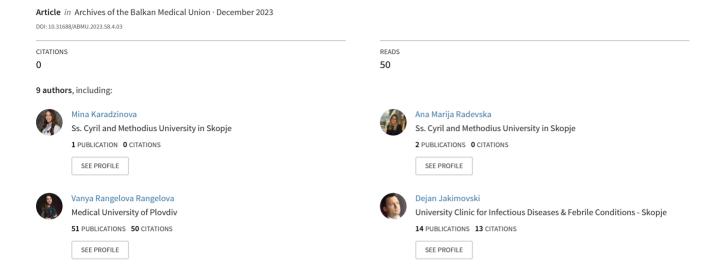
## HIV/AIDS KNOWLEDGE AND STIGMATISING ATTITUDE TOWARDS PEOPLE LIVING WITH HIV/AIDS AMONG STUDENTS OF MEDICAL SCIENCES IN THE REPUBLIC OF NORTH MACEDONIA



#### **ORIGINAL PAPER**

# HIV/AIDS KNOWLEDGE AND STIGMATISING ATTITUDE TOWARDS PEOPLE LIVING WITH HIV/AIDS AMONG STUDENTS OF MEDICAL SCIENCES IN THE REPUBLIC OF NORTH MACEDONIA

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Received 07<sup>th</sup> Sept 2023, Accepted 05<sup>th</sup> Nov 2023 https://doi.org/10.31688/ABMU.2023.58.4.03

#### **A**BSTRACT

**Introduction.** Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) have been significant global health issues for almost 40 years. However, stigma and discrimination against individuals with HIV/AIDS hinder testing, disclosure, and adherence to prevention and treatment strategies. Future healthcare workers' knowledge and attitudes toward people living with HIV/AIDS (PLWHA) are important for better care and prevention, necessitating appropriate education and training.

**The objective of the study** was to examine the general HIV/AIDS knowledge among students of health sciences in the Republic of North Macedonia and to assess medical students' overall stigmatising attitude towards PLWHA.

**Materials and methods.** A prospective cross-sectional study was conducted during November-December

#### RÉSUMÉ

Connaissance du VIH/SIDA et attitude stigmatisante envers les personnes vivant avec le VIH/ SIDA parmi les étudiants en sciences médicales en Macédoine du Nord

**Introduction.** Le virus de l'immunodéficience humaine (VIH) et le syndrome d'immunodéficience acquise (SIDA) constituent des problèmes de santé mondiaux importants depuis près de 40 ans. Cependant, la stigmatisation et la discrimination à l'encontre des personnes vivant avec le VIH/SIDA (PVVIH) entravent le dépistage, la divulgation et l'adhésion aux stratégies de prévention et de traitement. Les connaissances et les attitudes des futurs travailleurs de la santé à l'égard des PVVIH sont cruciales pour améliorer les soins et la prévention, ce qui nécessite une éducation et une formation appropriées.

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2022, surveying 743 medical sciences students in the Republic of North Macedonia with a 4-item questionnaire. The questionnaire collected demographic data, assessed knowledge, and explored stigmatizing attitudes toward PLWHA. Data underwent accuracy checks using the Kolmogorov-Smirnov test, and reliability analysis using Cronbach's  $\alpha$  coefficient.

**Results.** Among participants (77.7% female, median age 22 years), understanding of HIV/AIDS features related to contagiousness, prevention, and therapy was evident. No significant differences were observed between genders, age, ethnicity, and religion. However, approximately half were unaware of the possibility for prevention of vertical HIV transmission vertical HIV transmission, and over half were unaware of the timely postexposure prophylaxis importance. Over half of the participants rejected statements indicating HIV stigma and discrimination.

**Conclusion.** Students showed a good knowledge of HIV/AIDS transmission, diagnosis, and prevention but lacked understanding of treatment. Continuous education and training are essential. Encouragingly, no evidence of stigmatizing attitudes toward PLWHA was found among medical sciences students.

**Keywords:** HIV/AIDS, knowledge, attitudes, stigma, medical education.

#### List of abbreviations

AIDS - acquired immunodeficiency syndrome

ART - antiretroviral therapy

HIV - human immunodeficiency virus

MSM - men who have sex with men

PLWHA - people living with HIV/AIDS

PreP – pre-exposure prophylaxis

SUT - State University of Tetovo

UKIM - University of Ss. Cyril and Methodius in Skopje

WHO - World Health Organization

#### Introduction

Since its discovery almost 40 years ago, the human immunodeficiency virus (HIV) has been a major global health issue, affecting around 40 million people worldwide. According to the World Health Organisation (WHO), in 2021 there were 1.5 million new cases of HIV infection and 650000 cases of HIV-related deaths globally. By virtue of antiretroviral therapy (ART), millions of affected lives are prolonged, HIV-positive patients have a decreased transmission rate of the disease, and people can prevent getting infected by the proper use of pre-exposure prophylaxis (PrEP)<sup>2, 3</sup>. ART is truly a historical breakthrough and has made tremendous progress and improvements in

**L'objectif de l'étude** était d'examiner les connaissances générales sur le VIH/SIDA parmi les étudiants en sciences de la santé en République de Macédoine du Nord et d'évaluer l'attitude stigmatisante globale des étudiants en médecine à l'égard des PVVIH.

**Matériels et méthodes.** Une étude transversale prospective a été menée en novembre-décembre 2022, interrogeant 743 étudiants en sciences médicales en Macédoine du Nord avec un questionnaire en 4 éléments. Le questionnaire a collecté des données démographiques, a évalué les connaissances et a exploré les attitudes stigmatisantes envers les PVVIH. Les données ont été soumises à des contrôles d'exactitude à l'aide du test de Kolmogorov-Smirnov et à une analyse de fiabilité à l'aide du coefficient  $\alpha$  de Cronbach.

**Résultats.** Parmi les participants (77,7% femmes, âge médian de 22 ans), la compréhension des caractéristiques du VIH/SIDA liées à la contagiosité, à la prévention et à la thérapie était évidente. Aucune différence significative n'a été observée entre le sexe, l'âge, l'origine ethnique et la religion. Cependant, environ la moitié ignoraient la prévention de la transmission verticale du VIH, et plus de la moitié ignoraient l'importance d'une prophylaxie post-exposition en temps opportun. Plus, de la moitié des participants ont rejeté les déclarations faisant état de stigmatisation et de discrimination liées au VIH.

**Conclusion.** Les étudiants ont montré une bonne connaissance de la transmission, du diagnostic et de la prévention du VIH/SIDA, mais manquaient de compréhension du traitement. L'éducation et la formation continues sont essentielles. Il est encourageant de constater qu'aucune preuve d'attitudes stigmatisantes à l'égard des PVVIH n'a été trouvée parmi les étudiants en sciences médicales.

**Mots-clés:** VIH/SIDA, connaissance, attitude, stigmatisation, formation médicale.

HIV or acquired immunodeficiency syndrome (AIDS) patients' quality of life. Yet, approximately 75% of people living with HIV/AIDS (PLWHA) are receiving ART, and more than 10 million people do not have access to this life-prolonging treatment<sup>4</sup>.

The Republic of North Macedonia demonstrates a relatively low incidence of HIV infection, with 469 cases recorded as of December 31, 2019. However, there has been a noticeable upward trend in newly reported HIV infections in recent years. The year 2019 witnessed the most significant number of newly diagnosed cases, with a total of 66 individuals identified. In aggregate, among the cases that have been officially recorded, 413 individuals (88%) were male, while 56 individuals (12%) were female. ART is provided

at no cost to patients diagnosed with HIV infection, irrespective of their CD4 cell level<sup>5</sup>.

Besides the health aspects that PLWHA face, stigmatisation and discrimination represent major barriers<sup>6</sup>. HIV stigmatisation is defined as the negative attitudes and beliefs directed towards PLWHA<sup>7</sup>. Stigmatisation of PLWHA is believed to be one of the critical challenges leading to impediments to access and benefit from HIV testing, serum status disclosure, regular uptake, and adherence to prevention and treatment strategies<sup>8-11</sup>. HIV-related stigma is not only a barrier to HIV prevention, treatment, and care, but it also imposes unjust or prejudicial treatment of PLWHA in fields outside of healthcare, such as education, workplace, justice system, as well as within families and communities<sup>12</sup>.

The examination of stigmatizing behaviour towards PLWHA among the health sciences students holds potential for identifying deficiencies and shortcomings in medical education and clinical training programs<sup>13,14</sup>. Prior research has indicated that a deficiency in sufficient abilities and inadequate training may be linked to feelings of anxiety and avoidance, ultimately leading to a negative attitude<sup>15,16</sup>. Misconceptions about HIV transmission, such as the idea that it can happen through handshakes, hugs, or sharing utensils, can cause fear of being infected and associated stigmatising behaviour<sup>17</sup>. The knowledge and attitudes of future healthcare workers towards PLWHA will ultimately influence the quality of service provided, management and prevention of the disease, as well as treatment outcome and adherence. Hence, it is very important to invest in appropriate education by providing sufficient knowledge, adequate skills, and proper training for younger generations<sup>18,19</sup>. For this reason, studying stigma and discrimination against PLWHA is considered among the top priorities of HIV/AIDS research<sup>12,20,21</sup>.

**THE OBJECTIVE OF THIS STUDY** was to examine general HIV/AIDS knowledge (especially in terms of transmission and care) among students of health sciences in the Republic of North Macedonia, as well as to assess medical students' overall stigmatising attitude towards PLWHA.

#### **M**ATERIALS AND METHODS

#### Study design and participants

The self-administered online survey that served as the foundation for this cross-sectional study was prepared using Google Forms. The questionnaire was designed to assess HIV/AIDS knowledge and stigmatising attitudes among students of medical sciences in the Republic of North Macedonia. The survey was

distributed between November-December 2022 using a practical sampling method, starting with the contacts of the authors (five of whom were students of medical sciences at the University of Ss. Cyril and Methodius (UKIM), Skopje), who distributed the survey on Facebook and WhatsApp by posting the survey link on the major pages and groups targeting students of medical sciences. The online questionnaire targeted students who were associated with the three major faculties of medical sciences currently present in North Macedonia, UKIM, the State University of Tetovo (SUT), and the University "Goce Delcev", Shtip. There were no incentives offered to individuals who answered, therefore, participation was entirely voluntary. Since the official language of study in the faculties of North Macedonia is Macedonian, it was used in survey construction and distribution; however, there was an English translation as well, for the sake of other nationalities that participated in the study. The minimum required sample size was estimated at 500 respondents, considering a total number of 10,046 students of medical sciences<sup>22</sup>.

#### **Ethical considerations**

The study received an ethical exemption from the Ethics Committee as it met on of the criteria for exemption (an anonymous survey or interview that do not involve collection of identifiable data). The study instrument included three sections.

The first section collected information on socio-demographic characteristics of the participants by using six items: age, gender, ethnicity, religion, faculty, and academic year.

The second section was focused on the HIV/AIDS knowledge. There were 9 questions to assess the knowledge of the respondents regarding the possibilities for transmission of the virus, and 5 questions assessing the knowledge about the possibilities for prevention of the transmission of HIV. Each of the 14 items had the following three options as responses (Yes; No; I do not know).

The third section of the questionnaire assessed the stigmatizing attitude of the respondents towards PLWHA. For this purpose, a PLWHA Scale (SAT-PLWHA-S) was prepared based on questions adjusted from previous relevant studies<sup>23-28</sup>. It consisted of 15 questions about stereotyping, prejudices and affects, and discrimination of PLWHA – each scored on a 5-point Likert scale from 1 (strongly disagree) to 5 (strongly agree). Face validity and content validity of the items were checked by the first author and the senior author before survey distribution. For the first 14 questions, the scoring system was as follows: "highly disagree" (+2), "disagree" (+1), "neutral" (0), "agree" (-1), "highly agree" (-2); and for the last 15<sup>th</sup> question, the scoring system was reversed, meaning:

**Table 1.** Demographic characteristics of the participants (n=743)

Variable	Category	n, (%)				
	Curegory	11, (70)				
Age (year)						
Median	22					
25 <sup>th</sup> ; 75 <sup>th</sup>	20;23					
percentile	18-34					
Min-max	1031					
Sex	Male	168 (22.6)				
	Female	575 (77.4)				
Ethnic group	Macedonian	572 (77)				
	Albanian	104 (14)				
	Turkish	24 (3.2)				
	Serbian	15 (2)				
	Other	28 (3.8)				
	Other	20 (5.0)				
Religion	Christianity	550 (74)				
	Muslim	147 (19.8)				
	Other	46 (6.2)				
	University of Ss.					
	Cyril and Methodius	500 (05 A)				
	State University of	708 (95.3)				
University	Tetovo	22 (3)				
	University of Goce	13 (1.7)				
	Delchev					
	Medical Faculty	645 (86.8)				
Faculty	Dental Faculty	37 (5)				
raculty						
	Pharmacy Faculty	61 (8.2)				
	1st year	131 (17.6)				
Academic year	2 <sup>nd</sup> year	99 (13.3)				
	3 <sup>rd</sup> year	175 (23.6)				
	4 <sup>th</sup> year	127 (17.1)				
	5 <sup>th</sup> year	117 (15.7)				
	6 <sup>th</sup> year	94 (12.7)				

"highly disagree" (-2), "disagree" (-1), "neutral" (0), "agree" (+1), and "highly agree" (+2).

The total HIV stigma score was used to assess the stigmatising attitude of the respondents towards PLWHA. A score of +30 to +16 indicated a highly positive attitude, +15 to +1 a positive attitude, a score of 0 to -15 indicated a negative attitude, and a score of -16 to -30 respectively a highly negative attitude.

#### Statistical analysis

The presentation of quantitative variables in this study involved reporting the mean and standard deviation (mean  $\pm$  SD) or the median (25th percentile; 75th percentile), which were determined based on the distribution of the sample. The qualitative variables were represented using numerical values in the form of absolute and relative frequencies, as well as totals and percentages (n, %). The Kolmogorov-Smirnov test was utilized to ascertain the distribution of the sampled students. The Chi-Square test was employed to examine the presence of an association between two nominal variables. Statistical tests were considered to have statistical significance if the p-value was less than 0.05. The data was systematized, processed, and analysed using SPSS v.26 for Windows (IBM Corp. Released 2019. Armonk, NY: IBM Corp).

#### **R**ESULTS

#### **Demographic characteristics**

The total number of participants included in our study was 743, most females (n=575, 77.4%), Macedonians (n=572, 77.0%) (Table 1). The mean age

Table 2. Knowledge of students in regards to HIV transmission and methods for prevention.

Question		Incorrect n, (%)	I don't know n, (%)
HIV can be transmitted through sexual intercourse		5 (0.7)	3 (0.4)
HIV can be transmitted through infected syringes and needles	722 (97.2)	8 (1.1)	13 (1.7)
HIV can be transmitted from mother to foetus during pregnancy and/or delivery		17 (2.3)	39 (5.2)
HIV can be transmitted through breastfeeding		126 (17.0)	177 (23.8)
HIV can be transmitted through blood transfusion		16 (2.2)	16 (2.2)
HIV can be transmitted by handshaking with an infected person		35 (4.7)	41 (5.5)
HIV can be transmitted by mosquito bite		110 (14.8)	167 (22.5)
HIV can be transmitted through contact with an infected person's saliva	460 (61.9)	202 (27.2)	81 (10.9)
Only homosexuals and sexual workers are getting infected with HIV		28 (3.8)	29 (3.9)
Oral contraceptives can prevent HIV transmission		100 (13.5)	121 (16.3)
It is possible to prevent vertical HIV transmission (from mother to foetus)	417 (56.1)	53 (7.1)	273 (36.7)
Chances of HIV transmission through unprotected sexual intercourse can be lowered by taking post-exposure prophylaxis on time	363 (48.9)	124 (16.7)	256 (34.5)
It is possible to lower the chance of HIV transmission through infected needle stick by taking post-exposure prophylaxis	347 (46.7)	114 (15.3)	282 (38)
Antiretroviral therapy is a life-prolonging treatment for PLWHA	582 (78.3)	22 (3)	139 (18.7)

**Table 3.** Summary of the responses of the respondents to the stigmatizing statements regarding PLWHA (n=743)

Statement	Completely disagree	Disagree	Neither agree, nor disagree	Agree	Completely agree
PLWHA got what they deserved, n, (%)	564 (75.9)	84 (11.3)	74 (9.9)	10 (1.3)	11 (1.6)
My support and attitude towards PLWHA depend on the way they got infected, n (%)	457 (61.5)	98 (13.2)	121 (16.3)	33 (4.4)	34 (4.6)
I would never be friends with PLWHA, n (%)	514 (69.2)	113 (15.2)	79 (10.6)	18 (2.4)	19 (2.6)
I would be ashamed to share that I am HIV positive, n (%)	189 (25.4)	113 (15.2)	231 (31.1)	128 (17.2)	82 (11.1)
PLWHA are contaminated and I would avoid any type of contact with them, n (%)	459 (61.8)	152 (20.4)	96 (12.9)	19 (2.5)	17 (2.4)
As a health sciences student I would fee anxious if had to care for/treat a PLWHA n (%)	419 (56.4)	153 (20.5)	108 (14.5)	49 (6.6)	14 (2.0)
As a future HCW I would not want to work with or treat PLWHA, n (%)	529 (71.2)	111 (14.9)	73 (9.7)	11 (1.6)	19 (2.6)
I believe that a HCW who has HIV/AIDS should not be allowed to work, n (%)	413 (55.6)	134 (18.0)	122 (16.4)	35 (4.7)	39 (5.3)
The opportunity to be in contact and/or work with PLWHA would have an impact on my choice of residency or future workplace, n (%)	419 (56.4)	132 (17.8)	130 (17.5)	35 (4.7)	27 (3.6)
Relative and sexual partners of PLWHA should be informed about their HIV status even with- out the consent of the infected person, n (%)	197 (26.5)	86 (11.6)	159 (21.4)	105 (14.1)	196 (26.4)
PLWHA should be quarantined, n (%)	507 (68.2)	121 (16.3)	69 (9.3)	22 (2.9)	24 (3.3)
HIV positive women should not have children, n (%)	315 (42.4)	147 (19.9)	286 (25.0)	47 (6.3)	48 (6.4)
Transmission of HIV should be punishable by law, n (%)	333 (44.8)	110 (14.8)	179 (24.1)	65 (8.7)	56 (7.6)
PLWHA are to be blamed for acquiring the infection, n (%)	377 (50.7)	146 (19.6)	174 (23.4)	24 (3.3)	22 (3.0)
The university education I have received gave me enough information to confidently work with PLWHA, n (%)	67 (9.0)	80 (10.8)	185 (24.9)	195 (26.2)	216 (29.1)

of the respondents was 21.8 years (median 22 years, standard deviation (SD): 2.1; range 18-34 years). Most participants were studying in the UKIM, Skopje (n=708, 95.3%), and in the Faculty of Medicine, respectively (n=645, 86.8%). Regarding the year of study of the respondents, there was almost equal distribution.

#### **HIV** knowledge

The respondents demonstrated a general understanding of the features of HIV/AIDS infection in terms of contagiousness, prevention, and therapy (Table 2). Concerning knowledge about modes of HIV transmission, most participants correctly identified sexual contact (98.9%), infected syringes and needles (97.2%), vertical transmission from mother to foetus (92.5%), and blood transfusion (95.7%) as routes of transmission. A significant proportion (59.2%) acknowledged the possibility of mother-to-foetus transmission through breastfeeding. However, there was a

statistically significant difference in the respondents' knowledge in terms of study years regarding the questions about the possible transmission of HIV through handshaking, saliva, and mosquito bite. The higher the study year, the better the knowledge of the respondents was. The participants in our study showed some gaps in their knowledge in regard to the prevention of the infection. Almost 50% of the respondents correctly identified the possibility of prevention of infection after unprotected sex and needle stick injury by taking post-exposure prophylaxis.

### Analysis of the stigmatising attitudes of the respondents towards PLWHA

The responses of the respondents in our study to the stigmatizing statements are presented in Table 3.

The assessment of the stigmatising attitude of the respondents that took part in our study showed an overall positive (n=225, 30.3%), and highly positive (n=419, 56.4%) attitude towards PLWHA. A negative

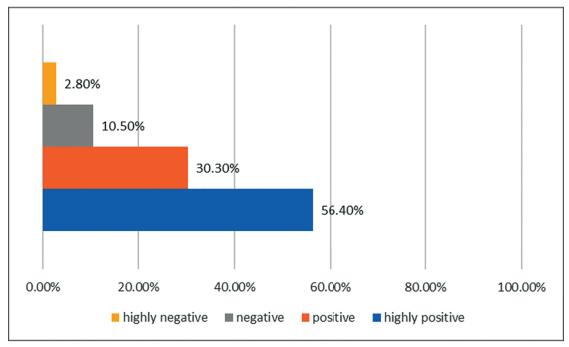


Figure 1. Overall stigmatising attitude of the respondents towards PLWHA.

attitude was observed among 99 respondents (n=99, 13.3%) (Figure 1).

We further explored the attitudes of the respondents according to some of their demographic characteristics. The female students tend to have significantly more often highly positive or positive attitude toward PLWHA compared to males (n=510, 88.7% vs. n=134, 79.7%, Pearson chi-square-9.882, p=0.020). A similar significant difference in the attitudes of the respondents was found according to study year. It was seen that with increasing the study year there was also an increase observed in the positive attitudes of the respondents towards PLWHA (80.1% –1<sup>st</sup> year students; 83.8%- 2<sup>nd</sup> year students; 84.5%-3<sup>rd</sup> year students; 87.4%-4<sup>th</sup> year students; 93.1%-5<sup>th</sup> year students; 93.6%-6<sup>th</sup> year students respectively, Pearson chi square-26.936, p=0.029).

#### **D**ISCUSSION

The significance of HIV as a global public health issue is evident from the establishment of World AIDS Day by the WHO, which aims to raise awareness about the importance of prevention, early detection, and treatment<sup>29</sup>. Young individuals appear to be particularly susceptible to HIV and other sexually transmitted illnesses in Croatia, as well as the larger region: 25% of HIV-positive people are between the ages of 20 and 29 years<sup>30</sup>. As a comparison, in the Republic of North Macedonia, 77% of the HIV positive individuals are aged between 20-39

years and 70% of them are men who have sex with men (MSM)<sup>31</sup>. The concern of unwanted pregnancy, rather than HIV infection, is the main reason why most of the students use condoms<sup>32, 33</sup>. The transmission of HIV is more common during anal intercourse compared to vaginal intercourse, as indicated by a meta-analysis and systematic review of published literature on the MSM population<sup>34, 35</sup>. Those people who are aware of risky behaviour often avoid getting tested, not because they are ignorant, but because they fear the results, lack trust in healthcare providers, and worry about discrimination and lack of support<sup>36</sup>.

To the best of our knowledge, the current study is the first to evaluate HIV/AIDS knowledge and to emphasise the possible stigmatising attitude towards PLWHA among students of medical sciences in North Macedonia. One of the reassuring findings of the study is that students of medical sciences in North Macedonia have a relatively good knowledge of HIV/AIDS transmission, diagnosis, and prevention. However, the paper also points out some gaps in knowledge regarding the management of HIV/AIDS, which could affect the quality of care provided to people living with the disease. A significant number of study participants, amounting to 40.8%, lacked awareness regarding the transmission of HIV from mother to child through breastfeeding, indicating a substantial deficiency in their knowledge about HIV/ AIDS. This outcome represents a significant lack of understanding about HIV/AIDS, which would have a detrimental effect on possible measures to prevent it<sup>23,37</sup>. More than half of the participants (51.2%) were not informed about the fact that the risk of HIV transmission through unprotected sexual intercourse can be reduced by promptly taking post-exposure prophylaxis. Additionally, only 46.7% of the participants were aware that it is currently possible to lower the likelihood of HIV transmission through an infected needle stick by promptly taking post-exposure prophylaxis. Such a result can be considered in another study in Iordan<sup>23</sup>. This finding highlights the importance of continuous education and training for students of medical sciences to ensure that they are equipped with the necessary knowledge and skills to provide the best possible care for people living with HIV/AIDS.

Another encouraging finding of the study is that most of the students held positive attitudes towards PLWHA. In contrast, previous studies showed that most participants held a negative stigmatising attitude towards PLWHA, which shows that this is a common problem worldwide<sup>38-41</sup>.

The study also highlights the importance of integrating HIV/AIDS education into the medical curriculum in North Macedonia. The use of Internet and social networks is increasingly being explored as a main advantage of preventing HIV, with users valuing the anonymity and confidentiality of information<sup>42</sup>. Another finding in this study, which was related to the attitude that HIV positive women should not have children, might be correlated to the absence of knowledge about the HIV treatment.

There were several limitations in this study. One limitation was the inherent bias of using the snowball sampling approach, which may have influenced the selection of participants. Additionally, there was a predominance of participants from only three medical schools, which could be attributed to the fact that these universities have larger numbers of enrolled students and are considered the biggest medical schools in the country. Another limitation was the possibility that respondents may have provided answers that they thought would be more favourable or suitable for the researchers.

#### **C**onclusions

The findings of this study have highlighted the need for continuous education and training of students of medical sciences, and the importance of interventions aimed at reducing stigmatising attitudes towards PLWHA. The study found that students of medical sciences in North Macedonia have good knowledge regarding HIV/AIDS transmission, diagnosis, and prevention, but there are gaps in their

knowledge regarding the treatment and post-exposure prophylaxis of HIV/AIDS. Additionally, the study did not find that there is a presence of stigmatising attitudes towards people living with HIV/AIDS. In summary, our research provides valuable insights into the level of HIV/AIDS knowledge and stigmatising attitudes among students of medical sciences. These findings are very important in addressing the HIV/AIDS in North Macedonia, ensuring that PLWHA receive the best possible care.

#### **Authors contributions:**

M.K., V.R, D.J., conceived the original draft preparation. M.K, K.P, V.R, D.J., M.H., were responsible for conception and design of the study. A.R., LRT.K., G.G., E.B., were responsible for the collection of data. All authors contributed equally to the present work. All authors contributed to the critical revision of the manuscript for valuable intellectual content. All authors have read and agreed with the final version of the manuscript.

#### **Compliance with Ethics Requirements:**

"The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.

#### **Acknowledgments:**

This paper was supported by the Ministry of Education and Science, Republic of Bulgaria (National Program "Young scientists and postdoctoral students-2")

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