The Croatian Translation of the Horne and Östberg Morningness-Eveningness Questionnaire With a Brief Review of Circadian Typology

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Abstract

Introduction: Horne and Östberg Morningness-Eveningness Questionnaire (MEQ) is a questionnaire widely used to assess the circadian typology.

The aim of this study was to translate the MEQ from English into Croatian.

Methods: The translation process included two independent forward translations, integration of the forward translation into a single translation, back-translation, back translation review and drafting of the final translation.

Results: No semantic differences were observed when comparing the original and the back-translation; thus, only minimal alterations were done to the final translation, compared to the first one.

Conclusions: The Croatian version of the MEQ is a reliable translation ready to be tested in a Croatian sample.

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Introduction

Human circadian rhythms are a result of an interaction of several factors, both external, such as light and temperature (1), and internal, determined by a circadian clock network consisting of molecular components where ARNTL, CLOCK, CRYs and PERs genes represent central nodes in the network (2, 3). The circadian system has a hierarchical structure. The suprachiasmatic nucleus (SCN) of the hypothalamus is the 'master clock' and controls the activity of the peripheral clocks (4). Two feedback loops, ARNTL/CLOCK and CRY/PER control expression of downstream transcription factors which regulate downstream target

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genes involved in different biochemical pathways (5).

The circadian rhythm has been shown to have an impact on human metabolism and several medical conditions, such as diabetes, myocardial infarction, or stroke (6). Different people tend to react differently to specific external factors, for example, with differences in the cyclic secretion of melatonin (7, 8), thus leading to differences in one's circadian rhythm.

Circadian typology shows important differences in biological and behavioral parameters and in circadian clock genes associated with sleep-wake rhythm (9).

The most common method of assessing human circadian preference is by using self-reported questionnaires in which the respondents choose the times in which they feel the best (10). The results of such questionnaires put the participants on a certain point of the Morningness-eveningness (ME) spectrum (11). On the one side of the spectrum there are the morning types. These individuals usually report early bedtimes and rise times, and they tend to perform better in the morning. On the other side of the spectrum there are the evening types, who report later bedtimes and rise times and tend to perform better later in the day (12). There are also intermediate or neither types, who fall somewhere in between the two extremes of the spectrum. Based on the obtained scores in the Morningness-Eveningness Questionnaire, individuals are classified into circadian typologies or chronotype (13). Large epidemiologic studies have demonstrated that chronotype has a normal Gaussian distribution that varies by age and gender, in that the young and old demonstrate earlier chronotype and adolescents and young adults demonstrate a later chronotype (1).

Several studies have confirmed the correlation of scores such as rMEQ, MESC and similar, with objective measures such as the timing of participants' body temperature, sleep-wake cycles, or cortisol or melatonin secretion rhythms (8, 14, 15). The genetics influences account for up to 50% of the variance in morningness, but other factors also have a significant influence, such as age and gender (16). The Morningness-Eveningness Questionnaire has been used in a wide range of research contexts, including circadian rhythm sleep disorders and studies of genetic influences on sleep patterns (17, 18).

One of the first validated, and still one of the most commonly used questionnaires is the Horne and Östberg Morningness-Eveningness Questionnaire (MEQ). Horne and Östberg adapted and validated the questionnaire previously developed by Öquist in 1970 (19). The questionnaire has since been translated into many different languages (19–21). To the authors' knowledge, the translation made in the present study is the first Croatian translation of this scale, which is significant when taking into account that this valuable instrument can be used for further research in this largely under-researched scientific area in the Croatian population. The complete Croatian version may be found in the supplement and can be freely used in other research.

Method

Morningness-Eveningness Questionnaire

The questionnaire consists of 19 questions dealing with individual preferred times of activity, time of day in which the participants go to sleep or wake up, as well as alertness after waking up (10, 19). Most answers are forced-choice - with no 'do not know/cannot decide' category. The summed scores can be used as a continuous variable, or they can be further divided into a five-point morningness-eveningness scale: definitely morning type (score 70-86), moderately morning type (score 59-69), neither type (score 42-58), moderately evening type (score 31-41), and definitely evening type (score 16-30) (10). The MEQ showed good internal consistency in several validation studies (20, 22).

Procedure

The translation was performed following the algorithm presented in Figure 1. The algorithm was decided upon after taking into account

procedures suggested in several articles (23–29). To simplify the data input of the filled in questionnaires, discrete item choices (multiple choice form ranging from A to E) have been substituted for continuous graphic scales (a visual scale in which the participants had to tick the appropriate hours) (30) in questions 17 and 18, with the scoring remaining identical to the original scale. The wording was also slightly altered, as suggested by Urbán et al. (30).

The questions in each stage of the translation procedure can be observed in Tables 1 and 2.

Results

The translation process can be viewed in Tables 1 and 2. Table 1 shows each question in the original, the translation obtained by combining the two independent forward translations, the back-translation into English, as well as the final version of the translation. Table 2 shows the two independent forward translations into Croatian. The translators aimed to provide a translation that is as close to the original as is possible, with no significant changes to the meaning, or style. When comparing the English version and the back-translated version, nosemantic differences were observed. The only differences between the original and the back translation were related to the use of different grammatical forms, which results in very similar meaning. The example of this may be seen in questions 1 and 2, where "Considering only your own 'feeling best' rhythm" of the original was back-translated into "Guided only by your own 'feeling best' rhythm". In question 11, the original construct "You wish to be at your peak performance" was back-translated into "You want to be at your best". Several other constructs also had a different wording, but with no relevant changes in meaning.

Discussion

The Horne and Östberg Morningness-Eveningness Questionnaire (MEQ) is one of the most widely used instruments in the area of circadian typology. It is easy to use, and the results of the test can be easily comparable to results of similar studies. In this study, the authors created a translation done in several steps, aiming to provide a reliable translation that can be applied in further research in the Croatian-speaking population.

Limitations of the study

This study failed to provide a validation of the final Croatian translation in a sample of Croatian-speaking participants, which should be performed in further research.

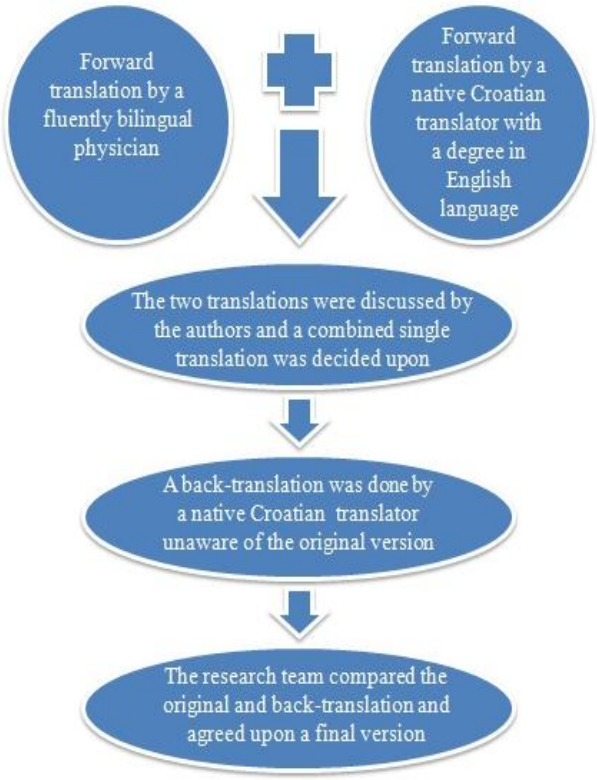

Figure 1. The algorithm of the translation protocol applied in this study

Table 1. Steps for the translation of the Morningness-Eveningness Questionnaire into the Croatian language and the final Croatian version

Original	Combined translation	Back-translation	Final version
1. Considering only your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day?	Kad biste se vodili samo svojim ritmom u kojem se najbolje osjećate, kada biste se ustali kada biste bili posve slobodni isplanirati svoj dan?	Guided only by your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day?	Vođeni samo osobnim ritmom u kojem se vi najbolje osjećate, kada biste se ustali kada biste bili posve slobodni isplanirati svoj dan?
2. Considering only your own "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your evening?	Kad biste se vodili samo svojim ritmom u kojem se najbolje osjećate, kada biste otišli spavati kada biste bili posve slobodni isplanirati svoju večer?	Guided only by your own "feeling best" rhythm, at what time would you go to sleep if you were entirely free to plan your evening?	Vođeni samo osobnim ritmom u kojem se vi najbolje osjećate, kada biste otišli spavati kada biste bili posve slobodni isplanirati svoju večer?
3. If there is a specific time at which you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?	Ako postoji specifično vrijeme kada morate ujutro ustati, u kojoj mjeri ste ovisni o tome da vas probudi budilica?	If there is a specific time at which you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?	Ako postoji specifično vrijeme kada morate ujutro ustati, u kojoj mjeri ste ovisni o tome da vas probudi budilica?
4. Assuming adequate environmental conditions, how easy do you find getting up in the mornings?	Ako se pretpostave adekvatni okolišni uvjeti, koliko vam se lako ujutro ustati?	Assuming that the environmental conditions are adequate, how easy do you find it to get up in the morning?	Ako se pretpostave adekvatni okolišni uvjeti, koliko vam se lako ujutro ustati?
5. How alert do you feel during the first half hour after having woken up in the mornings?	Koliko se budno osjećate u prvih pola sata nakon buđenja ujutro?	How alert do you feel in the first half hour after having woken up in the morning?	Koliko se budno osjećate u prvih pola sata nakon buđenja ujutro?
6. How is your appetite during the first half-hour after having woken in the mornings?	Kakav vam je apetit u prvih pola sata nakon buđenja ujutro?	How is your appetite in the first half hour after having woken up in the morning?	Kakav vam je apetit u prvih pola sata nakon buđenja ujutro?
7. During the first half-hour after having woken in the morning, how tired do you feel?	Koliko se umorno osjećate u prvih pola sata nakon buđenja ujutro?	How tired do you feel in the first half hour after having woken up in the morning?	Koliko se umorno osjećate u prvih pola sata nakon buđenja ujutro?
8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?	Kad nemate nikakvih obveza sljedeći dan, koliko kasnije odlazite u krevet u usporedbi s vašim uobičajenim vremenom?	When you have no commitments the next day, at what time do you go to bed, compared to your usual bedtime?	Kad nemate nikakvih obveza sljedeći dan, koliko kasnije odlazite u krevet u usporedbi s vašim uobičajenim vremenom?

9.	You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is between 7.0-8.0 AM. Bearing in mind nothing else but your own "feeling best" rhythm, how do you think you would perform?	Odlučili ste se baviti nekom fizičkom aktivnošću. Prijatelj vam predlaže da se njome bavite po sat vremena dva puta tjedno, a za njega je najbolje vrijeme između 7 i 8 sati. Imajući na umu isključivo vaš ritam u kojem se najbolje osjećate, što mislite kako biste to obavili?	You have decided to do a certain physical activity. A friend suggests that you do this for one hour twice a week, and the best time for him is between 7-8 a.m. Bearing in mind nothing else but your own 'feeling best rhythm', how do you think you would do?	Odlučili ste se baviti nekom fizičkom aktivnošću. Prijatelj vam predlaže da se njome bavite po sat vremena dva puta tjedno, a za njega je najbolje vrijeme između 7 i 8 sati. Imajući na umu isključivo vaš ritam u kojem se najbolje osjećate, što mislite kako biste to obavili?
10.	At what time in the evening do you feel tired and as a result in need of sleep?	U koje se doba večeri osjećate umorno i kao posljedicu toga osjećate potrebu za snom?	At what time in the evening do you feel tired and, as a result, in need of sleep?	U koje se doba večeri osjećate umorno i kao posljedicu toga osjećate potrebu za snom?
11.	You wish to be at your peak performance for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day and considering only your own "feeling best" rhythm, which ONE of the four testing times would you choose?	Želite biti u najboljem stanju za test za koji znate da će biti mentalno iscrpljujuć i trajati dva sata. Posve ste slobodni isplanirati svoj dan. Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, koje biste vrijeme testiranja, jedno od četiri ponuđena, izabrali?	You want to be at your best for a test that you know is going to be mentally exhausting and lasting two hours. You are completely free to plan your day. Considering only your 'feeling best rhythm',which one of the four testing times would you choose?	Želite biti u najboljem stanju za test za koji znate da će biti mentalno iscrpljujuć i trajati dva sata. Posve ste slobodni isplanirati svoj dan. Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, koje biste od četiri ponuđena vremena testiranja izabrali?
12.	If you went to bed at 11.0 PM at what level of tiredness would you be?	Kad biste pošli u krevet u 23 sata, na kojoj biste razini umora (pospanosti) bili?	If you went to bed at 23h/11 p.m., at what level of tiredness (sleepiness) would you be?	Kad biste pošli u krevet u 23 sata, na kojoj biste razini umora (pospanosti) bili?
13.	For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which ONE of the following events are you most likely to experience?	Iz nekog ste razloga otišli u krevet nekoliko sati kasnije nego inače, ali nema razloga za ustajanjem u neko posebno vrijeme sljedećega jutra. Koji ćete od ova četiri događaja najvjerojatnije iskusiti?	For some reason, you have gone to bed several hours later than usual, but there is no reason to get up at a particular time the next morning. Which one of the four following events are you most likely to experience?	Iz nekog ste razloga otišli u krevet nekoliko sati kasnije nego inače, ali nema razloga za ustajanjem u neko posebno vrijeme sljedećega jutra. Koji ćete od ova četiri događaja najvjerojatnije iskusiti?
14.	One night you have to remain awake between 4.00-6.00 AM in order to carry out a night watch.	Jedne večeri morate ostati budni između 4 i 6 ujutro da biste obavili noćnu stražu. Nemate obaveza	One night you have to remain awake between 4-6 a.m. in order to carry out a night watch. You	Jedne večeri morate ostati budni između 4 i 6 ujutro da biste obavili noćnu stražu. Nemate obaveza

	You have no commitments the next day. Which ONE of the following alternatives will suit you best?	sljedeći dan. Koja će vam od ponuđenih alternativa najbolje odgovarati?	have no commitments the next day. Which of the following alternatives would suit you best?	sljedeći dan. Koja će vam od ponuđenih alternativa najbolje odgovarati?
15.	You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own "feeling best" rhythm which ONE of the following times would you choose?	Morate odraditi dva sata teškog fizičkog rada. Posve ste slobodni isplanirati svoj dan. Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, koji biste od ponuđenih termina izabrali?	You have to do two hours of hard physical work. You are completely free to plan your day. Considering only your 'feeling best rhythm', which of the following times would you choose?	Morate odraditi dva sata teškog fizičkog rada. Posve ste slobodni isplanirati svoj dan. Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, koji biste od ponuđenih vremena izabrali?
16.	You have decided to engage in hard physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 10.0-11.0 PM. Bearing in mind nothing else but your own "feeling best" rhythm how well do you think you would perform?	Odlučili ste se baviti teškom fizičkom vježbom. Prijatelj vam predlaže da se njome bavite jedan sat dva puta tjedno, a za njega je najbolje vrijeme između 22 i 23 sata. Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, što mislite kako biste to obavili?	You have decided to engage in hard physical exercise. A friend suggests that you do this for one hour twice a week, and the best time for him is between 10-11 p.m. Considering only your 'feeling best rhythm', how do you think you would perform this?	Odlučili ste se baviti teškom fizičkom vježbom. Prijatelj vam predlaže da se njome bavite jedan sat dva puta tjedno, a za njega je najbolje vrijeme između 22 i 23 sata. Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, što mislite kako biste to obavili?
17.	Suppose that you can choose your own work hours. Assume that you worked a FIVE hour day (including breaks) and that your job was interesting and paid by results. Which FIVE CONSECUTIVE HOURS would you select?	Zamislite da sami možete birati svoje radne sate. Pretpostavite da radite pet sati u danu (uključujući stanke), da vam je posao zanimljiv i plaćen po učinku. Koje biste vrijeme izabrali za početak svog radnog vremena?	Imagine that you can choose your own work hours. Assume that you work five hours a day (including breaks) and that your job is interesting and paid by the results. At what time would you choose to begin your workday?	Zamislite da sami možete birati svoje radne sate. Pretpostavite da radite pet sati u danu (uključujući stanke), da vam je posao zanimljiv i plaćen po učinku. Koje biste vrijeme izabrali za početak svog radnog vremena?
18.	At what time of the day do you think that you reach your "feeling best" peak?	U kojem dobu dana smatrate da se najbolje osjećate?	At what time of the day do you think you feel your best?	U koje doba dana smatrate da se najbolje osjećate?
19.	One hears about "morning" and "evening" types of people. Which ONE of these do you consider yourself to be?	Ako čujete za izraz „jutarnji“ i „večernji“ tipovi ljudi, kako biste sebe svrstali?	If you ever heard of the terms 'morning' and 'evening' types of people, how would you categorize yourself?	Ako biste ikad čuli za izraze „jutarnji“ i „večernji“ tipovi ljudi, kako biste sebe svrstali?

Table 2. The comparison of two forward translations into Croatian from the English original

Forward translation 1	Forward translation 2
1. Kad biste se vodili samo svojim ritmom u kojem se najbolje osjećate, kada biste se ustali kada bi bili posve slobodni isplanirati svoj dan?	Kad biste se vodili samo svojim ritmom u kojem se najbolje osjećate, kada biste se ustali kada bi bili posve slobodni isplanirati svoj dan?
2. Kad biste se vodili samo svojim ritmom, u koliko sati biste se probudili kad biste mogli potpuno samostalno planirati dan?	Kad biste se vodili samo svojim ritmom u kojem se najbolje osjećate, kada biste otišli spavati kada biste bili posve slobodni isplanirati svoju večer?
3. Ako postoji točno vrijeme u koje se morate probuditi ujutro, koliko ste ovisni o alarmu budilice da vas probudi?	Ako postoji specifično vrijeme kada morate ujutro ustati, u kojoj ste mjeri ovisni o tome da vas probudi budilica?
4. Ako se pretpostavi da je danas prosječan, uobičajen dan, koliko vam je jednostavno ustajanje ujutro?	Ako se pretpostave adekvatni okolišni uvjeti, koliko vam se lako ujutro ustati?
5. Koliko se osjećate koncentrirano prvih pola sata nakon jutarnjeg buđenja?	Koliko se budno osjećate u prvih pola sata nakon buđenja ujutro?
6. Kakav imate apetit tijekom prvih pola sata nakon jutarnjeg buđenja?	Kakav vam je apetit u prvih pola sata nakon buđenja ujutro?
7. Koliko se umorno osjećate tijekom prvih pola sata budnosti?	Koliko se umorno osjećate u prvih pola sata nakon buđenja ujutro?
8. Kad nemate nikakvih obveza sljedeći dan, koliko kasnije odlazite u krevet u usporedbi s vašim uobičajenim vremenom odlaska u krevet?	Kada sutradan nemate obaveza, kada odlazite u krevet u usporedbi sa uobičajenim odlaskom u krevet?
9. Odlučili ste se uključiti u umjereno fizičko vježbanje. Prijatelj vam predloži da vježbate jedan sat dva puta tjedno i najbolje vrijeme za to jest između 7:00 i 8:00 ujutro. Ako razmatrate samo svoj ritam, koliko mislite da biste dobro vježbali?	Odlučili ste se baviti nekom fizičkom aktivnošću. Prijatelj vam predlaže da se njome bavite po sat vremena dva puta tjedno u, a za njega je najbolje vrijeme između 7 i 8 sati. Imajući na umu isključivo vaš ritam u kojem se najbolje osjećate, što mislite kako biste to obavili?
10. U koje se doba noći osjećate umorno i pospano?	U koje se doba večeri osjećate umorno i kao posljedicu toga osjećate potrebu za snom?
11. Želite biti u najboljoj formi za ispit za koji znate da će biti mentalno iscrpan i da će trajati dva sata. Kad biste mogli slobodno planirati cijeli	Želite biti u najboljem stanju za test za koji znate da će biti mentalno iscrpljujuć i trajati dva sata. Posve ste slobodni isplanirati svoj dan.

dan i vodeći se samo svojim ritmom, koje biste vrijeme pisanja ispita izabrali?	Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, koje biste vrijeme testiranja, jedno od četiri ponuđena, izabrali?
12. Ako biste legli u 23:00, koliko biste bili umorni?	Kad biste pošli u krevet u 23 sata, na kojoj biste razini umora (pospanosti) bili?
13. Zbog nekog ste razloga legli nekoliko sati kasnije nego inače, ali ne postoji razlog za buđenje u neko određeno vrijeme sljedećega dana. Koji ćete od sljedećih događaja vjerojatno doživjeti?	Iz nekog ste razloga otišli u krevet nekoliko sati kasnije nego inače, ali nema razloga za ustajanje u neko posebno vrijeme sljedećega jutro. Koji od ova četiri događaja ćete najvjerojatnije iskusiti?
14. Jedne noći morate ostati budni između 4:00 i 6:00 da biste odradili noćnu stražu. Nemate obaveza sljedeći dan. Koja vas od sljedećih opcija najbolje opisuje?	Jedne večeri morate ostati budni između 4 i 6 ujutro da biste obavili noćnu stražu. Nemate obaveza sljedeći dan. Koja će vam od ponuđenih alternativa najbolje odgovarati?
15. Morate odraditi dva sata teškog fizičkog rada. Možete potpuno slobodno planirati svoj dan. Uzimajući u obzir samo svoj ritam, koje biste vrijeme odabrali?	Morate odraditi dva sata teškog fizičkog rada. Posve ste slobodni isplanirati svoj dan. Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, koji biste od ponuđenih termina izabrali?
16. Odlučili ste se uključiti u tešku fizičku aktivnost. Prijatelj vam predloži da vježbate jedan sat dva puta tjedno i najbolje vrijeme za to jest između 10:00 i 11:00 ujutro. Ako razmatrate samo svoj ritam, koliko mislite da biste dobro vježbali?	Odlučili ste se baviti teškom fizičkom vježbom. Prijatelj vam predlaže da se njome bavite jedan sat dva puta tjedno, a za njega je najbolje vrijeme između 22 i 23 sata. Isključivo s obzirom na vaš ritam u kojem se najbolje osjećate, što mislite kako biste to obavili?
17. Pretpostavite da možete birati svoje radno vrijeme. Zamislite da radite pet sati dnevno (uključujući pauze), da vam je posao zanimljiv i da se plaća po učinku. Kojih biste pet UZASTOPNIH sati odabrali?	Zamislite da sami možete birati svoje radne sate. Pretpostavite da radite PET sati u danu (uključujući stanke), da vam je posao zanimljiv i plaćen po učinku. Kojih biste PET UZASTOPNIH SATI izabrali?
18. U koje doba dana mislite da se osjećate najbolje?	U kojem dobu dana smatrate da se najbolje osjećate?
19. Ako čujete za izraz „jutarnji“ i „večernji“ tipovi ljudi, kako biste sebe svrstali?	Često se može čuti o „jutarnjim“ i „noćnim“ tipovima ljudi. Što mislite, koji ste vi tip?

Appendix 1. The final version of the Croatian Morningness-Eveningness Questionnaire

MORNINGNESS/EVENINGNESS QUESTIONNAIRE

Upute:

1. Pažljivo pročitajte svako pitanje prije nego odgovorite na njega.
2. Odgovorite na SVA pitanja.
3. Na pitanja odgovarajte numeričkim redom.
4. Na svako bi pitanje trebalo odgovoriti neovisno o odgovorima na druga pitanja. NE vraćajte se unatrag i ne provjeravajte već dane odgovore.
5. Većina pitanje ima nekoliko ponuđenih odgovora. Kod svakoga pitanja križić stavite uz samo JEDAN odgovor. Pojedina pitanja imaju skalu umjesto ponuđenih odgovora. Stavite križić na prikladno mjesto na skali.
6. Na svako pitanje odgovorite što je iskrenije moguće. Vaši odgovori i rezultati držat će se u strogoj tajnosti.
7. Slobodno ostavite komentare ispod svakoga pitanja na mjestu predviđenome za to.

1. Vođeni samo osobnim ritmom u kojemu se najbolje osjećate, u koje biste se vrijeme ustali kada biste bili posve slobodni isplanirati svoj dan?

A) 5:00 – 6:30
B) 6:30 – 7:45
C) 7:45 – 9:45
D) 9:45 – 11:00
E) 11:00 – 12:00

2. Vođeni samo osobnim ritmom u kojemu se najbolje osjećate, u koje biste vrijeme otišli spavati kada biste bili posve slobodni isplanirati svoju večer?

A) 20:00 – 21:00
B) 21:00 – 22:15
C) 22:15 - 00:30
D) 00:30 – 1:45
E) 1:45 – 3:00

3. Ako postoji određeno vrijeme kada morate ustati ujutro, u kojoj ste mjeri ovisni o tome da vas budi budilica?

A) Nimalo ovisan/ovisna
B) Pomalo ovisan/ovisna
C) Poprilično ovisan/ovisna
D) Veoma ovisan/ovisna

4. Pod pretpostavkom da su okolišni uvjeti odgovarajući, koliko vam se lako ujutro ustati?

A) Nimalo lako
B) Ne veoma lako
C) Poprilično lako
D) Veoma lako

5. Koliko se ujutro budno osjećate unutar prvih pola sata nakon buđenja?

A) Nimalo budno
B) Pomalo budno
C) Poprilično budno
D) Veoma budno

6. Kakav vam je ujutro apetit unutar prvih pola sata nakon buđenja?

A) Veoma slab
B) Poprilično slab
C) Poprilično dobar
D) Veoma dobar

7. Koliko se ujutro umorno osjećate unutar prvih pola sata nakon buđenja?

A) Veoma umorno
B) Poprilično umorno
C) Poprilično osvježeno
D) Veoma osvježeno

8. Kad nemate nikakvih obveza sljedeći dan, koliko kasnije odlazite u krevet u usporedbi s uobičajenim vremenom vašega odlaska na spavanje?

A) Rijetko ili nikad kasnije
B) Manje od jedan sat kasnije
C) 1-2 sata kasnije
D) Više od dva sata kasnije

9. Odlučili ste se baviti nekom fizičkom aktivnošću. Prijatelj vam predlaže da se njome bavite po sat vremena dva puta tjedno, a za njega je najbolje vrijeme između 7 i 8 sati. Imajući na umu isključivo vaš ritam u kojemu se najbolje osjećate, što mislite – kakva bi bila razina vaše izvedbe?

A) Dobro bih to obavio.
B) Relativno bih to dobro obavio.
C) Bilo bi mi naporno.
D) Bilo bi mi veoma naporno.

10. U koje se doba večeri osjećate umorno i kao posljedicu toga osjećate potrebu za snom?

A) 20:00-21:00
B) 21:00-22:15
C) 22:15-00:45
D) 00:45-2:00
E) 2:00-3:00

11. Želite biti u najboljemu stanju za test za koji znate da će biti mentalno zahtjevan i da će trajati dva sata. Posve ste slobodni isplanirati svoj dan. Uzevši u obzir isključivo ritam u kojemu se najbolje osjećate, koje biste od četiri ponuđena vremena testiranja izabrali?

A) 8:00-10:00
B) 11:00-13:00
C) 15:00-17:00

D) 19:00-21:00

12. Kad biste pošli u krevet u 23 sata, na kojoj biste razini umora (pospanosti) bili?
A) Nimalo umoran/umorna
B) Pomalo umoran/umorna
C) Poprilično umoran/umorna
D) Veoma umoran/umorna

13. Iz nekog ste razloga otišli u krevet nekoliko sati kasnije nego inače, ali nemate razloga ustati u neko posebno vrijeme sljedećega jutra. Koji je od četiri navedena događaja najvjerojatniji u vašemu slučaju?
A) Probudit ću se u uobičajeno vrijeme i neću ponovno zaspati.
B) Probudit ću se u uobičajeno vrijeme i nakon toga zadrijemati.
C) Probudit ću se u uobičajeno vrijeme, ali ću ponovno zaspati.
D) Probudit ću se nakon uobičajenog vremena.

14. Jedne večeri morate ostati budni između 4 i 6 ujutro da biste obavili noćnu stražu. Nemate obaveza sljedeći dan. Koja će vam od ponuđenih alternativa najbolje odgovarati?
A) Ne bih otišao/otišla u krevet dok straža ne bi bila gotova.
B) Odrijemao/odrijemala bih prije i spavao/spavala poslije.
C) Dobro bih se naspavao/naspavala prije i odrijemao/odrijemala poslije.
D) Spavao/spavala bih samo prije straže.

15. Morate odraditi dva sata teškog fizičkog rada. Posve ste slobodni isplanirati svoj dan. Uzevši u obzir isključivo ritam u kojemu se najbolje osjećate, koje biste od ponuđenih vremena izabrali?
A) 8:00 - 10:00
B) 11:00 - 13:00
C) 15:00 - 17:00
D) 19:00 - 21:00

16. Odlučili ste se baviti teškom fizičkim treningom. Prijatelj vam predlaže da se time bavite dva puta tjedno po jedan sat, a za njega je najbolje vrijeme između 22 i 23 sata. Uzevši u obzir isključivo ritam u kojem se najbolje osjećate, što mislite – koliko kvalitetno biste to obavili?
A) Dobro bih to obavio.
B) Obavio bih to relativno dobro.
C) Bilo bi mi naporno.
D) Bilo bi mi veoma naporno.

17. Zamislite da možete sami birati svoje radno vrijeme. Pretpostavite da radite pet sati u danu (uključujući stanke), da vam je posao zanimljiv i plaćen po učinku. Koje biste vrijeme izabrali za početak svog radnog vremena?
A) 4:00 – 8:00 sati
B) 8:00 – 9:00 sati
C) 9:00 – 14:00 sati
D) 14:00 – 17:00 sati
E) 17:00 – 4:00 sata

18. U koje doba dana smatrate da se najbolje osjećate?
A) 5:00 – 8:00 sati
B) 8:00 – 10:00 sati
C) 10:00 – 17:00 sati
D) 17:00 – 22:00 sata
E) 22:00 – 5:00 sati

19. Moguće je čuti za „jutarnji“ i „večernji“ tip ljudi, u koji biste od njih svrstali sebe?
A) Zasigurno „jutarnji“ tip
B) Više sam „jutarnji“ nego „večernji“ tip.
C) Više sam „večernji“ nego „jutarnji“ tip.
D) Zasigurno sam „večernji“ tip.

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To Biofilm or Not to Biofilm?

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Abstract

Aim: The goal of this research is to examine the biofilm forming ability of Staphylococcus aureus and Pseudomonas aeruginosa clinical isolates in different in vitro conditions using Meuller-Hinton and Luria-Bertani broths.

Material and methods: 30 strains of Pseudomonas aeruginosa and 30 strains of Staphylococcus aureus obtained from clinical specimens were used. After preparing the suspensions of bacteria inoculated on broths, they were set on microtiter plates and the biofilm production was measured using the spectrophotometric reader on 550 nm. Strains were classified into four categories: non-producing, weak producers, moderate and strong producers, based on the comparison of optical density of samples and negative control.

Results: Both tested species successfully formed a biofilm in both broths ($p<0.01$). P. aeruginosa strains had a higher percentage of strong producers in both in vitro conditions, in comparison with S. aureus strains (3.3% vs 50%). Nevertheless, there is no statistically significant difference in biofilm formation between the strains, regardless the used broths, and there is no statistically significant difference between the biofilm forming ability of both species observed separately regarding in vitro conditions either.

Conclusion: Both species have an ability to produce biofilm, which likely contributes to the pathogenicity and virulence of these bacteria and also leads to a better understanding of their in vivo characteristics to cause infections related to biofilm.

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Introduction

Biofilm formation is one of the additional bacteria virulence factors which is still an interesting subject for numerous researches. Biofilm infections are becoming a major health problem in chronic infections and implants. Biofilm is a multicellular structure that protects bacteria from adverse environmental factors, making them highly resistant to different antibiotics. It also stores nutrients, which serve the bacteria to survive, protects them from phagocytosis, and secures survival in the host organism. Resistance to disinfectants is a very important characteristic of biofilm because it prevents removing bacteria from the surface, enabling such microorganisms to permanently colonize the human organism with pathological consequences. Biofilm should be considered as a mobile functional community with the features of a complete microorganism because, among other things, they have homeostasis, circulatory system, genetic material exchange and metabolic activity, which ensure their further development (1). In addition, biofilm-protected bacteria are capable to disperse individual bacterial cells and decomposing parts of biofilm into the surrounding tissues and circulation system. But most importantly, on the surfaces of medical devices or in the human body, biofilm is made by microorganisms with the ability to produce an extracellular polymeric substance. These polymeric substances have an ability to incorporate a large amount of water into their structure and become highly hydrated (2). These solid-liquid barriers between the surface and the aqueous environment allow the community of biofilms optimal conditions for the growth and survival of microorganisms. Also, biofilm is formed exclusively by the cells that produce polysaccharides in sufficient quantity (3). Several environmental and genetic signals control each step of biofilm development and dispersal. Accumulation of signal molecules in the environment allows each bacterial cell to estimate cell density or the total number of bacteria at that time – the quorum detection or quorum sensing phenomenon.

Colonization of medical devices is proportionally increased by surface irregularity and microorganisms bond more rapidly to hydrophobic surfaces such as plastic, rather than hydrophilic ones. (2,4). The appearance of biofilm on implants and various surgical implantable devices causes chronic infections, rejection of implants, ineffectiveness of the embedded device, organ damage, and sometimes even lethal outcome for the patient.

The aim of this research is to examine the biofilm forming ability of Staphylococcus aureus and Pseudomonas aeruginosa clinical isolates in different in vitro conditions using Mueller-Hinton and Luria-Bertani broths.

Material and methods

Sample preparation

This study included 60 bacterial strains, 30 Pseudomonas aeruginosa and 30 Staphylococcus aureus strains, obtained from different clinical specimens from 2007 to 2015 and isolated in microbiological laboratories at University Hospital Center Osijek, Croatia and in the General Hospital Slavonski Brod, Croatia. All bacterial strains are part of the collection of microbial strains kept at the Department of Microbiology and Parasitology, Faculty of Medicine, University of Osijek. Microorganisms were identified according to standard microbiological methods and biochemical tests to the species level (5). After the bacteria had been grown on the blood agar plate during 18-24 hours incubation, two to three individual colonies of bacterial cultures were taken and inoculated into vials with 3 ml of Mueller-Hinton (MH) (Becton Dickinson and Co., Cockeysville MD, USA) and Luria-Bertani (LB) (Difco R Luria-Bertani broth, Becton Dickinson, USA) broth. The suspensions were incubated in the thermostat at 37˚C for another 18-24 hours. After incubation, the tubes were well mixed (vortexed) and 20 μ l from each suspension was transferred into new tubes with 2 ml MH and LB broth, which yielded suspensions of approximately 5x105 CFU/ml concentrations. After the preparation, suspensions were planted on flat bottom

polyester microtiter plates (Copan, Brescia, Italy). Wells with 100 μl of uninoculated MH and LB were used as the negative control and the remaining wells had 50 μl MH or LB broths which were planted with 50 μl of the prepared suspensions. The biofilm-producing strain Acinetobacter baumannii ATCC 19606 was used as a positive control. The microtiter plate was incubated in the thermostat for 18-24 hours at 37˚C. After the incubation, the broth was shaken out and wells were washed three times with distilled water. At the end of the experiment, coloring with 0.1% crystal violet and solubilization with 95% ethanol was done (6). All measurements were done in triplicate.

Quantification of biofilm

The final step was a spectrophotometric measurement of biofilm production on an enzyme immunoassays plate reader (BioRad 93200 PR3100 TSC Microplate Reader) at 550 nm. The optical density (OD) values were measured in every well of the plate and they represent biofilm production. The final results were reported as the optical density cut-off value (ODc), which was calculated as average OD for each sample made in triplicate increased by three standard deviations of negative controls. The results were classified into the following categories: non-producers, weak, moderate and strong biofilm producers (6,7) according to the criteria presented in Table 1.

Table 1. The criteria for evaluating biofilm production

$OD < ODc$	Non-producers
$ODc < OD < 2 \times ODc$	Weak producers
$2 \times ODc < OD < 4 \times ODc$	Moderate producers
$4 \times ODc < OD$	Strong producers

OD = average optical density value of biofilm production in a single well; ODc = limit value of biofilm production (at least some biofilm produced)

Statistical Analysis

The results were processed using the statistical software package SPSS 19.0 (IBM Corp., Armonk, NY, USA), and the data processing was carried out by checking normality distribution and calculation of descriptive data, including the frequencies, percentages, median and interquartile ranges. Wilcoxon test of equivalent pairs, $\chi 2$ test with Fisher's exact test and Cramer's V (ϕ) coefficient were utilized for the statistical significance testing of the differences between two or more independent groups.

Results

The biofilm production ability data for both bacterial species regarding the in vitro nutrient condition (incubation in Mueller-Hinton and Luria-Bertani broths) are shown in Table 2. Data are presented as the average of triplicate measurement of optical density and includes medians and interquartile ranges for each variable used.

Table 2. The amount of biofilm formed, presented as the average optical density for *S. aureus* and *P. aeruginosa* using Luria-Bertani and Mueller-Hinton broths, in comparison to control

Staphylococcus aureus		C	Q
Luria-Bertani broth	control	0.059	0.014
	OD (AR)	0.085	0.031
Mueller-Hinton broth	control	0.076	0.011
	OD (AR)	0.097	0.043
Pseudomonas aeruginosa			
Luria-Bertani broth	control	0.032	0.017
	OD (AR)	0.318	0.481
Mueller-Hinton broth	control	0.070	0.07
	OD (AR)	0.330	0.602

Legend: C = median; Q = interquartile range; OD (AR) = average optical density

By comparing the data for both bacteria and cultivation media (Table 2), it can be seen that interquartile dispersal is greater for P. aeruginosa than for S. aureus strains.

Table 3. Distribution of biofilm production in *S. aureus* and *P. aeruginosa* strains using Luria-Bertani and Mueller-Hinton broth (p<0.01, Wilcoxon's Equivalent Pair Test)

Staphylococcus aureus	**non-producers**	**weak producers**	**moderate producers**	**strong producers**
	f (%)	f (%)	f (%)	f (%)
LB	17 (56.7)	13 (43.3)	0 (0)	0 (0)
MH	19 (63.4)	9 (30)	1 (3.3)	1 (3.3)
Pseudomonas aeruginosa	**non-producers**	**weak producers**	**moderate producers**	**strong producers**
	f (%)	f (%)	f (%)	f (%)
LB	0 (0)	8 (26.7)	4 (13.3)	18 (60)
MH	4 (13.3)	6 (20)	5 (16.7)	15 (50)

Legend: LB = Luria-Bertani broth; MH = Mueller-Hinton broth; f = frequency

It has been found that both bacterial species, S. aureus and P. aeruginosa successfully (to a statistically significant degree) created biofilm in both cultivation media (p<0.01, Wilcoxon's Equivalent Pair Test).

Table 4. The contingency table for biofilm production of *P. aeruginosa* and *S. aureus* strains in Luria-Bertani and Mueller-Hinton broth.

Pseudomonas aeruginosa		**Luria-Bertani broth**			
		weak producers	moderate producers	strong producers	**Total**
Staphylococcus aureus	non-producers	**2** **6.7%**	**2** **6.7%**	**13** **43.3%**	**17** **56.7%**
	weak producers	**6** **20.0%**	**2** **6.7%**	**5** **16.7%**	**13** **43.3%**
	total	**8** **26.7%**	**4** **13.3%**	**18** **60.0%**	**30** **100.0%**

Pseudomonas aeruginosa		**Mueller-Hinton broth**				
		non-producers	weak producers	moderate producers	strong producers	**Total**
Staphylococcus aureus	non-producers	**3** **10%**	**3** **10.0%**	**3** **10.0%**	**10** **33.3%**	**19** **63.3%**
	weak producers	**0** **0%**	**2** **6.7%**	**2** **6.7%**	**5** **16.7%**	**9** **30.0%**
	moderate producers	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**
	strong producers	**0** **0%**	**1** **3.3%**	**0** **0%**	**0** **0%**	**1** **3.3%**
	total	**4** **13.3%**	**6** **20.0%**	**5** **16.7%**	**15** **50.0%**	**30** **100.0%**

The correlation between the tested bacterial species according to their biofilm production ability is shown in Table 4. There is no statistically significant difference in biofilm formation between S. aureus and P. aeruginosa strains in Luria-Bertani (Fischer's exact test, p=0.075) or in Mueller-Hinton broth (Fischer's exact test, p=0.359).

The ability to produce biofilm depending on different cultivation conditions is shown in Figures 1 and 2. Staphylococcus aureus strains had very modest biofilm production in both broths: 43.3% of the strains seem to be weak producers and the remaining are non-producers in Luria-Bertani broth. There is even a smaller number of biofilm weak producers (30%) in Mueller-Hinton broth, and almost all remaining ones are biofilm non-producers, with the exception of one moderate (3.3%) and one strong (3.3%) producer. P. aeruginosa strains belong to strong biofilm producers in both in vitro conditions. In Luria-Bertani broth, all tested strains were shown as biofilm producers. Weak producers accounted for 26.7% of the strains, moderate ones accounted for 13.3%, and 60.0% were strong producers. There was 20.0% of weak producers, 16.7% of moderate producers and 50.0% of strong producers in Mueller-Hinton broth, and 13.3% of the strains were biofilm non-producers.

There was no statistically significant difference between cultivation conditions and the ability to form biofilm either in S. aureus or in P. aeruginosa strains.

Figure 1. Biofilm production ability of S. aureus in Luria-Bertani and Mueller-Hinton broths (Fischer's exact test, p=0.664).

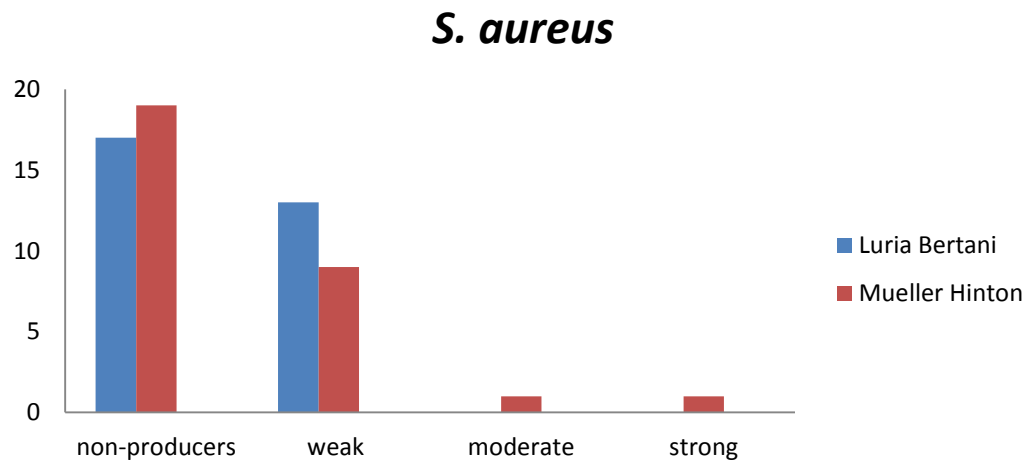
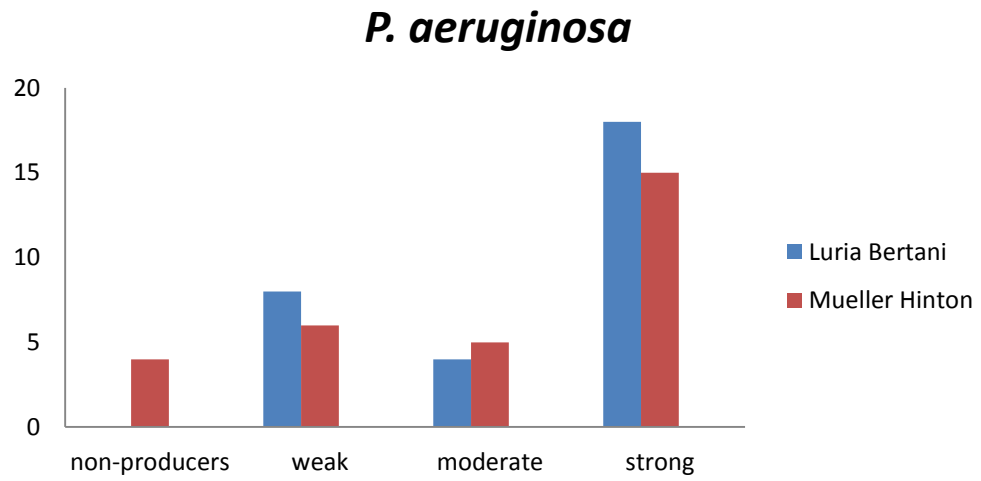


Figure 2. Biofilm production ability of P. aeruginosa in Luria-Bertani and Mueller-Hinton broths (Fischer's exact test, p=0.476).



Discussion

The main observation which arises from this study is that both bacterial species, gram-positive S. aureus and gram-negative P. aeruginosa, successfully and to a statistically significant degree form biofilm in both tested broths (Table 4). Another important observation is that interquartile dispersal is greater in P. aeruginosa compared to S. aureus strains. P. aeruginosa strains have a higher incidence of extreme values and thus a greater range of results. However, no statistical difference was observed with regard to the medium in which biofilm production was measured. Both species showed that biofilm production is more pronounced in Luria-Bertani medium by comparing the percentage, but no statistical significance has been established in statistical tests. Although Luria-Bertani medium is a medium in which higher production is expected, other authors have also pointed out the possibility that biofilm production may be unexpectedly expressed depending on the conditions of bacterial growth. Biofilm formation can be strongly affected both by growth media and by temperature (8,9). Another study (10) has also shown that both of these bacterial species are biofilm producers, independently of the clinical specimen isolation origin (sputum, urine, urine catheter, etc.). In this study, which involved the application of Congo agar and Tube method,

influence of the different in vitro conditions on biofilm forming ability of these two bacterial species was visible. (10) Both of the bacterial species have been shown to be strong producers of biofilm, with more than 80% of strong producers found (10). In our study, P. aeruginosa strains were strong producers in 55% cases, equally in both broths, whereas S. aureus strains had only one strong producer (3.3%). By comparing the results of this small series of experiments, it is reasonable to assume that the biofilm forming ability is greatly influenced by cultivation conditions, that it is nutrient dependent and also has a significant role in antimicrobial susceptibility of biofilms. (11,12,13)

Also, it is very important to emphasize that the role of biofilm in the genesis of infections associated with medical devices is indisputable. Microorganisms isolated from the samples of patients with these infections often exhibit the apparent ability to generate biofilms, as has been shown in many studies. (14,15) Additionally, it is known that multiple bacterial species can cooperate and form complex networks with many defending mechanisms and built-in sophisticated protection against the human immune system and antimicrobials as well. (16) Such polymicrobial biofilms are nowadays recognized as a significant factor in the pathogenesis of multiple infections in humans.

Conclusion

The obtained results are in agreement with previous medical and microbiological knowledge of biofilm formation, which plays a pivotal role in numerous infections such as periodontitis, chronic prostatitis, bacterial vaginosis, chronic otitis media, osteomyelitis, chronic pulmonary infections in cystic fibrosis patients, and chronic wound infection, considering that the investigated bacterial species, S. aureus and P. aeruginosa, are the most common etiological pathogens of these infections.

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Transparency declaration
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Suicidality in Depressive Patients

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Abstract

Aim: The aim of this research was to examine the incidence of suicides in patients with depressive disorders who were hospitalized at the Psychiatric Clinic of the Clinical Hospital Centre Osijek during 2015 and 2016, and to determine the relationship between suicidality and parameters measured in this research.

Methods: This research included 325 depressive patients hospitalized in the Psychiatric Clinic of the Clinical Hospital Centre Osijek during 2015 and 2016. Data were collected from medical records of patients diagnosed with depressive disorders. To collect data, the authors used a questionnaire drafted for the purposes of this research requiring the following information to be filled in: age, gender, employment status, marital status, qualifications, number of children, the existence of suicide attempts or repeated suicide attempts, the way in which suicide was attempted, number of hospitalizations and treatment duration in years, number of suicide attempts, motivation for attempted suicide, psychiatric heredity, comorbidity.

Results: Of the total number of respondents (n=325), 119 (36.6%) patients had suicidal behavior pattern in the past, significantly more in 2015 (Fisher's exact test, P<0.001), at present the suicidal behavior pattern had 134 patients, significantly more in 2015 (Fisher's exact test, P=0.04). Eighty (24.6%) respondents had attempted suicide. Thirty-three (41.3%) out of the 80 (24.6%) respondents who had attempted suicide were men and 47 (58.8%) were women. The existence of psychiatric heredity or attempted suicide in the family does not affect the suicide attempt of the respondents.

Conclusion: Suicidal behavior patterns in depressive patients hospitalized in the Psychiatric Clinic of the Clinical Hospital Centre Osijek during 2015 and 2016. are common.

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KEYWORDS: depressive disorders, suicidal ideation, hospitalization

Introduction

According to available data from the registers of the Croatian Institute of Public Health, one can see that in 2013 there were more than forty thousand hospitalizations associated with mental disorders registered, while depressive disorders were the cause of more than 13% of hospitalizations and the cause of 11% of day hospital treatments used due to mental disorders. (1). According to the Criminal Code of the Republic of Croatia, suicide as a criminal act is prosecuted in the case when the suicide is encouraged by another person. The criminal processing is conducted exclusively on a person who encouraged another person to the attempts to commit suicide. (2) A number of risk factors that can lead to suicide and attempts to commit suicide have been identified, and. affective disorders or personality disorders are mostly addressed (3). There is a specific sequence of behavior which occurs before attempting suicide, i.e. the pre-suicidal behavior syndrome and appeal-phenomenon which are often neglected and are often ignored (4). Ringel defined the phenomena of parasuicidal and pre-suicidal behavior, and thus gave an insight into the events of psychopathological changes that lead up to suicide attempts. The first phase, which Ringel termed "insufficiency and narrowing", is the period in which fear and sadness arise and are present to an extent which limits the individual in performing everyday activities. From such feelings, the second phase is developed, a phase of aggression, when an individual directs aggression towards him/herself because of isolation from the environment. Fantasy about suicide or the escape phase constitutes the third phase, which develops from a relief mechanism into concrete ideas by which the suicidal person seeks to escape to a better world. At that stage, he/she considers the techniques and methods of committing suicide and increasingly thinks of oneself as the late one. The fourth phase, the phase of mental anesthesia, is the period in which dissociation of the individual's personality is noticed. One person performs the tasks of daily life, and the other is preparing for suicide (5). The rate of committing suicide in the general population is 20 : 1, while in clinical cases, in individuals with depressive episodes, the rate is much higher, ranging from 5-10 : 1(6). Risk factors for committing suicide that most commonly occur in individuals suffering from depressive disorder are most commonly associated with symptoms of the disease or with specific situations, features of personality, the specifics of family medical history and similar. The groups affected by these disorders are melancholic depressive individuals with a high level of self-criticism and low level of self-esteem, as well as adolescents and older adults (7). In the Republic of Croatia, in the period from 2000 to 2014, the suicide rate dropped from 20.9 to 16.3 per 100000 of citizens, but then the suicide rate grew to 722 (17 committed suicides per 100000 persons) in 2015. The ratio of men and women in the observed period ranges from 2.2 - 3.7 : 1. The rate of suicide in the general population grows with age, with the highest rates being found in the population older than 65. The most common way of committing suicide in both sexes within the general population is hanging (8). Beside the risk factors, that there are also protective factors that reduce the possibility of suicide. This includes family and social support, pregnancy, postpartum period, a larger number of children in the family and strong religious beliefs. More protective factors are the care for health and regular physical check-ups, regular application of therapies and optimal physical activity (9). The aim of the present study is examine the incidence of suicides in patients with depressive disorders who were hospitalized at the Psychiatric Clinic of the Clinical Hospital Centre Osijek during 2015 and 2016, and to determine the relationship between sociality and parameters measured in this research.

Methods

This research included 325 depressive patients hospitalized in the Psychiatric Clinic of the Clinical Hospital Centre Osijek during 2015 and 2016. Data were collected from the medical records of patients diagnosed with depressive disorders. To collect data the authors used a questionnaire made for the purpose of this research with the following information: age,

gender, employment status, marital status, qualifications, number of children, the existence of suicide attempts or repeated suicide attempts, motivation for attempted suicide, whether they live alone or with their families, psychiatric heredity, comorbidity. All data were collected from 15 January 2017 to 1 May 2017 and recorded so as not to reveal the identity of the patient.attempts, the way in which the suicide was attempted, number of hospitalizations and treatment duration in years, number of suicide.

Statistical analysis

Categorical data are presented with absolute and relative frequencies. Numerical data are described by the median and the boundaries of the Interquartile range. The differences in categorical variables were tested by the chi-squared test and, if necessary, by Fisher's exact test. The normality of distribution of numerical variables was tested by the Shapiro-Wilk test. The differences between the numerical variables between the two independent groups were tested by Mann-Whitney in the test (23.24). All P values are two-sided. The significance level (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014) was used is set to Alpha = 0.05. For statistical analysis, the MEDCALC statistical software version 14.12.0.

Results

Out of the total number of respondents (n=325), 119 (36.6%) patients exhibited a suicidal behavior pattern in the past, significantly more in 2015 than in 2016 (Fisher's exact test, P<0,001), at present the suicidal behavior was present at 134 patients, significantly more in 2015 than in 2016 (Fisher's exact test, P=0.04). Eighty (24.6%) respondents had attempted suicide. From 80 (24.6%) respondents who had attempted suicide, 33 (41.3%) were men and 47 (58.8%) were women (Table 1).

Table 1. Suicidal behavior pattern in the past and in the present, suicide attempt according to the sex of respondents

	Number (%) of respondents			P*
	2015	2016	Total	
Suicidal behavior pattern in the past				
Yes	71 (47.3)	48 (27.4)	119 (36.6)	**< 0.001**
No	79 (52.7)	127 (72.6)	206 (63.4)	
Suicidal behavior pattern in the present				
Yes	71 (47.3)	63 (36)	134 (41.2)	**0.04**
No	79 (52.7)	112 (64)	191 (58.8)	
Suicide attempt				
Yes	38 (25.3)	42 (24)	80 (24.6)	0.79
No	112 (74.7)	133 (76)	245 (75.4)	
Total	150 (100)	175 (100)	325 (100)	
Suicide attempt according to sex				
Male	17 (44.7)	16 (38.1)	33 (41.3)	0.65
Female	21 (55.3)	26 (61.9)	47 (58.8)	
Total	38 (100)	42 (100)	80 (100)	

* Fisher's exact test

There were no significant differences in 2015 and 2016 in terms of the number of suicide attempts (Table 2).

Table 2. Number of suicide attempts in years 2015 and 2016

Number of suicide attempts	Number (%) of respondents			P*
	2015	2016	Total	
One	23 (60.5)	30 (71.4)	53 (66.3)	0.82
Two	9 (23.7)	7 (16.7)	16 (20)	
Three	1 (2.6)	1 (2.4)	2 (2.5)	
Four	0 (0)	1 (2.4)	1 (1.3)	
Five	1 (2.6)	1 (2.4)	2 (2.5)	
>five	4 (10.5)	2 (4.8)	6 (7.5)	
Total	38 (100)	42 (100)	80 (100)	

* Fisher's exact test

Place of residence, marital status, the basic diagnosis and treatment duration do not seem to be connected with the respondent's attempted suicide. In terms of number of suicide attempts, there was a statistically significant difference between subjects who had had suicidal ideas in the past or who presently had such ideas on the one side and those who never had such ideas on the other, with the former subjects being the ones who attempted suicide more often (Fisher's exact test, P < 0.001) (Table 3).

Table 3. Respondents according to the basic diagnosis, suicidal behavior pattern and suicide attempt

	Number (%) of respondents according to suicide attempts			P*
	No	Yes	Total	
Location				
Rural	128 (52.2)	43 (53.8)	171 (52.6)	0.89
Urban	117 (47.8)	37 (46.3)	154 (47.4)	
Marital status				
Married	159 (64.9)	48 (60)	207 (63.7)	0.39
Single	42 (17.1)	11 (13.8)	53 (16.3)	
Divorced	40 (16.3)	19 (23.8)	59 (18.2)	
In a relationship	4 (1.6)	2 (2.5)	6 (1.8)	
The basic diagnosis				
Depressive episode (F32)	75 (30.6)	29 (36.3)	104 (32)	0.41
Recurrent depressive disorder (F33)	170 (69.4)	51 (63.8)	221 (68)	
Treatment duration				
1 year	32 (13.1)	11 (13.8)	43 (13.2)	0.66
2 years	13 (5.3)	4 (5)	17 (5.2)	
3 years	9 (3.7)	6 (7.5)	15 (4.6)	
4 years	5 (2)	0	5 (1.5)	
5 years	9 (3.7)	3 (3.8)	12 (3.7)	
>5 years	177 (72.2)	56 (70)	233 (71.7)	
Suicidal ideas in the past				
Yes	51 (20.8)	68 (85)	119 (36,6)	< 0.001
No	194 (79.2)	12 (15)	206 (63.4)	
Suicidal ideas in the present				
Yes	74 (30.2)	60 (75)	134 (41.2)	< 0.001
No	171 (69.8)	20 (25)	191 (58.8)	
Total	245 (100)	80 (100)	325 (100)	

* Fisher's exact test

The median age of the respondents who tried to commit suicide was 53 years of age (interquartile range from 43 to 60 years). The age of respondents was from 17 to 76 years, similar to those who have not tried suicide (Table 4).

Table 4. Age of the respondents according to suicide attempt

	The median age (interquartile range) of the respondents who tried to commit suicide			P*
	No	Yes	Total	
Age of the respondents [years]	55 (49 - 62)	53 (43 - 60)	54 (46 - 61)	0.05

*Mann Whitney U test

The median age of respondents with one single attempted suicide was 54 (interquartile range from 45 to 60 years old), while the median of respondents with more than one attempt was 51 (interquartile range from 37 to 62 years) without statistically significant differences between those two groups (Table 5).

Table 5. Age of the respondents with one single attempted suicide and respondents with more than one attempt

	The median age (interquartile range) of the respondents according to the number of suicide attempts			P*
	One single attempted suicide	More than one attempt	Total	
Age of the respondents [years]	54 (45 - 60)	51 (37 - 62)	54 (46 - 61)	0.28

*Mann Whitney U test

For 38 (11.7%) respondents, family problems were the motive to attempt suicide, interpersonal problems motivated 33 (10.2%) of the respondents, and there were 7 (2.2%) of the respondents without a clear motive. The existence of psychiatric heredity was present in 94 (28.9%) of the respondents, significantly more among respondents who attempt suicide in the 2015 than in 2016 (Fisher's exact test, $P < 0.001$), and 20 (6.2%) of the respondents had had experience with attempted suicide in their families (Table 6).

Table 6. Motive to attempt suicide, psychiatric heredity and attempted suicide in families in years 2015 and 2016

	Number (%) of respondents			P*
	2015	2016	Total	
Motive to attempt suicide				
Family problems	17 (11.3)	21 (12)	38 (11.7)	0.87
Interpersonal problems	19 (12.7)	14 (8)	33 (10.2)	0.20
Without a clear motive	2 (1.3)	5 (2.9)	7 (2.2)	0.46
Psychiatric heredity				
Yes	59 (39.3)	35 (20)	94 (28.9)	
No	84 (56)	138 (78.9)	222 (68.3)	**<0.001**
Unknown	7 (4.7)	2 (1.1)	9 (2.8)	
Total	150 (100)	175 (100)	325 (100)	
Attempted suicide in family				
Yes	11 (7.4)	9 (5.1)	20 (6.2)	0.49
No	137 (92.6)	166 (94.9)	303 (93.8)	
Total	148 (100)	175 (100)	323 (100)	

** Fisher's exact test*

The existence of psychiatric heredity or suicide attempts in their families does not affect the suicide attempts of the respondents themselves. The number of respondents who attempted suicide and who had positive psychiatric heredity was 53 (66.3%), while 169 (69%) of the respondents did not have any experience with attempting suicide in their families or positive psychiatric heredity (Table 7).

Table 7. Respondents according to psychiatric heredity and attempted suicide in the family

	Number (%) of respondents according to suicide attempts			P*
	No	Yes	Total	
Psychiatric heredity				
Yes	68 (27.8)	26 (32.5)	94 (28.9)	
No	169 (69)	53 (66.3)	222 (68.3)	0.55
Unknown	8 (3.3)	1 (1.3)	9 (2.8)	
Total	245 (100)	80 (100)	325 (100)	
Attempted suicide in the family				
Yes	16 (6.6)	4 (5.1)	20 (6.2)	0.79
No	228 (93.4)	75 (94.9)	303 (93.8)	
Total	244 (100)	79 (100)	323 (100)	

* Fisher's exact test

Discussion

As the authors stated at the very beginning, affective diseases are most common among persons who commit suicide. The lifetime risk of suicide in patients with depression is 15% (10). It should be emphasized that in patients suffering from depressive disorders, the risk of suicide is twenty times higher than in the general population (11, 12). In present study, 9.2% of respondents indicated deliberate self-harm and suicide attempts as the reasons for their last hospitalization. On the other hand, data from literature show that, out of the total number of respondents, a quarter of them had attempted suicide either recently or in the past, without statistically significant differences according to the sex of the respondents. Suicide is three times more common in men, which was not found within our selected patient population. The authors can see from the results that there are no statistically significant differences regarding sex of the person who committed suicide (13). In addition, from the literature authors could see that the ratio of suicide attempts in men and women varied based on sociodemographic characteristics, which indicates that in countries with a higher standard of living there is a higher frequency of suicide attempts in males, while the frequency of suicide attempts among women shows a higher percentage in countries of medium and low living standards (14). In present study, however, there was no statistically significant relationship found between the place of living, so we can conclude that, in the case of present population, the living standard according to the place of living did not have any influence on whether the respondent attempted suicide or not. The lowest rate of attempted suicides was found in married people

and that single life increases the risk by 2 times. The same applies to divorced and widowed persons, whose risk is also twice as high as the risk found in singles (13). On the other hand, in present study there was no statistically significant relationship between the marital status of the patients or whether they lived alone or in a young society in his /her own family and the intention for committing suicide. Published studies show that people suffering from depression with the intent to commit suicide are of the average age of 55 (15). In present population, the average age of respondents who had attempted suicide was 53 years of age, with no significant differences in relation to those who had not attempted suicide, which coincides with data from the literature and is included in their interquartile age range. In present study, we hypothesized that the number of hospitalizations is connected with the attempts of suicide, however, this was not shown to be statistically significant and corresponds to the data from literature, which indicates that the number of psychiatric consultations did not correlate with increased risk of suicide in depressed patients (16). The most common motives that we can find in literature are interpersonal relationships, lack of love in the family, and loss of control over their disease (17). Present research has shown that the most common motives for suicidal behavior are family problems and interpersonal reasons. For example, Kieholz's scheme of judgement and determination of suicidal tendencies has shown that the presence of suicide(s) in the patient's immediate family or among other close relatives increases the risk of suicide possibility (18). We can notice how this scheme can be applied to our study because people with suicidal ideas in the past or those with suicidal ideas at the present time attempted suicide more often. Numerous studies, including family studies, studies of twins and adoptive researches, associate family communication and the possibility of inheritance of suicidal behavior (reviewed by 19). It is mentioned that the risk for a depressive episode is an independently inherited factor (20). In present research, based on the selected population, the existence of psychiatric heredity or suicide attempts in the family did not affect the attempted suicide of the respondents. In the actual attempted suicide, the most common method of execution of the act involves deliberate self-harm by using drugs/intoxication, which is also the most persistent in the form of suicide attempts, with deliberately intoxication and the effects of alcohol being found much more often in 2015. Deliberate self-harm by hanging, strangulation, and asphyxiation showed no significant differences during monitoring period. Data from the World Health Organization show that the methods of attempted suicides are different in certain parts of the world. The authors can distinguish three most common methods of attempted suicides in the world: hanging, poisoning with organophosphates and pesticides, and suicides by firearms. Given the fact that Croatia is a part of Europe, we have compared the obtained data with the rest of Europe, where it has been shown that the most common methods of suicide attempts, in the respondents in this study, were deliberate self-harm by using drugs and suicide attempt with a firearm, which coincides with the results obtained in present study (21).

Summary of the findings of present study are: a) Recurrent depressive disorder (F33) was more common in 2016, while depressive episode (F32) was more common in 2015 (out of the total number of patients suffering from a depressive disorder); b) The most common comorbidity diagnoses of the respondents were in the area of mental disorders and disorders of behavior, secondly there were diagnoses in the fields of circulatory system diseases, while the least comorbidity was found in the area of skin and subcutaneous tissue diseases; c) Suicidal forms of behavior in terms of suicidal ideas in the past and suicidal ideas in the present were significantly more present in 2015; d) There were no significant statistical differences with regard to gender and age of the respondents in relation to their attempted suicides, e) Place of residence, marital status, the basic diagnosis and treatment duration were not found to be connected with whether the respondent attempted suicide or not; e)Subjects with suicidal ideas in the past or in the present

attempted suicide more frequently, to a statistically significant degree, f) The most common motives of suicidal behavior patterns were family problems and interpersonal considerations; g) The existence of psychiatric heredity or attempts of suicide in the family were not found to be connected with suicide attempts of the respondents; h) The number of psychiatric hospitalizations was not found to be connected with suicide attempts of depressed patients.

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