Kovačević J, Miškulin M, Matić Ličanin M, Barać J, Biuk D, Palenkić H, Matić S, Kristić M, Biuk E, Miškulin I. Quality of life in road traffic accident survivors. Zdr Varst. 2020;59(4):202-210. doi: 10.2478/sjph-2020-0026.

QUALITY OF LIFE IN ROAD TRAFFIC ACCIDENT SURVIVORS

KAKOVOST ŽIVLJENJA PREŽIVELIH V CESTNOPROMETNIH NESREČAH

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Received: Apr 26, 2020
Accepted: Aug 19, 2020

Original scientific article

ABSTRACT

Introduction: The loss of quality of life is the major consequence following a non-fatal road traffic accident (RTA). Previous research regarding quality of life did not include uninjured RTA survivors. The research aim was thus to evaluate the quality of life of the RTA survivors regardless of whether or not they sustained injures, and to identify factors associated with decreased quality of life after the RTA.

Methods: A cohort of 200 RTA survivors with and without injuries was followed after experiencing an RTA. The quality of life and mental health outcomes were assessed 1 month following RTA. A vast range of sociodemographic, pre-RTA health-related, RTA related, RTA injury-related, compensation-related factors and mental health outcomes were investigated.

Results: Decreased quality of life following an RTA showed an association with the low socioeconomic status of the RTA victims, poor pre-RTA health, injury-related factors, compensation-related factors and psychological disorders after the RTA.

Conclusions: Identifying predictors of decreased quality of life following an RTA will enable planning interventions targeting the most important factors that influence recovery of RTA victims. Assessing and recording of self-reported quality of life should be a part of the routine protocol in RTA survivors' health-care.

Keywords:
road traffic accident, quality of life, injury severity, health status, compensation

IZVLEČEK

Uvod: *Poslabšanje kakovosti življenja je glavna posledica cestnoprometne nesreče (CPN), ki nima smrtnega izida. Pretekle raziskave kakovosti življenja niso vključevale preživelih CPN, ki se niso poškodovali. Cilj raziskave je bil oceniti kakovost življenja preživelih CPN, ne glede na to, ali so bili poškodovani, in prepoznati dejavnike, povezane z zmanjšano kakovostjo življenja po CPN.*

Metode: *Spremljali so kohorto 200 ljudi, ki so preživeli CPN, s poškodbami ali brez njih. En mesec po CPN so ocenili kakovost življenja in izide duševnega zdravja. Raziskali so širok spekter socialno-demografskih dejavnikov; dejavnikov, povezanih z zdravjem pred CPN; dejavnikov, povezanih s poškodbami v CPN; dejavnikov, povezanih z odškodnino, in izidov duševnega zdravja.*

Rezultati: *Izkazalo se je, da je zmanjšana kakovost življenja po CPN povezana z nizkim socialno-ekonomskim statusom žrtev CPN, slabim zdravjem pred CPN, dejavniki, povezanimi s poškodbami, dejavniki, povezanimi z odškodnino, in psihološkimi motnjami po CPN.*

Sklepi: *Prepoznavanje napovednih znakov zmanjšane kakovosti življenja po CPN bo omogočilo načrtovanje intervencij, usmerjenih v najpomembnejše dejavnike, ki vplivajo na okrevanje žrtev CPN. Ocenjevanje in evidentiranje samoocenjene kakovosti življenja bi morala biti del rutinskega protokola zdravstvenega varstva preživelih CPN.*

Ključne besede:
cestnoprometna nesreča, kakovost življenja, resnost poškodbe, zdravstveno stanje, odškodnina

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1 INTRODUCTION

About 50 million people around the world suffer from non-fatal road traffic accident (RTA) injuries every year (1). In 2018, 13,989 people were injured in RTAs in Croatia (2). The non-fatal consequences of RTAs are numerous: functional and cognitive impairments, psychological consequences and a decrease in the quality of life of the survivors (3).

Decreased quality of life (QoL) is the major consequence of RTAs (4). Research shows that the physical and mental components of health-related QoL are decreased in the long-term, even in RTA survivors with minor injuries (5-7). QoL consistently and independently predicts return to pre-injury employment and life participation among RTA survivors who sustained mild/moderate injuries (8). Research also shows that psychiatric disorders in RTA survivors decrease QoL (9), especially PTSD (4, 7, 10, 11), depressive disorder (4, 9) and anxiety disorder (9). Other potential factors that influence QoL in RTA survivors are expectations regarding recovery, pain level, social support, perceived life-threat in the RTA (4, 12, 13), level of education, injury severity, compensation claim, early medical complications and socioeconomic factors, especially financial problems (11). The research regarding the impact of RTA injury severity on the QoL following the RTA is inconsistent, some studies found that injury severity does not predict later QoL (4, 6, 9), while others found the contrary (11, 14).

However, all published studies on QoL following an RTA only include the injured RTA survivors, and thus there is no knowledge on the QoL of the uninjured survivors. Furthermore, there are no QoL studies conducted among RTA victims in Croatia.

The research aim was therefore to evaluate the quality of life of the RTA survivors regardless of whether or not they sustained injures, and to identify factors associated with decreased quality of life after the RTA.

2 METHODS

A cohort of 200 RTA survivors recruited at the Institute of Emergency Medicine of the Vukovar-Srijem County in Croatia were included in a prospective study. They were assessed 1 month following the RTA. Inclusion criteria for participating were being an RTA survivor and aged 18 and older. RTA survivors with cognitive dysfunctions and inability to give consent were excluded, as well as those aged under 18. Recruitment of the participants and data collection was done by a medical doctor.

During the research period, from October 2016 to December 2017, 640 people were involved in RTAs. Figure 1 gives details on the formation of the prospective cohort.

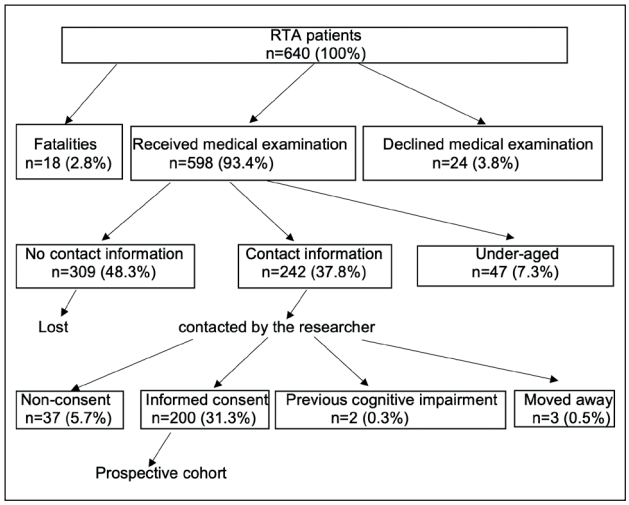

Figure 1. Cohort recruitment diagram.

Using a specially designed questionnaire the following data about RTA survivors was collected in this study: age, gender, place of residence, education, employment, marital status, self-assessed economic status and religiousness. Using the same questionnaire, data regarding pre-RTA health-related factors such as smoking, alcohol consumption, drug abuse, earlier road crash experience, traumatic exposures, prior PTSD, chronic diseases, psychiatric diseases and previous permanent pain was also collected.

Finally, the RTA-related factors explored through the same questionnaire were the type of road user, number of motor vehicles that were involved in the RTA, the injured and fatalities, fault for causing the RTA, compensation status, memory loss after the RTA, loss of consciousness in the RTA, injury status and severity, hospitalization, surgery and rehabilitation, self-assessed life-threat and pain following the RTA. The classification and detailed description of all collected data are shown in Tables 1, 2 and 3.

Based on medical records related to the RTA, the injury severity was assessed using the Abbreviated Injury Scale (AIS) (15). The final score was assigned using the New Injury Severity Scale (NISS). NISS classifies injuries as minor, moderate, serious, severe and critical (16).

The PTSD Checklist for Civilians (PCL-C) was used for the assessment of PTSD symptoms following the RTA (17). A cut-point of 30 was used, as suggested for general population (18).

The Beck Anxiety Inventory (BAI) was used for the assessment of anxiety symptoms following the RTA, with the cut-point of 22 (19).

The Beck Depression Inventory—I (BDI—I) was used for the assessment of depression symptoms following the RTA, with the cut-point of 11 (20).

The Short Form-36 (SF-36) was used for the assessment of QoL following the RTA (21). In this QoL is a multidimensional concept describing a satisfactory, balanced and healthy life comprising both biopsychosocial and socioeconomic aspects (22), such as physical health, psychological status, independence level, social relations, personal beliefs and relations within a specific environment (11). SF-36 is the most widely used instrument for assessing health-related QoL, and has been standardized and validated. This self-reporting measure contains 36 items and is used for the assessment of health status across eight domains: physical function (PF), role limitations due to physical health (RP), role limitations due to emotional problems (RE), vitality (VT), mental health, referring to the absence of anxiety and depression, (MH), social functioning (SF), bodily pain (BP) and general health (GH). The result scores range from 0 to 100, and higher obtained values present better perception of the QoL (23). The Croatian version of SF-36 was validated and found reliable in the Croatian population (24).

The normality of data distribution was tested with the Kolmogorov-Smirnov test; thereafter descriptive statistics were applied. The Mann-Whitney U test and Kruskal-Wallis test were applied for the comparison of numerical variables. Spearman's correlation was applied to test the correlation between the QoL domains and psychological outcomes following the RTA. The statistical significance level was set at $p<0.05$. The statistical package Statistica for Windows 2010 (version 10.0, StatSoft Inc., Tulsa, OK, USA) was used for the analysis.

3 RESULTS

3.1 RTA Survivors' Characteristics

Participants' median age was 42.5 years (interquartile range 28.3-56.0), 54.0% were males, 56.5% had rural residence, 62.5% finished high-school education, 58.0% were employed, 64.5% had a partner, 58.0% reported self-assessed average economic status, 90.5% were religious, 64.5% were non-smokers, 49.5% never used alcohol, 51.0% used medications, 42.0% had prior RTA experience, 52.0% reported previous traumatic exposures, 42.0% had previous chronic disease, 11.0% had previous psychiatric disease and 9.5% suffered previous permanent pain (Table 1). Non-participants and participants had similar age, gender and primary injury location.

Table 1. Sociodemographic factors and health status before the RTA.

Sociodemographic factors and health status	N	%
Sex		
Male	108	54.0
Female	92	46.0
Age group (years)		
Younger (18–41)	97	48.5
Older (≥42)	103	51.5
Place of residence		
Urban	87	43.5
Rural	113	56.5
Education		
Primary	38	19.0
Secondary	125	62.5
Higher education	37	18.5
Employment		
Employed	116	58.0
Out of work	52	26.0
Retired from work	32	16.0
Relationship status		
Single	71	35.5
Has a partner	129	64.5
Self-assessed economic status		
Under average	40	20.0
Average	116	58.0
Above average	44	22.0
Religiousness		
No	19	9.5
Yes	181	90.5
Smoking		
No	129	64.5
Yes	71	35.5
Alcohol use		
No	99	49.5
Yes	101	50.5
Drug abuse		
No	197	98.5
Yes	3	1.5
Use of medications		
No	98	49.0
Yes	102	51.0
Type of medications		
None	98	49.0
Non-psychiatric	78	9.0
Psychiatric	7	3.5
All types	17	8.5
Previous RTAs		
No	116	58.0
Yes	84	42.0
Traumatic exposures		
No	96	48.0
Yes	104	52.0

Sociodemographic factors and health status	N	%
Prior PTSD		
No	193	96.5
Yes	7	3.5
Chronic disease		
No	116	58.0
Yes	84	42.0
Psychiatric disease		
No	178	89.0
Yes	22	11.0
Permanent pain		
No	181	90.5
Yes	19	9.5

RTA details are shown in Table 2, and these reveal that 61.0% of the participants were drivers of motor vehicles, 53.5% were involved in the RTA involving two or more vehicles, 42.0% were in the RTA with one victim, 2.5% were in the RTA with fatalities, 61.5% of the participants were not at fault for causing the RTA, 43.5% of the participants claimed compensation and 10.0% received compensation.

Table 2. RTA details.

RTA details.	N	%
Type of road users		
Driver of motor vehicle	122	61.0
Co-driver or a passenger	61	30.5
Pedestrian or a cyclist	17	8.5
Motor vehicles crashed		
None	1	0.5
One	92	46.0
Two or more	107	53.5
Injured		
None	29	14.5
One	84	42.0
2–3	72	36.0
More than 3	15	7.5
Fatal outcomes		
No	195	97.5
Yes	5	2.5
At fault		
No	123	61.5
Yes	70	35.0
Unknown	7	3.5
Claimed compensation		
No	113	56.5
Yes	87	43.5
Obtained compensation		
No	180	90.0
Yes	20	10.0

The health status of the participants following the RTA is shown in Table 3. Multiples injures were sustained by 62.0% of the participants, 15.5% of the participants had no injuries, 48.0% sustained minor injuries, 18.0% sustained moderate injuries, 14.0% sustained serious injuries, 3.0% sustained severe injuries and 1.5% sustained critical injuries in the RTA. Threat to life was experienced by 46.0% of the participants, 16.0% reported unconsciousness, 14.0% reported amnesia, 32.0% were hospitalized, 10.0% underwent surgical treatment, 23.0% underwent rehabilitation procedures, 76.5% reported pain following the RTA, 35.5% reported PTSD symptoms, 20.0% reported depression symptoms and 4.5% reported anxiety symptoms following the RTA.

Table 3. Health status following the RTA.

Health status following the RTA	N	%
Number of injuries		
Without injures	31	15.5
One	45	22.5
Multiple	124	62.0
Injury location		
None	31	15.5
Head	18	9.0
Face	2	1.0
Neck	8	4.0
Chest	8	4.0
Abdomen	1	0.5
Spine	3	1.5
Upper extremities	3	1.5
Lower extremities	10	5.0
Several locations	116	58.0
Major injury		
Without injuries	31	15.5
Head	58	29.0
Neck	37	18.5
Chest	19	9.5
Abdomen	12	6.0
Upper extremities	17	8.5
Lower extremities	26	13.0
Injury level		
Without injury	31	15.5
Minor	96	48.0
Moderate	36	18.0
Serious	28	14.0
Severe	6	3.0
Critical	3	1.5
Self-assessed life-threat		
No	108	54.0
Yes	92	46.0
Loss of consciousness		
No	168	84.0
Yes	32	16.0

Health status following the RTA	N	%
Loss of memory		
No	172	86.0
Yes	28	14.0
Hospitalization		
No	136	68.0
Yes	64	32.0
Days in hospital		
None	136	68.0
1–3	27	13.5
4–10	19	9.5
11 and more	18	9.0
Surgery		
No	180	90.0
Yes	20	10.0
Rehabilitation		
No	154	77.0
Yes	46	23.0
Pain following the RTA		
No	47	23.5
Yes	153	76.5
Symptoms of PTSD		
No	129	64.5
Yes	71	35.5
Symptoms of depression		
No	160	80.0
Yes	40	20.0
Symptoms of anxiety		
No	191	95.5
Yes	9	4.5

3.2 Quality of Life After the RTA

The median values of QoL obtained across eight domains are presented in Table 4.

Table 4. Quality of life of the participants after the RTA.

QoL domains	Median	Interquartile range
Physical functioning	70.0	30.0–100.0
Role limitations due to physical health	0.0	0.0–100.0
Role limitations due to emotional health	100.0	67.0–100.0
Vitality	60.0	50.0–75.0
Mental health	68.0	52.0–76.0
Social functioning	75.0	50.0–100.0
Bodily pain	55.0	33.0–80.0
General health	70.0	55.0–84.0

The sociodemographic factors that were found to be associated with lower QoL domains were female gender, older age group, lower education level, unemployment, lower self-assessed economic status and irreligiousness. Pre-RTA health showed an association with lower QoL domains after the RTA, in terms of alcohol abstinence, previous traumatic exposure, previous chronic disease, previous psychiatric disease, previous permanent pain, use of medications and especially psychiatric medication use. Injury-related factors found associated with lower QoL domains were injury affliction, injury severity, self-assessed life-threat, pain following the RTA, hospitalization and its duration, surgery, unconsciousness in the RTA and rehabilitation following the RTA. Among RTA-related factors, not being at fault in the RTA, claiming compensation, obtaining compensation and vulnerable road users had lower QoL (Table 5).

Table 5. Factors influencing quality of life following the RTA.

	QoL domains							
Factors	**PF**	**PR**	**ER**	**VT**	**MH**	**SF**	**BP**	**GH**
Sociodemographic								
Gender	p=0.681[a]	p=0.651[a]	p=0.020[a]	p=0.072[a]	p=0.064[a]	p=0.408[a]	p=0.052[a]	p=0.118[a]
Age group	p=0.084[a]	p=0.204[a]	p=0.226[a]	p=0.078[a]	p=0.012[a]	p=0.079[a]	p=0.959[a]	p<0.001[a]
Place of residence	p=0.795[a]	p=0.098[a]	p=0.876[a]	p=0.427[a]	p=0.096[a]	p=0.069[a]	p=0.224[a]	p=0.469[a]
Education	p=0.478[b]	p=0.045[b]	p=0.182[b]	p=0.344[b]	p=0.152[b]	p=0.828[b]	p=0.118[b]	p=0.034[b]
Employment	p=0.087[b]	p=0.403[b]	p=0.037[b]	p=0.094[b]	p=0.067[b]	p=0.988[b]	p=0.007[b]	p<0.001[b]
Marital status	p=0.628[a]	p=0.965[a]	p=0.101[a]	p=0.465[a]	p=0.671[a]	p=0.874[a]	p=0.525[a]	p=0.708[a]
Self-assessed economic status	p=0.014[b]	p=0.032[b]	p=0.049[b]	p<0.001[b]	p<0.001[b]	p=0.245[b]	p=0.003[b]	p<0.001[b]
Religiousness	p=0.898[a]	p=0.407[a]	p=0.868[a]	p=0.010[a]	p=0.041[a]	p=0.130[a]	p=0.072[a]	p=0.862[a]
Pre-RTA health								
Body mass index	p=0.182[b]	p=0.496[b]	p=0.754[b]	p=0.319[b]	p=0.538[b]	p=0.141[b]	p=0.612[b]	p=0.182[b]
Smoking	p=0.620[a]	p=0.214[a]	p=0.260[a]	p=0.056[a]	p=0.096[a]	p=0.198[a]	p=0.510[a]	p=0.097[a]
Alcohol consumption	p=0.020[a]	p=0.007[a]	p=0.046[a]	p=0.009[a]	p=0.028[a]	p=0.036[a]	p=0.005[a]	p=0.003[a]
Drug abuse	p=0.637[a]	p=0.314[a]	p=0.969[a]	p=0.214[a]	p=0.566[a]	p=0.500[a]	p=0.425[a]	p=0.272[a]
Previous RTAs	p=0.840[a]	p=0.774[a]	p=0.709[a]	p=0.589[a]	p=0.083[a]	p=0.793[a]	p=0.864[a]	p=0.133[a]
Previous traumatic exposures	p=0.983[a]	p=0.510[a]	p=0.471[a]	p=0.032[a]	p=0.116[a]	p=0.394[a]	p=0.604[a]	p=0.031[a]
Prior PTSD	p=0.890[a]	p=0.612[a]	p=0.986[a]	p=0.941[a]	p=0.308[a]	p=0.882[a]	p=0.776[a]	p=0.206[a]
Chronic disease	p=0.039[a]	p=0.026[a]	p=0.410[a]	p=0.001[a]	p=0.001[a]	p=0.043[a]	p=0.073[a]	p<0.001[a]
Psychiatric disease	p=0.435[a]	p=0.061[a]	p=0.024[a]	p=0.057[a]	p=0.001[a]	p=0.452[a]	p=0.039[a]	p=0.010[a]
Previous permanent pain	p=0.828[a]	p=0.771[a]	p=0.069[a]	p=0.001[a]	p=0.033[a]	p=0.172[a]	p=0.052[a]	p=0.014[a]
Use of medications	p<0.001[a]	p<0.001[a]	p=0.058[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]
Types of medications	p<0.001b	p<0.001[b]	p=0.253[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]	p<0.001b	p<0.001[b]
RTA injury-related								
Injury affliction	p<0.001[a]	p<0.001[a]	p=0.228[a]	p=0.061[a]	p<0.001[a]	p=0.003[a]	p<0.001[a]	p=0.008[a]
Injury severity	p<0.001b	p<0.001[b]	p=0.347[b]	p=0.081[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]
Self-assessed life-threat	p=0.001[a]	p<0.001[a]	p=0.013[a]	p=0.002[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]
Pain following the RTA	p<0.001[a]	p<0.001[a]	p=0.001[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]	p=0.002[a]
Hospitalization	p<0.001[a]	p<0.001[a]	p=0.200[a]	p=0.177[a]	p=0.053[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]
Hospitalization duration	p<0.001[b]	p<0.001[b]	p=0.318[b]	p=0.244[b]	p=0.128[b]	p<0.001[b]	p<0.001[b]	p<0.001[b]
Surgery	p<0.001[a]	p<0.001[a]	p=0.281[a]	p=0.045[a]	p=0.036[a]	p<0.001[a]	p<0.001[a]	p<0.001[a]
Loss of consciousness	p=0.033[a]	p=0.088[a]	p=0.334[a]	p=0.393[a]	p=0.597[a]	p=0.298[a]	p=0.311[a]	p=0.355[a]
Loss of memory	p=0.226[a]	p=0.150[a]	p=0.962[a]	p=0.196[a]	p=0.097[a]	p=0.116[a]	p=0.984[a]	p=0.190[a]
Rehabilitation	p<0.001[a]	p<0.001[a]	p<0.001[a]	p=0.086[a]	p=0.095[a]	p<0.001[a]	p<0.001[a]	p=0.012[a]
RTA-related								
Fault	p=0.109[b]	p=0.659[b]	p=0.393[b]	p=0.322[b]	p=0.657[b]	p=0.537[b]	p=0.024[b]	p=0.461[b]
Fatalities	p=0.953[a]	p=0.541[a]	p=0.618[a]	p=0.762[a]	p=0.147[a]	p=0.157[a]	p=0.343[a]	p=0.421[a]
Compensation claim	p=0.072[a]	p=0.140[a]	p=0.001[a]	p=0.005[a]	p=0.178[a]	p=0.001[a]	p=0.031[a]	p=0.367[a]
Obtained compensation	p=0.474[a]	p=0.102[a]	p=0.001[a]	p=0.232[a]	p=0.537[a]	p=0.155[a]	p=0.370[a]	p=0.607[a]
Type of road users	p=0.007[b]	p=0.053[b]	p=0.502[b]	p=0.156[b]	p=0.070[b]	p=0.113[b]	p=0.017[b]	p=0.004[b]

[a]Mann-Whitney U test; [b]Kruskal-Wallis test; PF = physical health; RP = role limitations due to physical health; RE = role limitations due to emotional health; VT = vitality; MH = mental health; SF = social functioning; BP = bodily pain; GH = general health

Mental health outcomes showed a reverse correlation with all QoL domains after the RTA. The correlations between QoL domains and the symptoms of depression, anxiety and PTSD are presented in Table 6.

Table 6. Correlation between quality of life and mental health outcomes after the RTA.

	QoL domains							
Mental health	**PF**	**PR**	**ER**	**VT**	**MH**	**SF**	**BP**	**GH**
Depression symptoms	r_s=-0.410	r_s=-0.364	r_s=-0.430	r_s=-0.588	r_s=-0.586	r_s=-0.582	r_s=-0.438	r_s=-0.593
	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*
Anxiety symptoms	r_s=-0.274	r_s=-0.263	r_s=-0.523	r_s=-0.446	r_s=-0.378	r_s=-0.485	r_s=-0.427	r_s=-0.442
	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*
PTSD symptoms	r_s=-0.380	r_s=-0.345	r_s=-0.449	r_s=-0.476	r_s=-0.552	r_s=-0.549	r_s=-0.527	r_s=-0.437
	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*	p<0.001*

*Spearman's correlation; PF = physical health; RP = role limitations due to physical health; RE = role limitations due to emotional health; VT = vitality; MH = mental health; SF = social functioning; BP = bodily pain; GH = general health

4 DISCUSSION

The study explored the QoL following an RTA and the prospective cohort included RTA survivors both with and without injuries, unlike other studies exploring RTA outcomes only among the injured. The QoL of the RTA survivors one month after the experienced RTA was below the average scores for the general Croatian population in the following QoL domains: RP (0.0 vs. 61.5) and BP (55.0 vs. 64.6) (24). Other studies also found RTA survivors had lower QoL than general population norms (4, 6, 12, 23, 25).

The sociodemographic factors found associated with lower scores of the QoL domains in this study, i.e. older age, female sex, lower education level, unemployment, and lower economic level, are consistent with other research data. The Croatian general population sample reported lower QoL scores in the older age group and among females (24). Other studies of RTA victims also found older age to be associated with lower QoL after the RTA (4-6, 9, 11, 23, 25-27). A few studies also found female RTA survivors reporting lower QoL than males (5, 25, 28). Lower education level (7, 11, 12), unemployment (6, 9, 11) and lower economic status (11) of RTA victims were associated with lower QoL after the RTA in several studies. Irreligiousness has not been explored in the earlier studies, but was found relevant in this cohort.

Pre-RTA health status was found to be associated with lower QoL after the RTA. Other studies also found pre-injury health status (6, 9, 11, 12, 23, 27, 29), pre-injury chronic illness (6, 9, 11, 12, 23, 29) and pre-injury psychological history (7, 12, 23, 27, 29) to be associated with decreased QoL in the RTA victims. Use of medications and psychiatric medication use were found associated with all QoL domains except RE. This factor should be further explored, since people may not want to report a psychiatric disease due to stigmatization, but may report information regarding medications they use (30). This study also found previous traumatic exposure to be associated with lower VT and GH. Alcohol consumption was found to be positively associated with all QoL domains, indicating that moderate alcohol use has a positive effect on the QoL (31).

The most important injury-related factors associated with all QoL domains were pain following the RTA and self-assessed life-threat, with the latter also found to be significant in other studies (9, 12). Pain following an RTA is a well-known predictor of QoL (4, 6, 7, 9, 13, 27, 29). Given that a high baseline pain after the RTA is associated with poor long-term health outcomes, strategies to reduce pain levels should be introduced early following an RTA to reduce the risk of long-term health consequences (28). The results with regard to injury severity are inconsistent in the literature, some studies found it to be associated with lower QoL (3, 11, 12, 14, 23, 28, 32), while others found no association (5, 6, 9) or a negative association (4). However, in all studies where RTA survivors with mild injuries reported similar or worse QoL scores than RTA victims with more severe injuries, data were obtained from compensable injury claim registers restricted to a certain level of injuries i.e. mild to moderate. It can be argued that decreased QoL outcomes are the result of the compensable nature of the injury, leading to reporting bias for secondary gain (12). The current study showed not only that more severely injured had lower QoL scores, but also that RTA survivors without injuries had better QoL scores. Other research exploring traumatic injuries also showed that sustaining a traumatic (not RTA) injury was associated with lower QoL (33). Therefore, in order to obtain reliable data, research regarding the impact of RTA injury on health outcomes should include RTA victims with all injury levels along with those who are uninjured, possibly outside compensation settings.

Factors indirectly injury-related, i.e. hospitalization, duration of hospitalization, surgery, loss of consciousness during RTA and rehabilitation after the RTA, had a negative impact on the QoL after an RTA. Other research also found hospitalization to be associated with lower QoL scores (3, 9, 12, 27). Psychological consequences after the RTA,

namely PTSD, depression and anxiety symptoms, showed correlations with all QoL domains. Other studies also found psychological distress (27), PTSD (4, 7, 11, 29) and anxiety/depression (4-6, 9) to be independent predictors of QoL following the RTA. Vulnerable road users, i.e. pedestrians/cyclists reported lower scores of PF, BP and GH. This association is also probably injury-related, since there were no uninjured cyclists nor pedestrians in this study. These findings all corroborate that RTA injury and its severity are both relevant for the QoL outcomes.

Compensation claim is a thoroughly investigated factor showing a negative association with the QoL following an RTA in many studies (5, 7, 11, 27, 34, 35). In addition to the negative association of compensation claim with the QoL, this study showed that obtaining compensation was associated with a lower score of RE. Furthermore, not being at fault in the RTA was associated with higher scores of BP. Other research also found that not-at-fault RTA victims had lower QoL and reported significantly higher rate of neck and back pain than the at-fault group (28). This suggests that non-physical factors such as victimization, frustration and anger at being involved in an RTA that was the fault of someone else all affect well-being (28).

Most of the studies investigating the QoL of RTA survivors used the SF-36 questionnaire (4, 5, 14, 23, 28, 33-35) or its shortened version. the Short Form – 12 (SF-12) (6, 8, 9, 12, 13, 26, 27), while a minority used other available instruments for assessing QoL (7, 26, 31). Therefore, the data from this study are comparable with that in other QoL studies.

4.1 Strengths and Limitations

This study investigated a vast range of variables, sociodemographic, psychosocial, health-related, injury-related, RTA-related and compensation-related factors. Unlike other research, this one included uninjured RTA survivors with the traumatic experience of an RTA. Furthermore, the injured RTA victims included all levels of injury severity. Cohort recruitment was conducted outside any compensation scheme to eliminate secondary gain bias. All patients received immediate post-RTA healthcare in public healthcare settings irrelevant of their injury and health insurance status. Emergency and acute care, rehabilitation and other healthcare services for the RTA patients in Croatia are provided by public hospitals and are paid for by the state health insurance, and not like in some countries by third-party insurers in the scheme of a fault-based or no fault-based compensation system. As such, Croatian RTA victims receive equal healthcare and social benefits regardless of their involvement in compensation procedures. Given all the above, the studied cohort might be similar to general population in Croatia.

The limitations of this study include self-reported data collection. Pre-RTA physical and mental health problems were self-reported, without specific diagnostic tools or medical records. The recruited participants represent only 31.3% of registered RTA survivors, mostly due to the absence of contact information of the RTA victims (48.3%). The response rate was high, and 82.6% of the contacted RTA victims gave consent to participate. The telephone contact information of the patient is not a routinely obtained in Croatian emergency medicine institutes. Furthermore, there are objective reasons, such as the medical condition of the patients and absence of relatives, that hinder obtaining contact information. Nevertheless, given the relatively small sample size, more resources should be invested in obtaining RTA victims' contact information and implementing this procedure in the routine protocols.

5 CONCLUSIONS

Decreased QoL following an RTA showed associations with the low socioeconomic status of the RTA victims, poor pre-RTA health, injury-related factors and psychological disorders after the RTA. Identifying the predictors of decreased QoL following an RTA will enable planning interventions targeting the most important factors influencing health of RTA victims. Assessing and recording of self-reported QoL should be a part of the routine protocol in RTA survivors' healthcare.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

FUNDING

The study was funded by grants from Croatian Ministry of Science and Education dedicated to multi-year institutional funding of scientific activity at the Josip Juraj Strossmayer University of Osijek, Osijek, Croatia - grants number: IP12 and IP13.

ETHICAL APPROVAL

The research was approved by the Ethics Committee of the Faculty of Medicine Osijek, Croatia (Ethical Approval Code: 2158-61-07-17-211).

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SCREENING STRATEGY MODIFICATION BASED ON PERSONALIZED BREAST CANCER RISK STRATIFICATION AND ITS IMPLEMENTATION IN THE NATIONAL GUIDELINES – PILOT STUDY

SPREMENJENI MODEL PRESEJANJA GLEDE NA IZRAČUN INDIVIDUALIZIRANE OGROŽENOSTI ZA RAKA DOJK IN MOŽNA IMPLEMENTACIJA PRESEJANJA GLEDE NA KATEGORIJO OGROŽENOSTI V DRŽAVNE SMERNICE – PILOTNA RAZISKAVA

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Received: Mar 24, 2020
Accepted: Aug 31, 2020

Original scientific article

ABSTRACT

Background: One of the most consistent models for estimating personalized breast cancer (BC) risk is the Tyrer-Cuzick algorithm that is incorporated into the International Breast Cancer Intervention Study (IBIS) software. Our main objective was to provide criteria for the classification of the Slovenian population, which has BC incidence below the European average, into risk groups, and to evaluate the integration of the criteria in Slovenian guidelines. Our main focus was on women age <50 with higher BC risk, since no organized BC screening is available for these women.

Methods: Slovenian age-specific BC risks were incorporated into IBIS software and threshold values of risk categories were determined. Risk categories were assigned according to the individual's ten-year risk for women aged 40 and older, and lifetime risk for women between 20 and 39. To test the software, we compared screening strategies with the use vs. no use of IBIS.

Results: Of the 197 women included in the study IBIS assigned 75.1% to the BC risk group, and the rest to the moderately increased risk. Without IBIS 80 women were offered mammographic and 33 ultrasound screening. In contrast, 28 instead of 80 would have been offered mammographic screening and there would have been no referrals for ultrasound if IBIS had been used.

Conclusions: The Slovenian IBIS has been developed, tested and suggested for personalized breast cancer risk assessment. The implementation of the software with the consideration of Slovenian risk thresholds enables a more accurate and nationally unified assessment.

Keywords:
Tyrer-Cuzick model, breast cancer risk assessment, personalized breast cancer screening, breast cancer

IZVLEČEK

Uvod: *Trenutno je kot najdoslednejši model za oceno individualizirane ogroženosti za raka dojk razpoznan Tyrer-Cuzickov algoritem, vključen v program IBIS (International Breast Cancer Intervention Study), ki temelji na angleških podatkih o incidenci raka dojk. Glavni cilj naše raziskave je bil postaviti merila za razvrščanje slovenskih žensk, ki imajo ogroženost za raka dojk pod evropskim povprečjem, v skupine ogroženosti glede na izračun ogroženosti z uporabo programa IBIS. Prav tako smo želeli oceniti morebitno vpeljavo teh meril v slovenske smernice. Poseben poudarek je namenjen bolj ogroženim pod petdesetim letom starosti, saj za ženske v teh starostnih skupinah nimamo organiziranega presejanja.*

Metode: *V program IBIS smo umestili slovensko generacijsko specifično incidenco raka dojk in določili mejne vrednosti skupin ogroženosti (populacijska, zmerno povečana in visoka). Skupine ogroženosti so bile določene na podlagi 10-letne ogroženosti za ženske, ki so stare 40 let ali več, in doživljenjske ogroženosti za ženske, stare med 20 in 39 let. S programom IBIS smo izračunali ogroženost za raka dojk za ženske, ki so prišle na preventivni pregled v okviru primarnega in sekundarnega zdravstvenega varstva, in primerjali priporočila, ki so bila svetovana po pregledu, s priporočili, ki bi veljala, če bi uporabili program IBIS.*

Rezultati: *V raziskavo smo vključili 197 žensk in za vsako posameznico izračunali ogroženost za raka dojk s pomočjo programa IBIS. Program je 75,1 % žensk umestil v skupino populacijsko ogroženih, ostale pa v skupino zmerno povečane ogroženosti. Brez uporabe IBIS-a je bilo 80 žensk umeščeno v bolj ogroženo skupino, opravile so presejalno mamografijo, 33 ženskam pa so opravili ultrazvočno preiskavo dojk. Če bi uporabili nova merila razvrščanja v skupine ogroženosti s pomočjo izračuna programa IBIS, bi jih 28 namesto 80 opravilo presejalno mamografijo. Prav tako ne bi nobene ženske po novih merilih poslali na ultrazvočno preiskavo dojk.*

Zaključki: *Razvili smo program IBIS, ki vsebuje slovensko populacijsko incidenco raka dojk. Program smo testirali in predlagali za orodje izračunavanja individualizirane ogroženosti za raka dojk. Uvedba programa bi, ob upoštevanju enotnih mejnih vrednosti kategorij ogroženosti, omogočala natančnejšo in bolj poenoteno obravnavo žensk na državni ravni.*

Ključne besede:
Tyrer-Cuzickov model, ocena ogroženosti za raka dojk, individualizirano presejanje za raka dojk, rak dojk

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1 INTRODUCTION

Breast cancer is the most common cancer in women. Globally, more than 2 million women were diagnosed with breast cancer in 2018 (1). The average crude incidence rate in Slovenia has risen from 37.2/100,000 women in the period 1968–1972 to 127.2/100,000 in the period 2012–2016. Between the years 2012 and 2016 the average number of breast cancer in Slovenia was 1,279 annually (the average female population being 1,039,219 over this period) (2). The increase in the breast cancer burden is the result of the increasingly important role of reproductive risk factors (early menarche, later age at first full-term birth) and an aging population.

Evidence-based screening tests and early cancer detection followed by appropriate treatment decreases mortality and helps improve patients' quality of life. In Slovenia, a national breast cancer screening programme, DORA, offers biennial mammography to all women between the ages of 50 and 69 (3, 4). Recruitment is based solely on the participants' age, with a previous breast cancer diagnosis as an exclusion criterion. According to the current national regulation (Rules on Carrying Out Preventive Health Care at the Primary Level), women aged 20 to 50 are entitled to a clinical breast examination once every three years, performed by their gynaecologist at the primary level (5). If a woman is considered to be at higher risk, she is offered regular screening, as stated in the Table 1 (5).

Table 1. The summary of criteria for the higher risk women category and advised breast cancer screening tests according to current Rules on Carrying Out Preventive Health Care at the Primary Level (5).

Criteria for higher breast cancer risk category (at least one of the following)	**Screening tests at a Breast Unit for the higher breast cancer risk category** (after the age of 40)
First full-term birth >30 years	Clinical breast examination (every 12-24 months) and Mammography and/or ultrasound examination of the breast or No screening is performed according to clinician decision
At least one relative (mother, sister, daughter) with breast cancer	
Personal history of breast disease that increases breast cancer risk	
Personal history of breast cancer in the past	

However, these rules are in many ways outdated. Our data indicates that a substantial number of women are offered unnecessary mammography scans and are screened too often, especially those under the age of 50 (6). In 2018, the Slovenian breast cancer detection and treatment guidelines were therefore updated (7). These new guidelines recommend that women at higher risk should be screened according to their personal breast cancer risk. It is therefore very important to assess a woman's risk even before she gets the invitation from the national breast cancer screening programme, since she might be at higher risk and should start with screening before the age of 50.

At the moment there are several different mathematical models available for personalized breast cancer risk assessment (8-11). Historically, the two oldest models are the Gail and Claus models (9-11). Both have numerous limitations, however, which have to be taken into account when performing the assessment and interpreting the results (12). The first studies using the Gail model indicated there was a tendency to overestimate the risk in younger women and to underestimate that in older women. As such, the model has recently been improved in order to correct some of these shortcomings (12-14).

Subsequently developed mathematical models, such as BOADICEA (the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm), have proved to be more useful than their predecessors in the familial setting (15,16). At present, the Tyrer-Cuzick algorithm, which is incorporated into the IBIS (International Breast Cancer Intervention Study) software, is seen as the most consistent model for estimating breast cancer risk. IBIS includes both genetic and non-genetic risk factors in the assessment of breast cancer risk (17). Its algorithm is based not only on a woman's family history and data on generation-specific population breast cancer risks, but also information on women's personal history, such as age, age at menarche and first full-term birth, parity, body height and weight, age at menopause and use of hormone replacement therapy as well as breast density and polygenic risk score (17). At the moment, IBIS software and BOADICEA are the most thoroughly validated models for calculating individual breast cancer risk (15, 17-19).

The software S-IBIS (Slovenian IBIS) for determining personalized breast cancer risk in the Slovenian population-based on Tyrer-Cuzick algorithm was developed and suggested for individual risk calculation (20). It is also presented as a possible tool for assessing breast cancer risk in the updated Slovenian guidelines (7).

The main objective of our study was to provide criteria for the division of our population into groups according to their individual breast cancer risk and to evaluate the impact of the new grouping algorithms on screening procedures. A special emphasis was given to women under 50, since no organized breast cancer screening is available for this age group.

2 METHODS

2.1 Data Source

In order to test S-IBIS and the newly-proposed risk categories we used data from women who visited either the regional Breast Unit (BU) in Kranj or a general practitioner working in the field of breast cancer prevention at the Primary Health Care Centre (PHCC) in Logatec (21, 22). Combining data from both pilot studies, 197 women were included in our analysis, 100 from BU and 97 from PHCC. Both centres represent locations in Slovenia at the primary health level where women may be assessed and referred for breast cancer screening. All the personal and family history data necessary for the calculation of individual risk in the S-IBIS software (version-8) was collected during interviews. Additional information on the anticipated screening procedures (mammography, ultrasound examinations or just further appointments) was obtained from their health records. For each woman a personalized breast cancer risk was calculated with S-IBIS. According to the calculated 10-year or lifetime risk and age group, every woman was assigned to one of the Slovenian breast cancer risk categories: low risk, population risk, moderately increased risk, high risk. The McNemar test was used to determine if there were differences between the anticipated screening according to the current rules on carrying out preventive health care at the primary level and the screening according to the updated Slovenian guidelines (7).

SPSS version 24 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses.

2.2 Personalized Breast Cancer Risk Evaluation Tool IBIS and Its Adjustment to Slovenian Population

The Tyrer-Cuzick model has been shown in several independent studies to be the most consistently accurate when compared with other models, in other words it performs accurately in the identification of a moderately increased to high risk of developing breast cancer, and currently it is the tool that includes the largest number of established breast cancer risk factors (23). The model has been incorporated into a computer programme, the IBIS software, which gives a personalized breast cancer risk estimate (17). The Tyrer-Cuzick model (now in version-8) combines family history, endogenous hormonal factors, benign breast disease, and other risk factors such as age, body mass index, hormone replacement therapy use, and mammographic density, as well as genetic factors (including *BRCA* and a polygenic risk score) into a single statistical model. The program assumes that there is genetic but yet unknown gene predisposing to breast cancer, in addition to the *BRCA1/2* genes, to account for the remaining familial risk not explained by *BRCA1/2*. The woman's family history is used to calculate the likelihood of her carrying a pathogenic genetic variant, which in turn affects her likelihood of developing breast cancer. The phenotype of the woman can be modelled by (17):

$$Phenotype = \begin{pmatrix} no \\ BRCA1 \\ BRCA2 \end{pmatrix} \begin{pmatrix} no \\ yes \end{pmatrix}$$

where the first column contains information about the pathogenic variant in *BRCA* genes and may either contain the normal allele, a *BRCA1* mutated allele or a *BRCA2* mutated allele. The second column contains an adverse gene (the "low penetrance gene") which was created to act as a surrogate for the effect of all the other "unknown" genes and which causes an increase in the relative hazard of breast cancer. This low penetrance gene is dominant so that a woman with two copies will have the same phenotype as a woman with one copy. To estimate this risk from family history (caused by the adverse genes), the model fits the results of the study done by Anderson et al. (24).

The family history of breast and ovarian cancer of the woman's blood relatives is used to calculate the distribution of her genotype probabilities, which is used in calculation of the phenotypic probabilities. For a woman the absolute risk (*Pr*) of developing breast cancer between ages t_1 and t_2 is given by (17):

$$Pr(cancer) = 1 - \left(1 - \sum_{i=1}^{6} p_i F_i(t_1, t_2)\right)^{\alpha}$$

where pi is the probability of the woman having the relevant phenotype, $F_i(t_1,t_2)$ is the probability of getting breast cancer between ages t_1 and t_2 given the woman's phenotype i and α is the relative risk due to personal factors. The IBIS software routinely estimates the likelihood of a woman developing breast cancer specifically within 10 years of her current age and over the course of her lifetime. The tool is not intended to assess the risk for women who have already been diagnosed with breast cancer (25).

For the purpose of this study, IBIS software was adjusted using Slovenian specific population breast cancer risks. Breast cancer incidence and mortality rates for the period 2006–2010 were obtained from the population-based Slovenian Cancer Registry (2).

2.3 Calculation of the Threshold Values

The calculations of threshold values of breast cancer risk categories for Slovenian women followed the procedures used in the development of the English guidelines (NICE – National Institute for Health and Care Excellence) (26-28). The direct application of the NICE threshold values of breast cancer risk categories would not be appropriate, however, because the Slovenian population risk of breast cancer is about 10% lower compared to the English

population risk (29). The appropriate threshold values for Slovenian women were thus determined based on the data on the cumulative 10-year and lifetime breast cancer risks from the Slovenian Cancer Registry.

In the NICE guidelines, which are internationally available and therefore might be used by other countries, the decision to define moderately increased breast cancer risk with a threshold of 3% 10-year risk at age 40 years (or 17% lifetime risk) was taken as that is a level of risk equivalent to the average population risk of a 50-year old woman eligible for breast screening through English national breast screening programmes.

On the other hand, in Slovenia, a level of risk equivalent to the average population risk of 50 to 59 year-old women is lower, at about 2%, so the same 2% for 10-year risk was considered to be a threshold value for moderately increased risk at the age of 40. The upper threshold of moderately increased risk category for those aged 40 and 49 was set as five times the 10-year risk in the same age group. In the Slovenian female population aged 40-49 the 10-year risk was 1.3% in period 2011-2015, so the upper threshold value was set at 6.5% (the number is rounded up). For the age groups 50-59 and 60+ the lower threshold value of the moderately increased risk category was set as two times the 10-year relative risk (1.9% and 2.8%, respectively) yielding thresholds of 4.0% and 5.5% (the numbers are rounded for future use in practice); the upper threshold values remained the same as for the age group 40-49 (20).

For women in their twenties, it is more reasonable to report their lifetime risk. In our project, the calculation was done for a woman aged 25 years: first, using S-IBIS software we created a hypothetical case of a woman aged 40 with a family history of only one first- or second-degree relative diagnosed with breast cancer at older than age 40 who is at a 2% 10-year risk of developing breast cancer. By only changing the age from 40 to 25 the moderately increased risk threshold was obtained. The calculated lifetime risk of this woman was 16%, so the threshold for moderately increased risk in those aged between 20 and 39 was set at 16% or greater (20).

3 RESULTS

3.1 Characteristics of the Studied Women According to Breast Cancer Risk Factors

In the study we calculated breast cancer risk scores of 197 women that consecutively attended preventive visits at the Breast Unit or Primary Health Centre and agreed to participate in the study, using S-IBIS software. In Table 2 we present the characteristics of the women studied according to breast cancer risk factors (age, family history, age at menarche, age at first full-term birth).

Table 2. Characteristics of the studied women according to breast cancer risk factors.

	Number of women	Percent (%)
Age groups		
20-39 years	87	44.2
40-49 years	104	52.8
50-59 years	6	3.0
Family history		
positive	62	31.5
negative	135	68.5
Age at menarche		
<11 years	7	3.6
11-12 years	57	28.9
13-14 years	108	54.8
>15 years	25	12.7
Age at first full-term birth		
<25 years	81	41.1
25-29 years	54	27.4
>30 years	31	15.7
nulliparous	31	15.7

3.2 Breast Cancer Risk Categories for the Studied Women

For the division into breast cancer risk categories the newly calculated breast cancer risk thresholds for Slovenian women were used (Table 3). Table 3 presents the calculated breast cancer risk thresholds that are suggested for the division of women into risk categories according to the individual's ten-year risk for women age 40 and more, and lifetime risk for women between 20 and 39 (20).

Table 3. Breast cancer risk categories for Slovenian women.

	Breast cancer risk category			
	Low risk (%)	Population risk (%)	Moderately increased risk (%)	High risk (%)
Lifetime risk between ages 20 and 39	-	<16	16 and higher	>30
10-year risk between ages 40 and 49	-	<2.0	2.0 to 6.5	>6.5
10-year risk between ages 50 and 59	<1.3	1.3 to<4.0	4.0 to 6.5	>6.5
10-year risk from age 60+	<1.3	1.3 to<5.5	5.5 to 6.5	>6.5

Based on the population of Slovenia and its average lifetime risk to develop breast cancer (i.e. up to age 85), women with a lower risk were assigned to the lower risk category, women with a risk that was 2- to 3-fold that of the population (16% – 29% lifetime risk) were assigned to the moderately increased risk category, and women with a lifetime risk of 30% and above the population risk were assigned to the high breast cancer risk category. Since the risk may vary over a woman's lifetime, we also set the cut-off values for 10 year-risk groups (40–49, 50–60, and 60+ years) as defined in Table 3 (20).

By using S-IBIS and newly calculated breast cancer risk categories, a total of 197 women were assigned to breast cancer risk groups. Of these, 148/197 (75.1%) were assigned to the population risk category and 49/197 (24.9%) to moderately increased risk category, and none to the high or low risk categories.

3.3 Evaluation of the Impact of the New Grouping Algorithms on Screening Procedures

The comparison of anticipated mammographic and ultrasound screening (according to the current rules on carrying out preventive health care at the primary level) in women who visited preventive centres, and the foreseen screening according to the new Slovenian guidelines, are presented in Tables 4 and 5, respectively.

Table 4. Comparison of anticipated mammographic screening and screening foreseen in the new Slovenian guidelines by breast cancer risk categories and age groups.

	Anticipated mammographic screening (number of women)		Mammographic screening according to the new Slovenian guidelines (number of women)	
Breast cancer risk category*	**Population risk**	**Moderately increased risk**	**Population risk**	**Moderately increased risk**
Ages between 20 and 39	5	4	0	0
Ages between 40 and 49	43	27	0	34
Ages between 50 and 55	1	-	6	-
All ages	49	31	6	34

*Risk category was determined by S-IBIS calculation, and division into risk groups was performed as proposed in Table 3.

Table 5. Comparison of anticipated ultrasound screening for breast cancer prevention and screening foreseen in the new Slovenian guidelines by breast cancer risk categories and age groups.

	Anticipated ultrasound screening (number of women)		Ultrasound screening according to the new Slovenian guidelines	
Breast cancer risk category*	**Population risk**	**Moderately increased risk**	**Population risk**	**Moderately increased risk**
Ages between 20 and 39	7	3	According to the new guidelines ultrasound screening is recommended only upon referral from a radiologist	
Ages between 40 and 49	14	9		
Ages between 50 and 55	0	-		
All ages	21	12		

*Risk category was determined by S-IBIS calculation, and division into risk groups was performed as proposed in Table 3.

Out of the 148 women who fall into the population risk category, 49 (33.1%) were deemed suitable for mammographic screening according to the current rules of primary practice, while 31 (63.3%) women out of 49 were in the moderate risk category. Thus, from all of the 197 women included in the pilot studies, 80 (40.6%) would be eligible for mammographic surveillance until the age of 55. In contrast, with the implementation of the new Slovenian guidelines, only six women from the population risk category and 34 (17.3%) from the moderate risk category would be eligible for mammographic screening. All women above the age of 50 would be offered mammographic screening in the population-based organized screening program that is already available.

When comparing both approaches, 28 women of 80 who were offered mammographic screening according to the current rules, would also be offered mammographic screening according to the new guidelines. On the other hand, 12 women out of 117 that were not offered mammographic screening by current rules would be offered it with the implementation of the new guidelines. An exact McNemar's test determined that there was a statistically significant difference between current practice and the new Slovenian guidelines for mammographic screening, at $p<0.001$.

In the population risk category, 21 (14.2%) women out of 148 had ultrasound screening, in the moderately increased risk category it was 12 (24.5%) women out of 49, giving a total of 33 (16.8%) out of 197 women. With the implementation of the new Slovenian guidelines, ultrasound would only be performed on referral from a radiologist.

4 DISCUSSION

Our study results will help to update and re-design clinical pathways for the early detection of breast cancer in asymptomatic Slovenian women and to identify those women with an increased risk of breast cancer. Furthermore, these results will inform recommendations for the Breast Cancer Detection and Treatment Guidelines. In Slovenia, breast cancer screening procedures are currently defined in the national Rules on Carrying Out Preventive Health Care at the Primary Level that are authorized by the Ministry of Health (5). However, these are outdated, vague and non-specific, with an unreasonably broad definition of higher breast cancer risk, which results in unequal treatment of women who would have the same numerically assessed breast cancer risk. As a consequence of this unspecific risk categorization and broad definition of higher breast cancer risk, many women receive screening referrals too frequently and often unnecessarily. This, in return, leads to unnecessary cost and other negative effects, such as a psychological burden for the women and capacity issues for the screening units. The Institute of Oncology Ljubljana therefore implemented the guidelines of breast cancer diagnosis and treatment in 2018, where it is stated, among other points, that the breast cancer risk can be calculated with the help of specific risk assessment tools (7). A reliable personalized breast cancer risk assessment is essential for the provision of risk-benefit analysis prior to the initiation of any screening, preventive or diagnostics procedures (30).

In addition, it is of utmost importance to determine the Slovenian threshold values for the stratification of asymptomatic women into breast cancer risk groups, based on the S-IBIS software results, with these being the low, population, moderately increased and high breast cancer risk group. Our comparisons show that using the English population specific risks overestimates the risks for Slovenian women by up to 10%, but the comparison needs validation based on actual cases in the assessed cohort (29). We defined the threshold values in collaboration with experts from Slovenia and an expert from Great Britain who was involved in the development of the English guidelines (NICE – National Institute for Health and Care Excellence) (26-28).

We evaluated the use of the software and newly proposed thresholds. We measured probable changes in the existing work processes and evaluated the impact of the use of the software tool on screening procedures compared to the current practice under the existing rules. The S-IBIS was used for risk assessment on a sample of asymptomatic women who were mostly younger than 50 years and therefore not included in the national breast cancer screening programme, but were evaluated to be at moderately increased or high risk and so entitled to breast cancer screening under the existing rules.

The results clearly show it is reasonable to incorporate the use of S-IBIS into everyday practice. With regard to the current system, our study revealed that 40.6% (80 out of 197) women were referred to screening mammography. If the new guidelines were applied, by using risk category stratification, as explained in Table 3, only 14.2% (28) of women would be referred for further screening due to moderately increased or high breast cancer risk. It seems that we are currently offering mammography to women aged between 40–50 who are at population risk, mainly due to vague regulations. More than half of these women could wait until the age of 50 for the national screening program invitation. Based on our results, we expect that the number of screening mammograms that the public healthcare system is paying for would decrease if mammography were performed based on our proposed personal risk calculations. Such an approach would also offer equal opportunities to all Slovenian women.

The major limitation of our study was the sample size, since it represents only a small fraction of the Slovenian female population. On the other hand, it was the first attempt to numerically and systematically assess breast cancer risk in the Slovenian female population at the primary health care level, and to set the cut-off values for breast cancer risk categories in this group. These results provide us with the design of a clinical pathway for early detection of breast cancer among Slovenian asymptomatic women aged under 50 who are at an increased risk of breast cancer. Direct validation of these estimates based on 5- and 10-year risk will provide further validation of our findings.

In conclusion, the implementation of the developed and upgraded software S-IBIS for the calculation of the personalized breast cancer risk and implementation of new clinical pathways in the Slovenian health care system will bring more evidence-based referrals of asymptomatic women who are at increased risk of breast cancer. The number of unnecessary preventive procedures will decrease, and the waiting times for those who are eligible for higher risk screening will be reduced. This data is likely to be useful in other countries with lower than average European breast cancer incidence rates.

ACKNOWLEDGEMENTS

We thank prof. Jack Cuzick, PhD, FRS, CBE for providing support and comments that greatly improved the manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

FUNDING

The work was supported in part by the Ministry of Health of the Republic of Slovenia and Slovenian Research Agency within the Target research programme CRP 2016. DGE, is supported by the all Manchester NIHR Biomedical Research Centre (IS-BRC-1215-20007).

ETHICAL APPROVAL

The National Medical Ethics Committee at the Ministry of Health from the Republic of Slovenia (No. 0120-404 / 2016-2 KME 60 /07 /16) approved the study.

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MEASLES OUTBREAK IN A ROMA COMMUNITY IN THE EASTERN REGION OF SLOVAKIA, MAY TO OCTOBER 2018

IZBRUH OŠPIC V ROMSKI SKUPNOSTI NA VZHODNEM SLOVAŠKEM MED MAJEM IN OKTOBROM 2018

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Received: Feb 13, 2020
Accepted: Sep 10, 2020

Original scientific article

ABSTRACT

Background: Despite the effective National Immunization Programme of Slovakia, some population groups are incompletely vaccinated or unvaccinated. We aimed to describe the measles outbreak spread in Eastern Slovakia between May and October 2018, affecting the Roma communities in relation to the existing immunity gaps.

Methods: We defined a group of persons living in socially closed communities with low vaccination coverage.

Results: Of 439 measles cases (median age: 10 years), 264 (60.1%) were vaccinated, 137 (31.2%) received two doses and 127 (28.9%) one dose of measles vaccines, 155 (35.3%) were unvaccinated and 20 (4.6%) had an unknown vaccination status. Samples from 102 patients (with two-dose vaccination status) were additionally tested for antibodies against rubella and mumps. Of 102 cases, 68 (66.7%) cases had a positive IgM and 23 (22.5 %) IgG antibodies against measles. For rubella, only 20 (19.6%) cases had seropositive IgG levels, for mumps higher positivity was detected in 60 persons (58.8%). We could detect only a small percentage with positive serology results of rubella IgG antibodies across all age groups. We have assumed that rubella antibodies had to be produced following the vaccination. Their absence in the cases with two doses of MMR suggests that these vaccines could not have been administrated despite the fact that this data was included in the medical records. Sequential analysis of two samples showed measles genotype B3.

Conclusion: This outbreak can outline the existence of a vulnerable group of the Roma. Low vaccinate coverage represents a serious public health threat.

Keywords:
measles, Roma, outbreak, vaccination coverage

IZVLEČEK

Ozadje: *Kljub učinkovitemu nacionalnemu programu imunizacije na Slovaškem so nekatere skupine prebivalcev nepopolno cepljene ali necepljene. Želeli smo opisati izbruh ošpic na vzhodnem Slovaškem med majem in oktobrom 2018, ki je prizadel romske skupnosti v povezavi z obstoječimi vrzelmi v imunosti.*

Metode: *Opredelili smo skupino ljudi, ki živijo v socialno zaprtih skupnostih z nizko precepljenostjo.*

Rezultati: *Med 439 primeri ošpic (mediana starost: 10 let) je bilo 264 ljudi cepljenih (60,1 %), 137 (31,2 %) jih je prejelo dva odmerka in 127 (28,9 %) en odmerek cepiva proti ošpicam, 155 (35,3 %) jih je bilo necepljenih, pri 20 (4,6 %) pa status cepljenja ni bil znan. Vzorce 102 bolnikov (s statusom cepljenja z dvema odmerkoma) smo dodatno testirali za protitelesa proti rdečkam in mumpsu. Od 102 primerov jih je 68 (66,7 %) imelo pozitiven rezultat za protitelesa IgM in 23 (22,5 %) za protitelesa IgG proti ošpicam. Kar zadeva rdečke, smo samo pri 20 (19,6 %) primerih ugotovili seropozitivne ravni IgG, medtem ko smo za mumps odkrili večjo pozitivnost pri 60 posameznikih (58,8 %). V vseh starostnih skupinah smo odkrili samo majhen odstotek posameznikov s pozitivnimi serološkimi rezultati za protitelesa IgG proti rdečkam. Predpostavili smo, da po cepljenju zagotovo nastanejo protitelesa proti rdečkam. Njihova odsotnost v primerih z dvema odmerkoma cepiva MMR nakazuje, da to cepivo ni moglo biti uporabljeno, čeprav so bili ti podatki vključeni v zdravstveno kartoteko. Sekvenčna analiza dveh vzorcev je pokazala genotip ošpic B3.*

Sklep: *Ta izbruh lahko kaže na obstoj ranljive skupine Romov. Nizka precepljenost pomeni resno grožnjo za javno zdravje.*

Ključne besede:
ošpice, Romi, izbruh, precepljenost

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1 INTRODUCTION

For more than three decades of high reported coverage (≥98% at the national level) with two doses of schedule (Measles-containing-vaccine first-dose (MCV1) and Measles-containing-vaccine second-dose (MCV2)) in the routine immunization programme, Slovakia has eliminated measles and rubella according to established criteria of the Regional Verification Commission for Measles and Rubella Elimination in Europe (1). Measles outbreaks continue to occur in Europe, however, and several countries have reported outbreaks affecting the general population due to inadequate vaccination coverage and the persistent circulation of the measles virus (2).

One of many factors contributing to a decline in immunization rates is a growing community of Roma population (3). About 500,000 (9.2% of the total population) Roma live in Slovakia, mainly in three of eight Regions of Slovakia (the Košice, Prešov and Banská Bystrica). The communities are mostly sedentary groups living in poor, isolated living sites (4). The measles outbreak between May 4 and October 16 2018 was detected in the Košice Region in the Michalovce and Sobrance Districts.

The aim of the study is to better understand the role of vaccination gaps in Roma communities in Slovakia with regard to the epidemic spread of measles. The results can contribute to more effectively implementing preventive measures in the future.

2 MATERIALS AND METHODS

2.1 Study Design

The study is designed as a case study analysing the epidemic outbreak of measles in the defined community.

2.2 Case Description and Outbreak Investigation

The data from the patients diagnosed with measles during the outbreak was collected from the mandatory case notifications that were transmitted to the Epidemiological Information System of the Slovak Republic (EPIS SR). The patients were ambulatory or inpatients admitted to the Michalovce Hospital's Department of Infectious Diseases and the Children's Faculty Hospital in Košice between May to October 2018. The necessary data was obtained by interviewing the patients' relatives, their paediatricians and the GPs as well as by reviewing the inpatients' medical records.

Measles cases were classified according to the classification of measles cases as defined by the Commission Implementing Decision (EU) 2018/945 (5).

The suspected clinical cases were defined as having a fever with a body temperature ≥380C, maculopapular rash, and at least one of the following symptoms: conjunctivitis, coryza, cough, or Koplik's spots (6).

The case-based reports provided data for disease onset, date of birth, sex, confirmation of diagnosis (i.e. laboratory confirmed, epidemiologically linked, or clinically compatible case), vaccination status, hospitalization and complications.

An outbreak of measles was defined as the occurrence of a chain of transmission of three or more cases linked in time and place and was declared by the Regional Public Health Authority (RPHA) in Michalovce and the National Public Health Authority of the Slovak Republic (NPHA of SR).

2.3 Vaccination Coverage and Incidence Data

The data about vaccination status (MCV1, MCV2) was collected from the annual administrative monitoring in Slovakia. A retrospective review of vaccination coverage of measles was carried out for the years 1999 to 2018. The vaccination status of 24-month-old children was analysed according to date of birth from 1999 to 2016 (MCV1). The epidemiological data about measles transmission was obtained from the EPIS SR and the data about vaccination from the annual regular controls in Slovakia.

2.4 Laboratory Data

The ELISA tests were used to measure immunoglobulin M and G (IgM, IgG) antibodies. Tests were performed at the Alpha medical, s.r.o. (microbiological laboratory) and the National Reference Laboratory for Measles, Mumps and Rubella (NRL MMR) of the NPHA SR in Bratislava.

Measles were tested semi-quantitatively for IgM and IgG antibodies with Enzygnost Anti-Measles-Virus IgM/IgG (Siemens) sets, and cut-off values for a positive IgM/IgG>0.100 were calculated as the index of optical densities (OD) of the cut-off/OD of the sample, Rubella was tested with Enzygnost Anti-Rubella-Virus IgG (Siemens) semi-quantitatively (OD cut off/OD sample), with cut-off values for a positive IgG>0.100. Mumps IgG was tested quantitatively with RIDASCREEN Mumps Virus IgG (R-Biopharm AG) with cut-off values for a positive IgG>244 U/ml.

Clinical specimens of four laboratory-confirmed cases were submitted to the WHO European Regional Reference Laboratory for Measles and Rubella at the Robert Koch Institute in Berlin, Germany to determine the genotype of the measles virus (MV) circulating during the outbreak as well as to identify its likely origin. The serum was sent for confirmatory testing and the throat swabs were submitted for virus detection, sequencing and genotyping of the MV RNA according to standard instructions.

2.5 Outbreak Control Measures

Specific control measures for the target populations affected by the outbreak were implemented by the RPHA in Michalovce in accordance with the national guidelines, including conducting outbreak and contact investigation

for cases, implementing isolation measures, catch-up and post-exposure vaccination and prophylaxis with immunoglobulin for people at high risk of developing severe disease, as well as providing information about the epidemiological situation. Proactive communication was carried out with parents, healthcare workers, microbiologists, hospitals, healthcare institutions, state administrations, the NPHA SR, the Ministry of Health of the Slovak Republic (MoH SR) and the Crisis Staff of the Michalovce District Office (7). Eligibility for prophylactic MMR vaccination and human normal immunoglobulin (HNIG) was assessed for all contacts and the healthcare workers. Those who had not been vaccinated appropriately for their age (i.e. the first dose of MMR vaccine at 15 months, the second dose at 10 years) were eligible for prophylactic MMR vaccination, in which case they were administered the vaccine within 72 hours of the contact with an infectious measles case. HNIG was considered for immunocompromised contacts, unvaccinated pregnant women, and selected infants under six months of age if they had been in contact with an infectious case in the previous six days (7, 8).

The letters with recommended control measures were sent out immediately: GPs were requested to check the immunization status of all contacts (children, adolescents and adults) and to immunize all those unvaccinated with one dose of MMR vaccine. The medical specialists in hospitals and outpatient settings were requested to be alert to the early detection, timely reporting, isolation, diagnosis, and treatment of measles cases. It was also recommended to hospitalize the patients with measles living in crowded households to ensure better conditions for treatment and care and to minimize the spread of the disease in communities with poor living conditions. The close Roma contacts with measles cases were isolated for 21 days at home.

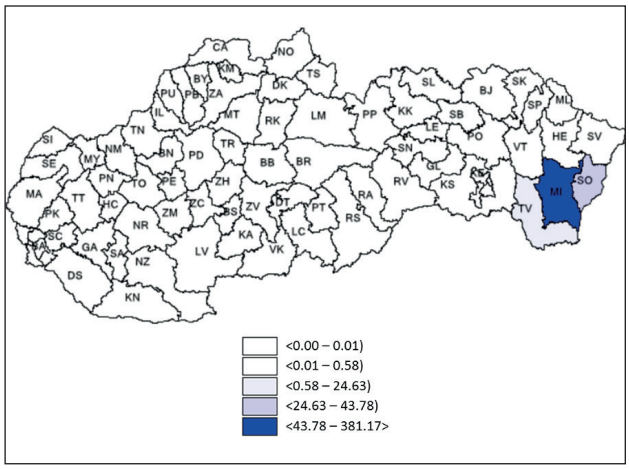
2.6 Methods of Data Analysis

The study uses descriptive data to characterize epidemic outbreak of measles, which is then used as a basis for the qualitative analysis of the implemented epidemic measures.

3 RESULTS

3.1 Outbreak Description

From May to October 2018 a measles outbreak was confirmed in Eastern Slovakia, specifically the region of Košice. First cases were imported from the UK (three imported and 1 imported-related cases). Measles spread to the Roma settlements in the Michalovce (416 cases from May 4 to September 20) and Sobrance (19 cases from May 25 to October 16) districts and affected the Roma population. During the outbreak period, four healthcare workers (HCWs) also developed measles in the Michalovce Hospital. A total of 439 measles cases were reported to the EPIS SR (Figure 1).



Source: EPIS SR, © Public Health Authority of the Slovak Republic

Figure 1. Map of the reported measles cases by districts, Slovakia, May 4–October 16, 2018 (n=439).

Figure 2 shows the measles incidence rate and level of vaccination coverage from 1997 to 2018 in Slovakia. The long-term high-performing immunization programme at national and sub-national (regional) levels showed a significant drop from 99.6% to 95.2% and from 99.5% to 95.4%. The vaccination coverage (MCV1) in the monitored districts ranged from 99.7% to 97.3% and from 100.0% to 96.3%.

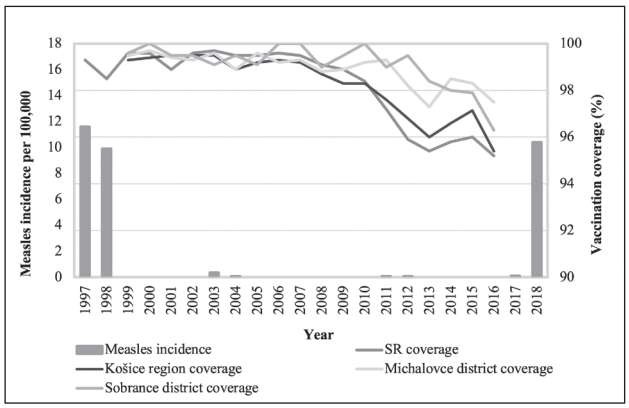


Figure 2. Measles incidence rate and measles vaccination coverage according to the cohorts 1999-2016, the Michalovce and Sobrance districts in the Košice region, Slovakia, 1997-2018.

Figure 3 shows the number of the reported measles cases by date of symptoms onset. The outbreak reached its peak by July 2018 (week 28/2018), with 80 (18.2%) reported cases.

A total of 439 cases were reported with the dates of rash onset from May 4 to October 16, 2018 (weeks 19/2018 to 43/2018).

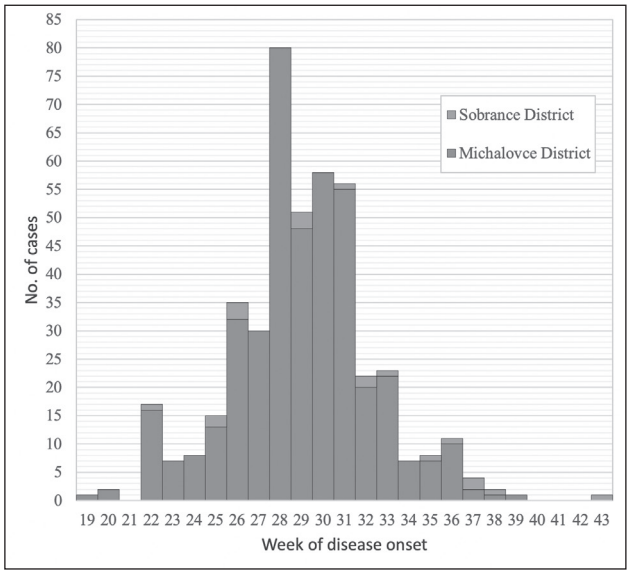


Figure 3. The epidemic curve of the total measles cases, May–October 2018, for the Michalovce and Sobrance districts, Slovakia (n=439).

The median age for the total of 439 measles cases was 10 years (range: 0 month–51 years), while in 278 measles cases (63.3%) it was ≤14 years.

Measles was diagnosed after two or more days (from two to 12) following rash onset. 274 cases were laboratory confirmed and 165 were classified as probable cases. The reported complications included 39 cases of pneumonia. Out of total 439 cases, 288 patients were admitted to hospital with median hospitalization of 6.5 days (range: 1–10 days). The most common age group affected by measles were children <1 year of age and then adolescents in 15-19 years of age (157 cases; 35.8%). Out of 288 hospitalized cases, a ten-day stay in hospital was recorded in 67 patients, of whom 64 were children and adolescents aged 0-19 years.

Table 1 shows the characteristics of all cohorts (439 cases) and the characteristics of the 102 measles cases cohort with positive two doses vaccination status by gender, age and type of applied vaccine (monovalent, bivalent and trivalent vaccines). More than 90% of the cohort patients were vaccinated with two doses of trivalent MMR (measles, mumps, and rubella) vaccine.

Within the Roma cohort, 175 out of 435 cases were unvaccinated or had unknown vaccination status for measles. Four sick healthcare workers were vaccinated with two doses of bivalent measles-mumps vaccine. Out of 264 vaccinated cases, 137 (31.2%) received two doses and 127 (28.9%) only one dose of measles vaccine in their vaccination records. 155 measles cases (35.3%) were unvaccinated and 20 (4.6%) of unknown vaccination status. We additionally tested antibodies against rubella and mumps in a set of 137 patients with two doses of vaccine. Samples from 102 patients were examined.

Table 1. Characteristics of all patients (n=439) and of patients vaccinated with two MCV doses (n=102) in the measles outbreak in Michalovce and Sobrance districts, Slovak Republic 2018.

	Characteristics of all patients in the measles outbreak, the Michalovce and Sobrance districts (n=439)									
	Age group									
Characteristic	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-34**	**35-44**	**45-54**	**TOTAL**
Males	33	27	30	35	38	16	19	10	4	212
Females	48	33	42	30	38	13	12	8	3	227
TOTAL (%)	81 (18.5)	60 (13.7)	72 (16.4)	65 (14.8)	76 (17.3)	29 (6.6)	31 (7.1)	18 (4.1)	7 (1.6)	439 (100.0)
Hospitalized	72 (16.4)	51 (11.6)	52 (11.8)	31 (7.1)	47 (10.7)	18 (4.1)	13 (3.0)	2 (0.5)	2 (0.5)	288 (65.6)
Complications	10 (2.3)	8 (1.8)	6 (1.4)	2 (0.5)	8 (1.8)	1 (0.2)	3 (0.7)	0	1 (0.2)	39 (8.9)
	VACCINATION STATUS (%) (n=439)									
MCV1										
MMR*	0	21 (4.8)	58 (13.2)	15 (3.4)	13 (3.0)	10 (2.3)	6 (1.4)	0	0	123 (28.0)
MM**	0	0	0	0	0	0	1 (0.2)	0	0	1 (0.2)
Mo***	0	0	0	0	0	0	0	2 (0.5)	1 (0.2)	3 (0.7)
All MCV1	0	21 (4.8)	58 (13.2)	15 (3.4)	13 (3.0)	10 (2.3)	7 (1.6)	2 (0.5)	1 (0.2)	127 (28.9)

	Age group									
Characteristic	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-34**	**35-44**	**45-54**	**TOTAL**
MCV2										
MMR*	0	0	0	37 (8.4)	52 (11.8)	17 (3.9)	10 (2.3)	2 (0.5)	0	118 (26.9)
MM**	0	0	0	0	0	0	0	1 (0.2)	0	1 (0.2)
Mo***	0	0	0	0	0	0	9 (2.1)	7 (1.6)	2 (0.5)	18 (4.1)
All MCV2	0	0	0	37 (8.4)	52 (11.8)	17 (3.9)	19 (4.3)	10 (2.3)	2 (0.5)	137 (31.2)
All vaccinated	0	21 (4.8)	58 (13.2)	52 (11.8)	65 (14.8)	27 (6.2)	26 (5.9)	12 (2.7)	3 (0.7)	264 (60.1)
Unvaccinated	0	23 (5.2)	14 (3.2)	12 (2.7)	5 (1.1)	1 (0.2)	0	0	3 (0.7)	58 (13.2)
Unvaccinated for age	81 (18.5)	16 (3.6)	0	0	0	0	0	0	0	97 (22.1)
All unvaccinated	81 (18.5)	39 (8.9)	14 (3.2)	12 (2.7)	5 (1.1)	1 (0.2)	0	0	3 (0.7)	155 (35.3)
Unknown	0	0	0	1 (0.2)	6 (1.4)	1 (0.2)	5 (1.1)	6 (1.4)	1 (0.2)	20 (4.6)
	Characteristics of patients vaccinated with 2 MCV doses in the measles outbreak in the Michalovce and Sobrance districts (n=102)									
Males	0	0	0	13	22	7	6	4	1	53
Females	0	0	0	10	23	5	7	3	1	49
TOTAL (%)	0	0	0	23 (22.5)	45 (44.1)	12 (11.8)	13 (12.8)	7 (6.9)	2 (2.0)	102 (100.0)
	VACCINATION STATUS (%) (n=102)									
MCV1										
MMR*	0	0	0	0	0	0	0	0	0	0
MM**	0	0	0	0	0	0	0	0	0	0
Mo***	0	0	0	0	0	0	0	0	0	0
All MCV1	0	0	0	0	0	0	0	0	0	0
MCV2										
MMR*	0	0	0	23 (22.5)	45 (44.1)	12 (11.8)	13 (12.7)	0	0	93 (91.2)
MM**	0	0	0	0	0	0	0	0	0	0
Mo***	0	0	0	0	0	0	0	7 (6.9)	2 (2.0)	9 (8.8)
All MCV2	0	0	0	23 (22.5)	45 (44.1)	12 (11.8)	13 (12.7)	7 (6.9)	2 (2.0)	102 (100.0)

*TRIMOVAX, PRIORIX,
M-M-RVAXPRO
**MOPAVAC
***MOVIVAC

3.2 Laboratory Confirmations

A total of 274 measles cases were laboratory confirmed at Alpha Medical, s.r.o. (microbiological laboratory) from May 4 to October 16.

One hundred and two samples obtained from the measles cases with two-dose vaccination status were tested for IgG antibodies against measles, mumps and rubella at the NRL MMR of the NPHA SR.

For measles, 68 (66.7%) cases had positive IgM antibodies, 33.3% of them were equivocal and 23 (22.5%) had positive IgG. For rubella, only 20 (19.6%) cases had seropositive IgG levels, while for mumps higher positivity was detected in 60 persons (58.8%). The remaining cases had negative test results.

Table 2 contains the proportion of persons with positive and negative serology results for a rubella IgG antibody test. Across all age groups of the measles cases, we can detect only a small percentage with a positive serology result of rubella IgG antibody test, ranging from 10.0% in the age groups of 10–14 and 45–54, and up to 30.0% in the 35-44 group.

Clinical specimens of four laboratory confirmed cases were tested in RKI Berlin. Sequential analysis of two samples showed measles genotype B3, dist. sequence in MeaNS: 5258, identical to Nmed Strain MVs/Bradford. GBR/13.18.

Table 2. The antibodies against measles, mumps and rubella by age group in the measles outbreak, May–October 2018, the Michalovce and Sobrance districts in the Košice region, Slovakia (n=102).

	Positive and cut-off values (n=102) (%)						
Characteristic	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-34**
All cases	23	45	12	13	7	2	102
				MEASLES			
IgM n=68	13 (12.7)	31 (30.4)	8 (7.8)	11 (10.8)	4 (3.9)	1 (1.0)	68 (66.7)
IgG n=23	1 (1.0)	7 (6.9)	3 (2.9)	4 (3.9)	6 (5.9)	2 (2.0)	23 (22.5)
				MUMPS			
IgG n=60	10 (9.8)	28 (27.5)	8 (7.8)	6 (5.9)	6 (5.9)	2 (2.0)	60 (58.8)
				RUBELLA			
IgG n=20	2 (2.0)	3 (2.9)	3 (2.9)	4 (3.9)	6 (5.9)	2 (2.0)	20 (19.6)
	Positive antibodies against rubella and the type of applied combination vaccines (n=20) (%)						
MMR+MMR	2 (10.0)	3 (15.0)	3 (15.0)	1 (5.0)	0	0	9 (45.0)
MM+MMR	0	0	0	3 (15.0)	1 (5.0)	0	4 (20.0)
Mo+Mo	0	0	0	0	5 (25.0)	2 (10.0)	7 (35.0)
TOTAL	2 (10.0)	3 (15.0)	3 (15.0)	4 (20.0)	6 (30.0)	2 (10.0)	20 (100.0)
	Negative antibodies against rubella and the type of applied combination vaccines (n=82) (%)						
MMR+MMR*	21 (25.6)	42 (51.2)	9 (11.0)	9 (11.0)	0	0	81 (98.8)
MM+MMR**	0	0	0	0	0	0	0
Mo+Mo***	0	0	0	0	1 (1.2)	0	1 (1.2)
TOTAL	21 (25.6)	42 (51.2)	9 (11.0)	9 (11.0)	1 (1.2)	0	82 (100.0)

*MMR+MMR: two doses of trivalent vaccine against measles, mumps and rubella
**MM+MMR: one dose of bivalent measles-mumps vaccine, and one dose of trivalent measles-mumps-rubella vaccine
***Mo+Mo: two doses of monovalent vaccine against measles

3.3 Outbreak Control Measures

Staff of the RPHA in Michalovce visited the Roma settlements several times, together with Roma health mediators (RHM), to identify contacts and search for additional cases and close contacts. Close contacts (and especially those 18 years or younger) were invited to GPs for post-exposure prophylaxis. The RPHA informed the local hospital, emergency departments, paediatricians, GPs and the local media, and urged physicians to investigate all suspected measles cases, to isolate them if infectious and to notify cases immediately. Simultaneously, they highlighted a need for verification of the measles immunity status of all unvaccinated hospital staff, and for vaccination of all those susceptible to infection.

Following the risk assessment of the situation related to the reported rising numbers of measles cases in many European countries (mainly in the Czech Republic, May 2017) and the permanent threat of importation of measles cases, the NPHA SR sent out an official warning letter to all RPHAs on May 2017.

During the measles outbreak in 2018, letters with recommended control measures were prepared by the RPHA in Michalovce (repeatedly from May 7 2018), the NPHA SR (May 28 2018), the Crisis Staff of the Michalovce District Office (July 13 2018).

4 DISCUSSION

The measles outbreak in Slovakia in 2018, involving 439 reported measles cases from two Roma settlements in Eastern Slovakia, represents the most serious episode in the country since the elimination of measles in 1999. This revealed and confirmed that pockets of low vaccination coverage do exist in some areas of Košice, particularly among the Roma communities. The estimated total Roma population in Slovakia is around 500,000 (4). They live in overcrowded and often extremely poor sanitary conditions. Another characteristic trait of the Roma is frequent travelling throughout the country and abroad (9, 10).

This outbreak points to the insufficient vaccination coverage of the Roma population in Slovakia. The analysis of the vaccination coverage status of the cases shows a prevalence of individuals having received at least one dose of measles-containing vaccine. It is difficult to monitor the uptake of MMR vaccine among the Roma, but it is known to be low (3). In this outbreak a high level of susceptibility was found among children and adolescents (0–14 age group), confirming a low vaccination uptake. Barriers to the vaccination of the Roma have been previously described (4). The outbreak in their settlements did not spread widely within the communities.

The majority of cases were hospitalized, with the median hospitalization of six days (1–10 days). The same higher proportion with five-day hospitalization (5–6 days) was described in the Irish outbreak in 2016. Of the complications only pneumonia (39 cases) was reported. There were no reported cases of seizures, meningitis or encephalitis, and there were no deaths (11).

The measles monovalent vaccine was introduced in Slovakia in 1969. The combined bivalent vaccine (MM–measles, mumps) was implemented in 1987 and trivalent MMR (measles, mumps, rubella) in 1992. Currently, in accordance with the National Immunization Programme (NIP) of the Slovak Republic, two doses of MMR vaccine are recommended for children (15–18 months and 10 years old). We have noticed a gradual decrease in vaccination coverage, particularly in the cohorts from 2000–2016. However, this decrease is not homogenous and there are differences across the regions. A similar situation was also described in other countries of the European Union (12).

Analysis of IgG antibodies against rubella showed the relatively low prevalence rate of positive values not corresponding with medical records. This finding points to the importance of immunological examinations carried out on a regular base to get a valid picture on the actual situation. We assume that rubella antibodies had to be produced following the vaccination. Their absence in those cases with a stated history of two doses of MMR vaccine suggests that these vaccines could not have been administered, despite the fact that this data was included in the patients' medical records.

A high immunogenicity of PRIORIX (MMR) was demonstrated in the clinical trials with infants and children aged from 12 months to two years. The vaccination with a single dose of PRIORIX induced antibodies against measles in 98.1% of the vaccinated individuals, against mumps in 94.4% and against rubella in 100% of previously seronegative ones. Two years after primary vaccination seroconversion rates were 93.4% for measles, 94.4% for mumps and 100% for rubella. All results after M-M-RVAXPRO and TRIMOVAX were similar (13).

Recently, mumps outbreaks have been reported in these localities. The positivity rate of 54.9% for antibodies may be induced by natural infection, although no case history of illness was either confirmed or disproved.

Sequential analysis of the samples obtained from the early cases in Michalovce showed measles genotype B3, which has been dominantly circulating in Europe (France, Italy and Romania) over the past three years. Mean data from the last two years (2017, 2018) show that measles virus genotype B3 was endemic in several countries worldwide (11, 14, 15).

All contacts received a dose of MMR vaccine as post-exposure prophylaxis within 72 hours of last exposure. The contacts born before 1969 were not advised to get MMR vaccination due to the high probability that they had received natural immunity from childhood infection. Paediatricians and GPs vaccinated 6,076 persons (family members, co-workers and others in contact with patients), out of whom 4,648 were children.

Thanks to relatively high immunization rates (from 97.3% to 100%) among the cohorts of small children (born between 2010 and 2016 in the districts of Michalovce and Sobrance) and to the work done by the RPHA in Michalovce in conducting the contact investigation for cases, vaccination of susceptible persons and household contacts, as well as in implementing isolation measures, the outbreak did not affect the whole region and remained limited.

As a potential limitation of the study it should be mentioned that we were not able to include into the analysis all factors determining epidemic spread. However, the analysis is based on immunological examinations and thus provides a valid picture on the actual situation.

5 CONCLUSION

High infectivity combined with a decrease in vaccine coverage and a dramatic increase in disease prevalence present a risk of a measles outbreak in many EU countries (16). The outbreak examined in this study has highlighted the immunity gaps existing in the Roma population in Slovakia. The risk should not be underestimated, and represents a more serious threat than sporadic cases of susceptible individuals. Susceptible population groups may reintroduce indigenous measles transmission even in those countries confirmed as disease-free or in a population with high vaccine coverage (17).

Efforts are thus needed to improve the methods used to identify the high risk areas and to develop specific strategies within target susceptible groups. An improvement in health services is essential to reach the Roma communities. To achieve this, a more effective

communication strategy should be defined to address the subjects objecting to vaccination, and placing this within a wider discussion on their responsibility towards the community. The implementation of catch-up campaigns targeting children and adolescents should also be considered.

CONFLICT OF INTEREST

The authors declare no conflicts of interest exist.

FUNDING

The study was partially supported by the Research and Development Support Agency under Contract No. APVV-0096-12 (Biomathematical modelling and evaluation of indicators of vaccination and their impact on the epidemiological situation of selected vaccine-preventable diseases, EPIBIOMAT).

ETHICAL APPROVAL

The patient information used in this research was collected following ethical principles. All patients signed informed consent forms for each diagnostic intervention and research purpose, according to the related act of the Ministry of Health. Supplementary immunological examinations were approved by the Ethics Committee of the Ministry of Health of the Slovak Republic.

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TRAINING FAMILY DOCTORS AND PRIMARY CARE NURSES IN EVIDENCE-BASED PREVENTION, SCREENING AND MANAGEMENT OF CARDIOVASCULAR RISKS IN WESTERN UKRAINE: A LONGITUDINAL STUDY

USPOSABLJANJE SPECIALISTOV DRUŽINSKE MEDICINE IN MEDICINSKIH SESTER V OSNOVNEM ZDRAVSTVENEM VARSTVU ZA PREVENTIVO, KI TEMELJI NA DOKAZIH, PRESEJANJE IN OBVLADOVANJE KARDIOVASKULARNIH TVEGANJ V ZAHODNI UKRAJINI: LONGITUDINALNA ŠTUDIJA

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Received: Nov 9, 2019
Accepted: Sep 12, 2020

Original scientific article

ABSTRACT

Keywords:
continuing medical education, readiness to change practice, motivation, screening

Introduction: The Ukrainian primary healthcare programme of preventive and screening recommendations has not been evidence-based. The traditional system of continuous medical education in Ukraine places participants in the role of passive listeners. This study explored the effects of an interactive training course on evidence-based prevention and screening of cardiovascular risks, on changes in Ukrainian family doctors' (FDs) and primary care nurses' (PCNs) knowledge and readiness to change practice over time.

Methods: Three hundred and seven FDs and PCNs participated in the study. Changes in participants' knowledge were assessed with 20 multiple choice questions, and their readiness to change practice with a five-item questionnaire. These were administered before, immediately after, three and twelve months after training.

Results: The mean pre-course knowledge score was 6.1 (SD 1.8) out of 20, increasing to 14.9 (SD 2.3) immediately afterwards ($p<0.001$). Three months later it was 10.2 (SD 3.2) and at one year it was 10.4 (SD 3.3), both of which were significantly higher than the pre-training level ($p<0.005$). The percentage of participants that were highly motivated to change their practice increased from 18.4% before the training to 62.3% immediately afterwards ($p<0.001$). Three months later, this fell to 40.4%. At 12 months it further reduced to 27.4%, but was still significantly higher than the baseline level ($p<0.001$).

Conclusions: The interactive training was effective in increasing both participants' knowledge and their readiness to change their clinical practice. The impact of the training diminished over time, but was still evident a year later.

IZVLEČEK

Ključne besede:
stalno medicinsko izobraževanje, pripravljenost na spremembo prakse, motivacija, presejanje

Uvod: *Ukrajinski program osnovnega zdravstvenega varstva s priporočili za preventivo in presejanje ni temeljil na dokazih. Tradicionalni sistem stalnega medicinskega izobraževanja v Ukrajini postavlja udeležence v vlogo pasivnih poslušalcev. V tej študiji so preučili učinek interaktivnega tečaja usposabljanja za preventivo, temelječo na dokazih, in presejanje kardiovaskularnih tveganj na spremembe v znanju ukrajinskih družinskih zdravnikov in medicinskih sester v osnovnem zdravstvenem varstvu ter na njihovo pripravljenost, da sčasoma spremenijo prakso.*

Metode: *V študiji je sodelovalo 307 družinskih zdravnikov in medicinskih sester v osnovnem zdravstvenem varstvu. Spremembe v znanju udeležencev so ocenili z 20 vprašanji izbirnega tipa, njihovo pripravljenost za spremembo prakse pa z vprašalnikom s 5 točkami. Ta vprašanja/vprašalnike je bilo treba izpolniti pred usposabljanjem, takoj po njem ter čez 3 in 12 mesecev.*

Rezultati: *Povprečna ocena znanja pred tečajem je bila 6,1 (SD 1,8) od 20, takoj po tečaju pa se je povečala na 14,9 (SD 2,3; $p < 0,001$). Čez 3 mesece je bila ocena 10,2 (SD 3,2) in po enem letu 10,4 (SD 3,3), kar je v obeh primerih pomembno višje od ravni pred usposabljanjem ($p < 0,005$). Odstotek udeležencev, ki so bili zelo motivirani za spremembo prakse, se je z 18,4 % pred usposabljanjem povečal na 62,3 % takoj po usposabljanju ($p < 0,001$). V 3 mesecih se je ta delež zmanjšal na 40,4 %. Po 12 mesecih se je dalje zmanjšal na 27,4 %, vendar je bil še vedno pomembno višji od izhodiščne ravni ($p < 0,001$).*

Sklepi: *Interaktivno usposabljanje je učinkovito povečalo tako znanje udeležencev kot njihovo pripravljenost za spremembo klinične prakse. Učinek usposabljanja se je sčasoma zmanjšal, vendar je bil po enem letu še vedno očiten.*

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1 INTRODUCTION

The global burden of chronic non-communicable diseases is rising, and achieving an adequate balance between addressing modifiable risk factors and avoiding over-treatment is a international priority (1-3). In the past, chronic disease screening systems in post-Soviet countries were not evidence-based (4). Following Ukrainian Ministry of Health Care legislation in 2018, the post-Soviet screening protocol was cancelled (5), and it has not been replaced by a new state screening system (4). Individual primary care providers (mainly family doctors, FDs) have their own systems of screening for their patients, which are not always evidence-based, but there are now plans for a state cardio-vascular risk screening programme (blood pressure, cholesterol and blood glucose measurements, body mass index evaluation) (4). The future role of primary care nurses (PCNs) in screening for chronic disease in Ukraine is, as yet, undecided (4), but there is a move to involve them in screening management. There is, therefore, a need to increase the knowledge of both FDs and PCNs regarding evidence-based screening recommendations and to increase their readiness to implement them (4).

Continuing medical education (CME) increases clinician knowledge, as well as improving their performance and patient outcomes (6). The effect size is larger when the educational interventions are interactive (7) and use multiple methods (6). Traditional lectures and seminars are less effective than interactive teaching (8, 9), yet passive learning with minimal trainee participation still dominates the medical education curriculum in Ukraine and other post-Soviet Eastern European countries (10-12). Innovative training techniques may thus improve FD and PCN training in these countries and be an effective means to induce changes in medical practice (13, 14). Interactive teaching techniques are recommended by the European Academy of Teachers in General Practice / Family Medicine (EURACT) and other medical organizations, and are part of the blended teaching model used with FDs in most developed European countries (15-17). Interactive training seminars combine various methods which draw participants' attention and involve them in practical interactions (18-21). Interactive pedagogical methods include "brainstorming" (a group activity that encourages participants to focus on a topic and contribute to the free flow of ideas), work in small groups, demonstrations, presentation of clinical cases, role-play and feedback. Active teaching/learning techniques can be used to develop creative thinking and establish practical skills and competencies (8, 22, 23). Interactive methods of education in CME of doctors and nurses, such as blended learning, may help to raise their levels of knowledge (15, 17). While interactive training, with the assessment of FDs' knowledge levels before and immediately after the training, is being introduced into the system of education for primary health care providers in different regions of Ukraine, it mostly relates to emergency care (24, 25).

We aimed to evaluate the effectiveness of interactive training sessions for FDs and PCNs on evidence-based prevention screening and management of cardiovascular risks. We describe our experience in introducing such a training programme in the western (Transcarpathian) region of Ukraine, and we report the changes in participants' level of knowledge and their readiness to implement the evidence-based strategies they learned over time.

2 METHODS

2.1 Setting

The Transcarpathian region is a mountainous area of western Ukraine which includes some remote and distant districts. Primary medical health care is provided by 662 FDs and by approximately 1,000 PCNs for the region's population of 1.25 million people, about two-thirds of whom live rurally. All FDs and PCNs work at state-supported family medicine clinics, subordinated to local Primary Care Centres.

CME, in the form of a one-month academic course every five years, has been mandatory for FDs and PCNs wishing to confirm or upgrade their qualification category. These courses are conducted at the Postgraduate Faculty of the Uzhhorod National University (UzhNU), and are funded by the practitioners themselves, or by their Primary Care Centres (10). The courses have mainly been based on traditional didactic lectures, with the teaching provided by non-FD specialists. FDs from remote districts often work in solo practices without replacements available and thus cannot complete the full one-month training course. Shorter practical training sessions can, therefore, potentially become an alternative to the current system.

2.2 The Training Sessions

Our team at the UzhNU Family Medicine Training Centre organized an eight-hour practical training course on "Evidence-based steps for the prevention, screening and management of cardiovascular risks in FD's practice". We used a blended model of teaching with interactive pedagogical techniques that were developed from descriptions of similar programmes in other countries (22, 23). We held 20 identical training sessions for FDs and PCNs in the 12 districts of the Transcarpathian region during 2016-2017, and each participating FD and PCN attended a single session. PCNs were invited to the sessions to increase their understanding of their role in screening management, as we considered that they could be involved in counselling at-risk patients (smoking cessation, lifestyle changes, etc.), measurement of body mass index (BMI) and blood pressure (BP), glucose and cholesterol testing, and organizing follow-up appointments.

At the beginning of each training session we gave a short interactive introductory talk, aimed at increasing participants' readiness to implement changes in their medical practice. To allow for multiple points of view, we then divided the participants into five small groups (usually with six participants in each). To ensure that each group could benefit from a wide range of skills and experience, the groups were made heterogeneous in terms of age, profession (FD/PCN) and experience. Trainers from our training centre (members of EURACT who were trained in, and had experience of, using interactive training methods for FDs) facilitated a series of four 45-minute workshops, leading discussions on the prevention, screening and management of cardiovascular diseases. The trainers encouraged the participants to express their thoughts and suggest solutions to perceived barriers. Every group rotated through each of the four workshops, with short breaks (5-15 minutes) in between. An overview of the modules is given in Table 1.

Table 1. Module aims and teaching methods.

Module name	Key learning aims	Teaching methods used
Evidence-based approaches to effective counselling for patients with cardiovascular risks	Evidence-based counselling methods for patients with cardiovascular risks	• Participant discussion of clinical cases • Work in small groups • Role-play
Metabolic syndrome	Screening and management of components of metabolic syndrome	• Brainstorming • Demonstrations of practical skills (determination of BMI, waist circumference, glucose measurement) • Clinical cases discussion • Work in small groups
Rational statin therapy	Evidence-based steps for dyslipidemia screening and rational statin therapy for patients with cardiovascular risks	• Brainstorming • Hands-on training in taking blood samples for lipid profile and glucose measurement • Clinical cases discussion • Work in small groups
Essential hypertension and its complications	Evidence-based steps for management of patients with essential hypertension and its complications	• Brainstorming • Work in small groups • Participant discussion of clinical cases

Module 1 ("Evidence-based approaches to effective counselling for patients with cardiovascular risks") employed participant role-play. Participants were offered situational tasks, as close as possible to real life situations, which gave them the opportunity to apply their knowledge and skills regarding effective counselling of patients with cardiovascular disease risks.

Module 2 ("Metabolic syndrome") included a presentation of a simulated clinical case, with use of "brainstorming" and demonstrations of practical skills (determination of BMI, waist circumference, glucose measurement). An online resource for FDs that had been developed by the organizers, the "Primary Prevention and Screening Calculator", was presented to the participants for use in their practice (26). This resource gives practitioners clear evidence-based algorithms for the prevention of cardiovascular disease, and advice on how to develop a cardiovascular disease screening plan.

Module 3 ("Rational statin therapy") was conducted as a clinical case demonstration of evidence-based management of a patient with dyslipidemia in an FD's practice. During this session the PCNs had an opportunity to get hands-on training in taking blood samples for lipid profile and glucose measurement.

Module 4 ("Essential hypertension and its complications") was the final session, in which all participants brainstormed techniques and discussed clinical cases.

2.3 Questionnaires

Participants completed two paper questionnaires in which they were asked to state their age, profession (FD or PCN), number of years in practice, and location of their practice (rural or urban). We assessed participants' knowledge levels by using 20 multiple choice questions (MCQs) about the training topic, chosen by the course trainers from a bank of MCQs used for the certification of doctors and nurses (31). Each correctly answered question was given a score of 1 point, and a wrong answer scored no points, giving a possible range of 0-20 points. We categorized the results as 'high' or 'low' levels of knowledge, depending on whether individuals answered more than half of the questions correctly (≥11 points).

We assessed participants' readiness to implement evidence-based prevention and screening with a five-item questionnaire (see Appendix 1). The items were adapted from an IDEA Health and Fitness Association questionnaire (27) and piloted by 20 FDs and 20 PCNs. No changes needed to be made as a result of the pilot. Respondents were asked to assess their levels of readiness to use evidence-based screening of cardio-vascular risks in their own clinical practice, using a five-point scale (ranging from 1, not ready/unmotivated, to 5, very ready/motivated). For each respondent, the sum of the five scores gave a total "readiness to change" score, giving a possible range of 5 to 25. Scores of ≥21 were categorized as "Very ready to change practice".

We administered the questionnaires immediately before and after the training, as well as three months and one year after the training days. For the latter, we emailed the questionnaires to each of the regional departmental heads, who sent paper copies to the practitioners in their regions. The completed questionnaires were returned to us by post.

2.4 Statistical Analysis

Participants' baseline characteristics were analysed using percentages for categorical variables, with means and standard deviations (SD) for continuous variables. The scores for each round of questionnaires and the proportion of those who achieved high scores are presented as means with 95% confidence intervals (CI). Chi square tests were used to compare proportions, and t-tests to compare means. Statistical data were processed using Stata, version 15.1 IC (StataCorp LP, College Station, Texas).

3 RESULTS

Overall, 600 FDs and PCNs took part in the training sessions, and of those 307 (51.2%) (211 FDs, 96 PCNs) agreed to participate in the study. All study participants completed the survey immediately after and three months after the training, but 12 months afterwards only 218 participants out of the 307 (71%) did so. The mean study participant age was 45.4 years (SD 12.8), and most (73.4%) worked in rural areas (Table 2).

Table 2. Characteristics of study population (N=307).

Mean age (SD)	45.4 (12.8)
Age <45years (%)	146 (47.6)
Profession (%)	
Primary care nurse	96 (31.3)
Family doctor	211 (68.7)
Years in practice (%)	
<10	59 (19.1)
10-19	48 (15.7)
20-29	85 (27.8)
≥30	115 (37.4)
Working in rural practices (%)	225 (73.4)

The mean participant knowledge level before the training was 6.1 (SD 1.8) (see Figure 1 and Table 3), and significantly higher immediately after the training: 14.9 (SD 2.3, p<0.001). Three months after the training it was 10.2 (SD 3.2), and one year after the training it was 10.4 (SD 3.3), both of which were significantly higher than the pre-training level (p<0.005).

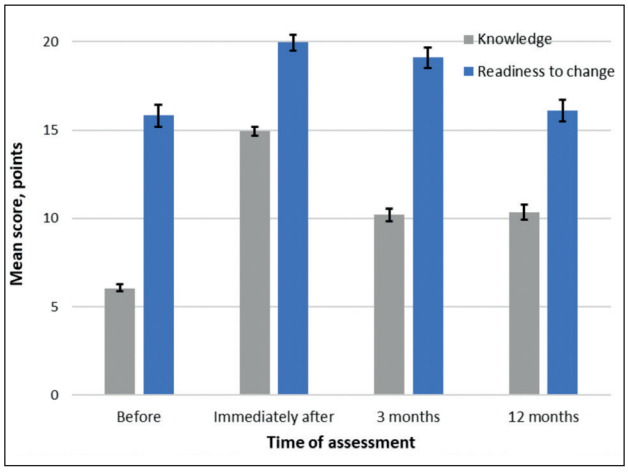

Figure 1. Mean participant knowledge and levels of readiness to change at different times, with 95% confidence intervals.

The mean level of readiness to implement evidence-based prevention, screening and management of cardiovascular risks at the beginning of the training was 15.8 (SD 3.4) (see Figure1 and Table 3). Immediately after the training, it increased to 20.0 (SD 2.4), significantly higher than the pre-course results ($p<0.005$). While three months after the training this increase had been maintained, at 19.1 (SD 3.2), and was still significantly higher than before the training, twelve months after the training it had fallen to 16.1 (SD 4.5), similar to the pre-training level.

Table 3. Results of the knowledge and readiness to change questionnaires before and after the training sessions.

Characteristic	Knowledge		Readiness to change	
	Mean score (SD)	High level of knowledge, % (95% CI)	Mean score (SD)	High level of motivation, % (95% CI)
Before training	6.1 (1.8)	2.4 (1.0-4.7)	15.8 (3.4)	18.4 (11.8-26.7)
Immediately after training	14.9 (2.3)	93.7 (90.5-96.1)	20.0 (2.4)	62.3 (52.7-71.2)
After 3 months	10.2 (3.2)	51.7 (46.1-57.1)	19.1 (3.2)	40.4 (31.3-50.0)
After 12 months	10.4 (3.3)	48.2 (41.4-55.1)	16.1 (4.5)	27.4 (21.6-33.8)

The proportion of FDs and PCNs who had a high level of knowledge (≥11 points) before the training was 2.4%, immediately after the training it was 93.7%, three months after the training it was 51.7%, and after 12 months it was 48.2%. All these values were significantly higher than that for the pre-training course ($p<0.001$), (Figure 2).

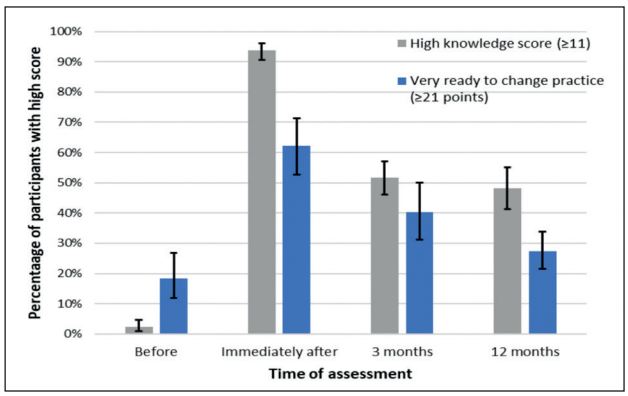

Figure 2. Percentage of participants who answered more than half of the questions correctly at different times, and who were very ready to make changes to their practice, with 95% confidence intervals.

The percentage of FDs and PCNs that were very ready to implement evidence-based preventive measures in their own practices increased significantly from 18.4% before the training to 62.3% immediately after ($p<0.001$) (see Figure 2 and Table 3). Three months later, this had fallen to 40.4%, and after 12 months it had further reduced to 27.4%, though this was still significantly higher than the pre-training level ($p<0.001$).

4 DISCUSSION

4.1 Summary of Important Findings

In a training programme for FDs and PCNs in western Ukraine, the level of knowledge of evidence-based prevention, screening and management of cardiovascular risks increased substantially after a one-day interactive training course and, while it reduced in subsequent testing, remained significantly higher than baseline one year later. While the percentage of participants who were very ready to make changes to their clinical practice was significantly higher at all stages after the training, overall readiness-to-change scores had fallen back to near-baseline levels one year later.

4.2 Strengths and Limitations

This is the first report of interactive training for FDs and primary care nurses in this region. The study included almost a third of all the FDs working in the Transcarpathian region. We were able to explore the effects of our training programme over twelve months of follow-up. While just over half of the training session participants completed the questionnaires, we did not have consent to collect data on the characteristics of those that did not complete these. The knowledge level questions were chosen by experienced FDs from a pre-existing MCQ question bank and therefore have face validity. The "readiness to change" questionnaire was piloted before use in the study. However, we did not assess the reliability of either questionnaire.

We also did not assess whether the programme had an impact on actual patient care. There was no comparison

of the effect of the interactive training seminars with traditional Ukrainian medical teaching methods, such as didactic lectures. Moreover, it should be noted that other, unmeasured factors could have influenced participants' knowledge and readiness to change during the year following their training seminars.

4.3 Comparison with Existing Literature

A Spanish study assessed the effect of an interactive course comprising four half-day sessions designed to develop the knowledge and skills required to practice evidence-based care (22). Similar to our study, it demonstrated a significant increase in participants' knowledge. However, there was no follow-up beyond the questionnaire administered at the end of the course. In a study in China, a weekly face-to-face evidence-based medicine training course was found to increase knowledge and future anticipated use (24). Participants' subsequent management of hypertension in the community was significantly better than that of a control group that had followed a self-instruction course instead. In a South African five-day interactive training programme for family physician clinical trainers, participants showed an increase in self-reported competencies three months after the course, but an objective assessment showed no change in their capabilities (25).

In a manual for motivational interview training, there are recommendations to assess participants' levels of interest and confidence in their skills, their readiness to learn those skills, and their self-perceived skill levels (26), all of which were assessed in our interactive training programme. The majority of General Practitioners (GPs) consider evidence-based medicine to be a positive concept (32). However, barriers have been reported that limit its use (33, 34). Research showed that audits, feedback information and group discussion positively contributed to Dutch GPs' motivation to change their practice (35).

4.4 Interpretation of the Findings and Recommendation for Further Research

An interactive teaching method for Ukrainian primary care clinicians resulted in an increase in their knowledge and readiness to change clinical practice, which was to a varying extent still present a year later. Barriers to changing practice identified by the participants included lack of time for motivational interviewing, low levels of patient motivation for lifestyle modification and limited access to screening investigations, especially in rural areas, and these may have limited the extent to which the participants' own motivation increased.

While there was an immediate increase in the levels of motivation, this reduced over time, which may reflect the limited effect of the single, eight-hour intervention. More research is thus needed to find out whether the increased readiness to change results in real changes in clinical practice, and, if so, what those changes are. There is also a need to find out how, in the post-Soviet Eastern European countries, the increased readiness to change clinical practice can be sustained in the long term.

5 CONCLUSIONS

Interactive training is an effective way to increase primary care clinicians' level of knowledge of evidence-based prevention, screening and management of their patients' cardiovascular disease risks, and their short-term readiness to make changes. Although participants' level of knowledge increased and remained stable between three and 12 months after training, their initial increase in levels of readiness to change reduced over that time. Further research should thus identify methods to attain long-lasting effects from CME, as well as explore the effects of this course on practitioners' satisfaction and patients' health outcomes.

ACKNOWLEDGMENTS

We would like to thank the participants who took part in this study, the Regional Health Care Department of Transcarpathian Regional Administration in support of training, the Postgraduate Faculty of UzhNU for the use of their teaching facilities, the trainers of the Family Medicine Training Centre of Postgraduate Faculty, and the doctors and nurses who piloted the survey.

FUNDING

The training was supported by the Regional Health Care Department of Transcarpathian Regional Administration, which helped reimburse the participants' expenses. The Regional Health Care Centres and Uzhgorod University paid the trainers' salaries.

COMPETING INTERESTS

The authors declare no conflicts of interest.

ETHICS APPROVAL

Research ethics approval was not required as no biomedical intervention was performed.

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Appendix 1. "Readiness to change" questionnaire.

Please estimate your level of readiness to use evidence-based screening of cardio-vascular risks in your own clinical practice, using a 5-point scale (where 1 is not ready/unmotivated, 2 is slightly ready/motivated, 3 is moderately ready/motivated, 4 is well motivated/ready, 5 is very ready/motivated).

	Rating, from 1 to 5
1. From a personal point of view, how motivated are you to conduct evidence-based screening of cardio-vascular risks in your own clinical practice?	
2. Taking external factors into account, how motivated are you to conduct evidence-based screening of cardio-vascular risks?	
3. How ready will you be to implement the planned new state programme of screening for cardio-vascular risks?	
4. How ready are you, in terms of your own clinical abilities, to conduct evidence-based screening of cardio-vascular risks?	
5. How ready are you to manage the full range of evidence-based screening tests for cardio-vascular risks? *	

* Evidence-based screening of cardiovascular risks includes:
- every 1-2 years, measuring the blood pressure of all your patients who are aged 18 or older;
- regular assessment of cardio-vascular risk factors (smoking, alcohol intake, calculation of BMI, family history) for all your patients who are aged 18 or older;
- lipid profile measurement every 5 years, in all your male patients who are aged 40 or older, and all your female patients who are aged 45 or older;
- blood glucose measurement every 5 years for all your patients who are aged 45 or older, or sooner in patients with BMI ≥25 or who have additional risk factors.

Dermota U, Grmek Košnik I, Janežič S, Rupnik M. Changing epidemiology of presumptive community-associated-methicillin-resistant Staphylococcus aureus in Slovenia in 2014-2015 compared to 2010. Zdr Varst. 2020;59(4):236-244. doi: 10.2478/sjph-2020-0030.

CHANGING EPIDEMIOLOGY OF PRESUMPTIVE COMMUNITY-ASSOCIATED-METHICILLIN-RESISTANT *Staphylococcus aureus* IN SLOVENIA IN 2014-2015 COMPARED TO 2010

SPREMEMBA EPIDEMIOLOGIJE PRI PROTI METICILINU ODPORNEM *Staphylococcus aureus* DOMAČEGA OKOLJA V SLOVENIJI V LETIH 2014–2015 V PRIMERJAVI Z LETOM 2010

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Received: Jan 28, 2020
Accepted: Sep 16, 2020

Original scientific article

ABSTRACT

Introduction: Although the distinction between the Community-Associated-Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) and Hospital-Associated-Methicillin-Resistant *S. aureus* (HA-MRSA) has blurred in recent years, the CA-MRSA is an important group because of its potential to cause fulminant and severe infections. Its importance has further increased with the emergence of Livestock-Associated-Methicillin-Resistant *S. aureus* (LA-MRSA).

Methods: In the present study we analysed clonal distributions and virulence factors in presumptive CA-MRSA isolated from January 2014 to December 2015 and compared the results with our previous study from 2010. Phenotypic definition for presumptive CA-MRSA was based on resistance to cefoxitin and oxacillin and susceptibility to at least two of the following four antibiotics: ciprofloxacin, erythromycin, clindamycin and gentamicin.

Results: In 2014 and 2015 altogether 304 MRSA isolates fulfilled our screening phenotypic definition, 45 isolates were cultivated from clinical specimens and 259 from screening specimens. Sequence types ST398, LA-MRSA and *mec*C MRSA increased significantly in 2015 compared to 2010 (p-value <0.05) and were spread over Slovenia.

Conclusion: The clonal distribution of presumptive CA-MRSA has changed within the study period in Slovenia. In 2015 the most frequent clone among clinical and screening specimens was a pig-associated clone, ST398, but the number of confirmed ST398 infections remains low. While previously ST398 and *mec*C positive MRSA strains were geographically limited, they have spread throughout the country since 2010.

Keywords:
Slovenia, CA-MRSA, LA-MRSA, *mec*C, *spa* types, clones

IZVLEČEK

Uvod: *Čeprav je ločevanje med proti meticilinu odporno Staphylococcus aureus domačega okolja (CA-MRSA) in proti meticilinu odporno Staphylococcus aureus bolnišničnega okolja (HA-MRSA) oteženo, je v zadnjih letih CA-MRSA pomembna skupina, ki povzroča resne in ogrožajoče okužbe. Njen pomen se je povečal še s pojavom proti meticilinu odpornim Staphylococcus aureus rejnih živali (LA-MRSA).*

Metode: *V tej raziskavi smo analizirali zastopanost klonov in prisotnost virulenčnih dejavnikov pri sevih, sumljivih za CA-MRSA, ki so bili osamljeni od januarja 2014 do decembra 2015, in rezultate primerjali s študijo iz leta 2010. Fenotipska definicija za sumljive CA-MRSA je temeljila na odpornosti proti cefoksitinu in oksacilinu ter občutljivosti za vsaj dva od naslednjih štirih antibiotikov: ciprofloksacin, eritromicin, klindamicin in gentamicin.*

Rezultati: *V letih 2014 in 2015 je skupno 304 izolatov MRSA ustrezalo naši fenotipski definiciji. 45 izolatov je bilo osamljenih iz kliničnih kužnin, 259 iz nadzornih kužnin. Sekvenčni tip ST398, LA-MRSA in mecC MRSA, sta se v letu 2015 v primerjavi z letom 2010 signifikantno povečala (p-vrednost <0,05) in se razširila po Sloveniji.*

Zaključki: *Zastopanost klonov pri sevih, sumljivih za CA-MRSA, se je v obdobju raziskave v Sloveniji spremenila. V letu 2015 je bil najpogostejši klon med kliničnimi in nadzornimi kužninami klon ST398, MRSA rejnih živali, vendar je število potrjenih okužb s ST398 še vedno nizko. V letu 2010 so bili ST398 in mecC pozitivni sevi MRSA geografsko omejeni, v kasnejših letih pa so se razširili po vsej državi.*

Ključne besede:
Slovenija, CA-MRSA, LA-MRSA, mecC, tipi spa, kloni

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1 INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens responsible for hospital-associated (HA-MRSA) and community-associated (CA-MRSA) infections. The epidemiological distinction between HA-MRSA and CA-MRSA is blurred, because CA-MRSA is increasingly invading into the health care setting and consequently the epidemiology of MRSA has changed (1-3). MRSA infections in the community can also be caused by livestock-associated MRSA (LA-MRSA) (1, 4-7). In recent years, LA-MRSA, specifically sequence type (ST) ST398 has emerged in farm animals, and human infections caused by MRSA ST398 have been increasingly documented in Europe (1, 4-9). In 2011 a novel divergent *mec*A gene homologue (*mec*A_{LGA251}), designated *mec*C, was discovered. *mec*C MRSA was reported in humans, in animals (farm, companion and wildlife animals), and also from non-animal sources (e.g. water and urban wastewater), indicating the existence of *mec*C MRSA in several different reservoirs (1, 10, 11).

No systematic surveillance of MRSA isolates and epidemiology of MRSA clone distribution in humans has been performed in Slovenia, and only the surveillance of presumptive CA-MRSA has been established. Antibiotic susceptibility patterns with partially available epidemiological data (colonization or infection with MRSA and hospitalization history in the previous year) was used as a screening tool to select the presumptive CA-MRSA in an earlier study (12), and isolates were analysed on a national level for the first time in 2010 (13). To be consistent with inclusion criteria and for comparative purposes we have retained the previous antibiotic susceptibility based criteria for strain selection and are referring to the strains as presumptive CA-MRSA.

Among 92 presumptive CA-MRSA in 2010 the most prevalent sequence types (STs) were ST45 (n=35, 38%), ST398 (n=14, 15.2%), ST5 (n=9, 9.8%) and ST22 (n=8, 8.7%) (13). *mec*C MRSA was confirmed previously in Slovenia and seven of 359 isolates (1.7%) were positive for *mec*C in a retrospective study covering the years 2006 to 2013 (14).

The aim of the present study was to determine possible changes in the clonal distribution of presumptive CA-MRSA in Slovenia. We performed a prospective study and compared the phenotypic and genotypic characteristics of presumptive CA-MRSA isolates collected from seven laboratories throughout Slovenia collected in 2014 and 2015 with isolates obtained from the same laboratories in 2010.

2 MATERIALS AND METHODS

2.1 Study Setting

The Centre of Medical Microbiology at the National Laboratory for Health, Environment and Food (NLZOH) covers seven laboratories throughout Slovenia. The same NLZOH laboratories that participated in the study in 2010 were invited to collect prospective MRSA isolates from routine diagnostics that fulfilled the screening phenotypic pattern for presumptive CA-MRSA during a two-year sampling period (1 January 2014 to 31 December 2014 and 1 January 2015 to 31 December 2015). Isolates were classified as presumptive CA-MRSA if they were resistant to oxacillin and cefoxitin and susceptible to at least two of the following four antibiotics: ciprofloxacin, erythromycin, clindamycin and gentamicin. Each MRSA isolate, with additional information (gender, sample material and date of sampling), was sent to the NLZOH Kranj for molecular analyses. Information was extracted from the laboratory information system (MBL, Src Infonet, Kranj). All isolates were identified by MALDI-TOF mass spectrometry (Bruker Daltonic GmBH, Bremen, Germany).

2.2 Antimicrobial Susceptibility Testing

The susceptibility patterns of the MRSA isolates were performed by the agar disk diffusion method according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (15, 16). The antibiotics tested were penicillin, cefoxitin, gentamicin, tobramycin, kanamycin, erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, rifampin, linezolid, mupirocin and fusidic acid (BD, Sparks, USA). Minimal inhibitory concentration (MIC) determination of oxacillin, cefoxitin and vancomycin was performed using the E-test (bioMerieux, Marcy-l'Etoile, France).

2.3 Molecular Characterisation

The presence of *mec*A or *mec*C and Panton-Valentine leukocidin (PVL) genes was tested by PCR using Genotype MRSA (Hain Lifescience GmbH, Nehren, Germany). Genes encoding for staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed* and *see*), toxic shock syndrome toxin (tst), locus enterotoxin gene cluster (*egc*) and staphylococcal exfoliative toxins (*eta*, *etb*, *etd*) were detected by multiplex PCR (17-19). SCC*mec* typing was performed by a multiplex PCR strategy previously described by Chen et al. and Petersdorf et al. (20, 21).

The DNA sequence-based methods, *spa* typing, is based on the number of tandem repeats and sequence variation in region X of the *S. aureus*-specific staphylococcal protein A gene. Amplification, sequencing and analysis of *spa* typing were performed according to a method described previously and analysed with Ridom SpaServer

(22). *Spa* clustering was performed using the spa-plugin in BioNumerics, and the results displayed with a minimum spanning tree. Multilocus sequence typing (MLST) is a highly discriminatory method of DNA sequence-based typing that relies on analysis of relatively conserved genes that encode essential proteins (seven housekeeping genes). MLST was performed for 19 MRSA isolates according to a method described by Enright et al. (23) and sequence types (ST) were determined with BioNumerics MLST-plugin (version 7.6; Applied Maths). All other STs were assigned based on *spa* types as published on http://spaserver.ridom.de and Kinross et al. (6).

2.4 Statistical Analyses

Data were analysed with the free online software MedCal'c (www.medcalc.org). Categorical variables were compared using the Chi-square test. A p-value ≤0.05 was considered as statistically significant.

3 RESULTS

3.1 Bacterial Isolates – Distribution Between Clinical and Screening Specimens

In 2010, 2014 and 2015, 92, 122 and 182 MRSA isolates had a positive susceptibility pattern of presumptive CA-MRSA, respectively. Of those, 335 MRSA isolates were cultivated from screening specimens and 61 from clinical specimens (year 2010, n=16, 17.4%; year 2014, n=20, 16.4%; year 2015, n=25, 13.7%) (Table 1).

3.2 Distribution of *Spa* Types among Presumptive CA-MRSA

Presumptive CA-MRSA strains isolated during 2014 were associated with 46 different *spa* types and two (1.6%) strains were non-typable. Strains from 2015 were associated with 71 different *spa* types and one (0.5%) strain was non-typable. Based on *spa* types were strains in 2014 and 2015 were assigned to 18 MLST STs (Table 2, Figure 1).

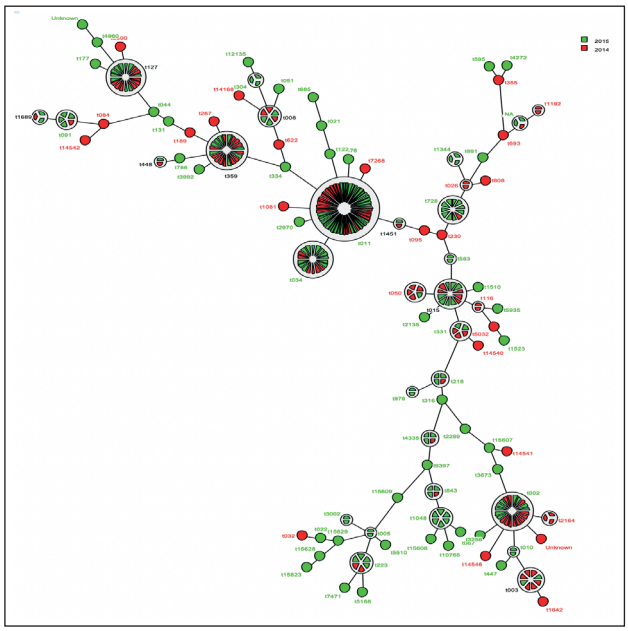

Figure 1. Distribution of CA-MRSA *spa* types circulating in Slovenia in 2014 and 2015.

Cluster analysis was performed using the *spa* typing plug-in tool of the BioNumerics program. Nodes indicate *spa* types and the number of sections within the node indicate number of strains for each *spa* type. Tree is color-coded according the year of isolation.

Table 1. Distribution of presumptive CA-MRSA isolates among clinical specimens.

Specimen	Results[a] for MRSA patients from		
	2010 (n=16)	2014 (n=20)	2015 (n=25)
Skin and soft tissue	12 (75)	13 (65)	13 (52)
Aspirate tracheae	1 (6.3)	4 (20)	4 (16)
Knee joint puncture	-	1 (5)	1 (4)
Urine	1 (6.3)	1 (5)	2 (8)
Nose swab	-	1 (5)	1 (4)
Blood culture	-	-	1 (4)
Bone	-	-	1 (4)
Faeces	-	-	1 (4)
Nasopharynx	-	-	1 (4)
Ear swab	2 (12.4)	-	-

[a] value is number (%)

Table 2. Most frequent *spa* types and sequence types of presumptive CA-MRSA isolates from year 2010, 2014 and 2015.

Rank	2010*			2014			2017		
	***spa* type**	**Frequency % (number)**	**MLST[a]**	***spa* type**	**Frequency % (number)**	**MLST[a]**	***spa* type**	**Frequency % (number)**	**MLST[a]**
1	t015	21.8 (20)	ST45	t011	17.1 (21)	ST398[b]	t011	17.0 (31)	ST398[b]
2	t011	13.0 (12)	ST398[b]	t359	9.8 (12)	ST97	t034	6.6 (12)	ST398[b]
3	t728	6.5 (6)	ST45	t002	8.1 (10)	ST5	t127	5.5 (10)	ST1
4	t091	6.5 (6)	ST7	t127	6.5 (8)	ST1	t728	5.5 (10)	ST45
5	t008	5.4 (5)	ST8	t034	4.9 (6)	ST398[b]	t002	4.9 (9)	ST5
6	t002	4.3 (4)	ST5	t003	4.9 (6)	ST5	t015	4.4 (8)	ST45
7	t005	4.3 (4)	ST22	t015	4.1 (5)	ST45	t359	3.8 (7)	ST97
8	t026	4.3 (4)	ST45	t050	3.3 (4)	ST45	t1048	3.3 (6)	ST130
9	t127	3.3 (3)	ST1	t008	2.4 (3)	ST8	t091	2.2 (4)	ST7
10	t020	2.2 (2)	ST22	t2164	2.4 (3)	ST5	t223	2.2 (4)	ST22
>10	t003, t006, t034, t041, t105, t108, t116, t174, t355, t595, t737, t791, t843, t846, t1079, t1094, t1111, t1179, t1218, t1231, t1509, t1911, t2032, t3824, t4365, t13070	28.4 (26)	ST1, ST5, ST7, ST8, ST22, ST30, ST45, ST72, ST88, ST130, ST152/377, ST225, ST228, ST398	t026, t032, t091, t095, t116, t189, t216, t223, t230, t267, t331, t355, t448, t622, t693, t728, t808, t843, t1081, t1094, t1192, t1340, t1451, t1689, t1842, t1943, t4335, t5032, t5500, t7268, t14168, t14540, t14541, t14542, t14546**	36.5 (45)	ST5, ST7, ST8, ST15, ST22, ST30, ST45, ST59, ST88, ST97, ST152/377, ST130, ST188, ST398, ST627, ST1774, ST3138	t003, t005, t008, t010, t021, t022, t044, t050, t051, t067, t122, t131, t177, t216, t304, t316, t331, t334, t447, t448, t583, t589, t595, t685, t786, t843, t976, t991, t1344, t1451, t1510, t1523, t1689, t1943, t2032, t2135, t2289, t2576, t2970, t3002, t3213, t3256, t3380, t3673, t3992, t4272, t4335, t4960, t5168, t5510, t9397, t10765, t12135, t15607**, t15608, t15609**, t15610**, t15628**, t15629**, t15773**, t15823**	44.6 (81)	ST1, ST5, ST8, ST22, ST30, ST45, ST59, ST80, ST88, ST97, ST152/377, ST130, ST188, ST247, ST398, ST913, ST3138

Legend: *Previously published (9); **New *spa* type; [a]Associated multilocus sequence types published on http://spaserver.ridom.de; [b]Previously published (15). Underlined *spa* type multilocus sequence types are those determined in this study.

The distribution of the most prevalent STs varied in different geographic regions (Figure 2). In 2014 12 *spa* types were determined among clinical specimens (t002, t008, t011, t015, t034, t050, t091, t223, t267, t359, t1842, t2164) while in 2015 20 *spa* types were determined (t002, t005, t008, t010, t011, t015, t034, t051, t122, t127, t131, t359, t1048, t1344, t2576, t3213, t4335, t15608, t15610, t15773).

The first major clone, ST45, detected in 2010 (n=35, 38%) decreased significantly overall to 15.5% (n=19) in 2014 (p-value=0.0003) and to 15.9% (n=29) in 2015 (p-value=0.0001).

The second major clone confirmed in 2010 and associated with LA-MRSA, ST398 (*spa* types t011, t034, t1344, t1451, t2576, t2970), increased significantly (p-value <0.05) from 15.2% (n=14) in 2010 to 27.5% (n=50) in 2015 (Figure 3). The association of these LA-MRSA with infections increased, as in 2010 it was reported for only one isolate from skin and soft tissue infection, while in 2015 LA-MRSA were associated with skin and soft tissue infection (n=3) and lower respiratory tract infection (n=1).

*mec*C positive MRSA, ST130 and ST3138 increased significantly (p-value <0.05) from 1.1% (n=1) in 2010 to 8.2% (n=15) in 2015 (Figure 3). In 2010 *mec*C was isolated from skin/mucosal (screening) swabs for MRSA colonization, in 2015 *mec*C MRSA were recovered from sputum (n=1) and blood culture (n=1), and all the remaining strains were recovered from skin/mucosal (screening) swabs for MRSA colonization.

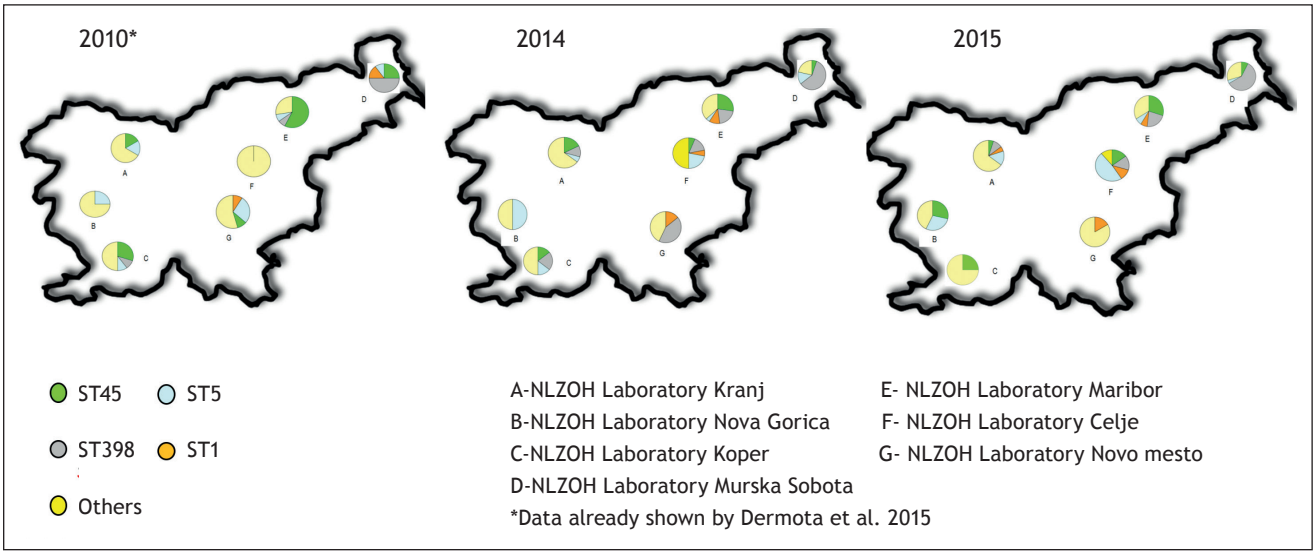

Figure 2. The distribution of presumptive CA-MRSA clones circulating in Slovenia for 2010, 2014 and 2015.

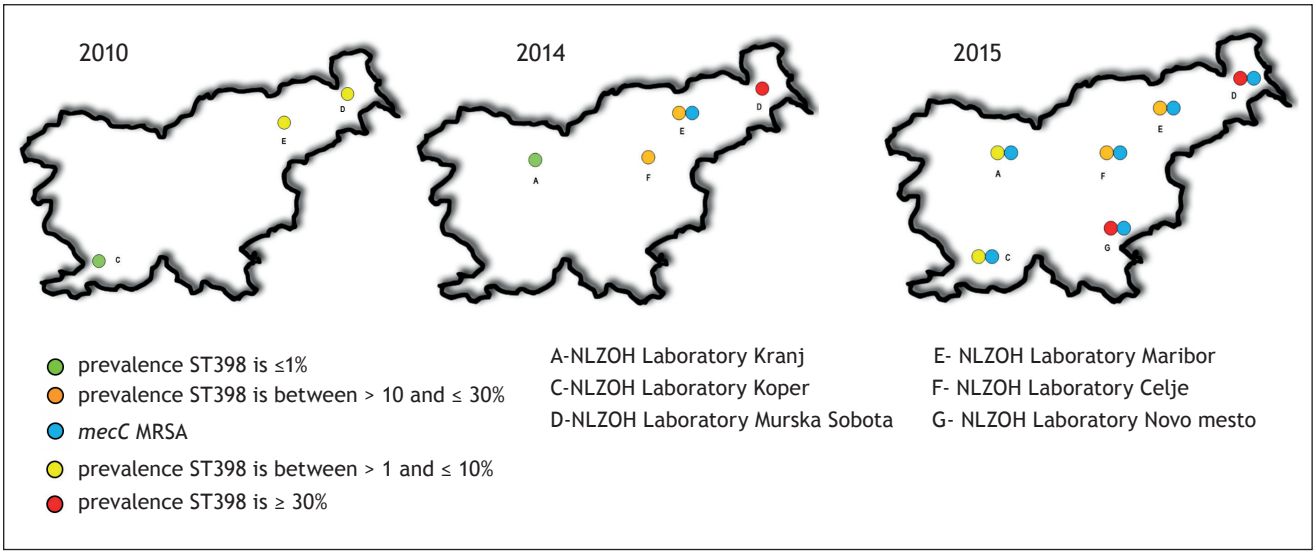

Figure 3. The distribution and prevalence of ST398 clone and *mec*C MRSA in Slovenia for 2010, 2014 and 2015.

Table 3. Frequencies of virulence associated genes and *spa* types in presumptive CA-MRSA isolates from 2010, 2014 and 2015.

Rank	2010*			2014			2017		
	Number of isolates	Frequency %	*spa* type (number)	Number of isolates	Frequency %	*spa* type (number)	Number of isolates	Frequency %	*spa* type (number)
sea	6	6.5	t008 (4), t041 (1), t2032 (1)	3	2.5	t008 (2), t003 (1)	15	8.2	t002 (5), t021 (1), t122 (1), t127 (1), t304 (3), t976 (2), t4335 (1), t12135 (1)
seb	2	2.2	t002 (1), t026 (1)	2	1.6	t216 (1), t1340 (1)	6	3.3	t216 (3), t976 (2), t2289 (1)
sec	33	35.8	t002 (1), t015 (18), t026 (3), t116 (1), t728 (5), t737 (1), t791 (1), t1079 (1), t1231 (1), t13070 (1)	13	10.7	t015 (2), t026 (1), t050 (1), t095 (1), t116 (2), t230 (1), t331 (3), t622 (1), t14540 (1)	41	22.5	t002 (4), t011 (1), t015 (6), t050 (1), t223 (2), t331 (2), t359 (1), t448 (1), t583 (2), t589 (1), t728 (10), t786 (1), t1048 (2), t1510 (1), t1523 (1), t3213 (1), t4335 (1), t5168 (1), t15628 (1), t15773 (1)
sed	14	15.2	t002 (2), t003 (1), t008 (4), t015 (2), t020 (1), t846 (1), t1094 (1), t1179 (1), t2032 (1)	18	14.7	t002 (9), t003 (3), t015 (1), t026 (1), t1192 (1), t2164 (2), t14541 (1)	17	9.3	t002 (4), t003 (2), t008 (2), t010 (2), t011 (1), t050 (1), t067 (1), t359 (1), t447 (1), t2032 (1), t15607 (1)
see	none	0	-	none	0	-	none	0	-
Locus *egc*	50	54.3	t002 (4), t003 (1), t005 (3), t006 (1), t015 (20), t020 (1), t026 (4), t041 (1), t105 (1), t116 (1), t728 (5), t737 (1), t791 (1), t1079 (1), t1094 (1), t1111 (1), t1231 (1), t3824 (1), t13070 (1)	53	43.4	t002 (10), t003 (6), t015 (5), t026 (2), t032 (1), t050 (4), t095 (1), t116 (2), t223 (2), t230 (1), t331 (3), t359 (2), t448 (1), t622 (1), t728 (1), t808 (1), t1081 (1), t1192 (2), t1340 (1), t1842 (1), t2164 (2), t5032 (1), t14540 (1), t14541 (1)	74	40.7	t002 (9), t003 (2), t005 (2), t010 (1), t011 (3), t015 (7), t021 (1), t022 (1), t050 (1), t067 (1), t091 (1), t122 (1), t127 (1), t216 (1), t223 (4), t331 (2), t359 (1), t447 (1), t583 (2), t589 (1), t685 (1), t728 (10), t1048 (1), t1510 (1), t1523 (1), t2135 (1), t2289 (1), t3002 (2), t3213 (1), t3673 (1), t4335 (1), t5168 (1), t5510 (1), t15607 (1), t15608 (1), t15609 (1), t15610 (1), t15628 (1), t15629 (1), t15773 (1), t15823 (1)
PVL	8	8.7	t002 (2), t005 (1), t008 (1), t091 (1), t355 (1), t791 (1), t4335 (1)	13	10.7	t002 (9), t050 (1), t127 (1), t2164 (1), t355 (1)	10	5.5	t002 (4), t010 (1), t044 (1), t067 (1), t127 (1), t131 (1), t595 (1)
tst	8	8.7	t026 (1), t105 (1), t728 (5), t1111 (1)	2	1.6	t015 (1), t728 (1)	23	12.6	t002 (4), t015 (1), t122 (1), t223 (3), t359 (1), t685 (1), t728 (10), t4335 (1), t15610 (1)
eta	none	-	-	1	0.8	t015 (1)	1	0.5	t991 (1)
etb	none	-	-	none	-	-	none	-	-
etd	none	-	-	none	-	-	3	1.6	t044 (1), t131 (1), t991 (1)

Legend: *Data already shown by Dermota et al. 2015; *sea* staphylococcal enterotoxin gene type A, *seb* staphylococcal enterotoxin gene type B, *sec* staphylococcal enterotoxin gene type C, *sed* staphylococcal enterotoxin gene type D, *see* staphylococcal enterotoxin gene type E, locus *egc* locus enterotoxin gene cluster, PVL Panton-Valentine leukocidin, *tst* toxic shock syndrome toxin gene, *eta* exfoliative toxin type A, *etb* exfoliative toxin type B, *etd* exfoliative toxin type D

3.3 Virulence Factors Among All Presumptive CA-MRSA

Two out of 11 tested virulence genes were not detected in the studied strain collection (*see*, *etb*). Enterotoxin genes (*sec*, *sed* and *egc*) were commonly present, while exfoliative toxins were rarely detected (Table 3). PVL was more common in 2014 (n=13, 10.7%) than in 2015 (n=10, 5.5%) and 2010 (n=8, 8.7%), but this result was not statistically significant (Table 4).

Associations of PVL positive MRSA with infections were reported in all study years. In 2010 PVL were detected in five presumptive CA-MRSA strains among 16 clinical specimens. All PVL positive strains were isolated from skin and soft tissue infections (n=5, 31.2%) and belonged to *spa* types t002 (n=1), t008 (n=1), t355 (n=1), t791 (n=1) and t4365 (n=1). Among 20 clinical specimens, six PVL positive presumptive CA-MRSA strains were isolated in 2014 from skin and soft tissue infections, belonging to spa types t002 (n=4), t050 (n=1) and t2164 (n=1). In 2015 four PVL positive out of 25 presumptive CA-MRSA strains were isolated from skin and soft tissue infections (n=3, 12%) and faeces (n=1, 4%). These PVL positive strains belonged to *spa* types t002 (n=1), t010 (n=1), t127 (n=1) and t131 (n=1).

3.4 SCC*mec* Types Among All Presumptive CA-MRSA

The most frequently confirmed type was SCC*mec* IV (2014, n=62, 50.1%; 2015, n=88, 48.4%) and SCC*mec* V (2014, n=42, 34.4%; 2015, n=66, 36.3%). SCC*mec* I was detected in 1.6% (n=2) isolates in 2014, and in 1.6% (n=3) in 2015, while SCC*mec* II was detected in 5.7% (n=7) isolates in 2014, and in 1.1% (n=2) in 2015. 5.7% (n=7) isolates in 2014 and 3.2% (n=6) isolates in 2015 were non-typable. SCC*mec* XI was detected in 1.6% (n=2) isolates in 2014, and in 8.2% (n=15) in 2015.

4 DISCUSSION

Several changes were observed in the clonal distribution and virulence properties of presumptive CA-MRSA strains during the years 2014 and 2015 in comparison to 2010 (13). Similar to as in 2010, clones ST5, ST45 and ST398 were most common during 2014 and 2015 study, but with different rankings.

In 2014 and 2015, the first major clone (2014, 28 strains, 22.9%; 2015, 50 strains, 27.5%) was related to a pig-associated clone, ST398, while in 2010 ST398 was found in 15.2% (n=14) and was the second most common clone. In 2010, most strains belonging to ST398 were predominantly found in rural areas of north eastern and southern Slovenia (regions D and E), where livestock breeding is an important agricultural activity. In 2014 and 2015, the density of ST398 was also confirmed in north eastern and southern Slovenia (regions D and E), but ST398 was also detected in non-agricultural regions, namely A, C, F and G (Figure 3). Since LA-MRSA has spread throughout Europe, the number of colonized people and infections is increasing (4, 7). The main reservoirs of LA-MRSA ST398 are pigs, poultry, cattle, and companion animals, and close contact with animals is a risk factor for LA-MRSA carriage (4, 7).

Table 4. Virulence factors of Slovenian presumptive CA-MRSA isolates from 2010, 2014 and 2015.

Virulence factor	2010* (n=92)	2014 (n=122)	2015 (n=182)	p-value (Chi-square test)	
	% (number) of presumptive CA-MRSA isolates			Year 2014 vs. 2010	Year 2015 vs. 2010
sea	6.5 (6)	2.5 (3)	8.2 (15)	0.15	0.61
seb	2.2 (2)	1.6 (2)	3.3 (6)	0.77	0.60
sec	35.8 (33)	10.7 (13)	22.5 (41)	< 0.0001	0.01
sed	15.2 (14)	14.7 (18)	9.3 (17)	0.92	0.15
see	0	0	0	-	-
Locus *egc*	54.3 (50)	43.4 (53)	40.7 (74)	0.11	0.03
PVL	8.7 (8)	10.7 (13)	5.5 (10)	0.63	0.31
tst	8.7 (8)	1.6 (2)	12.6 (23)	0.02	0.33
eta	0	0.8 (1)	0.5 (1)	0.61	0.79
etb	0	0	0	-	-
etd	0	0	1.6 (3)	-	0.39

Legend: *Data already shown by Dermota et al. 2015; *sea* staphylococcal enterotoxin gene type A, *seb* staphylococcal enterotoxin gene type B, *sec* staphylococcal enterotoxin gene type C, *sed* staphylococcal enterotoxin gene type D, *see* staphylococcal enterotoxin gene type E, locus *egc* locus enterotoxin gene cluster, PVL Panton-Valentine leukocidin, *tst* toxic shock syndrome toxin gene, *eta* exfoliative toxin type A, *etb* exfoliative toxin type B, *etd* exfoliative toxin type D

In recent years, LA-MRSA carriage was also reported in humans without any animal contact (7-9). MRSA ST398 has also been introduced into the health care setting, mainly in areas with a high density of livestock farming (5).

The second major clone (2010, 9 strains, 9.8%; 2014, 21 strains, 17.2%; 2015, 17 strains, 9.3%) was related to the ST5. Despite the difference in percentages, no significance in ST5 distribution was observed.

The third major clone (2010, 35 strains, 38.0%; 2014, 19 strains, 15.5%; 2015, 29 isolates, 15.9%) was related to the ST45. *Spa* type t015, SCC*mec* type IV decreased from 17.4% (n=16) in 2010 to 3.3% (n=4) in 2014 and 2.7% (n=5) in 2015. *Spa* type t728, SCC*mec* type IV also decreased from 6.5% (n=6) in 2010 to 0.8% (n=1) in 2014 and 5.5% (n=10) in 2015. Isolates with *spa* type t728 were found in 2010 in only one region in Slovenia, E, but in 2015 they were also found in two other regions, A and D.

European clone ST80 and Balkan clone ST152/377, which are circulating in Europe and our neighbouring countries (Italy, Austria, Croatia), are rare in Slovenia (24, 25). In 2010, PVL gene was detected in eight (8.7%) strains that belonged to ST5, ST7, ST8, ST22, ST72, ST88, ST152/377 and ST772. In 2014 and 2015, 23 (7.6%) PVL positive strains were associated with ST1, ST5, ST22, ST80 and ST152/377. PVL positive ST5 (t002) significantly increased from 1.3% (n=2) in 2010 to 7.4% (n=9) in 2014 (p-value=0.0466). PVL positive MRSA strains were also found in 2014 and 2015 in 10 clinical samples (nine samples from wounds, one from faeces), but in Slovenia PVL positive clones are surprisingly rarely distributed (25).

*mec*C positive MRSA strains were found in 1.1% in 2010 (14), while in 2014 and 2015 we observed an increase to 1.6% and 8.2%, respectively. *mec*C positive MRSA, associated with ST130 and ST3138, were observed in 2014 in the rural area of Southern Slovenia (region E), but in 2015 *mec*C MRSA were distributed throughout Slovenia, also in non-rural regions A, C, G and F (Figure 3). Most *mec*C positive MRSA were detected in asymptomatic carriers; in two patients, *mec*C were detected from clinical specimens (sputum, blood culture) (*spa* type t1048, t15608). In the literature, *mec*C MRSA have mainly been reported in humans from screening specimens, as well as from clinical specimens, and the range of infections caused by *mec*C-carrying MRSA is the same as seen in other *S. aureus*, including life-threatening diseases such as bacteraemia (7, 10, 26).

Our study has the following limitations. The first is that the definition of presumptive CA-MRSA is based on the susceptibility pattern. As we have written in the manuscript, in Slovenia no systematic surveillance of MRSA isolates is performed. To the best of our knowledge the HA-MRSA strains in our country are resistant to beta-lactam antibiotics, fluoroquinolones, macrolides and lincosamides (27). Because of the emerging CA-MRSA in Europe, in 2006 a definition for presumptive CA-MRSA as a screening tool, the same as in other countries, was set based on the susceptibility pattern. To compare the data from 2014 and 2015 with that from 2010, the same definition for presumptive CA-MRSA was used. The authors are aware, however, that our definition is not reliable without epidemiological data, as in 2010, due to incomplete epidemiological data of health care risk factors. The authors are also aware that our definition based on susceptibility pattern does not cover all CA-MRSA strains (e.g. more resistant CA-MRSA) and also includes more sensitive HA-MRSA strains. Another limitation of our study is that most isolates were recovered from screening specimens at mucocutaneous site.

In this work we confirmed the changes in clonal distribution of presumptive CA-MRSA in Slovenia. The most frequent sequence type during the 2014/2015 study period was ST398, a pig-associated clone, which is mostly distributed among asymptomatic carriers. While previously ST398 and *mec*C positive strains were geographically limited, they have spread throughout the country since 2010.

Future surveillance studies of molecular epidemiology with improved molecular methods and whole genome sequencing (WGS) of systematically collected MRSA are very important in Slovenia in order to monitor changes in clonal distribution.

CONFLICT OF INTEREST

The authors declare that no conflicts of interest exist in relation to this research.

FUNDING

The study was financed by internal funding sources.

ETHICAL APPROVAL

Not required.

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INTRODUCING THE EARLY TRAUMA INVENTORY SELF REPORT - SHORT FORM AND ITS QUALITATIVE AND QUANTITATIVE VALIDATION FOR THE SLOVENIAN GENERAL POPULATION

PREDSTAVITEV KVALITATIVNE IN KVANTITATIVNE VALIDACIJE SLOVENSKE RAZLIČICE KRATKE OBLIKE VPRAŠALNIKA ZA SAMOOCENO TRAVM IZ OTROŠTVA

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Received: Jul 7, 2020
Accepted: Sep 16, 2020

Original scientific article

ABSTRACT

Introduction: Traumatic experience in childhood or adolescence has a significant impact on the development of chronic mental and physical conditions in adulthood. Thus, it is very important for health professionals, especially primary care physicians to have an inventory in order to detect early trauma for planning appropriate treatment, such as the Early Trauma Inventory (ETI). The aim of this paper is to test the psychometric properties of the Slovenian translation of the short, self-rated version (ETISR-SF), and to further validate the instrument.

Methods: The research was done in two parts – qualitative and quantitative. In the qualitative part, a questionnaire was translated and culturally adapted using the Delphi method. For the quantitative part, 51 patients with substance use disorders hospitalized at the Centre for the Treatment of Drug Addictions were recruited, along with 133 controls. The psychometric properties of the questionnaire were checked. Internal consistency was calculated using Cronbach's alpha, test-retest reliability was examined graphically using a Bland-Altman plot. Discriminant validity between groups was gauged using the independent samples t-test.

Results: Consensus in the Delphi study was reached in the second round. Cronbach's alpha varied between 0.60 - 0.85. Of the four domains, physical abuse had the lowest Cronbach's alpha. The test-retest reliability is high for all domains, with correlation coefficients ranging from 0.82 to 0.96. The non-clinical sample differed significantly from the clinical sample.

Conclusion: The Slovenian translation of ETISR-SF is a satisfactory instrument for the evaluation of trauma before the age of 18.

Keywords: childhood trauma, Early Trauma Inventory, psychometric properties, validation studies, addictions

IZVLEČEK

Uvod: *Po poročilu Nacionalnega inštituta za javno zdravje smo v Sloveniji lani prvič ugotavljali razširjenost obremenjujočih izkušenj v otroštvu. Večina anketiranih (76 %) je v otroštvu doživela vsaj eno, dobra četrtina (27 %) pa štiri ali več obremenjujočih izkušenj. Travmatične izkušnje v otroštvu ali mladostništvu pomembno vplivajo na razvoj kroničnih duševnih in telesnih motenj v odrasli dobi in predstavljajo pomemben javnozdravstveni problem povsod po svetu. V Sloveniji še nimamo veljavnega in zanesljivega vprašalnika za presejanje, s katerim bi pri odraslih lahko ugotavljali zgodnje travmatične izkušnje. Vprašalnik za samooceno travm iz otroštva – kratka oblika (The Early Trauma Inventory Self Report – Short Form ETISR-SF) je eden takih vprašalnikov, ki omogoča hiter in zanesljiv pregled potencialnih travmatičnih dogodkov v otroštvu in mladostništvu. Z njim lahko hitro dobimo izhodišče za bolj poglobljeno raziskovanje v klinično-terapevtski praksi. Namen prispevka je predstaviti preverjanje psihometričnih lastnosti slovenskega prevoda kratke samoocenjevalne različice ETISR-SF in dodatno potrditi veljavnost vprašalnika.*

Metode: *Raziskava je bila izvedena v dveh delih. V kvalitativnem delu je bil vprašalnik preveden in kulturološko prilagojen s pomočjo študije delphi. Od povabljenih 51 specializantov psihiatrije se jih je v študijo delphi vključilo 8. V kvantitativnem delu so bile preverjene psihometrične lastnosti vprašalnika v klinični in neklinični populaciji. Vključenih je bilo 51 pacientov z motnjo odvisnosti od psihoaktivnih snovi, hospitaliziranih na Centru za zdravljenje odvisnih od prepovedanih drog, in 133 oseb v kontrolni skupini. Notranja konsistentnost je bila izračunana z uporabo koeficienta alfa Cronbach, zanesljivost preizkusnega testiranja je bila grafično preučena s pomočjo ploskve Blanda Altmana. Diskriminantna veljavnost med skupinami je bila ocenjena s t-testom za neodvisne vzorce.*

Rezultati: *V kvalitativnem delu raziskave je bilo soglasje s prevodom doseženo po dveh krogih delphi z nekaj manjšimi jezikovnimi popravki. V kvantitativnem delu raziskave se je 56 (42 %) oseb iz kontrolne skupine odzvalo na ponovno izpolnjevanje vprašalnika čez 14 dni. Zanesljivost preizkusa ponovnega testiranja je visoka za vsa področja s korelacijskimi koeficienti od 0,82 do 0,96. Koeficient Cronbach alfa se je gibal med 0,60 in 0,85. Del o fizični zlorabi je pokazal nižjo vrednost Cronbach alfe kot ostali deli (splošna travma, psihična in spolna zloraba). Zanesljivost dela o čustveni in spolni zlorabi je visoka (a = 0,85 in a = 0,82). Neklinični vzorec se je statistično značilno razlikoval od kliničnega vzorca.*

Zaključek: *Slovenski prevod ETISR-SF je zadovoljiv instrument za hitro oceno travmatskih izkušenj do 18. leta starosti.*

Ključne besede: *travmatske izkušnje v otroštvu, Vprašalnik za samooceno travm iz otroštva, psihometrične lastnosti, validacijske študije, odvisnosti*

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1 INTRODUCTION

According to the Diagnostic and Statistical Manual of Mental Disorders V, childhood trauma is defined as exposure to actual or threatened death, serious injury, or sexual violence (1). It has a significant impact on the development of chronic mental and physical conditions in adulthood, which results in increased use of medical services, especially emergency units (2-4). Childhood trauma is also associated with impaired psycho-social functioning and lower quality of life (2). Experiencing childhood trauma compromises both neural structure and function, rendering individuals susceptible to later cognitive deficits and psychiatric illnesses, such as schizophrenia, depression, bipolar disorder, anxiety, posttraumatic stress disorder (PTSD), substance abuse, and even suicide, in adolescence, young adulthood or later life (5, 6). Somatoform symptoms, which may relate to other psychological consequences of trauma, such as depression, anxiety, dissociation, and PTSD, are also linked to traumatic exposure (7). Stress is associated with a multiple immune and endocrine changes (8). Exposure to multiple and chronic stressors represents a particular risk for asthma, environmental sensitivity in the form of allergies to medications, cardiovascular health, gastrointestinal problems and migraine headaches (2, 4, 8, 9). The more severe the abuse is, the stronger is the association with poor outcomes in adulthood (3). Psychosomatic disorders, which in family practice are also called medically unexplained symptoms (MUS), are frequently associated with a history of traumatization (10). In a Slovenian family medicine practice it was found that 8.6% of patients had MUS attendees (11). A recent study by the Slovenian National Institute of Public Health showed that 76% of participants reported at least one and 27% four or more traumatic experiences in childhood (12). These are important findings for both psychiatrists and primary care physicians (9). To better understand patients' symptoms, it is important for health professionals to have a structured and comprehensive tool for measuring traumatic events in childhood (13). One of these is the Early Trauma Inventory (ETI) (14), created by Bremner et al. as a comprehensive expert-rated interview. A self-rated version (ETI-SR) was later developed and briefer self-rated short form (ETISR-SF) was made after a psychometric analysis identified redundant items (15). The ETISR-SF has been proven to be a valid instrument for retrospective self-assessment of childhood trauma in diverse populations (subjects with substance use disorders, war veterans, depressive patients and puerperae) (15-19), and has good test-retest reliability (17-19). It consists of 27 items in the four domains of physical, emotional, sexual abuse, and general trauma (15). It categorically assesses the existence of these events before the age of 18 (15).

The ETISR-SF has been translated with preserved psychometric properties to several cultural contexts and languages, including: Spanish (17), Korean (18), Brazilian Portuguese (19), Dutch (20) and Swedish (21). However, to the best of our knowledge, it has not yet been translated or psychometrically tested with regard to Slovenian. The benefits of this instrument lay in early, quick and economical screening for traumatic experiences, which could enable health care professionals to prevent more serious health and social consequences and also spare the system some costs due to unnecessary and often invasive diagnostic procedures. This represents a major advantage for the public health system, and gives a starting point for more in-depth exploration in clinical-therapeutic practice and research work.

2 METHODS

2.1 Aims

The aims of this study were to obtain semantic, cultural and conceptual translation and equivalence of the ETISR-SF questionnaire in Slovenian, examine the psychometric properties and further validate the instrument.

2.2 Instruments

2.2.1 Socio-Demographic Characteristics Questionnaire

The participants answered demographic questions assessing gender (male/female), age (years), marital status (single/married/in relationship/separated/widowed), children (yes/no), number of children, with whom currently living (alone/with parents/with child/with partner/with partner and child/with friends/homeless/other), level of education (several options were offered), employment status (several options were offered). The in-patients group gave additional data: participation in drug substitution programme, history of overdose, self-injury, attempted suicide and age of drug use.

2.2.2 The Early Trauma Inventory Self Report – Short Form

The ETISR-SF is a 27-item questionnaire, used for the assessment of physical, emotional, and sexual abuse, as well as general traumatic experience that may have occurred before the age 18, and it can be self-administered in about 15 min. Each of the items is answered 'yes' (coded as 1) or 'no' (coded as 0). There are an additional three items, which are at the end of questionnaire. One of these asks the subjects to choose one event that had the greatest impact on his/her life, and other two items measure the subsequent reactions, i.e. fear or depersonalization.

2.3 Design and Participants of the Study

Validation of the ETISR-SF questionnaire was done in two parts of the study, using qualitative and quantitative methods.

2.3.1 Qualitative Part of the Study

The ETISR-SF was translated from its original English version with permission from J. D. Bremner. It was first translated from English to Slovenian by two doctoral students, employed at the University Psychiatric Hospital Ljubljana, with fluent knowledge of English. In order to reconcile the equivalence of the translated versions of the inventory, these were compared and discussed by a team of six health care experts from the Centre for the Treatment of Drug Addiction (three psychiatrists, two psychologists and social worker), with all having good knowledge of English and Slovenian, resulting in few minor corrections. A sample of 51 residents of psychiatry at the University Psychiatric Hospital Ljubljana were invited via email to participate in the Delphi method to achieve consensus. All participants were provided with a written explanation of the aims and procedure of the study. Among those 51 invited experts, eight took part in the study. Each participant was asked to validate or reject a translation by rating each statement on a scale from 1 to 5, where 1 meant "strongly disagree" and 5 "totally agree". If they rated a translation with 3 or less they were asked to explain their disagreement and possibly propose more a suitable translation. The principal researcher evaluated the answers. Successful validation for each statement was obtained when at least 75% of the participants rated it 4 or above. If a statement did not meet this criterion, the principal researcher proposed a new translation, taking into account the participants' suggestions, which was then again sent to the group. Consensus was reached in the second round. Two independent English translators undertook back-translation. The back-translated inventory was very similar to the original version, and was confirmed by the author.

2.3.2 Quantitative Part of the Study

In the second part, ETISR-SF questionnaires were distributed to non-clinical and clinical populations. The non-clinical population was recruited among employees at the University Psychiatric Hospital Ljubljana, in order to examine the test-retest reliability. Questionnaires were distributed to 160 participants and were anonymous. In the first round 133 were returned, and after 14 days a further 56 questionnaires. Questionnaires were also completed by 51 in-patients at the Centre for the Treatment of Drug Addiction. Patients signed Consent Forms in which they were given all the related information about the study.

As the items were dichotomous, the means and standard deviations per item were calculated with means indicating the percentage of respondents who had experienced a particular traumatic event. Total score and the scores for each domain were obtained by counting the number of endorsed items (15). Discriminative validity was assessed by comparison of the following groups with regard to the ETISR-SF numerical score: patients with a history of drug abuse and healthy subjects (controls), and subjects experiencing severe fear, horror or out-of-body experiences at the time of traumatic the event, and subjects without such feelings. The independent samples t-test was used. Test-retest reliability was examined graphically by means of a Bland-Altman plot and the correlation between the two measurements was calculated. Cronbach's coefficient alpha was calculated as a measure of internal consistency of the questionnaire. Item-to-total correlation and Cronbach's alpha minus item reliability was calculated to identify items that influence the poorer validity and internal consistency of a particular subscale. P values<0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 26.

3 RESULTS

3.1 Qualitative Part of the Study

The first Delphi round for the four parts of ETISR-SF plus three additional questions at the end of the inventory showed acceptable agreement in most of the statements. The first part of the inventory, on general trauma, showed agreement in all except two statements (Q5, Q11). Q5 was rated as adequate by only 1/8 (12.5%) of participants and Q11 by 3/8 (37.5%) participants (Table 1).

Table 1. ETISR-SF part one – General trauma scale Likert scores, mean and median – Round 1 (N=8).

Results	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
>3 (n/8)	8	7	8	8	1	8	8	6	6	7	3
>3 (%)	100	87.5	100	100	12.5	100	100	75	75	87.5	37.5
Mean	4.25	4	4.37	4.37	2.875	4.25	4.25	4	4	4	3
Median	4	4	4	4	3	4	4	4	4	4	3

Legend: n – number of participants; Q – question

The, second part of the inventory, on physical abuse, showed agreement in all except one statement (Q4). Q4 was rated as adequate by just 1/8 (12.5%) of the participants (Table 2).

Table 2. ETISR-SF part two – Physical abuse scale Likert scores, mean and median – Round 1 (N=8).

Results	Q1	Q2	Q3	Q4	Q5
>3 (n/8)	8	7	6	1	6
>3 (%)	100	87.5	75	12.5	75
Mean	4.625	4.25	3	2.875	4.25
Median	5	4	3	3	4.5

Legend: n – number of participants; Q – question

The third part of the inventory, on emotional abuse, showed agreement in all except two statements (Q2, Q3). Q2 was rated as adequate by 2/8 (25%) of participants, and Q3 by only 1/8 (12.5%) of the participants (Table 3).

Table 3. ETISR-SF part three – Emotional abuse scale Likert scores, mean and median – Round 1 (N=8).

Results	Q1	Q2	Q3	Q4	Q5
>3 (n/8)	8	2	1	5	8
>3 (%)	100	25	12.5	62.5	100
Mean	4.25	3	2.75	3.75	4.625
Median	4	3	3	4	5

Legend: n – number of participants; Q – question.

The fourth part of the inventory, on sexual abuse, showed agreement in all except one statement (Q5). Q5 was rated as adequate by 2/8 (25%) of participants (Table 4).

Table 4. ETISR-SF part four – Sexual abuse scale Likert scores, mean and median – Round 1 (N=8).

Results	Q1	Q2	Q3	Q4	Q5	Q6
>3 (n/8)	5	8	8	8	2	7
>3 (%)	62.5	100	100	100	25	87.5
Mean	3.875	4.25	4.37	4.5	2.875	4.25
Median	4	4	4	4.5	2.5	4

Legend: n – number of participants; Q – question.

Three additional questions at the end of the inventory showed agreement in all statements (Table 5).

Table 5. ETISR-SF additional three items scale Likert scores, mean and median – Round 1 (N=8).

Results	Q1	Q2	Q3
>3 (n/8)	7	7	7
>3 (%)	87.5	87.5	87.5
Mean	4.125	4.125	4.25
Median	4	4	4.5

Legend: n – number of participants; Q – question.

In second Delphi round, concerning Q5 and Q11 for the part on general trauma, Q4 for the part on physical abuse, Q3 for the part on emotional abuse and Q5 for the part on sexual abuse, the participants proposed few alternative translations, which were discussed by the team from the Centre for the Treatment of Drug Addiction and then sent back to the participants for valuation. Consensus was thus reached in the second round (Table 6).

3.2 Quantitative Part of the Study - Study Subjects

Overall, 184 subjects agreed to participate in this research, 51 (27.7%) patients with substance use disorders and 133 (72.3%) healthy subjects (controls). Ninety-seven (52.7%) of the participants were female. The mean (SD) age of the participants was 37.8 (9.2) years. More than half (54.3%) had less than university education. The majority of participants (75%) were employed. Twenty-nine (56.9%) participants with substance use disorders were included in an opioid substitution programme, 21 (41.2%) reported the experience of overdose, 11 (21.6%) self-injury and 14 (27.4%) attempted suicide. The mean age (SD) of the patients with substance abuse disorders when trying their first drug was 15.1 (3) years.

Table 6. Mean and median: ETISR-SF – general trauma Q5, Q11, physical abuse Q4, emotional abuse Q3 and sexual abuse Q5 – Round 2 (N=8).

Results	GT - Q5	GT - Q11	PA - Q4	EA - Q3	SA - Q5
Mean	4.25	4.5	4	4	4.625
Median	4	4.5	4	4	5

Legend: GT – general trauma; PA - physical abuse; EA - emotional abuse; SA - sexual abuse; Q – question

Table 7. Participants' characteristics.

	n=184 (%)
Sex	
Female	97 (52.7)
Male	87 (47.3)
Mean age (SD) in years	37.8 (9.2)
Education	
Elementary school	27 (14.7)
Vocational school	22 (12)
High school	51 (27.7)
University or more	84 (45.7)
Working status	
Unemployed	40 (21.7)
Employed	138 (75)
Student	5 (2.7)
Retired	1 (0.5)
Group	
Controls	133 (72.3)
Patients	51 (27.7)
Drug substitution program	29 (56.9)
Overdosed	21 (41.2)
Self-injury	11 (21.6)
Suicide attempt	14 (27.4)
Mean age (SD) of 1st drug use	15.1 (3)

3.3 Quantitative Part of the Study - Validity

The validity of ETISR-SF was measured by its ability to distinguish between patients with known drug abuse and healthy controls. It was also assessed by comparing participants reporting experiencing severe fear, horror, frustration or having out-of-body experiences when experiencing any traumatic event described in the ETISR-SF and participants without such feelings.

The results of the comparison in all ETISR-SF subscales' scores between patients and healthy controls are summarized in Table 8. The controls had statistically significantly lower scores on all ETI domains.

Table 8. Mean ETI scores in healthy subjects (controls) and patients and results of an independent samples t-test.

Subscale (range)	Controls (n=133)	Patients (n=51)	t value	P
General trauma (0 - 11)	2.2 (1.9)	3.7 (2.2)	-4.66	< 0.001
Physical abuse (0 - 5)	2.2 (1.4)	2.8 (1.3)	-2.88	0.005
Emotional abuse (0 - 5)	1.1 (1.6)	2.6 (1.9)	-5.09	< 0.001
Sexual abuse (0 - 6)	0.4 (0.8)	1.1 (1.9)	-2.6	0.012

The results of the comparison in all ETISR-SF domains' scores between subjects that experienced severe fear, horror, out-of-body experiences during the traumatic event and those without such feelings are summarized in Table 9. Those without such feelings had statistically significantly lower scores on all ETI domains.

Table 9. Mean ETI scores in subjects experiencing severe fear or having out-of-body experiences and subjects without such feelings and the results of the independent samples t-test.

Subscale (range)	No severe fear or out of body experience (n=95)	Severe fear or out of body experience (n=89)	t value	P
General trauma (0 - 11)	1.7 (1.5)	3.7 (2.2)	-7.04	<0.001
Physical abuse (0 - 5)	2 (1.3)	2.8 (1.3)	-3.84	<0.001
Emotional abuse (0 - 5)	0.6 (1.2)	2.5 (1.8)	-8.49	<0.001
Sexual abuse (0 - 6)	0.2 (0.5)	1 (1.6)	-4.19	<0.001

3.4 Quantitative Part of the Study - Reliability

The internal consistency of the domains, as measured by Cronbach's alpha, is above Nunnally's proposed threshold of 0.7 (22) for the emotional abuse and sexual abuse domains (Table 4), and under this (around 0.60) for the general trauma and physical abuse domains. Values of Cronbach's alpha ≥0.60 are, however, considered acceptable (23,24). Test-retest reliability is high for all the domains, with correlation coefficients ranging from 0.82 to 0.96 (Table 10).

Table 10. Internal consistency and test-retest reliability.

Subscale	Cronbach's α (n=184)	Test - retest (n=58)
General trauma	0.64	0.84***
Physical abuse	0.60	0.82***
Emotional abuse	0.85	0.93***
Sexual abuse	0.82	0.96***

***p<0.0001

Descriptive statistics by item are provided in Table 11. The most commonly experienced events are being slapped in the face (81%) and being pushed or shoved (67%). The item-to-total correlation indicates items that threaten the domain's validity and internal consistency, while the Cronbach's alpha values are listed if an item is omitted from the domains. In the physical abuse domain one item that is poorly correlated with the total domain score, and for which its omission would result in a higher Cronbach's alpha, is the experience of being "burned with a cigarette". In contrast, the omission of none of the general trauma domain items would result in the higher internal consistency of the domain.

Table 11. Frequency of traumatic events, item-to-total correlation and value of Cronbach's alpha if item is omitted.

Subscale (range)	Mean (SD)	Item-to-Total Correlation	Cronbach's α (minus item)
General trauma			
Natural disaster	10% (31)	0.13	0.64
Serious accident	22% (41)	0.26	0.62
Serious personal injury	27% (45)	0.33	0.61
Serious injury/illness of parent	33% (47)	0.33	0.61
Separation of parents	22% (41)	0.24	0.62
Serious illness/injury of sibling	10% (31)	0.17	0.63
Serious injury of friend	41% (49)	0.29	0.62
Witnessing violence	37% (48)	0.49	0.57
Family mental illness	23% (42)	0.41	0.59
Alcoholic parents	28% (45)	0.34	0.60
See someone murdered	9% (29)	0.14	0.64
Physical abuse			
Slapped in the face	81% (39)	0.27	0.58
Burned with a cigarette	13% (34)	0.11	0.64
Punched or kicked	39% (49)	0.48	0.46
Hit with a thrown object	40% (49)	0.45	0.48
Pushed or shoved	67% (47)	0.44	0.49
Emotional abuse			
Often put down or ridiculed	27% (45)	0.67	0.82
Often ignored or made to feel you didn't count	39% (49)	0.65	0.82
Often told you are no good	26% (44)	0.65	0.82
Most of the time treated in cold or uncaring way	26% (44)	0.74	0.80
Parents fail to understand your needs	35% (48)	0.62	0.83
Sexual abuse			
Touched on intimate parts in a way that was uncomfortable	24% (43)	0.52	0.85
Someone rubbing their genitals against you	8% (27)	0.62	0.79
Forced to touch someone's intimate parts	8% (27)	0.67	0.78
Someone had genital sex with you against your will	7% (25)	0.71	0.77
Forced to perform oral sex	5% (23)	0.69	0.78
Forced to kiss someone in sexual way	5% (22)	0.53	0.81

The agreement between the first and second measurements was also examined graphically, using a Bland-Altman plot. The mean score for each domain on the two measurements for each participant was calculated and is shown on the x-axis, while the difference between scores is depicted on the y-axis. The expected mean difference between the scores of the two measurements is 0. The horizontal solid line shows the mean difference between the scores of the two measurements and is an estimate of bias, while the dotted lines represent 95% limits of agreement. Outliers lie above the upper and below the bottom dotted line. The indicator of high agreement between the two measurement occasions is if most points lie inside the mean±1.96 SD difference interval. The agreement between measurements is highest for the emotional abuse scale, but evident also for other domains.

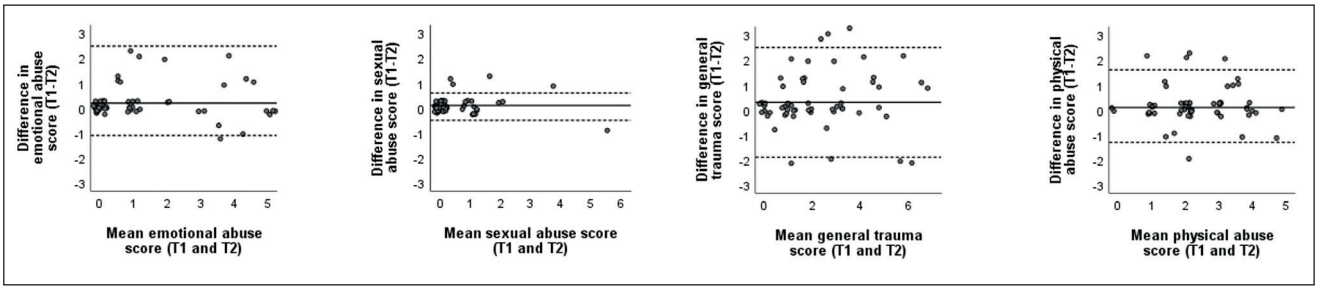

Figure 1. Bland-Altman plots for the test-retest reliability. Each plot illustrates the agreement between time 1 and time 2 measurements and identifies possible outliers. Each participant is represented on the graph with the mean value of the two assessments (x-axis) and difference between the assessments (y-axis). Reference lines show the mean difference between time 1 and time 2 (solid line), and 95% limits of agreement for the mean difference (dotted lines).

4 DISCUSSION

In this paper, we wanted to validate a Slovenian version of ETISR-SF and it was done in two parts, using both qualitative and quantitative methods.

The qualitative part of the study obtained semantic, cultural and conceptual translation and equivalence of ETISR-SF questionnaire in Slovenian using the Delphi method (Tables 1-5). Consensus was reached in the second round with minor adjustments (Table 6).

In the quantitative part of the study we examined the psychometric properties in clinical and non-clinical samples. The clinical sample was composed of in-hospital patients with substance use disorders (Table 7).

The ETISR-SF results showed statistically significant differences between these two groups, which is an indicator of discriminant validity. Patients with substance use disorders reported more traumatic events than the healthy control group, especially in the domains of general trauma and emotional abuse (Table 8). This was supported by comparing respondents experiencing extreme fear or out-of-body experiences and those without such feelings, which represent emotional responses to traumatic events (Table 9). More reported traumatic events in all domains were statistically significant related to emotional responses. The results from a study by Brajovic et al. showed that physical abuse is more strongly associated with alcohol use patterns than emotional abuse (25). Persons who had experienced four or more categories of childhood exposure to traumatic events, compared to those who had experienced none, had 4- to 12-fold increased health risks for alcoholism, drug abuse, depression, and suicide attempts, as well as 2- to 4-fold increases in smoking and poor self-rated health (26). The number of traumatic exposures showed a graded relationship to the presence of adult diseases, including ischemic heart disease, cancer, chronic lung disease, skeletal fractures, and liver disease (26). The Adverse Childhood Experiences (ACE) Study suggested that the impact of adverse childhood experiences on adult health status is strong and cumulative (26). Secondary prevention of the effects of adverse childhood experiences will first require increased recognition of their occurrence, and then effective understanding of the behavioural coping devices that are commonly adopted to reduce the emotional impact of these experiences (26).

The second psychometric property was reliability. Cronbach's alpha, as a measure of internal consistency, varied among the different domains. The general trauma and physical abuse domains exhibited lower internal consistency (α=0.64 and α=0.60, respectively) than emotional and sexual abuse (α=0.85 and α=0.82, respectively) (Table 10). Values were lower than those reported in other studies (15, 18, 19, 21) and similar for a Spanish version used with postpartum women (17). In the domain of general trauma, there are a few events which are obviously not typical for our cultural environment, like "natural disaster", "serious illness/injury of sibling" and "saw someone murdered" (Table 11). Within the physical abuse domain there is also an event which exhibits a low correlation with the overall score and reduces the Cronbach's alpha of the domain. Such events poorly correlate with the overall score on the scale and reduce its reliability, compared to the results found in other research, which indicate possible cultural differences in this regard. Similar findings can be found in a German validation of the inventory (27). The reliability of the scale for the domains of emotional and sexual abuse is very strong. Moreover, the values of the Cronbach's alpha coefficient are not problematic, and we have thus decided not to exclude items in order to maintain comparability of the questionnaire with other countries.

The test-retest reliability of the Slovenian version of the ETISR-SF is high for all subscales, with correlation coefficients ranging from 0.82 to 0.96 (Table 10). A graphical exploration of the test-retest reliability via

a Bland-Altmen plot also indicates high measurement stability on all subscales (Figure 1). That indicates the temporal stability of the measurement, which we want in practice. Similar findings were reported in other countries (17, 19, 21).

4.1 Limitations of the Study

One limitation of this research in the first, qualitative part was the poor response rate of the invited participants, although a higher number would probably not significantly affect the results of the translation. In the second, quantitative part a limitation is that there is no similar instrument in Slovenia to determine the external validity of the ETISR-SF. As yet there is nothing similar to the ETI interview, which can be used to ascertain trauma-related characteristics such as duration, start, frequency, seriousness, perpetrator relationship or subjective significance for trauma survivor. A disadvantage of questionnaires is that they only capture a few properties of potentially traumatic events, and not the subjective meaning that they have for the person who experienced them (28).

In spite of these limitations, this study's validation of the first questionnaire in Slovenian for the detection of early traumatic experience in adults showed that the instrument is valid for use in various part of the health care system.

5 CONCLUSION

Our findings showed that the Slovenian version of the ETISR-SF is a satisfactory instrument for the evaluation of childhood physical, emotional, and sexual abuse, as well as general trauma.

Traumatic experiences in childhood represent an important public health problem that has become a global epidemic in modern times. The consequences of early traumatic experiences can be indicated as psychosomatic and somatoform disorders, causing unnecessary, expensive and invasive diagnostics, and loss of time for starting proper treatment. Therefore, we urgently need a quick and economic measurement tool for early screening of traumatic events in childhood for use in the primary health care system. Such a tool would enable health care professionals to optimize the diagnostic process and refer patients to therapeutic and/or psychiatric treatment in a timely manner.

CONFLICTS OF INTEREST

The author declares that no conflicts of interest exist.

FUNDING

No funding has been received for the execution of this study and/or preparation of this manuscript.

ETHICAL APPROVAL

The study protocol was approved, as a part of PhD thesis by the Slovenian National Medical Ethics Committee No. 0120-121/2019/9 on 22nd October 2019.

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Appendix 1. Vprašalnik za samooceno travm iz otroštva – kratka oblika (ETISR-SF).

Prvi del. Splošne travme. Pred 18. letom.

1.	Ali ste bili kdaj izpostavljeni življenjsko nevarni naravni nesreči?	DA	NE
2.	Ali ste bili kdaj udeleženi v hudi nesreči?	DA	NE
3.	Ali ste kdaj imeli hudo telesno poškodbo ali bolezen?	DA	NE
4.	Ali ste kdaj doživeli smrt ali hudo bolezen starša ali skrbnika?	DA	NE
5.	Ali so se vaši starši razšli ali ločili?	DA	NE
6.	Ali ste kdaj doživeli smrt ali hudo poškodbo brata ali sestre?	DA	NE
7.	Ali ste kdaj doživeli smrt ali hudo poškodbo prijatelja?	DA	NE
8.	Ali ste bili kdaj priča nasilju nad drugimi, vključno z družinskimi člani?	DA	NE
9.	Ali je kdo v vaši družini imel duševno motnjo ali doživel "živčni zlom"?	DA	NE
10.	Ali so imeli vaši starši ali skrbniki težave z alkoholom ali drogami?	DA	NE
11.	Ali ste kdaj videli nekoga umorjenega?	DA	NE

Drugi del. Fizično kaznovanje. Pred 18. letom.

1.	Ali ste kdaj dobili klofuto?	DA	NE
2.	Ali so vas kdaj opekli z vročo vodo, cigareto ali čim drugim?	DA	NE
3.	Ali so vas kdaj tepli ali brcali?	DA	NE
4.	Ali vas je kdaj zadel predmet, ki ga je kdo vrgel v vas?	DA	NE
5.	Ali so vas kdaj porinili ali odrinili?	DA	NE

Tretji del. Čustvena zloraba. Pred 18. letom.

1.	Ali so vas pogosto poniževali ali zasmehovali?	DA	NE
2.	Ali so vas pogosto ignorirali ali pa ste se počutili neupoštevane?	DA	NE
3.	Ali so vam pogosto govorili, da niste za nič?	DA	NE
4.	Ali so pogosto z vami ravnali na hladen, neskrben način ali ste se počutili neljubljene?	DA	NE
5.	Ali vaši starši ali skrbniki pogosto niso uspeli razumeti vas ali vaših potreb?	DA	NE

Četrti del. Dogodki, povezani s spolnostjo. Pred 18. letom.

1.	Ali se je kdo kdaj dotaknil vaših intimnih ali drugih delov telesa (npr. prsi, stegen, spolovil) na način, ki vas je presenetil ali vam je bil neprijeten?	DA	NE
2.	Ali je kdaj kdo drgnil svoje spolovilo ob vas?	DA	NE
3.	Ali ste bili kdaj prisiljeni v dotikanje druge osebe po intimnih ali zasebnih delih njihovega telesa?	DA	NE
4.	Ali je kdo imel z vami spolni odnos proti vaši volji?	DA	NE
5.	Ali ste bili kdaj prisiljeni imeti oralni spolni odnos proti vaši volji?	DA	NE
6.	Ali ste bili kdaj prisiljeni poljubiti nekoga na seksualni in ne prijateljski način?	DA	NE

Če ste na katerokoli od zgornjih vprašanj odgovorili z "DA", odgovorite na naslednji vprašanji za tisti dogodek, ki je najbolj vplival na vaše življenje. Pri odgovarjanju upoštevajte, kako ste se počutili v času dogodka.

1.	Ali ste občutili hud strah, grozo ali nemoč?	DA	NE
2.	Ali ste imeli občutek, kot da bi bili izven svojega telesa ali kot da bi bili v sanjah?	DA	NE

Repič Lampret B, Remec ŽI, Drole Torkar A, Žerjav Tanšek M, Šmon A, Koračin V, Čuk V, Perko D, Ulaga B, Jelovšek AM, Debeljak M, Kovač J, Battelino T, Grošelj U. Expanded newborn screening program in Slovenia using tandem mass spectrometry and confirmatory next generation sequencing genetic testing. Zdr Varst. 2020;59(4):256-263. doi: 10.2478/sjph-2020-0032.

EXPANDED NEWBORN SCREENING PROGRAM IN SLOVENIA USING TANDEM MASS SPECTROMETRY AND CONFIRMATORY NEXT GENERATION SEQUENCING GENETIC TESTING

PROGRAM RAZŠIRJENEGA PRESEJANJA NOVOROJENCEV V SLOVENIJI S TANDEMSKO MASNO SPEKTROMETRIJO IN SEKVENCIRANJEM NASLEDNJE GENERACIJE ZA POTRDITVENO GENETSKO TESTIRANJE

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Received: Apr 17, 2020
Accepted: Sep 17, 2020

Original scientific article

ABSTRACT

Introduction: In the last two decades, the introduction of tandem mass spectrometry in clinical laboratories has enabled simultaneous testing of numerous acylcarnitines and amino acids from dried blood spots for detecting many aminoacidopathies, organic acidurias and fatty acid oxidation disorders. The expanded newborn screening was introduced in Slovenia in September 2018. Seventeen metabolic diseases have been added to the pre-existing screening panel for congenital hypothyroidism and phenylketonuria, and the newborn screening program was substantially reorganized and upgraded.

Methods: Tandem mass spectrometry was used for the screening of dried blood spot samples. Next-generation sequencing was introduced for confirmatory testing. Existing heterogeneous hospital information systems were connected to the same laboratory information system to allow barcode identification of samples, creating reports, and providing information necessary for interpreting the results.

Results: In the first year of the expanded newborn screening a total of 15,064 samples were screened. Four patients were confirmed positive with additional testing.

Conclusions: An expanded newborn screening program was successfully implemented with the first patients diagnosed before severe clinical consequences.

Keywords:
newborn screening, NBS, tandem mass spectrometry, MS/MS, next-generation sequencing, NGS, inborn errors of metabolism, IEM

IZVLEČEK

Uvod: *Uporaba tandemske masne spektrometrije je v zadnjih dvajsetih letih močno vplivala na diagnostiko vrojenih bolezni presnove, saj omogoča sočasno merjenje številnih acilkarnitinov in aminokislin iz posušenega madeža krvi in s tem prepoznavo različnih aminoacidopatij, organskih acidurij in motenj v metabolizmu maščobnih kislin. Program razširjenega presejanja novorojencev je bil v Sloveniji uveden septembra 2018. Obstoječemu programu presejanja kongenitalne hipotiroze in fenilketonurije je bilo dodanih 17 novih bolezni presnove, ob tem so bile vpeljane določene organizacijske spremembe.*

Metode: *Analiza vzorca posušenega madeža krvi poteka s tandemsko masno spektrometrijo. Za potrditveno testiranje je bila vpeljana metoda sekvenciranja naslednje generacije. Obstoječi heterogeni bolnišnični informacijski sistemi so bili povezani v enotni laboratorijski informacijski sistem, kar omogoča kodno identifikacijo vzorca, oblikovanje izvidov in dostop do vseh podatkov, potrebnih za interpretacijo izvida.*

Rezultati: *V prvem letu razširjenega presejanja novorojencev je bilo pregledanih 15.064 vzorcev. Z dodatnimi potrditvenimi testi je bila bolezen potrjena pri štirih bolnikih.*

Zaključki: *Razširjeno presejanje novorojencev je bilo uspešno vzpostavljeno. Prvi bolniki so bili prepoznani, diagnoza jim je bila postavljena pred pojavom kliničnih znakov.*

Ključne besede:
presejanje novorojencev, tandemska masna spektrometrija, sekvenciranje nove generacije, vrojene bolezni presnove, IEM, NBS, MS/MS, NGS

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1 INTRODUCTION

A newborn screening (NBS) program is essential for the detection of inborn errors of metabolism (IEMs) and other such disorders in infants. IEMs are inherited metabolic disorders in which genetic variants cause inactivity or lack of enzymes that are important in various chemical reactions in the body (1). The incidence of most IEMs is less than 1/10,000, which makes them rare (2). Nevertheless, early detection is crucial for the patient's clinical outcome. Patients with late diagnosis present with severe consequences, ranging from minor disabilities to lethal outcomes (3). Therefore, it is crucial to start the treatment (which often involves a specialized dietary intervention) as soon as possible to prevent unfavourable outcomes (4). In addition, genetic testing and counselling are offered to parents (3).

Expanded NBS is possible with tandem mass spectrometry (MS/MS), a method used for simultaneous testing of many different metabolites, including numerous acylcarnitines and amino acids on dried blood spots (DBS), which enable detection of many aminoacidopathies, organic acidurias and fatty acid oxidation disorders. Moreover, it is time- and cost-effective, with good sensitivity and specificity (5–9). There is also a lot of interest in implementing next-generation sequencing (NGS) into NBS programs, as it has been proven to be a valuable tool in confirming diseases, raising the possibility of family counselling, and influencing therapeutic decisions (10).

We aimed to describe the extended NBS program in Slovenia and to present the results of the first year of the program (11).

1.1 National Population-Based Newborn Screening Program in Slovenia

1.1.1 History of the Newborn Screening Program in Slovenia

The NBS program was first introduced in Slovenia in 1979 with screening for phenylketonuria (PKU) (12). Two years later, screening for congenital hypothyroidism (CH) was added (13). From 1993 to 2012, 358,831 newborns were screened for PKU, and 57 were diagnosed with the disease. A total of 427,396 newborns were screened for CH between the years 1991 and 2012,and the disease was confirmed in 184 cases (14). The incidence of PKU in the stated period was, therefore, 1:6769 for PKU and 1:2323 for CH, which meets the estimated incidence of those diseases in Europe (1:3000 – 1:30,000 for PKU and 1:1300 – 1:13,000 for CH) (14, 15).

In 2000, selective screening for IEMs was initiated, starting with analyses of amino acids and organic acids in symptomatic patients with suspected IEMs or patients with a positive family history for IEMs. Different chromatographic methods were used for the detection of metabolites, such as gas chromatography-mass spectrometry (GC-MS) for organic acid determination and ion exchange chromatography-post-column derivatization or liquid chromatography-tandem mass spectrometry (LC-MS/MS) for amino acid measurement. Liquid chromatography-tandem mass spectrometry was also used for acylcarnitine analysis. As of April 2014, a total of 168 patients with organic acid disorders and amino acid disorders (of them 140 with PKU diagnosed on NBS) and five with fatty acid oxidation disorders were in the Slovenian Register of Inborn Errors of Metabolism (16). Comparing these results with the data from those countries with expanded NBS screening programs showed that we should expect more identified cases (16). Therefore, a pilot study to estimate the incidences of IEMs and support the expansion of the NBS program in Slovenia was performed in 2014–2016 (10, 11).

1.1.2 Pilot Study of the Expanded Newborn Screening Program in Slovenia

To prepare an optimal strategy for the organization of the expanded NBS for IEMs in Slovenia, a pilot study was performed analysing 10,048 Slovenian newborns (10). In the initial stage, each IEM that was included in the study was identified by acylcarnitine and amino acid analysis with the MS/MS method for screening and confirmed with confirmatory testing. NGS was included for confirmatory testing in combination with other established tests for IEM detection, such as organic acid determination, amino acid analysis and enzyme activity testing. NGS allows the simultaneous analysis of all genes associated with IEMs included in the screening, and thus represents the method of choice for the confirmation of the diseases identified in the NBS. Out of 10,048 results, 113 follow-ups (newborns with the ten highest concentrations of metabolites included in the pilot study) were evaluated at an outpatient clinic, where additional confirmatory analyses were performed. With confirmatory tests, four new patients with IEMs were detected, namely a patient with glutaric aciduria type 1, a patient with very long-chain-acyl-CoA-dehydrogenase deficiency, and two patients with 3-methylcrotonyl-CoA-carboxylase deficiency. In all cases, the NGS approach identified causative genetic variants, such as those that have not yet been described in the literature (10, 11). Based on our results and comparisons with other European countries, the cut-off values and ratios of individual metabolites were determined.

2 METHODS

2.1 Expanded Newborn Screening in Slovenia

2.1.1 Protocol of Expanded Newborn Screening in Slovenia

The process begins with informing parents about the goals and the course of the NBS program. Parents are informed about the NBS procedure and its advantages by a neonatologist at the first medical examination of the newborn. At the same time, an online information leaflet is available (18). The collection of the samples and input of patient data into an electronic medical record (EMR) are performed next, with the generation of a barcode and QR code, followed by the transport of samples to the laboratory, where they are automatically processed, tested, analysed, and confirmed. In cases of a positive screening result, the family is informed by one of the paediatricians subspecialized in paediatric endocrinology and inborn errors of metabolism at the University Medical Centre (UMC) – University Children's Hospital Ljubljana (UCHL), who also provide family counselling, treatment and further management (9, 17).

Written informed permission is not required to perform the NBS in Slovenia, because the program is a mandatory nationwide population program, which is required by national legislation. If the parents refuse to take part in the program, the neonatologist performs additional counselling before the discharge of the newborn from the hospital. A written refusal statement must be signed on a blank sample card, and the child's paediatrician is informed. A blank card with maternal data is sent to the laboratory and archived (9).

A custom DBS card with space for barcode and QR code with the information of the newborn and the mother is used for blood sampling. Blood samples are taken 48 to 72 hours after birth from the newborn's heel or by venepuncture, according to the technical requirements (9).

Sample information and newborn identification data are entered into the hospital EMR, and an order for the NBS testing is created. The sample must be sent from the nursery within 24 hours by mail or courier service to the Department of Nuclear Medicine (DNM), UMC Ljubljana, where all the samples are accepted. Half of the sample card is used at DNM, where the fluorometric method is used for PKU screening and dissociation-enhanced lanthanide fluorescence immunoassay is used for CH screening, as described earlier (14). The other half is sent to the Clinical Institute for Special Laboratory Diagnostics, UMC – University Children's Hospital Ljubljana (CISLD), where the expanded NBS is performed. In the DNM and CISLD laboratory, sample barcodes are scanned, and the orders made by the hospitals are accepted and automatically transferred into the laboratory information system. Inadequate samples are declined, the nursery is informed, and a new sample is requested (9).

The algorithm for reporting and confirming results of the expanded NBS has been prepared, depending on the level of disease suspicion, mainly based on the degree of metabolite elevation and profiles of their combinations (Fig 1). The nursery is informed about the results of the screening automatically through the information system as soon as the laboratory specialist or/and clinician confirms the results. In the case of borderline or positive results, a new additional sample must be tested. For newborns with borderline results the nursery collects a second sample on the screening card and sample goes through the same initial screening procedure again. When the second testing is indicative of a disease, a newborn is referred to the UCHL for further diagnostics. Newborns with a positive result on initial screening are referred directly to the UCHL. Depending on the disease in question, a sample of urine, plasma or blood may also be requested. All positive results are tested using NGS and/or other confirmatory methods. Additionally, enzymatic activity is determined when indicated (performed at the Amsterdam Medical Center laboratory). The newly detected patients are treated and followed up at the UCHL (9).

Confirmatory testing for borderline and positive CH results is done at DNM, while PKU borderline results are retested at DNM, final confirmation of positive result is done at CISLD (14).

Samples are stored in the laboratory for an indefinite period and can be reused for the selective diagnostics if permitted by official state regulations. Quality indicators for the NBS program are evaluated once per year (9). CISLD is participating in different external quality control schemes, the Newborn Screening Quality Assurance Program (NSQAP) at the US Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/labstandards/nsqap.html) and European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism (ERNDIM) (https://www.erndimqa.nl/).

When the diagnosis is confirmed, the patient is included in the Slovenian Register of Rare Diseases, and the registration needs to be initiated by the physician who confirmed the diagnosis (9).

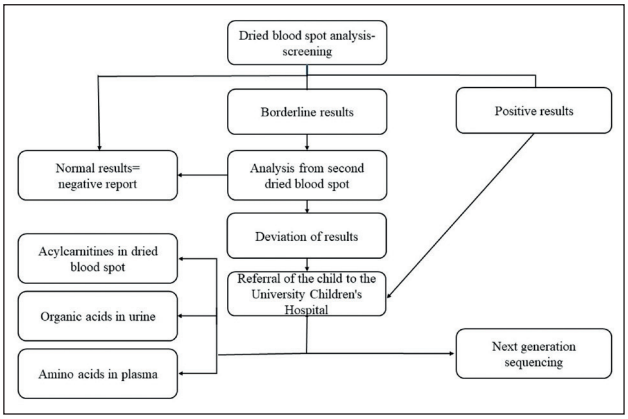

Figure 1. Screening algorithm for expanded NBS results.

A MS/MS (Waters Xevo TQD LC-MS/MS) is used for expanded screening (9). It quantifies the tested metabolites with stable-isotope-labelled internal standards, and allows the detection of many metabolites simultaneously in a single run (19). It can accommodate a high throughput of samples (2–3 min per sample) and has low reagent cost at the same time as being sensitive and specific (20).

For NGS confirmatory testing, an in-house panel of 72 genes has been developed by the newborn screening team, which includes a geneticist (Table 1). The NGS panel includes causative genes for the 18 diseases included in the NBS program (Table 2), with the addition of genes for the conditions that present with the increase of the same metabolites as the targeted disease and are therefore invaluable for differential diagnostics. Variants detected with NGS are confirmed by Sanger sequencing and reported to the NBS clinical team, which includes paediatricians subspecialized in the paediatric endocrinology and inborn errors of metabolism.

If one of the diseases not included in expanded screening is detected, the full NBS team (which includes both laboratory and clinical NBS teams) could make a case-by-case decision to proceed with diagnostics and reporting if that would be in the presumed clinical benefit of the newborn (21).

During the first year of the expanded NBS program, all 14 nurseries were gradually connected in the same laboratory information system.

Table 1. Panel of 72 genes for the NGS confirmatory testing in expanded NBS program.

ABCD1	ABCD4	ACAD8	ACAD9	ACADL	ACADM	ACADS	ACADVL
ACAT1	ADA	ALDH18A1	ARG1	ASL	ASS	AUH	BCAT2
BCKDHA	BCKDHB	BTD	CD320	CPS1	CPT1A	CPT2	DBT
DLD	ETFA	ETFB	ETFDH	ETHE1	FAH	GCDH	GCH1
GLUL	HADH	HADHA	HADHB	HLCS	HMGCL	HMGCS2	HPD
HSD17B10	IVD	LMBRD1	MCCC1	MCCC2	MLYCD	MMAA	MMAB
MMACHC	MMADHC	MTR	MTRR	MMUT	NAGS	OTC	PAH

Table 2. List of diseases included in expanded NBS program. * – already running newborn screening program for phenylketonuria; in agreement, the program is running in parallel for two years on both systems, and the reported results are from the existing program for PKU screening.

Tyrosinemia type 1	Carnitine palmitoyltransferase deficiency type 2
Maple syrup urine disease	3-methylcrotonyl-CoA carboxylase deficiency
Isovaleric acidemia	3-hydroxy-3-methylglutaric aciduria
Glutaric aciduria type 1	Holocarboxylase synthethase deficiency
Glutaric aciduria type 2	ß-ketothiolase deficiency
Propionic aciduria	Very long-chain acyl-CoA dehydrogenase deficiency
Methylmalonic aciduria	Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency
Carnitine uptake deficiency	Medium-chain acyl-CoA dehydrogenase deficiency
Carnitine palmitoyltransferase deficiency type 1	Phenylketonuria*

3 RESULTS

3.1 First Year of Experience

Altogether, 15,064 samples were screened: 14,743 were reported with negative results, 202 with borderline findings, and 68 with positive results, while 51 samples were rejected (Figure 2). Among the positive results, four patients were confirmed with additional diagnostic testing (Table 3). Additional diagnostic testing was done with the measurement of organic acids in urine, amino acids in plasma, enzyme activity determination, and genetic analysis, depending on screening results.

Positive predictive value was 5.8% (confirmed positives/ all positives).

The percentage of altered profiles that lead to borderline and false positive samples is distributed among all three groups of screened diseases as follows: 43.3% in the group of fatty acid oxidation disorders; 32.6% in the group of organic acid disorders; 17.4% in the group of amino acid disorders; and 6.8% results with profile of multiple elevated metabolites, that does not fit only one group.

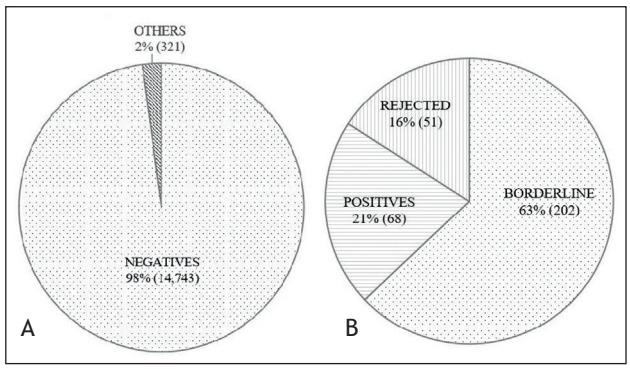

Figure 2. A – all samples (15,064); percentage of negative results compared to others (positive and borderline positive results and rejected samples). B – others – positive and borderline positive results and rejected samples (321); percentage among others (positive and borderline positive results and rejected samples).

Table 3. Values of acylcarnitines and amino acids at newborn screening and follow-up for confirmed patients. CoA – coenzyme A, DBS – dried blood spot, NBS – newborn screening, ref. – reference.

Module name	NBS results (from DBS) µmol/L	Follow-up (from DBS) µmol/L	Confirmation tests
Very long-chain acyl-CoA dehydrogenase deficiency	C14:1: 3.41 (<0.32) C14: 1.41 (<0.42) C14:2: 0.44 (0–0.05) C14:1/C2: 0.200 (<0.014) C14:1/C16: 0.84 (<0.08)	C14:1: 0.84 (<0.32) C14: 0.41 (<0.42)C14:2: 0.18 (<0.05) C14:1/C2: 0.090 (<0.014) C14:1/C16: 0.31 (<0.08)	One known heterozygous pathogenic variant and one likely pathogenic variant
Isovaleric acidemia	C5: 2.47 (<0.28) C5/C0: 0.132 (<0.018) C5/C2: 0.164 (<0.017) C5/C3: 1.91 (<0.21)	C5: 5.77 (<0.28) C5/C0: 0.222 (<0.018) C5/C2: 0.499 (<0.017) C5/C3: 5.84 (<0.21)	Elevated isovalerylglycine in urine Reduced enzyme activity: 0.1 nmol/(min mg prot) (ref. value: 0.94–1.94)
Medium-chain acyl-CoA dehydrogenase deficiency	C8: 0.27 (<0.16) C6: 0.27 (<0.12) C10: 0.11 (<0.26) C10:1: 0.13 (<0.09) C8/C10: 2.45 (<1.50) C8/C2: 0.07 (0.01)	C8: 0.96 (<0.27) C6: 0.477 (<0.15) C10: 0.14 (<0.32) C10:1: 0.285 (<0.25) C8/C10: 6.8 (<2.3) C8/C2: 0.07 (0.02)	One known heterozygous pathogenic variant and one variant of unknown significance Reduced enzyme activity: 0.20 nmol/(min mg prot) (ref. value: 0.43–1.63)
Hyperprolinemia	Proline: 623 (<261)	Proline: 794 (<441)	Two pathogenic variants. Elevated proline in plasma: 748 (110–417) µmol/L

4 DISCUSSION

The NBS was first introduced in 1963 in the USA with the Guthrie test for detecting PKU (22). Most developed countries already have an NBS program and screen for more diseases (9, 23–39). On the other hand, most of the countries worldwide do not have an expanded NBS (40, 41). In contrast with many other European countries, the Slovenian NBS until recently consisted only of testing for PKU and CH, which had put us in the same group as other countries in southeastern Europe (14, 40). Slovenia now has a spectrum of screened diseases comparable to those in most developed countries, with a total of 19 screened conditions (11, 20).

A study that assessed the NBS characteristics in 11 countries from southeastern Europe in 2014 showed that in most of only screening for PKU and CH were implemented, while four of the countries did not screen for PKU, and three of them did not screen for CH. A lack of financial resources was the main reason given for not introducing the expanded NBS, although the costs of the screening test per newborn varied from 1–12 euros in different EU countries, an average being 4 euros. In Slovenia the price of a screening test was calculated at 9.24 euros. The cost of confirmatory testing depends on tests needed for a certain condition. Other reasons given for not introducing the expanded NBS were the lack of staff and expertise. At that time, none of the countries from the region used MS/MS for the NBS. Slovenia was one of the three countries (together with Romania and Croatia) that reported plans to implement tandem mass spectrometry (MS/MS) in its NBS due to the expected expansion of screened diseases (40). In Croatia, the expanded NBS program was implemented in 2018 (42).

In the last few years, NBS with tandem mass spectrometry has become more available due to technological advances and has been proved to operate to a high standard. Therefore, it is already implemented in the NBS programs of many European countries as well as in the USA. MS/MS allows simultaneous screening for more IEMs from one dried blood spot on a filter test paper. It detects inborn errors of amino acid metabolism and urea cycle defects, most fatty acid oxidation disorders, and numerous organic acidurias (7). Due to high throughput, it is possible to test great numbers of samples in a timely fashion, which supports screening samples of dried blood spots from the whole population. Moreover, the reagent costs are low, the method has high sensitivity (90–100%) and is highly specific (99–100%) (6, 7, 20, 43–45).

In Slovenia, confirmational tests are performed using NGS, detecting gene sequences responsible for IEMs (9). In the future, it would be possible to use it for screening for a larger spectrum of genetic diseases and further expanding the NBS program panel. NGS can be used for parental and sibling testing, genetic counselling, and family planning (10, 46–48). The NGS result is unaffected by environmental factors, such as dietary intake and treatment, stress, which all influence metabolites levels, and could thus affect the first tier NBS results (49). The first pilot NBS programs are now reported to use genetic testing as a first-line diagnostics, and are expected to gradually become fully clinically implemented (50).

The newly screened diseases meet the World Health Organization (WHO) criteria for population-based screening (20, 51). Laboratory participates in external quality assurance schemes, organized by CDC and ERNDIM. The CDC scheme is organized as a quantitative analysis from DBS for newborn screening acylcarnitines and amino acids, and the interpretation of results is based on cut-offs. The ERNDIM scheme covers both quantitative analysis from DBS for selected metabolites and qualitative acylcarnitine measurement from DBS, where interpretation of the results is needed. The incidence of a specific IEM is essential when deciding on the inclusion of the disease in an NBS program. A pilot study was done to estimate the incidences for the Slovenian population, and the results of the study were also used for cut-off determination. CH will be tested as before, with testing of thyroid-stimulating hormone (TSH) values (9). All hospital information systems were connected to the same laboratory information system for more efficient and safer sample identification and data control. Approximately 20,000 newborns from Slovenia are screened per year. The cumulative incidence of the IEMs in Slovenia was established at 1:2762, including PKU with incidence 1:6769 (10).

The strength of this study is that we introduced NGS as a conformational test, which helps to get the final diagnosis as soon as possible. It is also important to get more information and knowledge if genetic testing will be used in the future for NBS. During the first year we were still optimizing our NGS procedure, DNA isolation, and protocols. With this information we could improve screening and conformational tests. The major downside of NGS for use for NBS is the turnaround time. One limitation of this study is the gradual inclusion of maternity wards during the first year of introduction of the expanded NBS program, so the total nationwide population of newborns was not included. In addition, due to the novelty of the program some planned features (such as reporting the parent refusals) were not yet implemented.

The first year of experience with the expanded NBS program gives us important feedback information and provides us with initial experiences that inform us about possible future improvements of the program. One goal is regularly informing and educating nurseries about importance of sample quality, to reduce rejected samples. Cut-off values will be re-evaluated according to external

control samples, and modified if necessary to reduce false positives. Second tier testing is planned for detection of propionic/methylmalonic acidemia, as it is known that the primary metabolite gives high number of false positive results. Isolation of DNA from DBS is another possibility, to reduce the need for additional sampling of blood.

5 CONCLUSIONS

In conclusion, the expanded NBS program with NGS as a confirmatory method was successfully implemented in Slovenia, and the first patients were appropriately detected through the program. Soon, we expect to expand the program further to also include relevant disorders which are not diagnosed with MS/MS. In the longer term, we expect that genomic testing will be gradually implemented as a first-line NBS test.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

FUNDING

The study was partly founded by the Slovenian Research Agency project V3-1505 and program P3-0343 and University Medical Centre Ljubljana's research project 20150138.

ETHICAL APPROVAL

The nationwide project to expand the NBS program in Slovenia with the tandem mass spectrometry and with confirmatory genetic testing was approved by the Slovenian National Medical Ethics Committee (#56/01/14). In 2018, the formal legislative framework was adopted as a basis of expanded NBS program in Slovenia, also requiring a yearly performance and quality analyses.

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AN INCREASING SCABIES INCIDENCE IN CROATIA: A CALL FOR COORDINATED ACTION AMONG DERMATOLOGISTS, PHYSICIANS AND EPIDEMIOLOGISTS

VEČANJE POJAVNOSTI SKABIESA NA HRVAŠKEM: POZIV K USKLAJENEMU UKREPANJU DERMATOLOGOV, SPLOŠNIH ZDRAVNIKOV IN EPIDEMIOLOGOV

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Received: May 12, 2020
Accepted: Sep 18, 2020

Original scientific article

ABSTRACT

Introduction: The aim of our study was to examine the scabies incidence in the Croatian population and to analyse potential related factors.

Methods: This mixed ecological study is based on a retrospective medical record review. National data from communicable disease reports was sourced and analysed for an 11-year period (2007-2017), with more focus on the period 2014-2017. Descriptive statistics were used to calculate trends. Differences between the groups were studied using Chi-square test and Kendall's tau (τ) correlation coefficient. Levels of significance were set at $p<0.05$ or $p<0.01$.

Results: From 2007 to 2017, scabies infestation in Croatia increased by 6-fold, particularly affecting children and young adults (19 years or younger). In the period 2014-2017, border counties which are part of migration flows were the counties with the highest average scabies incidences. A linear trend of increase in the number of tourists, immigrants and scabies infestations was noted on the national level for the analysed period, although a significant association was not observed. Regarding outbreaks of scabies within institutions, more than 80% of outbreaks occurred in institutions for adults. In the capital, Zagreb, the crude incidence rate increased 3-fold between 2014 and 2017.

Conclusions: The increased incidence of scabies, large disparities between counties, and prolonged outbreaks within families due to under-recognition and misdiagnoses points to a need for increased awareness among health practitioners. To the best of our knowledge, this is the first recent epidemiologic analysis on this topic, not only in Croatia but within the wider geographic region as well.

Keywords:
scabies, epidemiology, outbreaks, Croatia, neglected tropical diseases

IZVLEČEK

Uvod: *Cilj naše študije je bil preučiti pojavnost skabiesa pri hrvaški populaciji in analizirati morebitne povezane dejavnike.*

Metode: *Ta mešana ekološka študija je temeljila na retrospektivnem pregledu zdravstvenih kartotek. Pridobili in analizirali smo nacionalne podatke iz poročil o nalezljivih boleznih za 11-letno obdobje (2007-2017), pri čemer smo se bolj osredotočili na obdobje 2014-2017. Za izračun trendov smo uporabili opisno statistiko. Razlike med skupinami smo preučili s testom hi-kvadrat in Kendallovim korelacijskim koeficientom tau (τ). Stopnja pomembnosti je bila nastavljena na $p < 0,05$ ali $p < 0,01$.*

Rezultati: *Od leta 2007 do 2017 se je pojavnost okužb s skabiesom na Hrvaškem povečala za šestkrat, pri čemer je bolezen prizadela zlasti otroke in mlajše odrasle (stare 19 let ali manj). V obdobju 2014-2017 so bila obmejna okrožja, ki so del migracijskih tokov, tista z najvišjo povprečno pojavnostjo skabiesa. V analiziranem obdobju je bil na nacionalni ravni opažen linearen trend povečevanja števila turistov, imigrantov in okužb s skabiesom, čeprav ni bilo opaziti pomembne povezanosti. Kar zadeva izbruhe skabiesa v zavodih, se jih je več kot 80 % zgodilo v zavodih za odrasle. V prestolnici Zagrebu se je groba incidenčna stopnja med letoma 2014 in 2017 povečala za trikrat.*

Sklepi: *Povečana incidenca skabiesa, velika neskladja med okrožji in dolgotrajni izbruhi v družinah zaradi premajhnega prepoznavanja in napačnih diagnoz kažejo na potrebo po večji ozaveščenosti zdravstvenih delavcev. Po naših podatkih je to prva epidemiološka analiza, ki je bila pred kratkim opravljena na to temo, ne samo na Hrvaškem, temveč tudi na širšem geografskem območju.*

Ključne besede:
skabies, epidemiologija, izbruhi, Hrvaška, zanemarjene tropske bolezni

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1 INTRODUCTION

Scabies is a skin infestation caused by the mite *Sarcoptes scabiei var. hominis*. This disease most commonly presents as classic scabies with the intense, pruritic eruption at certain characteristic localizations such as the sides and webs of the fingers, wrists, axillae, areolae, and genitalia (1). The transmission of scabies usually happens through direct and prolonged skin-to-skin contact of at least ten minutes, thus scabies commonly occurs within the family and among people who live together or who are sexual partners. On the other hand, transmission via casual skin contact and fomites (clothing, bedclothes, or other objects) is rare (2-4). It is very difficult to contract scabies from brief, casual touching, like handshakes or hugs. A diagnosis is based on a patient's clinical picture and is confirmed by microscopic detection of the scabies mites, eggs, or faeces (5).

Epidemiological data shows that, as of 2015, scabies affects about 204 million people worldwide (2.8% of the population) (6). According to results from the Global Burden of Disease Study 2015, there was a 6.6% increase in the global incidence of scabies over the preceding 11-year period (2005 - 2015) (6). Recently published data confirm that scabies has a seasonal pattern, and most patients are infested in the winter and autumn (7).

Scabies infestation is more common in tropical regions (East Asia, Southeast Asia, Oceania, tropical Latin America, and South Asia), with prevalence estimates ranging from 0.2 to 71%, with the highest rates in the Pacific region and Latin America (8). Moreover, since European epidemiological studies are rare (8) and scabies is not a notifiable disease in many countries (2), it still represents a global and significant problem. Scabies is equally common in both sexes and among people of different ages, and is one of the three most common skin disorders in children (9, 10). However, lately there is growing data in the literature on the correlation between scabies incidence and population movements, particularly for refugees and asylum seekers coming to Western Europe, since they generally come from countries with a high prevalence of scabies (11, 12).

The direct effect of scabies on infected persons and its secondary effects on children, families, and communities worldwide is a strong impetus for advancing the agenda for global scabies control (13). Due to the important effects of this disease on global health, the World Health Organization's Department of Control of Skin Neglected Tropical Diseases (NTDs) has begun promoting the idea of a strategy for integrated scabies control and management (14).

In recent years there has been greater public discussion concerning the increased frequency of scabies in Croatia (15). Physicians and dermatovenerologists have seen this increase reflected in their practices, although to date there have been no wider epidemiologic analyses on this topic.

The objective of this study was thus to examine the time trends in incidence rates of scabies in Croatia and its capital, as well as its geographical patterns, in order to provide researchers and policymakers in Croatia and the broader region with relevant epidemiological data in order to develop effective disease control strategies and reduce current socioeconomic inequalities in treatment of scabies.

2 METHODS

2.1 Study Design

This is mixed ecological study that makes us of exploratory studies of both spatial and temporal patterns.

2.2 Units of Observation

There are two types of units of observation included in this study. In an exploratory study of temporal patterns in Croatia a single year is the unit of observation, while in the exploratory study of temporal patterns of scabies occurrence in Zagreb in the present study the unit of observation is a month. In our exploratory study of spatial patterns, the unit of observation is a county and there are 21 units included in the research.

2.3 Sources of Data

We analysed data from communicable disease notifications for scabies cases in Croatia for the period between 2007 and 2017, with more detailed analysis by the counties, age, facility and month in the period from 2014 to 2017. No identifiable personal were used for this study, and the dataset used is not openly available.

Reporting communicable diseases is mandatory in Croatia and regulated by the Health Care Act, the Act on the Protection of the Population against Communicable Diseases, the list of communicable diseases the control and prevention of which is of interest to Croatia and the ordinance on the method of reporting communicable diseases (16-18). Licensed health care professionals and health care institutions are required to report selected communicable diseases through the standardized questionnaire to the epidemiology service (part of the Croatian network of public health institutes), which is finally received and stored at the Croatian Institute of Public Health. The routinely collected health data used in this study were sourced from the state-owned Croatian Institute of Public Health database. This nationally representative database is available only on request. Therefore, in order to address our research objectives, our multidisciplinary team (consisting of epidemiologists,

public health specialists and dermatovenereologists) utilized different mechanisms for sourcing and analysis of the data.

2.4 Methods of Data Analysis

A scabies case is defined as an individual whose skin scrapings reveal mites, mite eggs or mite faeces, or as an individual with typical clinical signs epidemiologically linked to laboratory-confirmed cases. The average annual incidence rate per 100,000 inhabitants in Croatia was calculated by county using data from The Census of Population, Households and Dwellings 2011 from the Croatian Bureau of Statistics (19). Age specific annual incidences, along with seasonal trends, were both calculated on the national level. We also analysed outbreak reports by number of recognized and reported outbreaks, duration and facilities involved. In addition, we analysed percentile changes in relative (per capita) number of tourists, immigrants and patients with scabies by counties. The change over time is represented by standardized regression coefficient (beta) – ranging from -1 (complete negative linear association) over 0 (no association) to 1 (complete positive linear association).

For the analyses, we used SPSS FOR WINDOWS ver. 20.0 (SPSS, Chicago, IL, USA). Descriptive statistics were used to process data, calculate trends and create tables and graphs. Differences between the counties in the number of patients with scabies, relative to county size (The Census of Population, Households and Dwellings 2011 from the Croatian Bureau of Statistics) (19) were studied using Chi-square test, while Kendall's tau (τ) correlation coefficient was used to calculate the correlations between the time changes in relative number of tourists, immigrants and patients with scabies by Croatian counties. Kendall's tau was used due to small sample size (n=21) and non-normality of the parameter distributions.

Levels of significance were set to $p<0.05$ or $p<0.01$.

3 RESULTS

3.1 Temporal Pattern of Scabies Incidence in Croatia, 2007-2017

Our results show an increasing trend in scabies incidence across Croatia between 2007 and 2017. The incidence rate increased 6-fold during that 11-year period, with the steepest increase in the last four years (2014-2017) (Figure 1).

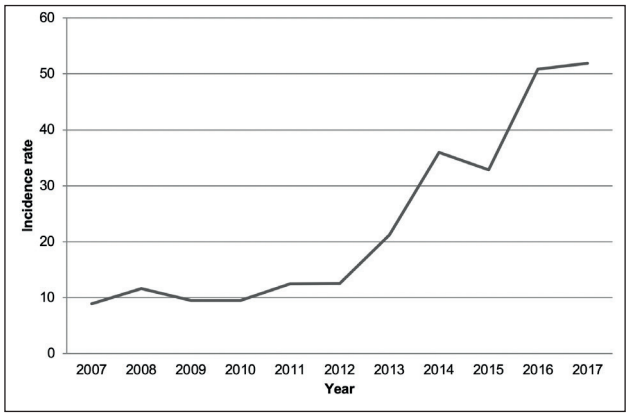

Figure 1. The scabies incidence rates in Croatia in the period 2007-2017.

Within the 2014-2017 period, the highest average incidences in Croatia were recorded among children younger than 4 years old, preschool and elementary school children aged 5-9 years and adolescents of ages 15-19 (Table 1). From 2014 to 2017, we observed a total of 106 outbreaks of scabies within families and within adult foster care homes, nursing homes and other facilities throughout Croatia. Health care professionals and assistants were among those infected.

3.2 Temporal Pattern of Scabies Incidence in Zagreb, 2014-2017

In the City of Zagreb, the crude incidence rate increased 3-fold between 2014 and 2017 (Figure 2, Table 2), compared with the same period in Croatia. The majority of cases were recorded during winter and autumn.

Table 1. The scabies incidence rates by age group in Croatia in the period 2014-2017.

Year	Age								
	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-59	>60
2014	79.92	75.37	64.57	61.43	31.96	18.99	26.24	23.72	31.41
2015	81.33	81.74	50.98	57.74	32.68	21.40	20.02	20.40	24.82
2016	94.97	95.44	79.01	97.88	49.75	38.14	36.50	37.32	38.78
2017	110.01	98.87	87.93	124.50	60.47	33.83	29.27	34.32	34.61
Average	**91.56**	**87.85**	**70.62**	**85.39**	**43.72**	**28.09**	**28.01**	**28.94**	**32.41**

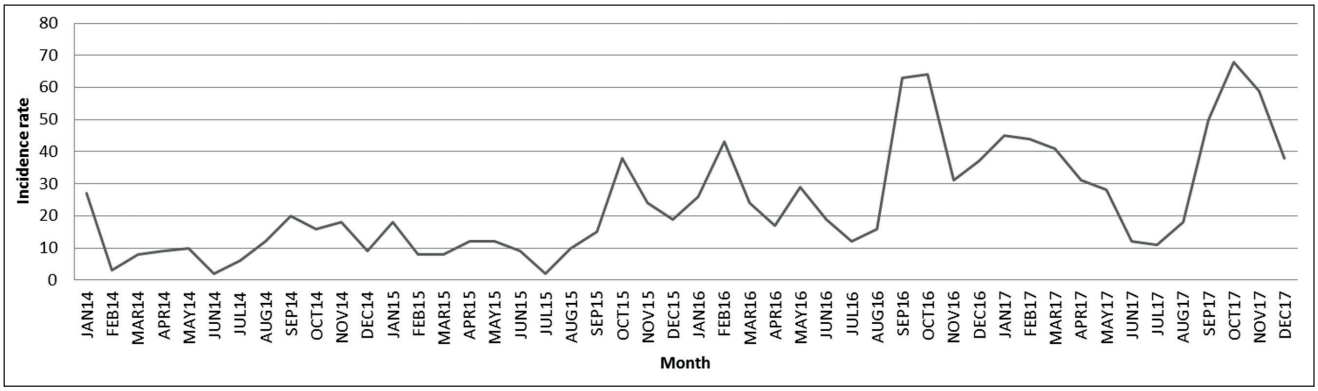

Figure 2. The scabies incidence rates by month in the City of Zagreb, Croatia in the period 2014-2017.

3.3 Spatial Pattern of Scabies Incidence by County and its Relationship to Migration and Tourism

Between 2014-2017, large discrepancies were observed at the county level, with some counties continuously having notably higher incidence rates (Sisak-Moslavina, Vukovar-Srijem, Osijek-Baranja and Međimurje County) or lower rates (Varaždin and Bjelovar-Bilogora County) than the national rate during the same period. In 2017, 2,224 cases were reported in Croatia, corresponding to a crude incidence rate of 51.90/100,000. Notably, four counties (Sisak-Moslavina, Vukovar-Srijem, Osijek-Baranja and Međimurje County) had almost twice the national incidence rate for 2017 (Table 2, Figure 3).

Table 2. The scabies incidence rates by county in Croatia in the period 2014-2017.

	Year			
	2014	**2014**	**2014**	**2014**
Sisak-Moslavina County	43.49	29.58	118.88	109.60
Vukovar-Srijem County	81.88	93.03	118.09	98.59
Osijek-Baranja County	46.55	55.08	96.71	95.73
Brod-Posavina County	45.40	75.04	94.59	20.81
Međimurje County	60.63	97.54	62.39	80.84
Virovitica-Podravina County	30.65	60.12	128.48	56.58
City of Zagreb	17.34	22.53	48.23	53.54
Lika-Senj County	41.24	39.27	35.34	49.09
Zadar County	18.23	26.47	17.06	52.94
Koprivnica-Križevci County	44.12	41.53	18.17	46.72
Karlovac County	83.79	20.17	29.48	42.67
Požega-Slavonia County	39.73	7.69	38.44	35.88
Dubrovnik-Neretva County	48.14	39.16	51.40	39.16
Zagreb County	18.61	20.82	30.28	38.48
Istria County	20.67	13.46	22.59	36.53
Krapina-Zagorje County	21.07	27.84	71.49	34.61
Šibenik-Knin County	28.34	12.80	22.86	32.00
Split-Dalmatia County	45.29	32.98	39.14	33.42
Primorje-Gorski Kotar County	48.95	19.24	22.96	21.95
Varaždin County	22.73	6.82	7.39	15.35
Bjelovar-Bilogora County	17.53	5.84	21.71	27.55
Croatia	**36.00**	**32.90**	**50.60**	**51.90**

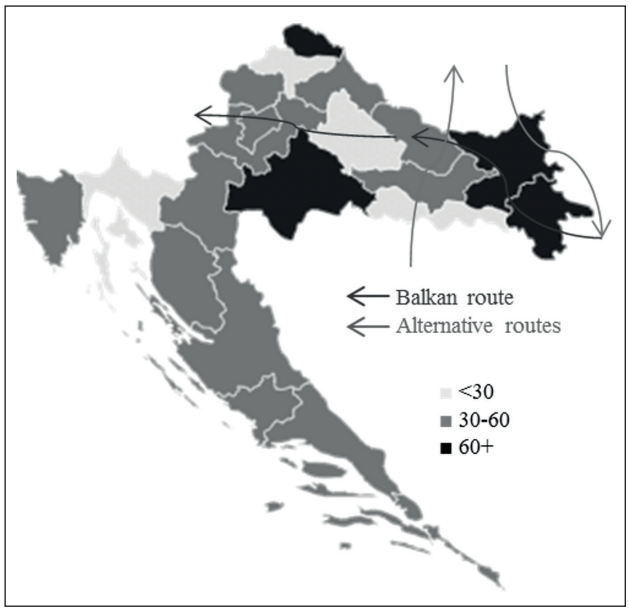

Figure 3. Scabies incidence by county and migration flows, Croatia, 2017. Migration flows data available from Arsenijević et al. 2017 (20) and Arsenijević et al. 2018 (21).

On average, around 40% of outbreaks in the observed period (2014-2017) lasted for several months due to late recognition and diagnosis, particularly among families. Regarding outbreaks of scabies within institutions, more than 80% were in institutions for adults and nearly half of them were recognized and resolved within one month. In Zagreb during the same period we detected 13 outbreaks, two-quarters within families. During autumn 2016, the first outbreak of scabies in an asylum centre (as part of the migrant crisis) was epidemiologically investigated and resolved within three months.

In each of the observed years (2014-2017) there was a large difference in scabies incidence among counties. According this statistical analysis of available data, the scabies incidence was not evenly distributed among counties, and this irregular distribution was statistically significant. Thus, some counties significantly deviated from the expected epidemiological data. In the 2014 $x2=416.95$, $df=20$, $p<0.01$; in 2015 $x2=679.44$, $df=20$, $p<0.01$, in 2016 $x2=919.71$, $df=20$, $p<0.01$, and in 2017 $x2=556.14$, $df=20$, $p<0.01$. The largest discrepancy between the observed and theoretically expected cases of scabies in 2014 were found in Karlovac County (fo=104, ft=46.4), in 2015 in Vukovar-Srijem County (fo=167, ft=59), in 2016 in Varaždin County (fo=13, ft=89.1) and in 2017 in Vukovar-Srijem County (fo=177, ft=93.2).

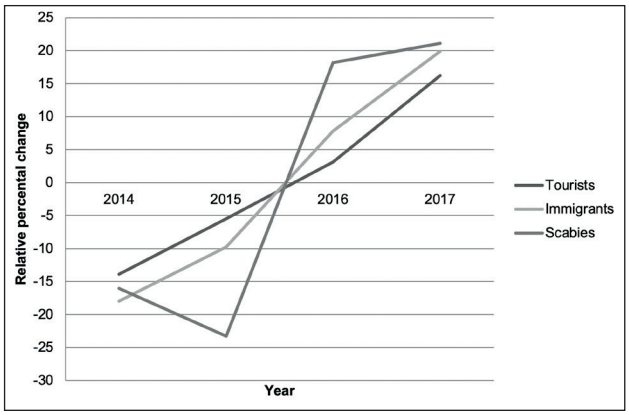

Figure 4. Relative percental dynamics of number of tourists, immigrants, and scabies over the four-year period in Croatia.

Figure 4 represents the percentile change in relative (per capita) number of tourists, immigrants and patients with scabies in Croatia. An almost linear trend of increase can be observed for the number of tourists and immigrants. On the other hand, for the number of patients with scabies, there is a small decrease from 2014 to 2015, then sharp increase from 2015 to 2016, followed by relative stability from 2016 to 2017.

Table 3. Linear approximation of change over time (2014 – 2017) in relative number (per capita) of tourists, immigrants and patients with scabies by counties.

County	Tourists	Immigrants	Scabies
Zagreb County	1.00	0.99	0.92
Krapina-Zagorje County	0.99	0.91	0.74
Sisak-Moslavina County	0.89	0.99	0.92
Karlovac County	0.99	0.96	-0.57
Varaždin County	0.96	0.92	-0.49
Koprivnica-Križevci County	0.88	0.98	-0.46
Bjelovar-Bilogora County	0.93	0.96	0.64
Primorje-Gorski Kotar County	1.00	0.95	-0.70
Lika-Senj County	0.97	0.72	0.15
Virovitica-Podravina County	0.59	0.99	0.70
Požega-Slavonia County	0.97	0.95	0.26
Brod-Posavina County	0.93	0.94	0.96
Zadar County	0.98	0.93	0.48
Osijek-Baranja County	0.98	0.99	0.99
Šibenik-Knin County	0.96	-0.03	0.25
Vukovar-Srijem County	0.99	0.78	0.85
Split-Dalmatia County	0.99	0.96	-0.51
Istria County	1.00	0.97	0.64
Dubrovnik-Neretva County	0.98	0.18	-0.01
Međimurje County	0.65	1.00	0.00
City of Zagreb	0.99	0.95	0.98
Croatia	**0.99**	**0.99**	**0.92**

The results in Table 3 show that the change in the relative number of tourists over observed time period is positive in all counties. In most of the counties the trend is almost linear (close to 1). The time trend of the relative number of immigrants by counties is also positive in most of the Croatian counties. The exceptions are Šibenik-Knin County, with no change (ß=-.033), and Dubrovnik-Neretva County with a small and insubstantial change (ß=-.181). However, the trend of change in the relative number of patients with scabies is not uniform, and varies greatly across the counties – from an evident increase (i.e. Osijek-Baranja County - ß=.990) to relative stability (i.e. Međimurje County - ß=-.003) to a substantial decrease (i.e. Primorje-Gorski Kotar County - ß=-.704).

The correlations between trends of the relative number tourists and relative number of patients with scabies was $\tau=-.052$ ($p=.878$), and between those of the relative number of immigrants and relative number of patients with scabies was $\tau=.153$ ($p=.345$). These insignificant ($p>.05$) correlations indicate that time trends of relative change in number of tourists or immigrants by counties are unrelated to the time trends of relative change in number of patients with scabies in the respective counties.

4 DISCUSSION

4.1 Summary of the Most Important Findings

According to our research and the obtained data, our initial suspicion on the increasing trend in scabies incidence in Croatia was confirmed on the national level in this study. Thus, in the period between 2007 and 2017, a 6-fold increase of the scabies incidence rate was found, and the steepest increase was in the last four 4 years of the analysed period. Most of the scabies cases were among children and adolescents. These results indicate the need to raise awareness among health care providers, especially those working with children and adolescents with itches, with the aim not to misdiagnose scabies.

Concerning the possible associations among scabies incidence, tourists and immigrants, we confirmed an increasing number of tourists and immigrants in the period 2014-2017. However, time trends of the relative change in number of tourists and immigrants by counties were not related to the relative change of scabies infestations. Although statistical significance was not present, the highest scabies incidence rates were recorded in Međimurje and Vukovar-Srijem counties, which indicates moderate influence of the proximity of the border and influence of movements of people on scabies outbreaks.
Concerning seasonal variations of scabies, our data for the City of Zagreb (monitored over a period of four years, 2014-2017) showed that infestation counts multiplied in winter and autumn months, thus confirming an influence of seasonal trends on scabies outbreaks. Therefore, our results may be a basis for the planning of further preventive measures.

4.2 Comparison of Study Findings with Reports in the Literature

According to the data in the literature, worldwide, scabies affects an estimated 100 million people across all continents and countries. Regardless, scabies is not a priority on the global health agenda, even though interest in the disease is increasing (11). However, outbreaks and management vary from country to country, and thus priority and management do as well (22).

Several trends and related factors have been found to be important for scabies outbreaks. Thus, a strong connection among scabies, migration and travel has been reported (11, 12, 15, 23). Since scabies moves with people, mass migration due to political and socioeconomic factors can promote its spread, as seen recently with the influx of asylum seekers arriving in Western Europe from high-prevalence countries (11, 12). Since scabies is transmitted by close person-to-person contact, it is logical that outbreaks have been reported in reception centres for asylum seekers across Europe (11). Previous studies claim that immigrants are at a higher risk of acquiring common communicable diseases compared to the native Western European population (23). Although most refugees in Croatia are only in transit, refugee and migration flows can still have an impact on the occurrence of scabies in the country. A recent study suggested that scabies cases rose at the time when refugees and migrants were intensively coming from the Middle East to Western Europe via the Southeastern European route (Turkey-Greece-Serbia-Croatia-Slovenia-Austria) (15). Data presented in this study supports this observation, since an approximately 6-fold increase in scabies incidence was confirmed in Croatia between 2007-2017. According to our results, local geography could have a great influence on this, as indicated by the increased scabies incidence in areas close to the borders with Serbia and Bosnia and Herzegovina, and those along refugee migration routes. Late recognition and the delay of proper treatment, along with an increase in regular and irregular migration from neighbouring countries with high incidence rates of scabies (Bosnia and Hercegovina, Kosovo, Serbia), likely caused this upward trend in scabies cases in Croatia during this time.

According to data from Germany, the prevalence of scabies is higher among refugees than in the general population; however, the risk of outbreaks themselves is not high (24). Recent analysis of the health status and disease burden of unaccompanied asylum-seeking adolescents in Germany (cross-sectional pilot study) showed the highest prevalence of infestation in sub-Saharan Africans (86.7%), including the highest prevalence of parasites in general (46.7%), with a higher disease burden among females (25). Similar results were obtained by Kortas et al., who found a total of 47.7% of the subjects with scabies infestations originated from Afghanistan and 25.0% from Eritrea (26). Consequently, there is a need for thorough medical and psychological screenings after the arrival of such individuals in order to reduce the individual disease burden and risk of infestation for others. It is also necessary to lower barriers to health care access for unaccompanied asylum-seeking minors and allow for need-specific health care and prevention (27).

Significantly, another possible cause for the increase could be Croatia's 2013 admission into the European Union and the consequent increase in travel to the country (23). An important, related factor could be the increase in Croatia's tourism rate over the last few years, which might indicate the influence of travel and tourism on the scabies rate. Our results indicate linear increases in the number of tourists, immigrants and scabies infestations on the national level for the analysed period, although with no statistically significant association. According to a previous study of skin disorders among travellers returning from tropical and non-tropical countries, more than 20% of all such disorders were caused by arthropods

and about 50% by infectious pathogens (27). As such, pre-travel consultations should include specific prophylaxis and consider the most common risk factors for each destination (28).

Moreover, close contact among young people during travel, concerts and festivals can influence scabies rates. Large summer concerts and festivals have become popular in Croatia in recent years, and it is possible that casual sex and poor hygiene in these crowded conditions contribute to incidence rates (29). This corresponds to our results, as they have confirmed that young people predominated among scabies patients. More public attention should thus be directed to the prevention of scabies among youth, especially due to their lifestyles. Appropriate hygiene in overcrowded places, such as mass gathering events, is also important. According to a previous retrospective analysis (data from 145 countries) of the infection burden from inadequate water, sanitation and hygiene in low- and middle-income settings, there is a need for better risk reduction, including the provision of reliable piped water, treatment of community sewage and awareness of hand hygiene (30).

Seasonal variations should be also mentioned, since a connection between scabies occurrence and weather conditions and the seasons was observed (31). According to previously published data, scabies infestations are especially common in winter (31). Thus, Liu et al. monitored the influence of temperature on scabies incidence and observed that, overall, the incidence of scabies was negatively correlated with temperature but positively correlated with humidity (30). Our results support the seasonal character of scabies, as the majority of cases were recorded during winter, but also in autumn (Zagreb). It is possible that increased scabies infestation in winter is a result of closer contact between people in indoor environments. It should also be considered that scabies cases are often reported weeks or even months after the patient has contracted it. For example, a case is only reported in winter months when the patient first sees their physician, when the symptoms are troublesome enough, but the infestation could actually have started in the summer or autumn, coinciding with the patient's travel during vacation.

Considering the data in the literature, it is also important to mention an increased risk for outbreaks at nursing homes and extended-care facilities since elderly persons tend to develop crusted scabies due to disease- or medication-related immunosuppression (24, 32). According to a previous smaller study conducted in the City of Zagreb, 10% of scabies cases were found in medical health personnel, predominantly in those working in nursing homes and psychiatric wards (33). In our study, we found a prominent number of scabies outbreaks within families, adult foster care homes, nursing homes and other facilities throughout Croatia. Our results indicate a need for increased caution and preventive measures for these populations and settings. Since health care professionals and assistants were among those infected, focus should be also on appropriate hygienic measures while working with patients.

Unfortunately, although scabies is a major public health problem and causes a significant disease burden worldwide, there are no agreed-upon international diagnostic guidelines (34). Thus, we can mention that due to the frequent setting of the diagnosis based only on the clinical picture, it is difficult to know the real number of scabies cases. Thompson et al. found that only 56% of clinical trials in medical databases specified which clinical findings were used for diagnosis (predominantly rash, rash distribution, pruritus and mite burrows) (35). According to their findings, parasitological testing was used in 63% of trials, more frequently in clinic-based than in field studies, and nearly one-quarter of trials (24%) did not perform diagnostic methods at all, which can lead to the further spread of scabies. However, in field conditions, the psychological and physical profiles of scabies patients may help in the early recognition of scabies, which is very useful, especially at peripheral care centres (36).

4.3 Limitations and Strengths of the Study

This retrospective, observational study, based on the official data presents the first national research regarding scabies incidence in Croatia, with findings based on the obligatory report data collected from all health care institutions in Croatia (for the years between 2007 and 2017). To the best of our knowledge, this is the first recent epidemiologic analysis on this topic, not only in Croatia but within the wider geographic region as well.

However, the limitation of this study is the lack of detailed data regarding the trend of scabies incidence throughout the years, since health care providers who send the report about communicable disease do not always answer all the questions in the report form. Therefore, we provided a more detailed analysis for the City of Zagreb (the capital of Croatia), since it accounts for one-quarter of whole Croatian population and has the highest number of hospitals and medical professionals at the national level. This study also found a possible impact of changes in the Croatian population, for example due to migration. In addition, we could only assume that health care providers reported all the scabies cases for the analysed period, although this might not have happened.

4.4 The Importance of the Study for Public Health and Other Professions in Croatia

The importance of this study is to emphasize the need for the implementation of existing standardized guidelines

with the aim early recognition and timely reporting of scabies. Furthermore, despite a universal health care system that provides mandatory health insurance for all residents, there is some inequality in health status in Croatia, similar to as in neighbouring countries such as Slovenia (37).

Late recognition of scabies by physicians also presents a problem, and is likely linked to the country's previously low rate – some medical doctors had not even seen scabies cases before, so did not think to suspect this infestation. It should also be remembered that some infected persons can have false negative results, leading to problems in practice when patients are treated ineffectively (38). When initial cases of scabies go undetected, these infected persons are then not able to prevent the spread of the disease in their immediate environments. Furthermore, there are many examples of non-compliance with treatment in practice. It is thus necessary to train general practitioners and medical staff on the early recognition, proper and timely diagnostics and notification of first cases. Special attention should be directed to the age groups with increased occurrence of scabies, such as children and adolescents. In addition, knowledge of basic hygienic measures is necessary from early childhood, and should be provided by kindergarten teachers (39), parents and other members of society. The epidemiology service has an important role in identifying contacts and applying preventive measures, stressing the necessity of conducting therapy and scabies management. Likewise, epidemiologists can do this only if they receive timely notification of the first sporadic case or early notification of an outbreak. Therefore, coordinated efforts between different sectors and collaboration among a range of health care providers (dermatologists, physicians and epidemiologists) is crucial (40).

4.5 Possibilities for Future Research in the Field

This study opens a new perspective on the transmission dynamic for this important but neglected communicable disease. Further research should be focused on the relationship between scabies incidence and other factors, such as tourism, both in Croatia and elsewhere. More attention should be directed to the research of scabies incidence in those countries with an increased number of movements, especially those with a rapidly growing annual number of tourists and migrants. In addition, wider epidemiological studies should be conducted internationally in order to compare trends among countries and regions. More research on scabies in clinical practice is recommended, with the focus on other possible related factors on scabies outbreaks and misdiagnosing of this disease.

5 CONCLUSION

The increased incidence of scabies, large disparities between counties, and prolonged outbreaks within families due late recognition and misdiagnoses points to a need for increased awareness among health practitioners on the occurrence of scabies in Croatia. For this, timely collaboration among general practitioners, health care professionals in resident and nursing homes, dermatologists and epidemiologists is crucial. The provision of timely diagnostics and treatment is a necessity, along with strict adherence to infestation control measures. Patient follow-ups and the prophylactic treatment of household members, patients, and staff who have had prolonged skin-to-skin contact with scabies cases is crucial in order to avoid outbreaks.

DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

FUNDING

None.

ETHICAL APPROVAL

No identifiable human data were used for this study, and the dataset used is not openly available.

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BARRIERS AFFECTING THE ORAL HEALTH OF PEOPLE DIAGNOSED WITH DEPRESSION: A SYSTEMATIC REVIEW

OVIRE, KI VPLIVAJO NA USTNO ZDRAVJE LJUDI Z DIAGNOZO DEPRESIJE: SISTEMATIČEN PREGLED

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Received: Dec 4, 2019
Accepted: Sep 17, 2020

Review

ABSTRACT

Introduction: The problems of oral health of people diagnosed with depression are not adequately recognized, either in developed or developing countries. Social stigma, lack of self-interest, or even inadequate approaches of dental doctors towards the unique situation of this group of people this lead to excessive oral health problems.

Methods: The bibliographic database PubMed/Medline, Google Scholar, and Whiley online library were searched using the following text and MeSH as separate key terms and in combination: depression and oral health/dental caries/periodontal disease/tooth loss/utilization of oral health services/and barriers. The content of documents was analysed using qualitative methodology.

Results: Twenty-six original studies were included in the review. Level/severity of depression, medication and medical comorbidity are the most important medical barriers influencing the oral health of people diagnosed with depression. Dental fear and anxiety are mostly combined with low oral hygiene and bad oral health. Socio-economic status, dental insurance, bad habits and education also have important roles in the oral health status of people diagnosed with depression.

Conclusion: Including individuals with depression and oral health problems in national health programs, creating specific prevention programs, or subsidizing the cost of treatment are some of the recommendations suggested as solutions.

Keywords:
depression, oral health services, oral health, barriers

IZVLEČEK

Uvod: *Težave z ustnim zdravjem pri ljudeh z diagnozo depresije niso ustrezno prepoznane niti v razvitih državah niti v državah v razvoju. Družbena zaznamovanost, pomanjkanje zanimanja zase ali celo neustrezen pristop zobozdravnikov do edinstvene situacije pri tej skupini ljudi povzročajo čezmerne težave z zdravjem ustne votline.*

Metode: *Bibliografska podatkovna zbirka PubMed/Medline, Googlov učenjak in spletna knjižnica Whiley so bili preiskani z uporabo naslednjega besedila in izrazov MeSH kot ločenih ključnih izrazov in v kombinaciji: depresija in ustno zdravje/zobni karies/parodontalna bolezen/izguba zob/uporaba zobozdravstvenih storitev/in ovire. Vsebina dokumentov je bila analizirana s kvalitativno metodologijo.*

Rezultati: *V pregled je bilo vključenih 26 izvirnih študij. Stopnja/resnost depresije, zdravila in pridružene bolezni so najpomembnejše zdravstvene ovire, ki vplivajo na ustno zdravje ljudi z diagnozo depresije. Tesnoba in strah pred zobozdravstvenimi posegi sta večinoma povezana s slabo ustno higieno in slabim zdravjem ustne votline. Pri ustnem zdravju ljudi z diagnozo depresije imajo pomembno vlogo tudi socialno-ekonomski status, zobozdravstveno zavarovanje, slabe navade in izobrazba.*

Sklep: *Vključitev posameznikov z depresijo in težavami z ustnim zdravjem v nacionalne zdravstvene programe, oblikovanje posebnih preventivnih programov ali zagotavljanje ugodnejših cen storitev so nekatera priporočila, ki so bila predlagana kot rešitev.*

Ključne besede:
depresija, zobozdravstvene storitve, zdravje ustne votline, ovire

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1 INTRODUCTION

According to the World Health Organization, more than 300 million people of all ages are suffering from depression, but mainly people between 15 and 30 years, and this contributes in a significant way to the global disease burden (1, 2).

Depression is a mood disorder that may be caused by diverse internal and external factors: changes in brain chemistry, family history, or traumatic life experiences (3). This mood disorder is more than just a of feeling sadness, as it includes insomnia, weak concentration, and lost interest in everyday activities that could diminish concern for a one's general and oral health. Moreover, depression is often linked with bad habits like eating disorders, smoking, and drug and alcohol abuse (4-6).

The bad oral hygiene in people with depression is linked with the fact that dental fear is more common in this group (7, 8). There are many studies showing that the level of oral hygiene is insufficient among people with depression, who thus have a higher rate of dental cavities compared to a general population. The inflammation of the gums caused by accumulated dental plaque can lead to problems like halitosis, periodontal disease development and eventually tooth loss (9). Antidepressants used in the treatment of this mental disorder may cause xerostomia, trouble in swallowing, bruxism, and an increase the number of salivary lactobacilli, which causes tooth decay and consequent gum diseases (10). As such, keeping good oral health can improve the quality of life in people with depression, and also have a positive effect on the government budget and out-of-pocket health care spending (5).

The treatment of depression could be improved by investing more resources (11), and adopting an interdisciplinary approach to this condition (12, 13). However, data about barriers influencing the oral health of people with depression, and the possibilities for improvement, is still lacking (14-17). Existing systematic research papers and original research have reported on one or a few contributing factors of weak oral health status in depressed people (18, 19). However, most such studies are based on cross-sectional data giving information just on the current situation, and most do not follow the long-term expression of depression as a chronic illness (20, 21). In order to provide evidence for opportunities to improve oral health in patients with depression, the objective of the current study was to summarize the literature on barriers that could lead to an increase in pathological dental conditions of people in this group.

2 METHODOLOGY

2.1 Document Sources

The bibliographic database PubMed/Medline was searched using the following text and MeSH as separate key terms and in combination: depression and oral health/dental caries/ periodontal disease/tooth loss/utilization of oral health services/and barriers (22). Google Scholar and Whiley online library were searched for more related articles (23, 24). All articles returned after every combination of keywords were reviewed, initially by abstract content and then if they were in line with the subject of this study they were included in the data.

2.2 Method of Document Identification and Assessing the Quality of Studies

The analyses of articles was done using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement checklist guidance and study flow diagram produced based on the PRISMA recommendations. We used a qualitative methodology, as tis is highly recommended for systematic reviews, such as this one (25).

2.3 Methods of Inclusion/Exclusion of Documents

We included studies with a focus on a primary diagnosis of depression that also considering oral health conditions and barriers. Lifetime diagnoses and current diagnose of depression were both included. We considered studies on the adult population, and thus all participants in the included studies had to be 18 years and over, without any other psychiatric disorder. In the search strategy used with the online medical libraries, the following inclusion criteria were defined: clinical trials, abstracts and original articles (excluding review articles) available in full text, performed on humans, in the English language and not older than twenty years.

2.4 Content for Extraction

Information extracted from the original articles were references, country, year of the study, study design, age of the observed population, number of depressed participants, medical barriers, psychological barriers and other barriers.

3 RESULTS

3.1 Presentation of Observation Units

The search of PubMed returned 671 articles. This was carried out using a combination of different keywords with a Boolean operator for each word combination, as presented in the study flow diagram. By following the inclusion and exclusion criteria explained in the method section the number of articles fell to 135, and

after carefully examining the results all duplicates were removed. The final number of articles from the PubMed search was thus 13. The search of the Whiley online library and Google Scholar was performed, and an additional 13 articles were found. The final number of studies included in the research was thus 26.

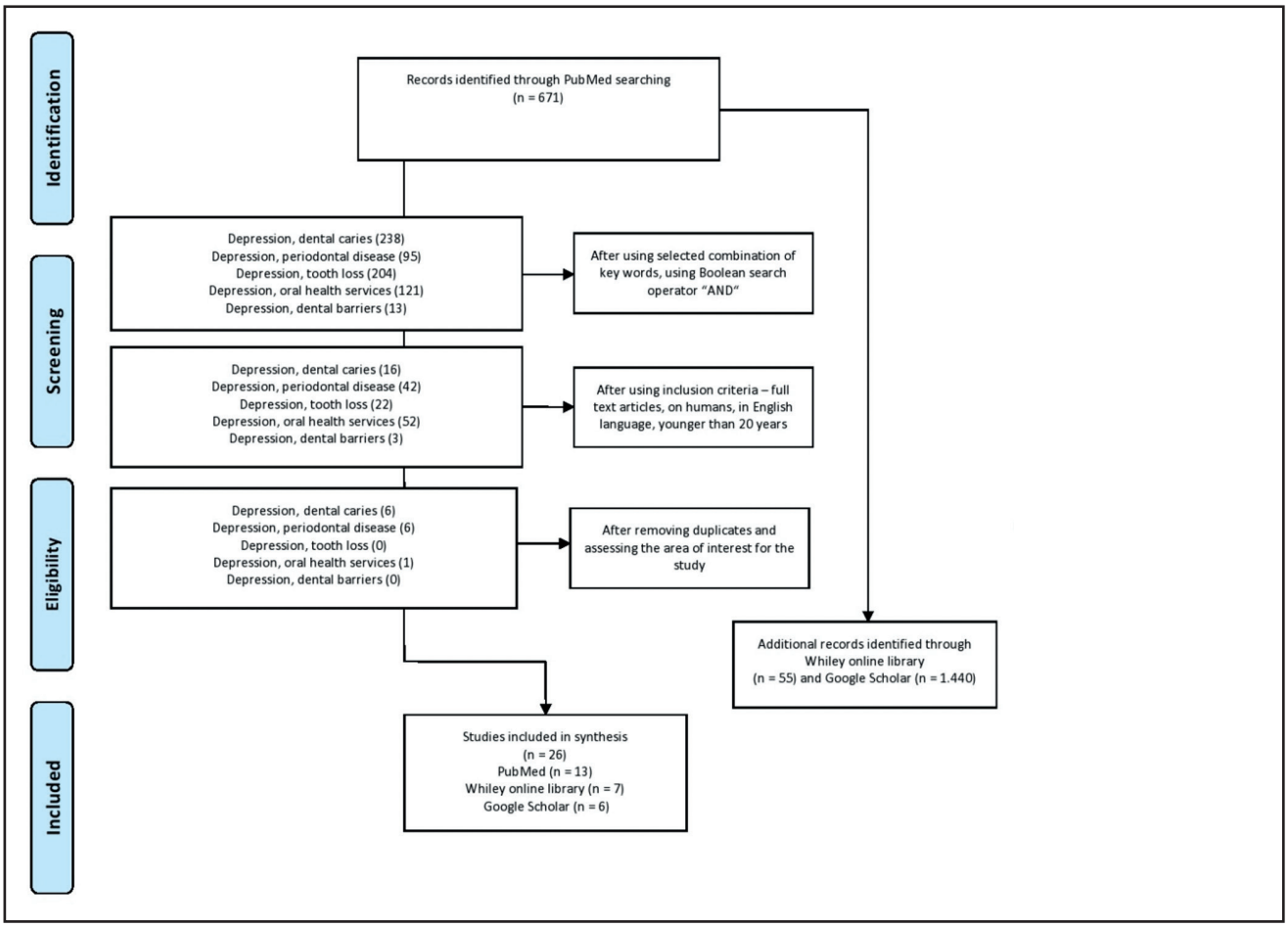

Figure 1. Flowchart of the course of the selection of documents in the systematic review of the literature on barriers affecting the oral health of the people diagnosed with depression, following the PRISMA.

3.2 Characteristics of the Studies Included in the Systematic Review

We examined the year of research, population age, number of participants, and country where the research was conducted, along with barriers. For this research, we sorted the barriers that influence oral health in depressed people into three groups – medical, psychological and other (Table 1). Medical barriers are those affecting the oral health that are an integral part of depression: duration of the depressive episode, the clinical expression of depressive symptoms, the number of depressive episodes and number of hospitalizations, response to treatment, side effects of prescribed medications and observed comorbid somatic states. The psychological barriers are connected to the individual patient opinions, emotions and decisions: lack of self-esteem, dental fear, opinions about dental health, self-perceived general and oral health. The other barriers are the widest group, and include the socio-demographic and socio-economic characteristics as well as the physical barriers and habits of the individual, such as last dental check-up, possessing any kind of dental insurance, oral health habits, eating habits, smoking or drinking, education level, material and marital status, gender, cost of dental treatment, geographical availability of the service and similar.

Table 1. Selection of documents in a systematic review of the literature on barriers affecting oral health of people diagnosed with depression listed by reference, country, year of study, study design, age, number of participants, and observed barriers.

Module name	Country	Year of the study	Study design	Age of the observed population	No. of depressed participants	Medical barriers	Psychological barriers	Other barriers
Delgado-Angulo et al. (26)	Finland	2015	cross-sectional study	30 years and older	1.229	level/severity of depression	/	consummation of sugar, number of tooth brushing, non-smokers, number of regular check-ups
Jin Park et al. (27)	Korea	2014	cross-sectional study	19 years and older	6.139	level/severity of depression	self-perceived oral health	/
Marques-Vida et al. (28)	Portugal	2006	cross-sectional study	average age 21	388	level/severity of depression	anxiety	/
Skośkiewicz-Malinowska et al. (29)	Poland	2018	cross-sectional study	over 65 years	500	level/severity of depression	/	socio-demographic
Houtjes et al. (30)	Netherlands	2011	cross-sectional study	58 years and older	99	level/severity of depression, physical health, medication	self-perceived oral health	socio-economic characteristics
McFarland et al. (31)	US	2010	cross-sectional study	19 years and older	399	level/severity of depression	self-efficacy	oral health behaviour
Heaton et al. (32)	US	2013	cross-sectional study	21 years and older	2.024	/	/	age, income, insurance
Teng et al. (33)	Taiwan	2016	cross-sectional study	18 years and older	7.625	/	/	income, dental insurance
Okoro et al. (34)	US	2012	cross-sectional study	18 years and older	19.397	level/severity of depression	/	/
Pohjola et al. (35)	Finnish	2011	cross-sectional study	30 years and older	644	/	dental fear	socio-economic and socio-demographic
Persson et al. (36)	Sweden	2010	cohort study	18 years and older	10	/	dental fear, low self-care	/
DiMatteo et al. (37)	-	2000	cohort study	-	-	/	noncompliance	/
Bernson et al. (38)	Sweden	2012	case-control study	18 years and older	404	/	dental anxiety	socio-demographic
Hugo et al. (39)	Brazil	2006	cross-sectional study	50 years and older	230	/	lack of self-care, stress	bad oral habits
Sasaki (40)	Tokyo	2005	case-control study	average age 50	36	medication	lack of self-care	/
Yamamoto et al. (41)	Japan	2017	cohort study	65 years and older	872	/	self-esteem	/
Anttila et al. (42)	Finland	2006	cohort study	31–32 years old	1.920	/	self-perceived oral health	education, income, tooth brushing frequency
Hybels et al. (43)	US	2015	cohort study	65 years and older	944	level/severity of depression	self-perceived oral health	/
Mago and Thyvalikakath (44)	Canada	2014	cross-sectional study	45 years and older	2.162	/	/	age, marital status, education level, dental insurance, income
Adeniyi et al. (45)	Nigeria	2011	cross-sectional study	mean age of 39.2	105	/	/	inadequate approach, income, socio-demographic

Module name	Country	Year of the study	Study design	Age of the observed population	No. of depressed participants	Medical barriers	Psychological barriers	Other barriers
Shippee et al. (46)	Minnesota	2012	cross-sectional study	18 years and older	475	/	self-perceived oral health	appointment problems, work/ family responsibilities, transportation, education
Nguyen et al. (47)	US	2018	cross-sectional study	18 years and older	1.778	general health status	/	without insurance
Malecki et al. (48)	Wisconsin	2015	cross-sectional study	21 years and older	263	level/severity of depression	/	insurance, oral habits
Wiener et al. (49)	West Virginia	2018	cross-sectional study	21 years and older	2.374	/	/	socio-economic, socio-demographic, insurance, oral habits
Anttila et al. (50)	Finland	2001	case-control study	55 years	780	/	self-perceived oral health	smoking
Simon and Von Korff (51)	US	2006	cross-sectional study	Mean age of 50	439	medical comorbidity	/	/

4 DISCUSSION

4.1 Effects of Medical Barriers

Recent research on the oral health of people with depression showed that this group suffers from high levels of caries, periodontal disease and tooth loss. Due to the effects of depression, including lack of self-interest, applied medications, difficulty of accessing dental services, fear and inappropriate approach of dental doctors, dental caries and periodontal disease are the most frequent oral and dental diseases (19).

Depression severity is a very important factor and has a direct correlation with an increased number of dental caries. Delgado-Angulo found that adult depressed people had 25% more decayed teeth than non-depressed adults, and the highest incidence of dental caries was among depressed adults in the age group of 35-44 (69% more dental caries than non-depressed adults). Certain habits (less consumption of sugar, an average number of tooth brushings, not smoking) were at a satisfactory level in this group, but the number of regular dental check-ups should be higher and the amount of visible dental plaque should be lower (26). Jin Park et al. compared depressed people with a control group and showed that those with a lifetime diagnoses of depression brushed their teeth once or less a day (15.8%), had bad self-perceived oral health (52.3%), had a toothache more (31.5%) and a higher rate of periodontal bleeding (in both jaws) (27). Other research concluded that depressed respondents had higher odds of gum bleeding (4.96 times) (28).

There are many studies dealing with oral diseases among elderly depressed people. It is important to be note here that depression is not a condition that occurs among older people as an integral part of the aging process. However, depression in the elderly is usually followed by bad overall health and could also lead to serious damage to the oral system (high number of dental caries, more progressive periodontal disease and more tooth loss). Skośkiewicz-Malinowska et al. found that all depressed adults aged over 65 years had dental caries, and that as the severity of depression increased the number of missing and decayed teeth became larger (29). In Houtjes et al. most of the subjects were female (67%) and the major finding was that people with late-life depression had a higher percentage of unmet needs, which was explained by the impact of depression severity (30).

The study conducted by McFarland et al. concluded that the severity of depression had a significant impact on general oral health and oral health behaviour, with an average of 1.52 decayed teeth, 8.41 filled teeth and 3.23 extracted teeth, and the depressed subjects' oral health was poorer than in non-depressed patients, while the frequency of brushing and flossing teeth was significantly lower (31). Additionally, mental illness contributes to the lower utilization of dental services, which is in correlation with the severity of depression. Other studies suggest that only 40% of people with mental illnesses visit dentists, among which the most regular visitors were patients with mood disorders, but they also were the group with the lowest utilization of dental services (32, 33). In Okoro et al. 24% of respondents had depression, 8% of them had current depression and 16% with lifelong depression, and the results shows that people with lifelong depression had a higher likelihood of having at least one tooth extracted, a1.38 times greater chance not visiting a dentist or having dental cleaning in the past few years in comparison with the control group (34).

From a physiological perspective, sympathetic stimulation could also reduce the salivary flow and is observed as a part of the underlying mechanisms in depression

pathophysiology. Additionally, the tricyclic antidepressant affects the salivary process by blocking parasympathetic stimulation of the salivary gland. Similar patterns were observed in some of the medications for the treatment of comorbid cardiovascular and metabolic diseases, and they could all lead to xerostomia and thus oral diseases. Anhedonia in depression also leads to the low oral hygiene and accumulation of dental plaque that causes caries and periodontal disease (20).

4.2 Effects of Psychological Barriers

A high association between excessive dental fear in patients with the diagnosis of depression was found in many types of research (35, 36). Moreover, depressed patients don't visit a dentist regularly and have poor adherence to dental treatment due to their lack of self-care (37, 38). Root caries are a common localization of caries in older depressed patients and that could be explained not only by the insufficient amount of tooth brushing, but also by an inadequate tooth brushing technique due to a general lack of self-care (39, 40).

Lost and decayed teeth may cause problems with talking and chewing, and also affect the self-esteem of those with depression, which is already low, which are further contributing factors to social isolation, and Kisely et al. noted that all these factors eventually have an adverse impact on the life quality of patients with depression (21). Finally, having problems with smiling because of missing teeth as well as any other oral health problems may also reduce self-esteem and have effects on communications, which all may lead to worsening of depressive symptoms, especially in elderly people (41).

The association of dental health behaviour and self-perceived dental needs with depression were investigated in a population aged 31 and over (mild depression and depression summed to 22%), and symptoms of depression (mild symptoms), gender (female), education level (college degree) and family income (low) were associated with poorer frequency of teeth brushing and regularity of dental check-ups (42). Similar results were also found in a group aged 65 and older, which showed that even a moderate level of depression affects the self-perceived oral health of patients (health of the gum, decayed tooth, lost tooth, periodontal problems) (43).

4.3 Effects of Other Barriers

Mago et al. concluded that depressed patients had a 1.34 greater likelihood of never utilizing oral health services, especially males, in older age, widows/divorced, with post-secondary education, without dental insurance and with a self-perceived low income (44). Adeniyi et al. noted a high level of unmet dental needs in people with depression, identifying a potential problem in the oral health system with insufficient attention being paid to this group (45). Another study also found that about 25% of people with diagnosis of depression had unmet oral needs, with the main reasons being that 36.4% couldn't get a dental appointment, 27.7% had some work/family responsibilities and 26.6% had transportation issues (46).

Using a different methodological approach, i Nguyen et al. reported that 62.6% of people who did regularly see dentists reported some problem with their teeth and gums (toothache, sensitive tooth, bleeding gum, missing tooth) in the previous six months, and about 54% of all examinees had some mental illness (including depression) (47). The same study found that 27% of people with depression did not have insurance that covers dental health needs, while they had a 1.6 times greater chance of having some acute dental needs (47). In Malecki et al. 47.7% of respondents did not have insurance that covers dental needs and 29.4% did not have any kind of insurance, with every indicator of poor oral health being higher as the depression severity progressed (48). The main reason for the high unmet dental needs in this group was the cost of dental care.

A recent study by Wiener et al. concluded that female gender, low income, education level under college degree, irregular dental visits and a large number of untreated crown dental carries are more likely to be associated with more severe symptoms of depression (49). Lifestyle and eating habits are also common variables that are examined in those studies where a connection between depression and oral health was found. Depressed people often smoke for comfort, as well as consume more sweets. Smoke reduces the salivary flow and the regular consumption of sweets can decrease pH in the mouth, thus increasing the chances of dental and oral diseases (50). If depression is unrecognized by healthcare professionals then this will result in a higher rate of dental diseases, as one of the many health issues that depression may lead to if it's not prevented or/and adequately treated (51).

4.4 Limitations and Strengths of the Systematic Review

One limitation of this systematic review is the lack of standardized oral health indicators in the studies it examined (mostly expressed as the number of cavities, missing tooth or tooth with periodontal disease, rather than universal indexes) and insufficient comparison of the oral health status of people with depression between countries at different development levels. An additional limitation is that only some of the relevant bibliographic databases were searched.

wfurther research, and may also suggest some necessary improvements in health policy systems for the benefit of those with depression, or help in the development of special programs that will cover the specific needs of this group in a more effective way. It is also recommended that

a larger study with a longer follow-up period is conducted that collects information about the oral health status of this vulnerable category of people, which can then be used to drive better health care policies. Studies like this are currently lacking, but could lead to the creation of preventive programs for depressed individuals.

4.5 Importance of the Systematic Review for Oral Public Health

This systematic review has considerable public health importance because it presents a summation of the latest research in this area, and highlights the most important barriers that lead to inadequate or insufficient use of oral health care by people diagnosed with depression.

4.6 Possibilities for Future Research in the Field

This review article presents many possibilities and ideas for future research with regard to the oral health of people with depression, noting the preventability of the barriers that influence the oral health of this group. Using the standardized oral health indicators in future research would be helpful in collecting the numerical data that could be useful for a better comparison of results between countries, as well as inside countries, and these are two possibilities for future research.

5 CONCLUSION

It is important that most of the barriers affecting the oral health of people with depression be identified and then mitigated depending on their preventability. Many of the barriers identified in this study can be predicted and prevented through adequate and interdisciplinary and multidisciplinary approaches from both doctors and governments.

Raising the awareness of people with depression in the area of oral health through continuous education of such individuals, along with medical staff and doctors, would also result in a higher number of regular dental check-ups, which would consequently reduce the number of more radical and expensive dental treatments needed, and benefit the quality of the patients' lives overall.

The health care policies adopted by governments should be adapted for the specific needs of people with depression, including consideration of them in national programs for oral health or providing dental service benefits to reduce the costs. It should be the responsibility of every country, depending on the level of development and resources, to work towards reducing the burden of depression.

CONFLICTS OF INTREST

The authors declare that no conflicts of interest exist.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ETHICAL STATEMENT

Not required.

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Priporočamo uporabo videoposnetka z navodili za avtorje. Prispevke oddajte v elektronski obliki s pomočjo spletne aplikacije Editorial Manager, ki se nahaja na spletnem naslovu http://www.editorialmanager.com/sjph/. V uredništvo sprejemamo po pošti le še Izjave o avtorstvu in avtorskih pravicah, ki zahtevajo lastnoročni podpis. Prosimo, da jih pošljete hkrati z elektronsko oddajo prispevka na naslov: Nacionalni inštitut za javno zdravje, za revijo Zdravstveno varstvo, Trubarjeva 2, 1000 Ljubljana.

V spletno uredniško aplikacijo se prijavite kot ‘avtor’. Prva prijava zahteva vnos podatkov o avtorju, vse naslednje prijave pa le še vnos podatkov za prijavo, ki jih na svoj elektronski naslov prejmete po prvi prijavi v sistem.

Po uspešni prijavi izpolnite vsa zahtevana strukturirana polja. Potrdite izjavo, da vaš prispevek še ni bil objavljen ali poslan v objavo kakšni drugi reviji, da so prispevek prebrali in se z njim strinjajo vsi avtorji, da so raziskave na ljudeh oz. živalih opravljene v skladu z načeli Helsinško-Tokijske deklaracije oz. v skladu z etičnimi načeli.

Avtorji, ki v objavo pošiljate raziskovalno delo, opravljeno s pomočjo nekega podjetja, to navedite na koncu rokopisa v izjavi o financiranju. Navedete lahko tudi do dva neželena recenzenta.

Polje 'Comments' je namenjeno obveznemu predlogu treh recenzentov z imeni, nazivi, e-naslovi in zaposlitvijo. Navedete lahko tudi do dva neželena recenzenta.

Podatke o avtorju in soavtorjih vnesite kar se da natančno in popolno. Naveden naj bo korespondenčni avtor (s polnim naslovom, telefonsko številko in elektronskim naslovom), ki bo skrbel za komunikacijo z uredništvom in ostalimi avtorji.

Jezik prispevka je angleščina. Objavljamo izvirne znanstvene članke, sistematične pregledne znanstvene članke, metodologije raziskav in vabljene uvodnike. Pri izvirnih, metodoloških in sistematičnih preglednih znanstvenih prispevkih morajo biti naslov, izvleček in ključne besede prevedeni tudi v slovenščino.

Naslov, ključne besede in izvleček se oddajajo dvojezično v angleščini in slovenščini v strukturirana polja. Posebno polje za zapis v drugem jeziku obstaja le za izvleček, preostale podatke vnesite v obeh jezikih v ustrezno isto polje. Prvi izvleček je vselej v angleškem jeziku (do 250 besed - sistem vam besede sproti šteje), drugi pa v slovenskem jeziku (razširjen izvleček - do 400 besed).

Po vnosu strukturiranih podatkov oddajte še priponko - rokopis (od 1 Uvod naprej), ki ne sme zajemati podatkov, ki ste jih vnesli že pred tem v strukturirana polja, zlasti ne podatkov o avtorjih. Ime datoteke ne sme vključevati avtorjevih osebnih podatkov, prav tako ne imen ustanov, vključenih v pripravo rokopisa. Grafično in slikovno gradivo je kot ves rokopis v angleškem jeziku. Vključite ga v besedilo na mesto, kamor le-to sodi in ga opremite z naslovom. Oddate torej le en sam dokument, eno priponko. V Wordu uporabite možnost Postavitev strani/Številke vrstic (tako bo na robu vsake vrstice dokumenta dodana številka vrstice).

Pri oddaji sledite napotkom, ki vam jih ponuja sistem, pomagate pa si lahko tudi z 'Editorial Manager's Tutorial for Autors'.

Sistem najbolje deluje, če uporabljate zadnjo različico Acrobata.

Če pri oddajanju rokopisa naletite na nepremostljive težave, se za pomoč obrnite na naslov uredništva: zdrav.var@nijz.si.

V nadaljevanju podajamo še nekaj natančnejših napotkov.

ROKOPIS

Besedila naj bodo napisana z urejevalnikom Word for Windows 97-2003. Robovi naj bodo široki najmanj 25 mm. Znanstveni članki naj imajo naslednja poglavja: uvod, metode, rezultati, razpravljanje in zaključek. Uvodniki in sistematični pregledni članki so lahko zasnovani drugače, vendar naj bo razdelitev na poglavja in podpoglavja jasno razvidna iz velikosti črk naslovov. Poglavja in podpoglavja naj bodo številčena dekadno po standardu SIST ISO 2145 in SIST ISO 690 (npr. 1, 1.1, 1.1.1 itd.).

DOLŽINA PRISPEVKOV

Zahtevana dolžina prispevka je za vabljen uvodnik od 250 do 1000 besed, za znanstveni članek (originalni, metodološki ali sistematični pregledni) pa od 2000 do 4500 besed s slikovnim gradivom in literaturo vred. Revizija sme obsegati 5000 besed.

NASLOV IN AVTORSTVO

Naslov v angleškem in slovenskem jeziku naj bo kratek in natančen, opisen in ne trdilen (povedi v naslovih niso dopustne). Navedena naj bodo imena piscev z natančnimi akademskimi in strokovnimi naslovi ter popoln naslov ustanove, inštituta ali klinike, kjer je delo nastalo. Avtorji morajo izpolnjevati pogoje za avtorstvo. Prispevati morajo k zasnovi in oblikovanju oz. analizi in interpretaciji podatkov, rokopis morajo intelektualno zasnovati oz. ga kritično pregledati, strinjati se morajo s končno različico rokopisa. Samo zbiranje podatkov ne zadostuje za avtorstvo.

IZVLEČEK IN KLJUČNE BESEDE

Izvleček v angleškem in slovenskem jeziku naj bo pri znanstvenem in metodološkem članku strukturiran in naj ne bo daljši od 250 besed v angleščini in 400 besed v slovenščini, izvlečki ostalih člankov so lahko nestrukturirani. Izvleček naj vsebinsko povzema in ne le našteva bistvene vsebine dela. Izogibajte se kraticam in okrajšavam. Napisan naj bo v 3. osebi.

Izvleček znanstvenega članka naj povzema namen dela, osnovne metode, glavne izsledke in njihovo statistično pomembnost ter poglavitne sklepe (struktura IMRC - Introduction, Methods, Results, Conclusions).

Navedenih naj bo 3-10 ključnih besed, ki nam bodo v pomoč pri indeksiranju. Uporabljajte izraze iz MeSH - Medical Subject Headings, ki jih navaja Index Medicus.

KATEGORIJA PRISPEVKA

Kategorijo prispevka predlaga z vnosom v ustrezno polje avtor sam, končno odločitev pa sprejme urednik na osnovi predlogov recenzentov. Objavljamo izvirne znanstvene članke, metodološke članke, sistematične pregledne znanstvene članke in vabljene uvodnike.

REFERENCE

Avtorjem priporočamo, da pregledajo objavljene članke na temo svojega rokopisa v predhodnih številkah naše revije (za obdobje zadnjih pet let).

Vsako navajanje trditev ali dognanj drugih morate podpreti z referenco. Reference naj bodo v besedilu navedene po vrstnem redu, tako kot se pojavljajo. Referenca naj bo navedena na koncu citirane trditve. Reference v besedilu, slikah in tabelah navedite v oklepaju z arabskimi številkami ((1), (2, 3), (4-7)). Reference, ki se pojavljajo samo v tabelah ali slikah, naj bodo oštevilčene tako, kot se bodo pojavile v besedilu. Kot referenc ne navajajte izvlečkov in osebnih dogovorov (slednje je lahko navedeno v besedilu). Seznam citirane literature dodajte na koncu prispevka. Literaturo citirajte po priloženih navodilih, ki so v skladu s tistimi, ki jih uporablja ameriška National Library of Medicine v Index Medicus. Uporabljajte numerično citiranje. Imena revij krajšajte tako, kot določa Index Medicus (popoln seznam na naslovu URL: http://www.nlm.nih.gov).

Navedite imena vseh avtorjev, v primeru, da je avtorjev šest ali več, navedite prvih šest avtorjev in dodajte et al.

Če ima članek/knjiga DOI številko, jo mora avtor navesti na koncu reference.

PRIMERI ZA CITIRANJE LITERATURE

primer za knjigo:

1. Anderson P, Baumberg P. Alcohol in Europe. London: Institute of Alcohol Studies, 2006.
2. Mahy BWJ. A dictionary of virology. 2nd ed. San Diego: Academic Press, 1997.

primer za poglavje iz knjige:

3. Urlep F. Razvoj osnovnega zdravstva v Sloveniji zadnjih 130 let. In: Švab I, Rotar-Pavlič D, editors. Družinska medicina. Ljubljana: Združenje zdravnikov družinske medicine, 2002:18-27.
4. Goldberg BW. Population-based health care. In: Taylor RB, editor. Family medicine. 5th ed. New York: Springer, 1999:32-6.

primer za članek iz revije:

5. Florez H, Pan Q, Ackermann RT, Marrero DG, Barrett-Connor E, Delahanty L, et al. Impact of lifestyle intervention and metformin on health-related quality of life: the diabetes prevention program randomized trial. J Gen Intern Med. 2012;27:1594-601. doi: 10.1007/s11606-012-2122-5.

primer za članek iz revije, kjer avtor ni znan:

6. Anon. Early drinking said to increase alcoholism risk. Globe. 1998;2:8-10.

primer za članek iz revije, kjer je avtor organizacija:

7. Women's Concerns Study Group. Raising concerns about family history of breast cancer in primary care consultations: prospective, population based study. Br Med J. 2001;322:27-8.

primer za članek iz suplementa revije z volumnom in s številko:

8. Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect. 1994;102(Suppl 2):275-82.
9. de Villiers TJ. The role of menopausal hormone therapy in the management of osteoporosis. Climacteric. 2015; 18(Suppl 2):19-21. doi: 10.3109/13697137.2015.1099806.

primer za članek iz zbornika referatov:

10. Sugden K, Kirk R, Barry HC, Hickner J, Ebell MH, Ettenhofer T, et al. Suicides and non-suicidal deaths in Slovenia: molecular genetic investigation. In: 9th European Symposium on Suicide and Suicidal Behaviour. Warwick: University of Oxford, 2002:76.

primer za magistrske naloge, doktorske disertacije in Prešernove nagrade:

11. Shaw EH. An exploration of the process of recovery from heroin dependence: doctoral thesis. Hull: University of Hull, 2011.

primer za elektronske vire:

12. EQ-5D, an instrument to describe and value health. Accessed January 24th, 2017 at: https://euroqol.org/eq-5d-instruments/.

TABELE

Tabele v angleškem jeziku naj bodo v besedilu prispevka na mestu, kamor sodijo. Tabele naj sestavljajo vrstice in stolpci, ki se sekajo v poljih. Tabele oštevilčite po vrstnem redu, vsaka tabela mora biti citirana v besedilu. Tabela naj bo opremljena s kratkim angleškim naslovom. V legendi naj bodo pojasnjene vse kratice, okrajšave in nestandardne enote, ki se pojavljajo v tabeli.

SLIKE

Slike morajo biti profesionalno izdelane. Pri pripravi slik upoštevajte, da gre za črno-beli tisk. Slikovno gradivo naj bo pripravljeno:

- črno-belo (ne v barvah!);
- brez polnih površin, namesto tega je treba izbrati šrafure (če gre za stolpce, t. i. tortice ali zemljevide);
- v linijskih grafih naj se posamezne linije prav tako ločijo med samo z različnim črtkanjem ali različnim označevanjem (s trikotniki, z zvezdicami...), ne pa z barvo;
- v grafih naj bo ozadje belo (tj. brez ozadja).

Črke, številke ali simboli na sliki morajo biti jasni, enotni in dovolj veliki, da so berljivi tudi na pomanjšani sliki.

Ročno ali na pisalni stroj izpisano besedilo v sliki je nedopustno.

Vsaka slika mora biti navedena v besedilu. Besedilo k sliki naj vsebuje naslov slike in potrebno razlago vsebine. Slika naj bo razumljiva tudi brez branja ostalega besedila. Pojasniti morate vse okrajšave v sliki. Uporaba okrajšav v besedilu k sliki je nedopustna. Besedila k slikam naj bodo napisana na mestu pojavljanja v besedilu.

Fotografijam, na katerih se lahko prepozna identiteta bolnika, priložite pisno dovoljenje bolnika.

MERSKE ENOTE

Naj bodo v skladu z mednarodnim sistemom enot (SI).

KRATICE IN OKRAJŠAVE

Kraticam in okrajšavam se izogibajte, izjema so mednarodno veljavne oznake merskih enot. V naslovih in izvlečku naj ne bo kratic. Na mestu, kjer se kratica prvič pojavi v besedilu, naj bo izraz, ki ga nadomešča, polno izpisan, v nadaljnjem besedilu uporabljano kratico navajajte v oklepaju.

UREDNIŠKO DELO

Prispelo gradivo z javnozdravstveno tematiko mednarodnega pomena posreduje uredništvo po tehnični brezhibnosti v strokovno recenzijo trem mednarodno priznanim strokovnjakom. Recenzijski postopek je dvojno slep. Po končanem uredniškem delu vrnemo prispevek korespondenčnemu avtorju, da popravke odobri in upošteva. Popravljen čistopis vrne v uredništvo po spletni aplikaciji Editorial Manager. Uredništvo dopušča obravnavo največ treh revizij. Če tretja revizija rokopisa ne upošteva vseh pripomb recenzentov, se rokopis umakne iz uredniškega postopka. Sledi jezikovna lektura, katere stroške krije založnik. Med redakcijskim postopkom je zagotovljena tajnost vsebine prispevka. Avtor dobi v pogled tudi prve, t. i. krtačne odtise, vendar na tej stopnji upoštevamo samo še popravke tiskarskih napak. Krtačne odtise je treba vrniti v treh dneh, sicer menimo, da avtor nima pripomb.

V uredništvu se trudimo za čim hitrejši uredniški postopek. Avtorji se morajo držati rokov, ki jih dobijo v dopisih, sicer se lahko zgodi, da bo članek odstranjen iz postopka.

Morebitne pritožbe avtorjev obravnava uredniški odbor revije.

Za objavo članka prenese avtor avtorske pravice na Nacionalni inštitut za javno zdravje kot založnika revije (podpiše Pogodbo o avtorstvu in avtorskih pravicah). Kršenje avtorskih in drugih sorodnih pravic je kaznivo.

Prispevkov ne honoriramo in tudi ne zaračunavamo stroškov uredniškega postopka.

Avtor dobi izvod tiskane revije, v kateri je objavljen njegov članek.