.

2. *Knowledge of pharmacotherapy of FXS.* This section had seven questions that aimed to assess the participants’ practical knowledge of pharmacotherapy of FXS. For example, they were asked to choose the best medicine for treating symptoms such as attention deficit, hyperactivity, sleep problems, anxiety, aggressiveness, etc. The experts who composed the questions were child psychiatrists and it is assumed they follow the psychiatric

guidelines for the treatment approach.

3. *Self-assessment of education in NDDs during their medical studies.* Finally, the participants were asked to assess their education in NDDs during medical studies.

As presented above, two sections (*General and Pharmacotherapy knowledge*) were designed to assess knowledge of FXS (15 questions in total). Score higher than 9 (60%) would represent passing score “cut-off” [38,39]. The last section assessed the participants’ attitudes towards their education in NDDs during medical studies. Overall, this survey could assess their familiarity with NDDs in general.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The data were analyzed using descriptive and analytical statistics. Descriptive statistics included frequency (percent) of nominal variables and the measures of dispersion focusing on standard deviation (SD) for continuous variables. Parametric and nonparametric tests were used to test differences between variables. As for the latter, chi-square and Mann-Whitney *U* (M-W) tests were used to examine for the differences between nominal and ordinal variables, respectively. Kruskal-Wallis *H* test (K-W, “one-way ANOVA on ranks”), a rank-based nonparametric test, was also used to determine if there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable. Finally, the one-way analysis of variance (ANOVA) is used to determine whether there are any statistically significant differences between the means of three independent groups. Significance was indicated by $p \leq 0.05$ and internal consistency – “reliability” of the survey by Cronbach’s Alpha.

The study was approved by the Faculty of Medicine University of Belgrade Institutional Review Board (IRB) (reference number 1322/III-9).

RESULTS

General Knowledge of FXS

Table 1 depicts the number of correct answers among the participants to all questions in Section I.

The highest number of correct answers among the students was obtained for “symptoms in FXS that could be modified by pharmacotherapy” that ranged from 93% for students in Colombia to 82% for students in Serbia (q6 in Section I; Table 1). In contrast, the lowest number of correct answers was regarding “frequency of pharmacotherapy in FXS” that ranged from ~10% to 14% for students in Serbia and in Colombia, respectively (q4 in

Table 1. Level of General Knowledge of Fragile X Syndrome Among Senior Medical Students in Serbia, Georgia, and Colombia

Question (1-8) related to:	Correct answers, N (%)			χ^2	p
	Serbia	Georgia	Colombia		
1. Onset of FXS ^a symptoms	276 (79.32)	81 (72.32)	39 (67.24)	5.37	0.068
2. Early treatment in FXS	213 (61.21)	71 (63.39)	30 (51.72)	3.14	0.208
3. Beginning of pharmacotherapy in FXS	67 (19.25)	27 (24.11)	15 (25.86)	2.12	0.347
4. Frequency of pharmacotherapy in FXS	34 (9.77)	27 (24.11)	8 (13.79)	15.10	0.001*
5. Types of pharmacotherapy in FXS	277 (79.60)	88 (78.57)	53 (91.38)	4.84	0.089
6. FXS symptoms that could be modified by pharmacotherapy	286 (82.18)	97 (86.61)	54 (93.10)	5.04	0.080
7. Preclinical research in FXS	42 (12.07)	15 (13.39)	9 (15.52)	0.57	0.752
8. Conduct of clinical trials in FXS	217 (62.36)	61 (54.46)	44 (75.86)	7.46	0.024*

Abbreviation: ^afragile X syndrome; χ^2 : value of Chi-square test; *statistically significant p value: $p < 0.05$.

Table 2. Level of Knowledge of Pharmacotherapy of Fragile X Syndrome Among Medical Students in Serbia, Georgia, and Colombia

Question (1-7) related to:	Correct answers, N (%)			χ^2	p
	Serbia	Georgia	Colombia		
1. Treatment of ADHD ^a in FXS ^b	91 (26.15)	42 (37.50)	19 (32.76)	5.63	0.600
2. Treatment of sleep problems in FXS	112 (32.18)	78 (69.64)	26 (44.83)	49.17	<.0001*
3. Use of alpha-adrenergic agonists in FXS	53 (15.23)	18 (16.07)	8 (13.79)	0.02	0.926
4. Use of guanfacine in FXS	151 (43.39)	63 (56.25)	36 (62.07)	10.60	0.005*
5. Use of SSRI ^c in FXS	90 (25.86)	38 (33.93)	18 (31.03)	2.99	0.225
6. Treatment of anxiety in FXS	78 (22.41)	45 (40.18)	37 (63.79)	45.65	<.0001*
7. Treatment of aggressive behavior in FXS	111 (31.89)	33 (29.46)	24 (41.38)	2.61	0.271

Abbreviation: ^aAttention Deficit Hyperactivity Disorder; ^bfragile X syndrome; ^cSelective Serotonin Reuptake Inhibitors; χ^2 : value of Chi-square test; *statistically significant p value: $p < 0.05$.

Section I; Table 1), which was the only correct answer from Georgian participants that was significantly higher (24%) compared to the two other country participants ($p = 0.001$, Table 1). On the other hand, students from Georgia had the fewest correct answers regarding “preclinical research in FXS” (q7 in Section I; Table 1). Finally, students from Colombia had statistically significant higher number of correct answers regarding “conduct of clinical trials in FXS” than students from the two other countries (q8 in Section I; $p < 0.05$).

The median of correct answers among the three groups of participants was 4 (range 3-5) in Section I. Only one (0.9%) participant from Georgia and none from Serbia or Colombia had all 8 correct answers in the *General knowledge* section, whereas two participants from Serbia (0.57%), three from Georgia (2.68%), and none from Colombia answered all the items incorrectly. There was a strong internal consistency of the study for all three sites (Cronbach's Alpha 0.996 for Serbian, 0.978 for Georgian,

and 0.977 for Colombian participants, respectively).

Knowledge of Pharmacotherapy of FXS Among Senior Medical Students

Table 2 depicts the number of correct answers among the participants to questions in Section II.

The highest number of correct answers among the three countries was recorded for “treatment of sleep problems in FXS” in Georgia (~70%), “treatment of anxiety in FXS” in Colombia (~64%), and “use of guanfacine in FXS” in Serbia (43%), respectively. The lowest number of correct answers regarding “use of alpha-adrenergic agonists in FXS” was quite comparable among the three groups of students and ranged from 14% to 16% for students in Colombia and Georgia, respectively (q3 in Section II; Table 2, $p > 0.05$). Participants from Georgia had a statistically significant highest number (~70%) of correct answers to “treatment of sleep problems in FXS” (q2 in Section II, Table 2; $p < 0.0001$), whereas students

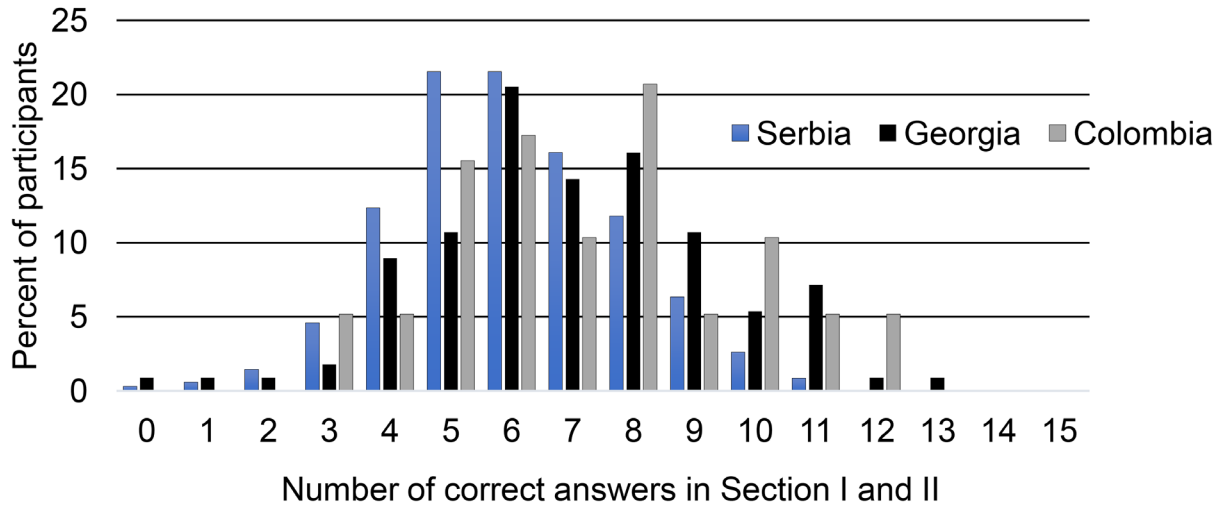


Figure 1. Total number of correct answers in Section I (*General knowledge of FXS*) and II (*Knowledge of pharmacotherapy of FXS*) among senior medical students in Serbia, Georgia, and Colombia.

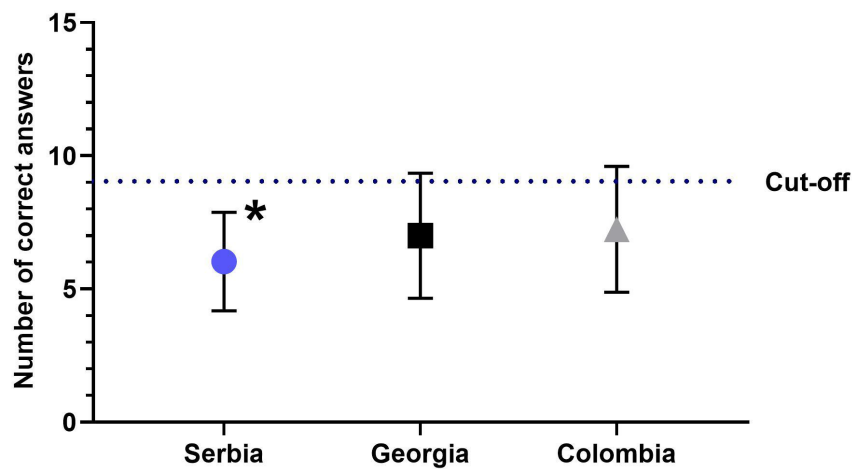


Figure 2. The average numbers of total correct answers in Section I (*General knowledge of FXS*) and II (*Knowledge of pharmacotherapy of FXS*) among senior medical students in Serbia, Georgia, and Colombia. There was statistically significant difference among the average values (* $p < 0.05$; ANOVA) among the cohorts in Serbia, Georgia, and Colombia. Error bars represent standard deviations.

from Colombia had a statistically significant highest number (62%) when asked about “guanfacine’s usage in FXS” (q4 in Section II; Table 2; $p < 0.01$) and (~ 64%) “treatment of anxiety in FXS” (q6 in Section II; Table 2; $p < 0.0001$). Only two (1.79%) participants in Georgia and none in the two other countries answered all the questions correctly.

The median number of correct answers in this section among participants 3 (range 2-4) in both Georgia and Colombia and 2 in Serbia (range 1-3). Overall, participants from Georgia and Colombia had statistically significant higher number of correct answers to questions in section

II than the students from Serbia (K-W test, $H = 44,349$, $p < .0001$; post hoc M-W U test: Serbia vs. Georgia: $U = 89.880$, $p < .0001$, Serbia vs. Colombia: $U = 93.099$, $p < .0001$). There was a strong internal consistency for those set of questions of the survey for all three sites (Cronbach’s Alpha ranged from 0.888 for Colombian to 0.930 for Serbian to 0.942 for Georgian participants, respectively).

Figure 1 depicts the number of correct answers to all questions included in both Sections related to FXS among senior medical students in Serbia, Georgia, and Colombia.

Table 3. Self-assessment of Participants from Serbia, Georgia, and Colombia of Knowledge of Fragile X Syndrome

During studies:	Answers, N (%)			χ^2	p
	Serbia	Georgia	Colombia		
1. I have never heard about FXS ^a	14 (4.02)	13 (11.61)	1 (1.72)	11.26	0.003
2. I have heard about FXS, but don't know much about FXS	168 (48.28)	56 (50.00)	33 (56.90)	1.49	0.47
3. I gained basic knowledge of FXS	162 (46.55)	39 (34.82)	25 (39.66)	4.74	0.09
4. I learned about FXS in detail.	4 (1.15)	4 (3.51)	1 (1.72)	2.91	0.23

Abbreviation: ^afragile X syndrome; χ^2 : value of Chi-square test; *statistically significant p value: $p < 0.05$.

As presented in Figure 1, the students from Serbia had in total 5 to 7 correct answers representing on average 1 in 5 of all their sample answering correctly (21%, 21%, and 16% of their cohort of students, respectively). In Georgia, similarly, their students had 6 to 8 correct answers (20.5%, 14.3% and 16.1% of their students, respectively), while students from Colombia most frequently had 5 to 8 correct answers (from 10% to 21% of their total students). We further compared distributions of the students with a top frequency of correct answers. As presented in Figure 1, the most frequent numbers of correct answers ranged from 5 to 10, capturing 1/3 (5/15) - 2/3 (10/15) of the survey's total number of questions. This category-based approach found no statistically significant difference among the cohorts (278/348, 87/112, and 46/58 in Serbia, Georgia and Colombia, respectively; $p = 0.88$, Chi-square test value: 0.25).

As presented in Figure 2, while there was a statistically significant difference among the average numbers of total correct answers (mean \pm SD, 6.03 ± 1.85 vs. 7.00 ± 2.35 vs. 7.24 ± 2.36 , $p < 0.05$; ANOVA) among the cohorts in Serbia, Georgia, and Colombia, respectively, neither cohort had an average value greater than 9 (60%) that would represent passing score "cut-off." None of the participants answered all the questions in Sections I and II correctly.

Self-assessment of Education in NDDs During Medical Studies Among Senior Medical Students

Table 3 depicts a self-assessment of medical students' knowledge of FXS.

Table 3 shows that roughly half of the students answered that they "have heard about FXS, but don't know much about FXS" (range from 48% in Serbia to 56% in Colombia; Table 3; $p > 0.05$) or "have basic knowledge of FXS" (range from 34% in Georgia to 46% in Serbia; Table 3; $p > 0.05$). The fewest number of students in the three countries claimed that they "learned about FXS in detail" (from 1.15% in Serbia to 3.51% in Georgia; Table 3; $p > 0.05$). In addition, most students (more than two-thirds of participants in each group, $p > 0.05$) concluded

that, during studies, they gained insufficient knowledge of pharmacotherapy of NDDs, including FXS and ASD (data not shown). Finally, almost all included participants from the three countries ($p > 0.05$) thought that education in the field of NDDs should be more intense (data not shown).

DISCUSSION

To our knowledge, this is the first study describing both general and more specific evidence-based level of knowledge currently recommended for the treatment of behavior problems in individuals with FXS conducted in senior medical students in Serbia, Georgia, and Colombia. In general, the study revealed a rather low level of knowledge of FXS among future rising medical doctors in these three developing countries as neither cohort reached an average value greater than 60% of the questions answered correctly as a passing score's "cut-off" [38,39]. Thus, we concluded that the students from three cohorts had insufficient knowledge of FXS. To illustrate, roughly 1 in 5 of the study respondents scored correctly on half or more facts about FXS from the survey. Moreover, some of the participants scored even lower when asked about if they have heard about preclinical studies in FXS. Importantly, many of those senior medical students did not have enough knowledge of pharmacotherapy of FXS, which was worse than their general knowledge of FXS. Indeed, unfamiliarity with the pharmacotherapy of symptoms associated with FXS was dominant, which prevailed in all aspects of pharmacotherapy covered by the survey. The results were corroborated by strong reliability analysis (Cronbach's $\alpha > 0.95$) for each part of the survey, which was examined separately for those three countries. Together, most participants have shown a low level of general and specific knowledge of FXS.

This study was conducted in the three developing countries with economies in transition [40,41], which could be at least in part generalized worldwide to countries with similar economies. There is a lack of data of frequency and services provided for individuals with

NDDs in many low- and middle-income countries [42]. Barriers to access and adequate care of those individuals with NDDs and their families include lack of knowledge, presence of stigma, systemic failures, and consequent poor quality of current services. The latter is in line with literature as 40%-80% of individuals with different mental disorders worldwide do not receive any kind of screening, treatment, or intervention [43]. For example, in Colombia and Serbia, clinical testing for *FMR1* mutations is rare resulting in an older age of diagnosis of FXS when compared to developed countries. These issues might be caused by restricted access to molecular testing through national health systems, a presence of negative stereotypes towards NDDs, and lack of knowledge among healthcare professionals about FXS and disorders related to *FMR1* mutations [33,34,44].

As widely reported, there is a major knowledge gap about NDDs such as ASD in different communities of those low- and middle-income countries [45,46]. A knowledge, attitude, and practice (KAP) survey conducted in Serbia in 2016 revealed a major gap in knowledge regarding the FXAD among medical professionals [33]. As FXS could be evaluated and treated by different medical specialties (ie, pediatrics, genetics, neurology, psychiatry, ophthalmology, orthopedics, ENT specialties), one might assume that would help with exposing senior medical students to the field during their medical studies and increase their knowledge about FXS and NDDs in general. The current study reveals that the education of medical professionals in the field of FXS, as a proxy of the field of NDDs, during their training is limited. Specialists that treat patients with NDDs come from different medical disciplines: psychiatry, neurology, pediatrics, genetics, etc. Each medical professional may have a different approach to treat those conditions. The knowledge of medical students about pharmacotherapy gets influenced by the source of knowledge, their personal preference for professional advancement, or the common use of available or less expensive medication. Importantly, the senior medical students in all three countries clearly indicated that they need additional education regarding FXS.

It is well-known that there is a cultural influence, at the macro- and micro-levels, on NDDs diagnoses, treatments, and treatment goals [47]. There is a possibility that the results of the applied survey in the current study could indicate the presence of stigma related to NDDs in these three different cultures/countries and a bias against the use of medications in children with FXS. Clinicians in those countries ought to be familiar with their own cultural biases of NDDs assessment and treatment. They need to have skills to deal with cultural norms in clinical practices [47]. According to results published by Mascayano and colleagues (2020), stigma toward mental illness could present a crucial limit for implementation

of mental health services in low- and middle-income countries. They analyzed interventions to reduce stigma toward mental illness that has been implemented in these countries through interpretation of articles published from 1990 to 2017. Based on their study, interventions are mostly based on improving attitudes and knowledge through the education of community members, consumers, as well as healthcare practitioners [48]. However, there are limited investigations on the cultural influences in this area and further research is needed [47].

Institutional collaborations between developed and developing countries could be crucial in education, research, provision of training and personnel in the field of NDDs, such as FXS. To illustrate, an excellent example is a collaborative agreement between the Kennedy Krieger Institute in Baltimore (<https://www.kennedykrieger.org>), an internationally recognized institution dedicated to improving the lives of individuals with disorders of the brain, spinal cord, musculoskeletal system, and the Faculty of Medicine in Belgrade in the field of FXAD. Similarly, the University of California Davis MIND Institute in Sacramento (<https://health.ucdavis.edu/mindinstitute/>) offers opportunities for international medical professionals through training programs such as The International Training Program in Neurodevelopmental Disorders (ITPND). In addition, the US-based National Fragile X Foundation dedicates a portion of its resources towards building international collaborations and holds a biennial international conference for families and professionals. The international collaboration between the world-renowned MIND Institute and relevant institutions in developing countries has inspired and helped promote education and research around the world. For example, in Colombia, the Ricaurte district contained a genetic cluster of FXS, which was uncovered and studied by the Universidad del Valle [49] in conjunction with the MIND Institute. The collaborative efforts included symposiums and academic events on FXAD in order to share information with the Ricaurte community, health professionals, and medical students. In addition to Serbia, Georgia, and Colombia, the field of FXAD is also currently developing in a number of countries including India, Mexico, the Republic of the Philippines, and Brazil [15,50-54]. The effort serves as a good example of an action plan towards a focus on increasing knowledge and awareness about FXS, with a potential for improving research, teaching, and education while increasing resources for patients with NDDs. Nevertheless, the current study reveals that the effort is not enough *per se* and ought to be expanded to include more of institutional support. Finally, this study might be applicable to other developing countries as a “jump-start” towards raising awareness about NDDs and improving the education of treatment and intervention professionals in this field.

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Appendix A

SURVEY

Please fill in this survey. The survey is anonymous. The collected data would serve exclusively for statistical analysis, and it would be published only in a summary form as a group to establish a baseline of knowledge related to Fragile X syndrome.

The Fragile X Syndrome (FXS) is the most common cause of inherited intellectual disability, and the most common cause of single gene mutation caused autism spectrum disorders (ASD). It is the result of a full mutation of the *FMR1* gene. The FXS is a rare disease, with the prevalence of 1:4,000 in males, and 1:8,000 in females. It is known that up to 60% of those with FXS are comorbid with ASD. Therefore, according to the international guidelines, individuals diagnosed with ASD of unknown cause and/or intellectual disability, should be tested for the *FMR1* gene mutation.

Gender: M F Age: _____ Academic year of medical studies: _____

Section I: General knowledge of FXS

1. The symptoms of the Fragile X Syndrome (FXS) can first be seen:
 - a. during intrauterine development.
 - b. in the early childhood (during the first few years of life).
 - c. during puberty.
 - d. in the adulthood.

2. Early treatment of the FXS is based on:
 - a. non-pharmacological methods.
 - b. pharmacotherapy.

3. Pharmacotherapy in children with the FXS is usually started:
 - a. during the first year of life.
 - b. between 2-5 years of age.
 - c. between 5-7 years of age.
 - d. during puberty.
 - e. in the adulthood.

4. According to the results of studies, the frequency of pharmacotherapy in individuals with FXS is:
 - a. 15-25% in adults
 - b. 25-40% in newborns
 - c. 40-55% in children aged 1-5 years
 - d. More than 60% in children aged 10 years and over.

5. Pharmacotherapy in patients with FXS is most commonly:
 - a. causal/targeted therapy.
 - b. symptomatic therapy.

6. Pharmacotherapy in individuals with FXS can modify:
 - a. behavioral disorders.
 - b. attention disorders.
 - c. hyperactivity.

- d. sleeping disorders.
 - e. All listed answers are correct.
7. Preclinical studies on animal models of FXS (e. g. experimental mice):
- a. cannot be conducted
 - b. can be conducted, but the results are not predictive for humans.
 - c. can be conducted, but they are expensive, slow and inadequate.
 - d. are conducted with great success.
8. Clinical studies, that would help develop new pharmacological agents in treating the fragile X syndrome:
- a. have never been conducted, and they are not planned to start.
 - b. have not been conducted yet, but they are going to start soon.
 - c. are currently being conducted, and some have already finished.
 - d. have been stopped due to negative results and severe side effects, and they will not be conducted again.

Section II: Knowledge of pharmacotherapy of FXS

1. Attention Deficit Hyperactivity Disorder (ADHD) is frequently seen in individuals with FXS. What should be used in treating this disorder?
- a. Anxiolytics.
 - b. Mood stabilizers.
 - c. Antidepressants.
 - d. Psychostimulants.
2. What should be used to treat sleeping disorders in individuals with FXS (regardless of the presence of autism spectrum disorders, ASD)?
- a. Anxiolytics.
 - b. Antihistamines.
 - c. Melatonin.
 - d. Low doses of antiepileptics.
3. Alpha-adrenergic agonist (e. g. clonidine) can be used in individuals with FXS to treat which of the following conditions?
- a. Mild to moderate hypertension.
 - b. Aggressiveness.
 - c. Anxiety.
 - d. Sleeping disorders.
4. Guanfacine, that is used to treat sleeping disorders and ADHD in individuals with FXS, is:
- a. Histamine receptors antagonist.
 - b. Serotonin receptors antagonist.
 - c. Alpha-adrenergic receptors agonist
 - d. Beta-adrenergic receptors agonist.
 - e. Muscarinic acetylcholine receptors antagonist.
5. In individuals with FXS, Selective Serotonin Reuptake Inhibitors (SSRI) should be used to treat which of the following conditions?
- a. Mood disorders
 - b. Anxiety

- c. Attention deficit
- d. Hyperactivity

6. Anxiety is a common symptom in individuals with FXS. What would you use to treat mild to moderate anxiety in these patients?

- a. Diazepam or lorazepam
- b. Clomipramine
- c. Sertraline
- d. Low doses of haloperidol

7. Which of the following drugs should not be used to treat aggressiveness, that is a common symptom of FXS?

- a. Risperidone
- b. Aripiprazole
- c. Olanzapine
- d. Methylphenidate

Section III: Self-assessment of education in neurodevelopmental disorders during medical studies

1. During studies:

- a. I have never heard about FXS
- b. I have heard about FXS, but don't know much about FXS
- c. I gained basic knowledge of FXS
- d. I learned about FXS in detail.

2. Do you think that, during studies, you gained sufficient knowledge about pharmacotherapy of neurodevelopment disorders, including FXS and ASD?

- a. Yes
- b. No

3. Do you think that education in the field of pharmacotherapy of neurodevelopment disorders should be more intense?

- a. Yes
- b. No