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.

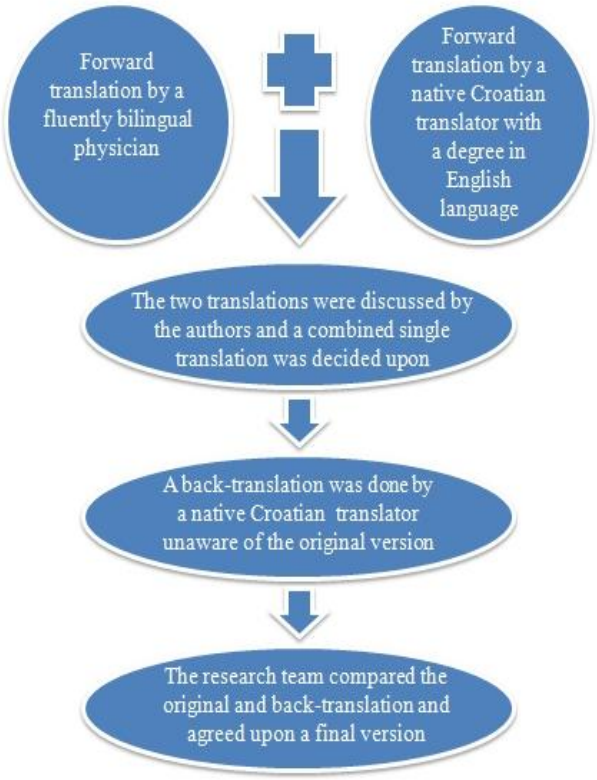

Figure 1. The algorithm of the translation protocol applied in this study

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).

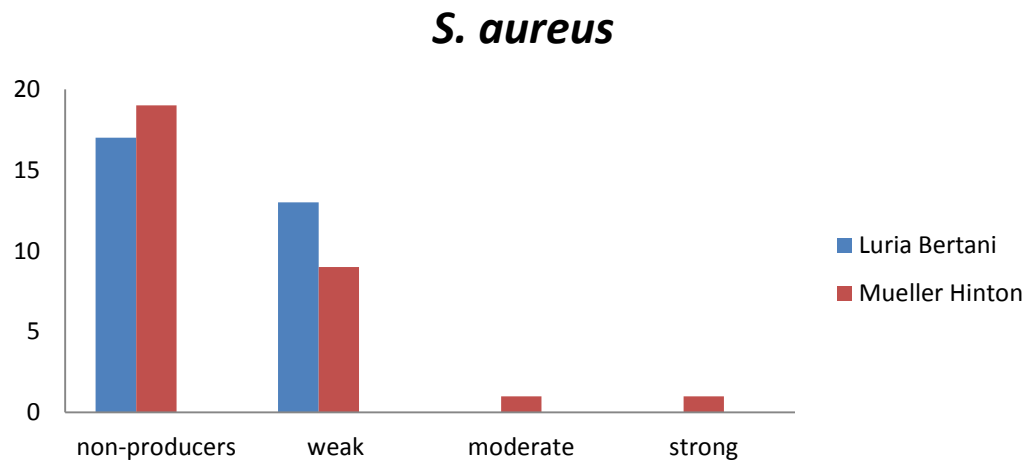
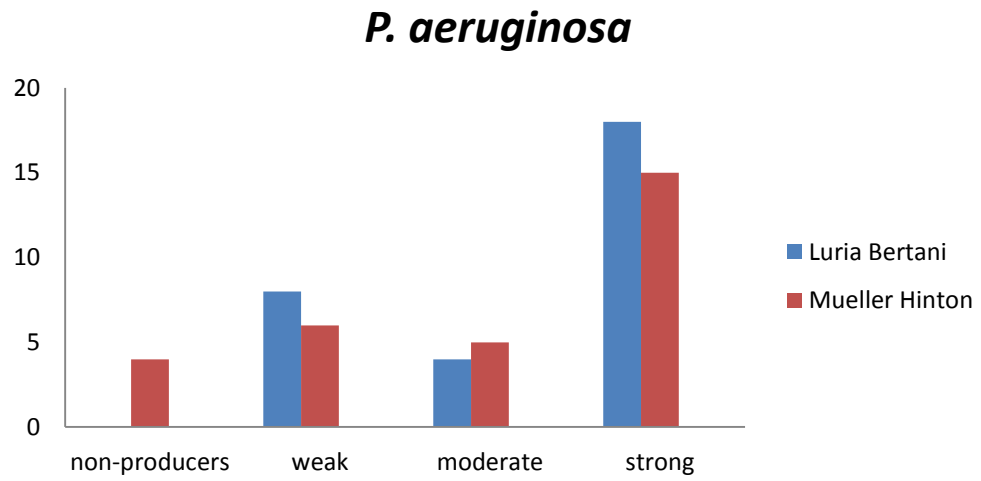


Figure 2. Biofilm production ability of P. aeruginosa in Luria-Bertani and Mueller-Hinton broths (Fischer's exact test, p=0.476).



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
Competing interests: None to declare.

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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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Impact of Delivery Room Resuscitation Efforts on Admission Temperatures in Infants Born < 32 Weeks Gestation

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Abstract

Aim: This study aimed to determine how delivery room resuscitation efforts influence admission temperatures in premature infants born before 32 weeks gestation.

Methods: We retrospectively analyzed a cohort of premature infants born before 32 weeks gestation from January 2014 until December 2016. We compared the impact of resuscitation efforts performed in the delivery room on the admission temperature. Hypothermia was defined as a core temperature of less than 36.5˚C on admission. The primary outcome was admission temperature in the Neonatal Intensive Care Unit. Secondary outcomes were Apgar scores in the first and fifth minute, pH on admission, respiratory distress syndrome requiring surfactant, persistent ductus arteriosus, necrotizing enterocolitis, late onset sepsis, kidney failure, intraventricular hemorrhage and death before hospital discharge. Exclusion criteria were inevitably lethal congenital malformations.

Results: We studied 147 infants born < 32 weeks gestation. In the delivery room, 66 (44.8%) of infants were given standard thermal care, 20 (13.6%) received standard care and continuous positive airway pressure (CPAP), 49 (33.3%) received standard care and IPPV, whereas 12 (8.1%) of infants received standard care and extensive resuscitation efforts (intubation and/or chest compressions and/or epinephrine). Patients receiving standard care and intermittent positive-pressure ventilation (IPPV) had significantly lower admission temperatures than those given standard care only (35.7˚C vs. 36.2 ˚C, $p < 0.02$). No correlation was found in infants receiving CPAP or extensive resuscitation efforts compared to those receiving standard thermal care only.

Conclusion: In our study, admission hypothermia was associated with IPPV in the delivery room. Application of CPAP or extensive resuscitation efforts in the delivery room did not influence admission temperatures.

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Introduction

Admission hypothermia is a common problem in preterm neonates and is reported in up to 78% of preterm neonates admitted to neonatal intensive care units (1). The World Health Organization classifies 36.0˚C to 36.4˚C as cold stress or mild hypothermia, 32.0˚C to 35.9˚C as moderate hypothermia, and lower than 32.0˚C as severe hypothermia (2). Admission hypothermia is associated with increased mortality in premature neonates (3), higher rates of late onset sepsis (4), respiratory distress syndrome (5) and intraventricular hemorrhage (6). For this reason, multiple strategies for preventing admission hypothermia have been proposed: the use of polyethylene wraps, heated mattresses, chemical warming packs and humidified and heated respiratory gases (7, 8). Miller et al. (9) reported decreased odds of hypothermia following no resuscitative efforts with the conclusion that this is due to the fact that very low birth weight infants are healthier and better able to maintain normothermia. On the other hand, Lyu et al. (3) reported that resuscitation in the first 30 minutes after birth was associated with increased admission temperature. They speculate "that an infant requiring resuscitation is likely to be sicker and require more care, which should result in the care team ensuring that all practices are followed, including attending to body temperature" (3).

This study aimed to analyze the impact of delivery room resuscitation efforts on admission temperatures in infants born < 32 weeks of gestation as well as short-term outcomes of these infants.

Materials and Methods

This retrospective cohort study was performed at a single, referral level III NICU of University Hospital Osijek (Osijek, Croatia). The medical charts of all infants of gestational age at birth ≥ 22 + 0/7 – 31 + 6/7 weeks born from January 2014 to December 2016 were retrospectively reviewed. Infants with major congenital anomalies were excluded. The study was approved by University Hospital Osijek Ethical Board.

Standard thermal care was provided for all infants immediately after birth, and included placing the infant in polyethylene wrap under a radiant warmer. The infants were divided in four groups with regards to resuscitation efforts undertaken. Infants who received only standard thermal care were assigned to "None" group, those who received standard care and continuous positive airway pressure (CPAP) were assigned to "CPAP" group, if intermittent positive pressure ventilation (IPPV) was applied (bag mask or T – piece), infants were assigned to the "IPPV" group and if at any time extensive resuscitation efforts (chest compression and/or epinephrine and/or delivery room intubation) were performed, infants were assigned to "CEI" group. Outcomes were compared among groups.

Our NICU was next to the delivery room and the transport time was within 5 minutes. The first temperature on admission was measured by the rectal method and was recorded immediately after admission to the NICU. We collected data regarding intrapartum and demographic variables. Antenatal steroids were defined as any doses of corticosteroids given before delivery. Chorioamnionitis was defined upon pathohistological analysis of the placenta. A low Apgar score was defined as an Apgar score < 7.

The short-term outcome variables included: respiratory distress syndrome (RDS) that required surfactant therapy, necrotizing enterocolitis at Bell's stage ≥ II, severe intraventricular hemorrhage (grades III or IV), patent ductus arteriosus requiring treatment, late onset sepsis, kidney failure and death before hospital discharge. Respiratory distress syndrome (RDS) was diagnosed by clinical and radiographic findings, and surfactant was delivered according to Croatian recommendations (10). Infants were diagnosed with sepsis if they had positive blood cultures for either bacteria or fungi. Severe intracranial hemorrhage was classified as IVH grade III – IV as classified by Papile et al. (11). Necrotizing

enterocolitis (NEC) was diagnosed in the presence of at least intestinal pneumatosis and/or portal venous gas (Bell's stage ≥2) (12). Kidney failure was diagnosed if the infant met the neonatal RIFLE criteria for kidney failure (13). Neonatal mortality was defined as infant death before hospital discharge.

Statistical analysis

Data are presented as arithmetic means, quartiles and standard deviations for continuous variables. Categorical variables were presented with frequency tables and cross tables. Due to the fact that variables were not normally distributed and some samples were small, nonparametric tests were used. For testing differences in distributions for two variables Mann-Whitney test was used and, in the case of three or more variables, Kruskal-Wallis ANOVA was used. For testing dependence between two categorical variables, 2 and Fisher exact tests were used. For the purposes of our study, p value of < 0.02 was considered to be statistically significant. Statistical analyses were performed by using statistical software Statistica 13.3.

Results

During the 3-year period, 147 infants met the inclusion criteria for the study. The maternal and infant variables of the four groups are listed in Table 1. Compared to "None" group, the infants in the "IPPV" group had lower birth weight (925g vs. 1207g, p < 0.02), and were more often born via C section (89.8% vs. 66.1%, p <0.02). Compared to the "None" group, the infants in the "CIE" group had lower birth weight (898 g vs. 1207g, p < 0.02), and were more often born following chorioamnionitis (96.1% vs. 46.9%, p <0.02).

Table 1. Comparison of maternal and infant variables among groups

	"None" (N=66)	"CPAP" (N=20)[a]	"IPPV" (N=49)	*p* value[b]	"CIE" (N=12)	*p* value[c]
Gestational age (weeks), median (IQR)	29.5 (27.1 - 30.5)	29.6 (28.7 - 30.9)	27.5 (25.7 - 30.0)	NS	27.0 (24.9 - 28.5)	NS
Birth weight, (g) median (IQR)	1207 (872 – 1545)	1323 (966 – 1422)	925 (697 – 1186)	<0.02	898 (721 - 1138)	<0.02
Female	33 (50%)	7 (35%)	27 (55.1%)	NS	2 (16.6%)	NS
Chorioamnionitis	31 (46.9%)	6 (30%)	25 (51.1%)	NS	11 (91.6%)	<0.02
Maternal hypertension	14 (21.2%)	6 (30%)	10 (20.4%)	NS	0 (0%)	NS
C section	44 (66.1%)	17 (85.1%)	44 (89.8%)	<0.02	6 (50%)	NS
Antenatal steroids	42 (63.6%)	15 (75%)	35 (71.4%)	NS	4 (33.3%)	NS

[a]No statistically significant *P* value: "CPAP" group compared to "None" group
[b]*p* value: "IPPV" group compared to "None" group
[c]*p* value: "CIE" group compared to "None" group

The median admission temperature was 36.3 ˚C (IQR range 35.9 ˚C – 36.7 ˚C). Admission hypothermia was found in 61.2% of the study population. Fifty-seven (38.7%) infants were normothermic, 51 (34.6%) were mildly hypothermic and 39 (26.5%) infants were moderately hypothermic. There were no severely hypothermic infants. The highest admission temperature was found in premature

infants with completed 30 weeks of gestation (36.5 ˚C) (Fig 1).

Compared to the "None" group, the "IPPV" group had lower admission temperatures (35.7 ˚C vs. 36.2 ˚C, p < 0.02). "CPAP" and "CIE" groups were not associated with higher rates of admission hypothermia compared to "None" group (Table 2). Compared to "None" group, infants in the "CPAP" group had fewer Apgar scores < 7 in the first (0%, vs. 30.3%, p <0.02) and fifth minute (0% vs. 25.7%, p <0.02) and lower mortality before discharge (0% vs. 18.1%, p < 0.02).

Fig 1. Admission temperatures (degrees centigrade) and gestational age

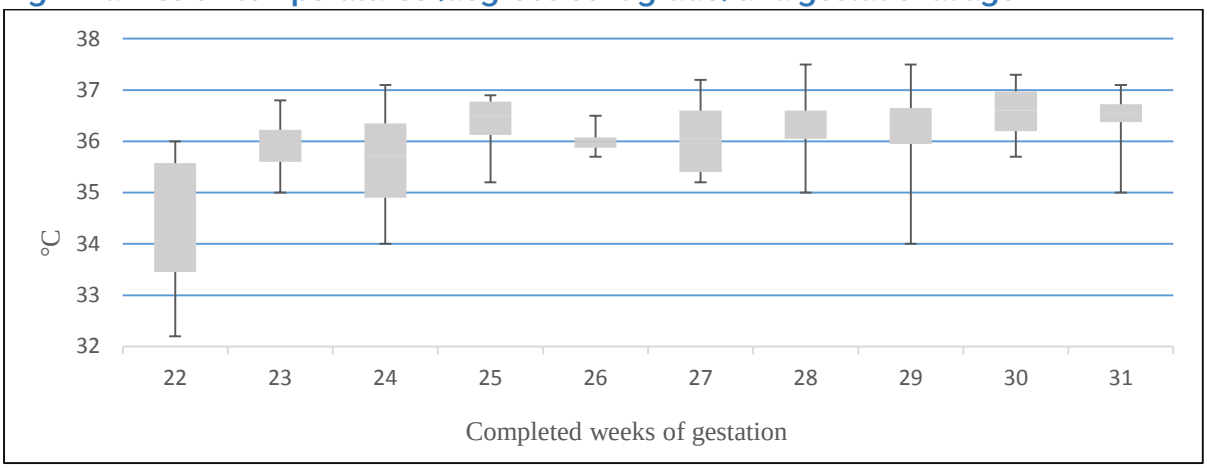

Table 2. Admission temperature and outcomes among groups

	"None" (N=66)	"CPAP" (N=20)	*p* value[a]	"IPPV" (N=49)	*p* value[b]	"CIE" (N=12)	*p* value[c]
Admission temperature, mean ± SD (˚C)	36.2±0.77	36.4± 0.49	NS	35.9±0.69	<0.02	35.7±1.44	NS
1-min. Apgar score < 7	20 (30.3%)	0 (0%)	<0.02	31 (61.2%)	<0.02	11 (91.6%)	<0.02
5-min. Apgar score < 7	17 (25.7%)	0 (0%)	<0.02	26 (53.0%)	<0.02	9 (75%)	<0.02
pH on admission, mean ± SD	7.24 ± 0.1	7.24 ± 0.9	NS	7.15 ± 0.13	<0.02	7.1 ± 0.16	<0.02
RDS requiring surfactant	51 (77.2%)	11 (55.0%)	NS	41 (83.6%)	NS	11 (91.6%)	NS
PDA	8 (12.1%)	1 (5%)	NS	4 (8.1%)	NS	4 (33.3%)	NS
NEC ≥2 grade	13 (19.7%)	3 (15%)	NS	10 (20.4%)	NS	7 (58.3%)	<0.02
LOS	28 (42.4%)	7 (35.0%)	NS	25 (51.0%)	NS	9 (75.0%)	NS
Kidney failure	7 (10.6%)	0 (0%)	NS	5 (10.2%)	NS	4 (33.3%)	NS
IVH ≥ grade III	16 (24.2%)	3 (15%)	NS	15 (30.6%)	NS	4 (33.3%)	NS
Mortality before discharge	12 (18.1%)	0 (0%)	<0.02	19 (38.7%)	NS	7 (58.3%)	<0.02

IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; LOS, late onset sepsis
[a]*p* value in the "CPAP" group compared to "None" group
[b]*p* value in the "IPPV" group compared to "None" group
[c]*p* value in the "CIE" group compared to "None" group

Compared to "None" group, infants in the "IPPV" group had more Apgar scores < 7 in the first (61, 2%, vs. 30.3%, p <0.02) and fifth minute (53.0% vs. 25.7%, p <0.02) and lower pH values (7.15 vs. 7.24) but did not have lower mortality and morbidity.

Compared to the "None" group, the "CIE" group had more Apgar scores < 7 in the first (91.6%, vs. 30.3%, p <0.02) and fifth minute (75.0% vs. 25.7%, p <0.02) and lower pH values (7.11 vs. 7.24). They also exhibited higher incidence of NEC grade ≥ 2 (58.3% vs. 19.7%, p <0.02) and higher mortality before discharge (58.3% vs. 18.1%, p <0.02).

Discussion

This study aimed to analyze the impact of delivery room resuscitation efforts on admission temperatures in infants born < 32 weeks of gestation as well as short-term outcomes in infants treated in a Croatian Neonatal Intensive Care Unit, using the WHO classifications of hypothermia: mild hypothermia, 36.0 to 36.4 1˚C; moderate hypothermia, 32.0 to 35.9 ˚C; and severe hypothermia, below 32 ˚C (2). Hypothermia rates in our cohort are similar to those reported in other studies (3, 14, 15).

In our study, lower admission temperatures were significantly associated with intermittent positive pressure ventilation (IPPV) but were not associated with application of continuous positive airway pressure (CPAP) or extensive resuscitation efforts which include delivery room intubation, epinephrine administration or chest compressions.

The finding that CPAP application in the delivery room does not lead to increased hypothermia is consistent with Miller et al., who speculate that very low birth weight are healthier and better able to maintain normothermia (9). This is also reflected in the finding that these infants had significantly better Apgar scores in the first and fifth minute and had a low mortality before discharge. Also, this is consistent with previous findings that delivery room application of CPAP is beneficial regarding outcomes in premature infants (16, 17).

On the other hand, the findings that extensive resuscitation efforts which include delivery room intubation, epinephrine administration or chest compressions do not lead (to a statistically significant extent) to lower rates of admission hypothermia are consistent with findings of Lyu et al. (3), who demonstrated that admission hypothermia is not associated with extensive resuscitation efforts despite the fact that these infants are sicker and in poor condition. This is consistent with our findings that infants who require extensive resuscitation efforts have significantly lower birth weights, lower Apgar scores and lower pH values on admission and have a high rate of death before discharge.

Compared to infants who received standard thermal delivery room care, infants who received IPPV in the delivery room had lower admission temperatures. We speculate that there are several factors leading to this. These infants had significantly lower birth weight than the group that received only standard thermal care, but, on the other hand, this did not influence admission temperature in the extensive resuscitation group.

We speculate that lower admission temperature in the IPPV group could be the result of ventilating these infants with unheated gases. Duration of resuscitation must also be taken into consideration. This is one of the limitations of our study: duration of delivery room resuscitation efforts is not taken into consideration because no accurate data is noted in the charts. Also, the temperature of the delivery room at the time of delivery is unknown and, just like in other institutions, these temperatures are known to be below the WHO-recommended 25˚C (2).

The limitations of the study are small sample size and a low number of infants receiving extensive resuscitation efforts in the delivery room. The effect of delivery room resuscitation procedures on admission temperatures in premature infants will require further investigation.

Promising results in maintaining normothermia in premature infants were recently published by

Pinheiro et al. (18) regarding the use of chemical warming packs in achieving above-average admission temperatures in neonates. Also, simple and inexpensive methods including preheating the delivery room in expectance of a premature birth have shown to reduce admission hypothermia rates (19).

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Competing interests. None to declare.

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The Use of Hydrogen Sulfide Donor in Cardiac Control During Low Doses of Ionizing Radiation (Experimental Study)

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Abstract

Aim: To analyze the influence of the hydrogen sulfide donor NaHS on the cardiac control in rats during low doses of ionizing radiation and characterize the predictors of imbalance in autonomic nervous system activities.

Methods: Adult male rats were treated once with NaHS 7.4 mg/kg, i.p. or vehicle (saline), without and with total body irradiation (TBI). The irradiation of animals in experimental groups was single-fractional, total, with total absorbed dose – 2 Gy. Heart rate variability (HRV) was recorded in rats after 30 minutes with NaHS pre-treatment, 24 h of TBI exposure and NaHS and TBI combination.

Results: HRV analysis revealed that low doses of radiation cause cardiac autonomic dysfunction. TBI rats exhibited signs of activation of sympathetic nervous system, while NaHS-treated rats showed mobilization of all parts of regulatory systems with predominant activation of the parasympathetic nervous system (PSNS). Overall, these results indicate that NaHS is involved in cardiac control and decreases sensitivity to low doses of radiation.

Conclusion: The gaseous messenger H2S under conditions of radiation modulates adaptive processes, acting as a regulator for imbalance of autonomic nervous system and may serve as a novel cardioprotective agent for ANS imbalance.

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Introduction

Recent data on extracellular and intracellular control of biological activities of gas transmitter

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KEYWORDS: hydrogen sulfide H2S, sodium bisulfide, radiation effects, autonomic nervous system (ANS), cardiovascular system (CVS), heart rate control

Hydrogen Sulfide (H2S) has shown that it plays multiple roles in several signaling pathways of physiological functions in almost all systems of the body [4, 12, 22]. According to the latest data, H2S is an important key regulator in the cardiovascular system [1, 17], neural signaling [27], gastrointestinal tract [13, 25], anti-inflammatory activity [28], as well as metabolic disorders [29]. Moreover, it has been established that the cardio-protective effect of hydrogen sulfide manifests in the reduction of myocardial damage during ischemia-reperfusion injury, oxidative stress, etc.[8, 20, 21]. H2S in physiological endogenous concentrations shows local [24, 25] and central effects (control of blood pressure and cardiac activity) [4, 12]. According to the other reports, H2S has been registered to have dose-dependent negative chronic and inotropic effects on the heart, which obviously depends on the initial state of the myocardium [23]. It is known that H2S can act as a free radical scavenger, reacting with radical oxygen species (ROS) and radical nitrogen species (RNS), including superoxide anion radical, peroxide, peroxynitrite and hypochlorite [5, 30, 14]. Several studies have also been conducted to show mechanisms of regulation of the physiological functions involving higher levels of control with the participation of H2S. Thus, the authors of the present study tried to analyze the impact of hydrogen sulfide on peripheral and central mechanisms involved in control of cardiac activity. One of the integrative tests for assessing the functional activity of different levels of regulatory systems was the monitoring of heart rate variability (HRV) [3, 7, 11, 16]. This non-invasive method enables not only high accuracy in assessing the activity of the autonomic nervous system (ANS) in experimental conditions, but also reflects the physiological and biochemical regulatory mechanisms, the capacity of aerobic metabolism, as well as the physiological reserve capabilities of the physiological system [15, 18, 19].

Due to its high reducing properties, H2S participates in the regulation of changes in oxidative and nitrosative stress [11, 14, 17]. One of the factors that causes oxidative stress is the effect of ionizing radiation. Natural and man-made sources of ionizing radiation contribute to human exposure and, accordingly, increase potential risk to human health. Much radiation is unavoidable, e.g., natural radiation, and represents a potential health risk [6]. As known, the human body responds to various physical, chemical and biological stimuli by developing a potent inflammatory response that triggers defense mechanisms crucial for maintaining tissue integrity and restoring tissue homeostasis and functions. Ionizing radiation-induced systemic effects usually arise from local exposure of an organ or part of the body [10]. The target for low doses of radiation are the membranes of subcellular and cellular structures, in which free radical processes are induced. For experimental rats, who are highly radioresistant animals (LD50 7 – 7.5 Gy), the dose of 2 Gy is considered low [2]. Relatively little work has been done on this topic associated with the effects of H2S and its main mechanisms involved in the systemic actions of ionizing radiation on the level of the whole body or on cellular level. Recently, it has been shown that low-dose radiation has a harmful systemic effect on the human body [6, 10, 26], but the influence of NaHS pretreatment during LDR injury is still not clear.

Therefore, the aim of this study was to analyze the effects of exogenous administration of hydrogen sulfide donor NaHS on cardiac control in rats under LDR and implement a comprehensive analysis of these changes in the parameters of HRV.

Materials and methods

Twenty-five male rats with an average weight 180-200 g that were used in our study were kept in conditions that were in accordance with university policies, and all experiments were approved by the Ethics Committee of Lviv National Medical University (20.01.2015; №1). The animals were selected into experimental groups (n=5): 1) control group – rats with single administration of 0.9% solution of NaCl at a dose 0.5 ml, i.p.; 2) group with single administration of NaHS at a dose of 7.4 mg/kg (Sigma Aldrich,

USA), i.p. and registration of HRV values in 30 minutes; 3) group with administration of NaHS at the same dose and determination of HRV in 1 day; 4) group that underwent irradiation in the total absorbed dose of 2 Gy; 5) group with introduction of NaHS + irradiation (2 Gy). Animals of the experimental group were irradiated with a single-fractional teletherapy machine device "Teragam" (source ^{60}Co) at a dose rate of 0.0393 R/s and a "source-surface" of 0.8 m. The total absorbed dose was 2 Gy. During irradiation, the animals were placed in individual cages-fixators.

We recorded a peripheral pulse non-invasively in non-anaesthetized animals for the purpose of HRV analysis (Hzhehotskyi MR, Patent UA, 2008) [9]. To record the parameters, 5 minutes after the rats stabilized, they were placed in a Plexiglas chamber and a photoplethysmographic transducer was attached to the base of each animal's tail [9]. Measuring of HRV parameters was carried out before the administration of NaHS, after 30 minutes and one day after the procedure, as well as one day after irradiation. Changes in HRV parameters were compared to their baseline values (before manipulations), which served as a control.

The duration of cardiointervals (AverVal Interv) was determined using software and a special fast-acting recording device [9]. During recording, dynamic series of cardiointervals were presented in the form of a cardiointervalograms. There was simultaneous analysis of dynamic series of cardiointervals and interpretation of HRV analysis data. The activity of the system-regulatory mechanisms of experimental animals was evaluated on the basis of spectral statistical methods, as well as variation pulsometry. Among the statistical parameters of the dynamic series of cardiointervals, the following indexes were determined: SDNN – the mean square deviation of the mean cardiointerval duration, CV – the coefficient of variation of the full array of cardiointervals, which characterizes the total effect of autonomous blood flow regulation; RMSSD – the square root of the sum of the differences in the series of cardiointervals, which was an indicator of activity of the parasympathetic link of autonomous regulation [3, 18].

Among the parameters of the variational pulsometry, the following ones were determined: the difference between the maximum and minimum values of cardiointervals (MxDMn) – the maximum amplitude of regulatory influences; mode (Mo) – the most likely level of functioning of the sinus node of the heart; mode amplitude (AMo) was a conditional indicator of the activity of the sympathetic link of regulation [3, 18]. With the help of spectral analysis, it was possible to characterize such parameters as the total power of the HRV spectrum (TP, 0.015 – 3.0 Hz) – the total absolute level of activity of regulatory systems [3, 18]. For the correct reproduction of the results of studies conducted in laboratory animals, the analysis of HRV was carried out using spectral components in the following frequency bands: Low Frequency – LF (0.015-0.25) Hz, which was associated with the level of activity of ergotropic slow influences, Mid Frequency – MF – (0.25-0.75) Hz, caused by sympathetic modulating effect on the cardiovascular system, High Frequency – HF – (0.75-3.0) Hz, which was characteristic of the activity level of the parasympathetic link of regulation [20, 21]. The digital results of all measurements were subjected to statistical variation analysis. The results were processed using Microsoft Excel spreadsheets and statistical software Statistica for Windows.

Results

As a result of the conducted analyses, it was found that the total power of the spectrum has increased to 78% in comparison to the basal level (control group) due to the increase of spectral power in all frequency bands, significantly 30 minutes after the administration of the H2S donor (Figure 1). Analysis of the internal structure of the spectrum has shown increased proportion of HF (24% ($p<0.05$)) and tendency of decreasing proportion of MF and LF. Correspondingly, the sympathovagal index

(MF/HF) decreased to 26% (<0.05) vs the control group (Figure 1).

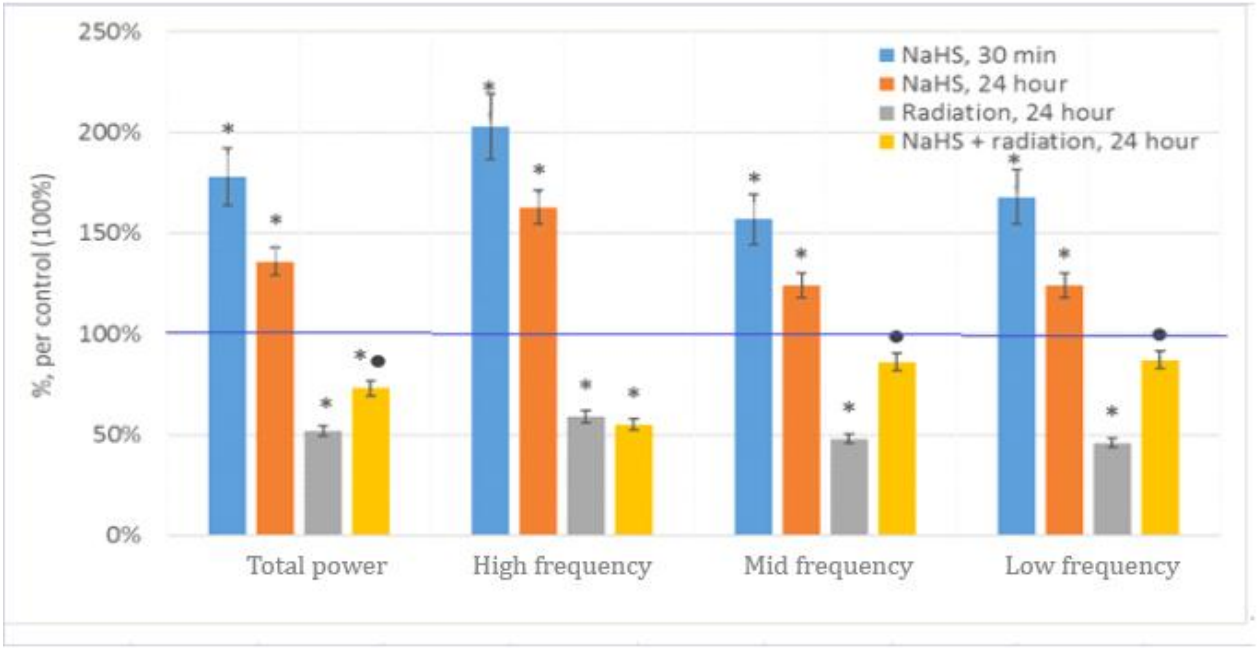

Figure 1. Changes in HRV spectrum indexes under the radiation exposure and previous administration of NaHS vs control group, which is marked as 100 % (blue line). Note: *– p<0.05 significance in relation to the control group; • – p<0.05 significance in relation to the irradiation group.

Also, the increased spectral power in the range of high frequency oscillations was detected, which correlates with rise of CV to 75% (p<0.05), SDNN to 32%, RMSSD to 41% (p<0.05), and MxDMn to 53% (p<0.05)). In general, this testifies to the activation of the autonomous circuit of regulation of cardiac activity (Table 1).

Decreased AMo (18% (p<0.05)), as well as MF/HF in comparison to control group indicates inhibition of sympathetic nervous system (SNS) activity. At the same time, a positive chronotropic effect was recorded, which may result in an increase in cardiac output (Table 1).

Table 1. Changes of HRV in rats

Parameters	Control	NaHS 30 min	NaHS 1st day	Irradiation 1st day	NaHS + irradiation 1st day
HR (heart rate), bpm	431.4±27.3	539.3±39.7*	444.7±29.7	433.1±31.2	406.1±24.7
SDNN (mean square deviation of the mean cardiointerval duration), s	0.030±0.003	0.039±0.003*	0.037±0.002*	0.024±0.002	0.029±0.002
CV (coefficient of variation), %	22.1±19	38.6±2.8*	27.9±2.3*	17.7±1.3*	19.0±1.5
RMSSD (square root of the sum of the differences in series of cardiointervals), s	0.031±0,003	0.043±0.004*	0.038±0.003	0.025±0.002	0.026±0.002
Mo (mode), s	0.151±0.012	0.151±0.014	0.151±0.013	0.147±0.012	0.151±0.013
AMo (mode amplitude), %	37.1±3.1	30.4±2.7	25.4±2.1*	36.3±3.2	37.2±3.4
MxDMn (maximum and minimum values of cardiointervals), s	0.128±0.011	0.196±0.017*	0.148±0.013	0.138±0.012	0.122±0.014

Note: * – $p<0.05$ significance in relation to the control group.

The results of HRV 24 h after administration of NaHS have shown noted maintenance of a high level of TP which exceeded the control value on 37% ($p<0.05$), however, it decreased on about 23% ($p<0.05$) after the influence of hydrogen sulfide on the 30th minute (Figure 1). As in the previous term, the spectral power increased the most in the range of high-frequency oscillations, both in comparison with the initial level and the level measured 30 minutes after the influence of NaHS. The increase of statistical parameters of HRV as CV, SDNN, RMSSD, as well as MxDMn (on average 25% ($p<0.05$) vs control data) was also noted, which indicates an increase in the tonus of the PSNS, but it was of a lesser degree of severity than data recorded at the 30th minute (Table 1).

One day after TBI at a dose of 2 Gy, the total power of spectrum (TP) decreased twofold compared to the control group (Figure 1). The decrease in power in all spectrum ranges was recorded: LF and MF to 54% and 52% ($p<0.05$) respectively, HF – to 40% ($p<0.05$). This data suggests a significant inhibition of the activity of all parts of cardiac regulatory systems. In that case, in the internal structure of the spectrum, the proportion of LF decreased to 16%, and MF practically did not change. The reduction of HRV time parameters (SDNN, CV, RMSSD) was marked (20% on average ($p<0.05$)), which indicates inhibition of PSNS activity (Table 1). In terms of the influence of irradiation accompanied by administration of NaHS, it was found that the values of time indexes have increased significantly relative to irradiation and almost reached the baseline (Table 1). Thus, the total power of the spectrum 1 day after irradiation accompanied by administration of a donor hydrogen sulfide was reduced to 27%

relative to the results of control, but it was significantly higher than at single irradiation (Figure 1). At the same time, the spectral power in the range of LF and MF, which was 86% vs control, was significantly increased compared to the rats with TBI effect.

Discussion

The obtained data are consistent with the results of other researchers, which have established the cardioprotective effect of H2S, manifested by an increase in the strength of cardiac contractions, as well as vasodilator effect in the period after the administration of a donor hydrogen sulfide [12, 13]. The increase in TP, as well as the spectral power in the HF and MF, RMSSD and other HRV statistical indexes, the decrease in MF/HF can be regarded as an increase in functional and metabolic reserves under the influence of H2S. Consequently, the revealed complex array of changes in the parameters of HRV indicates the mobilization of all regulatory systems with the predominant activation of the parasympathetic nervous system (PSNS) after the administration of NaHS [8, 11].

An elevated level of balanced autonomous components (HF + MF) and decrease of MF/HF index was a prominent marker of influence of peripheral regulatory system on central control. The obtained results of the study indicated a protective effect of NaHS under influence of LDR, which indirectly indicates improvement of processes of adaptation to the action of radiation under the influence of hydrogen sulfide biosynthesis modification [19], which consisted of activation of various links of regulatory systems with prevailing mobilization of the parasympathetic nervous system and moderate activation of peripheral control system regulated by H2S. On the 1st day after administration of NaHS, there was a prolonged effect in comparison to that found in the initial (30 min.) period of exposure to the hydrogen sulfide donor, NaHS, which ensures the maintenance of increased activity of regulatory processes. Thus, the reduction of the total effect of autonomic regulation by SDNN, RMSSD and CV correlated with the reduced total level of activity of regulatory processes by the spectral index of TP and indicated the activation of central regulatory systems. The previous administration of NaHS before irradiation led to a less pronounced reduction in TP than with irradiation alone, indicating the maintaining of a much higher level of regulatory processes in these conditions than with irradiation alone.

The effect of donor hydrogen sulfide NaHS against low-dose radiation injury, which was reflected in the modulation of adaptive processes, acting as a regulator of imbalance of autonomic nervous system, may serve as a novel cardioprotective agent increasing the capacity of restorative processes.

Conclusion

In conclusion, the data of the present study support that exogenous stimulation of peripheral H2S-dependent signaling processes by administration of NaHS significantly affected the systemic regulatory mechanisms of cardiac activity on different levels. After 30 minutes and 1 day following the administration of the donor hydrogen sulfide, activation of all the links of regulatory systems with the predominant mobilization of PSNS was marked. One day after irradiation at a dose of 2 Gy, the TP decrease was twofold, with decreasing power in all ranges, indicating significant inhibition of the activity of all regulatory systems. Finally, irradiation accompanied by previous administration of NaHS leads to a less pronounced reduction in TP than with irradiation alone, indicating the maintaining of a higher level of regulatory processes.

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Hemochromatosis Treatment by Venipuncture Through History

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Abstract

Hemochromatosis is a hereditary disease caused by the mutation of genes responsible for regulating iron metabolism in the body. The mutation results in increased absorption of iron from food, which is then deposited in various organs and tissues. Due to the excessive decomposition of iron, organs, most commonly liver, heart and pancreas are damaged. The standard therapeutic procedure for the treatment of hereditary hemochromatosis is phlebotomy or venipuncture, which removes excess iron from the blood. The therapeutic procedure is carried out until the iron level returns to the reference interval. Ancient, three thousand years old bloodletting skills are still used to this day. It was considered that the bloodletting establishes a good balance of bodily fluids referred to as eucrasia. Venipuncture was used to treat various diseases, with different amount of blood released, from half a liter to two liters, and sometimes even more. Venipuncture was applied not only for the treatment of existing diseases, but also as a preventive measure. To date, the use of therapeutic venipuncture has remained the gold standard for the treatment of hereditary hemochromatosis.

The aim of this paper is to present a historical review of venipuncture or phlebotomy as a therapeutic procedure for the treatment of hemochromatosis.

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Introduction

There are various expressions used to describe the procedure of therapeutic blood sampling, venipuncture, phlebotomy, venesection and bloodletting. A widely used expression across the English-speaking world is "phlebotomy", derived from the Greek word "phleb", which stands for "vein", and "tómos" which means "incision". Phlebotomy is the process of opening a vein by incision in order to take blood for therapeutic treatment or diagnosis. One of the first people who recognized the importance of veins and arteries was the Chinese Emperor known as the Yellow Emperor or Huang-Di (who ruled China in the 27th century BC). His famous remark was that "everything in the blood is under the control of the heart" (1).

The ancient skill of bloodletting dates back to about three thousand years ago. Ancient Greeks, Egyptians, the peoples of Mesopotamia, the Maya and the Aztecs all knew the practice of blood-shedding, or bloodletting, as a treatment

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for diseases. This practice began with the Egyptians 1000 years before Christ and was popular among doctors until the late 19th century. Bloodletting was performed by shavers at the recommendation of a physician. Barbershops that performed the procedure were recognized by a barber's pole with red and white stripes. Through history, bloodletting, especially with the help of leeches, was practiced as a treatment for existing diseases and as a method of prevention. Still, not everything was bad in the treatment by bloodletting. Medicinal leeches were used for improving circulation and blood flow during surgery. In some cultures, these treatments are still used today (2).

According to Hippocrates (460 BC - 380 BC), a person's personality (temperament) is determined by four body fluids (called "humors"): phlegm, yellow bile, black bile and blood. Hippocrates defined health as a good mixture (eucrasia), and disease as a bad mixture of these juices (dyscrasia). He used clinical observations to recognize the symptoms of certain diseases. All four classical elements: fire, earth, water and air were considered present in the blood, and it was believed that the letting of blood would restore the patient's general wellbeing. Blood loss was considered beneficial because it was a way of balancing the fluids. Hippocrates believed that the fluids had to be perfectly balanced in order for the individual to have good health. Any imbalance was treated by bloodletting (3).

In Greece, Galen of Pergamon (129-200 AD), a prominent Greek physician, surgeon and philosopher, first described that not only veins were filled with blood, but arteries as well, which were previously considered to be filled with air. He also believed in the humoral balance. He believed that blood should be released near the diseased part of the body. According to him, fever, termination of organ functions and headache are a result of excess blood, so the surgeon should tie the patient's hands to make the veins swell and become more pronounced in order to release a certain amount of blood (4).

In Hebrew teaching, specifically in the Talmud, it is said that the release of blood should be carried out on certain days of the week and month. Christianity adopts similar guidelines. Islamic scholars also believe that the release of blood is very useful for the treatment of fevers. Physical signs that required bloodletting were redness, swelling, skin warm to the touch, and any sign of pus. The treatment consisted of making numerous cuts on the body, and letting the blood flow into specially prepared receptacles. However, the treatment would be stopped, if the patient felt weak. Interestingly, there is no data on the number of deaths during these procedures (2,3).

Hemochromatosis

Primary hemochromatosis is a hereditary disease caused by HFE gene mutation on chromosome 6. This is the most common autosomal recessive hereditary disease of the Caucasian population in Northern European countries, with prevalence in Europe reported as 1: 300. It is equally represented amongst both male and female populations. Sickness rarely occurs in the African and African American population, as well as in the Asian population. Men develop a clinical picture more often than women, about 10 times more often, the reason for this being the loss of iron in women during periods, birth and breastfeeding. Hereditary hemochromatosis is divided into four subtypes, and only type 1 is clinically important. A total of 85-90% of patients with confirmed diagnosis of hemochromatosis are homozygotes for mutation C282Y (Y / Y genotype), while about 5% are heterozygotes for mutations C282Y, H63D and / or S65C. In individuals who did not demonstrate mutation C282Y, and who are heterozygotes for S65C and / or H63D, the possibility of developing a clinical picture of inherited hemochromatosis is extremely low (5.6).

Diagnoses of hemochromatosis include laboratory diagnostics, radiological diagnostics and histological treatment. The clinical picture of hemochromatosis includes tiredness, hyperpigmentation (bronze skin color), loss of

libido in men, diabetes, joint pain, cirrhosis of the liver and cardiomyopathy.

Laboratory diagnostics show elevated levels of serum iron and ferritin as well as increased transferrin saturation index.

Applying venipuncture in the treatment of hemochromatosis

Hemochromatosis was first described in Paris in 1865 by Armand Trousseau, a French physician, who outlined the disease as a set of symptoms involving diabetes, skin pigmentation, and liver cirrhosis. Trousseau did not name the disease (2).

Davis and Arrowsmith are attributed with the first report on the treatment of hemochromatosis which showed the value of venipuncture. In their report, they state that repeated venipunctures can remove harmful iron. To confirm the diagnosis of hemochromatosis, they carried out a liver biopsy. The first patient was a 69-year-old woman. Between March 1947 and February 1949, 40 liters of blood were drawn from this patient. According to their report, all patients treated by bloodletting showed a general improvement (7).

In 1954, Davey, Foxell and Kamp reported the case of a man at the age of 55 who was hospitalized in October 1950. The patient complained of abdominal pain that had lasted for 2 years, increased tiredness and gray and black pigmentation of the face, neck and upper arm. His liver was enlarged, spleen was not palpable, and free intra-abdominal fluid was found. He had not suffered from previous illnesses except for colon cancer surgery in 1936. He drank plenty of beer daily. The tested urine showed glucose in the urine. Skin biopsy showed iron deposits, especially around the sweat glands. The patient was treated with repeated venesections. Over the course of two years, 31 liters of blood were drawn from the patient. Heavy anemia developed, although the patient showed subjective and objective improvement. The patient was last registered in 1954 when his symptoms were pain in the joints. A report was published, claiming that venesection is an effective hemochromatosis treatment method (8). In 1965, a group of authors (Block, Moore, Wasi and Haiby) investigated a cohort of patients with hemochromatosis. In their research, a liver biopsy was performed prior to venesection, the findings of which suggested that liver lesions arise due to abnormal absorption of iron despite a normal diet (9). In an article published in 1969, Dr. R. Williamson reported that over a period of 5 years, the mortality of patients treated with repeat venesection was 11%, while the mortality of untreated patients was more than 60% (10). Sargent et al. report on two hypotheses regarding hereditary hemochromatosis. One hypothesis is that inherited hemochromatosis results in excessive absorption of iron from food consumed by the patient, which is the deposited in the organs, where it causes toxic organ damage. The second hypothesis is that hereditary hemochromatosis is actually a form of cirrhosis that is further complicated by excessive iron from the patient's diet. In eight patients with hereditary hemochromatosis, iron levels were tested before, during and after therapy (venipuncture). In all cases, serum iron levels after therapy were within normal limits, as were other blood indices. This is in favor of the hypothesis that inherited hemochromatosis is a disorder in the mechanism responsible for controlling iron absorption from food (11).

Untreated hemochromatosis can result in death due to cardiac complications. Heart damage is manifested as cardiomyopathy, arrhythmia and heart failure. A period of 12 months is required to restore normal liver function and up to 30 months to fully recover from angina. Continuous and controlled therapy (venipuncture) is needed to restore normal parameters (12).

An early diagnosis of hereditary hemochromatosis has a tremendous significance in preventing the development of diabetes mellitus as well as hepatocellular carcinoma. Niederau et al. claim that patients who received timely therapy prior to the onset of cirrhosis did not develop hepatocellular carcinoma later. Early treatment with venipuncture prevents the formation of cirrhosis

and attention should therefore be focused on early diagnosis of hereditary hemochromatosis (13).

A research conducted by a group of authors in Norway shows that the treating of hereditary hemochromatosis by venipuncture leads to a change in the level of some trace elements, including increased absorption of toxic elements such as copper. (14).

Conclusion

Therapeutic venipuncture or phlebotomy is the golden standard for the hemochromatosis therapy. In the procedure, 500 ml of blood containing 200 to 250 mg of iron is extracted. Standard procedures prescribe periodic venipuncture, approximately once a week, with the letting of 400-500 ml of blood, during the first 3 years, followed by 400-500 ml every 1-3 months. Venipuncture is carried out until the levels of iron and ferritin drop to the lower limit of the normal reference interval. Venipuncture can be performed on younger patients 2-3 times a week. The aim is to achieve ferritin values below 50 ng / ml and a ferritin saturation index under 50% in serum. Most patients can tolerate quite well a week of undergoing the procedure, which should be performed until hematocrit falls below 37%. To monitor the therapeutic response, it is recommended to check serum saturation and serum ferritin levels every 2 to 3 months (15).

Treatment of primary hemochromatosis by venipuncture is a safe and effective way of removing blood in order to reduce iron levels. Treatment is a lengthy process, usually lasting several years until iron values return to normal. Venipuncture improves the overall condition of the patient, alleviates the symptoms of fatigue, pain, and skin pigmentation. Regular therapy prevents liver complications, heart disease and diabetes (16).

Despite the advances in technology, bloodletting as a therapeutic procedure has survived to this day as a controlled, supervised, safe and efficient treatment option. In this procedure, it is important to follow all the guidelines for proper blood drawing. The role of medical laboratory personnel in diagnosing and monitoring primary hemochromatosis is vital. Laboratory tests allow the physician to make a proper diagnosis and evaluate the overall medical condition during the course of disease monitoring

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A Partner Relationship in Meeting the Needs of Patients and Their Families

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Abstract

A nurse is the first person who comes in contact with patients and their family members. She shares information with them by verbal and non-verbal communication, with the goal of establishing a good relationship and focusing her attention on the patient as a whole person. Nurse's sincere thoughts and feelings create a sense of security and open communication, which are key elements for providing healthcare and inclusion of the family in the decision-making process. A nurse who spends her time by the patient's side plans, implements, evaluates and documents the changes in the patient's condition. That way the healthcare is focused on meeting the physiological needs and maintaining stable condition of the patient. Struggle for life doesn't leave any time for the family to adjust to the new situation. Establishing the patient – family – nurse relationship is important not only in the beginning, but also during the whole treatment and decision-making process. When relationships within the family are stable, the family has an irreplaceable role in developing a sense of security and belonging for the patient.

Providing care for the patient is a priority for the healthcare team, whereas the most significant thing for the family are sincere and correct information about the patient's current condition, the effect of the applied therapy and the possible outcome of the treatment. Developing care philosophy focused on the family and the holistic approach includes assessment of the family's needs and the impact of the disease on its overall functioning. Cultural factors play a significant role in the ability to understand not only the patient's but also the family's point of view. Medical, ethical, legal and a whole range of other problems connected to the receiver and provider of services can be avoided by effective communication.

The purpose of this paper is to highlight the importance of a partner relationship, focusing on the family and the essential role of the nurse in decision-making.

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Introduction

Providing quality healthcare to the patient, cooperating with the family and establishing a good relationship in a multidisciplinary team are requirements nurses face in a holistic approach to the patient. Frequency and availability of information and discussion about the uncertainty of the prognosis help in creating a good working environment among healthcare team members and successful cooperation with the family. An illness is not stressful only for the suffering patient but also for family members and other persons from the patient's environment with whom he/she is in contact. The family's primary role is to support and protect its member throughout the course of the illness and the related decision-making process. This process is complex, the family considers it to be the most important one, but more importantly, the quality of service provision and the patient's quality of life depend on it. In deciding about procedures and interventions for the patient whose life is threatened, the recommended model is the joint decision-making, which includes clinicians, family members, relatives or a guardian, and it is important that all of them receive correct, valid and necessary information at the same time. Responsibility and decision-making are ethical issues. The family wants to actively participate in order to fulfil the wishes of the patient and expects open and sincere communication. In practice, a positive effect of family support on the outcome of a serious illness requiring intensive care has been recognised for decades. The role and participation of the nurse in decision-making is connected to the quality of health services, and the nurse's thoughtfulness and kindness towards the patient and the family contribute to the development of feelings of security and trust. Through her work by the patient's side and by providing comfortableness, gentle touch and information about any changes in the patient's condition, the nurse helps the family members develop an understanding of the patient's condition, as well as a perception of future development of the situation. By frequently following-up on the patient, the nurse becomes very well acquainted with the patient's personality.

Partnership Approach and Team Members

For the sake of cooperation within the team and a greater satisfaction of the patient and family members, members of a multidisciplinary team have to be responsible, properly trained, possess good communicational skills and be ready to hear other people and acknowledge their opinion. Nurses' skills imply more than just instrumental and technical skills and providing information to the patient - they also pertain to integration of affective and relational aspects of communication. Communication is the basis for ensuring satisfaction and understanding of expectations, it is to be conducted in detail, and often. Family members need to be given an opportunity to express their concern and seek clarification for anything that they do not understand during the discussion, in order to be able to make right decisions (1). Their needs and satisfaction depend on many factors, such as their social and economic situation, availability of health care or literacy. Anxiety that appears when someone is facing death can be alleviated through existential care, during which healthcare workers explore their own sense and values after witnessing the suffering of a patient whose life is threatened by an illness (2). In terminal patients, the comfort is made by sharing fears with the patient and the family (2), who have many unanswered questions, depending on their unique cultural, economic and religious background. Adequate and efficient communication among family members in the decision-making process protects the autonomy of the patient (3,4). In order for the nurses to be able to meet all the requirements in providing holistic care, they need to become actively involved in the discussion about the patient and in the actual decision-making process, which would result in greater satisfaction regarding communication with the family. It would also help alleviate patients' fear, and prevent burnout in nurses. It is necessary to implement continuous training in communicational skills and the Nursing Act

needs to be amended to include duties and competences, counselling and support for the patients' families and the development of a family-oriented concept (5,6). Without all-encompassing organizational dedication to the patient and the family, these challenges represent a barrier that prevents change of healthcare system culture. Care for the patient and the family is focused solely on the service provided to the users (7). Partnership approach acknowledges and attempts to use the knowledge and skills of both participants, and each brings a different, but potentially valuable and complementary relationship (8), which includes sharing ideas and mutual teaching (9).

Nurses and decision-making

Professionalism in nursing needs to reflect one's enjoyment in work. Nurses have to provide care by seeing the world through patients' eyes and meet their needs while sharing the experience of being hospitalised, developing a mutual partner relationship. Nurses have to be capable and willing to spend time with the patient and to be available to both the patient and their family. They should thoughtfully consider a person as an individual, not merely acquired compassion with the transfer of information in a non-verbal way, but also reacting to signs and expressing feelings of empathy. Nurses share their concerns and develop a feeling that each patient is an individual by actively listening to the patients and not by treating them as a number. They encourage inner strength and hope for accepting and implementing the therapy, increase motivation and raise the quality of their relationships.

Nurses are not always able to correctly asses and meet patient's needs (10), and they are often not included in the decision-making process (11,12). In their work, nurses encounter unsatisfactory communication and cooperation with doctors, which is a consequence of insufficient participation in the decision-making process and receiving information about the patient.

Nonparticipation in the decision-making and the lack of openness and dialogue arouses suspicion and distrust in the course and outcomes of the treatment, and deepens the feeling of anxiety and loneliness in family members in a time of a difficult existential situation. Nurses who devote more time to patients and members of their families have valuable information about the patient and the situation within the family. The family can share their concerns regarding the patient with them, and the outcomes of good communication are associated with stress reduction. Consequences of a lack of information are increased loneliness, insecurity and lack of understanding in the decision-making process. Nurses need more education in developing communication skills required for their work with a dying patient and his/her family (13). In those circumstances, a lack of trust and communication stimulate fear and patients become overwhelmed with anxiety and anger (2). Some nurses give patients and their families an opportunity to ask questions about issues that cause anxiety and hopelessness, or to talk about their feelings and desires for the future (2). When there is an increased likelihood that a patient will die, healthcare team members have to be ready to talk about it, because that is expected by the patient and his/her family.

Family and decision-making

A research conducted by Reesal Bath (2000) came to a conclusion that relatives need information but cannot always receive it from health professionals. Considering that they are in a state of physical and emotional exhaustion, it is unknown to what extent the patients are capable and competent to participate in the decision-making process, hence the responsibility is perceived as divided among those who are included in such process (14).

The lack of involvement of the family and lack of information, openness and reciprocity in decision-making leads to feelings of abandonment, inability to protect or support. A passive attitude in the process of decision-making can lead to anxiety and depression (15), whereas active involvement result in traumatic stress symptoms (16). Interaction between the family, nurses and doctors is of vital importance, where mutual trust is crucial (17,18). A systematic

approach enables expertise, communication skills, awareness, empathy and ability to adjust the plan to the relevant situation (19), which enables good cooperation with the family. The family needs to actively participate, plan and jointly make decisions regarding care provision to their loved-ones. Caregivers are faced with challenges regarding communication with the patients and providing information on their condition. The patient needs to choose to whom the information relating to his/her health can be provided. Providing information is a matter of privacy and wish which should be of utmost importance (20). Access to information about the patient's health condition, requirements and quality of relationship with the staff are the primary needs of the family, and meeting those needs is a primary responsibility of doctors and nurses in intensive care units, which is important for assessment of care quality (21). The family finds itself in a whirlpool of insecurity, shock, helplessness and confusion. The support is priceless (21). Little research has been conducted on interventions with families of critically ill patients, and almost no one has done anything to improve communication between the healthcare team and the patient's family (9). Nurses have a leading role in facilitating cooperation between the family physician, nurses, family members, applying a collaborative approach to the problem through planning and providing holistic care and integrating palliative care. Communication needs to be adjusted to suit each family individually. Monitoring of the protocol and application of standardized procedures in practice would help with the assessment for solving the patient's and the family's problems. Relatives of patients with carcinoma have different needs; however, their priority is quality care, in which they include their own perception and seek nurses' support. Relatives, in turn, provide immense support in reduction of stress and anxiety, and it is therefore important that the patient, while still able to do so, nominate a person who is to be informed about his/her condition. The family and the patient need time to realise that futile life-sustaining procedures are conduct to give time to the family to prepare itself for the final decision step by step (22-26). In such a way, providing care to the patient and caring for the family make up a unit in the process of joint decision-making (24).

Communication in the ER

Unexpected admission of a patient in the ER for the purposes of reanimation is a traumatic experience for the patient and his family, and it requires great support from the medical staff during and after the resuscitation. Important features of care for the family are presence and proximity of loved-one, meaningful information, support provided by the staff and comfort. After repeated but unsuccesful resuscitation, those who suddenly find themselves in mourning feel the need to see the body of their loved-ones and touch them, which enables them to better come to terms with their loss and ultimately helps them in the mourning process.. In such situations they want to hear (and ask for) an explanation regarding the circumstances of their loved-one's death but while they are in the state of shock, they cannot take in verbal information. Many authors also describe a psychological trauma experienced by the family members who were there when the resuscitation took place. In the aspect of healthcare providing, nurses meet basic human needs and consider that it is not possible to provide genuine holistic care in these situations. They often exhibit their inability to do so through insensitivity, disinterest, inhumanity and coldness (27). Determining the ability to make decisions on behalf of the weakened and exhausted patients depends on the assessment of the kind of help that caregivers can provide. The family is uncertain, they do not know what the patient knows or does not know, or what the patient him/herself would give their consent to or what they would be able to give their consent to. They often experience moments of hope related to improvement, but they also experience constant fear of deterioration or possible loss of loved-one, and confrontation with termination of treatment and acceptance of reality. Relatives suffer from mental disorders such as fear, depression, uncertainty, helplessness and hopelessness, but they also struggle with financial difficulties, problems with their work or education, problems with the

changing of roles in the family and social isolation. They often suffer from headache, back pain, sleeping disorders, fatigue, loss of appetite and reduction in physical strength (28) and they experience a high level of emotional stress (29,30).

Role of the Partner in Providing Support

Partners, relatives and close friends are not just advisors, but also a main source of support for patients with carcinoma (31) through provision of emotional stability and help during the treatment and in the patients' everyday functioning. For women suffering from breast cancer, partner's emotional support is of great significance and it alleviates their suffering. Not only does this feeling reduce stress, it also enhances the quality of life, boosts the self-esteem of these women and strengthens their trust in partner (32). Research has shown that in a situation in which one of the partners gets sick, it is the patient's partner who carries greater mental load than the patient him/her self (31), and their involvement in the decision-making process is the best way of providing support. Illness of a family member influences the balance in such a way that it comes to destabilisation and loss of security for all family members. They are faced with requirements caused by the disease, such as providing increased emotional support for the sick partner, redistributing life plans and taking on new tasks or roles within the family (33). Patients' partners seek and must receive emotional support in order to be able to carry their own burden and return to normal function.

Specificity of Communication with Elderly Patients

In an individual approach and contact with an elderly patient, a nurse needs to be familiar with what the patient believes, wants and whether he/she has any specific needs (34). The nurse encourages the patient not to lose hope, to accept the changes and to be persistent in order to achieve greater independence, (35) and also helps the patient in achieving his/her goals. Needs are changeable and dynamic, and in order to meet them, the nurse is expected to provide support as care coordinator (36). Life expectations of elderly patients are ever-growing, and conducting rehabilitation is a key factor in ensuring their independence and improvement of life quality after a traumatic event, in facing deterioration caused by a chronic health condition or in their preoccupation with the feeling of losing themselves (37). Developing a relationship of trust helps the patient in carrying out activities which he/she is capable of performing, in a right manner and in an environment in which he/she feels safe and has a sense of existence. A nurse recognises that through his/her knowledge and intuition, he/she has an irreplaceable role in assessing and providing motivation and support, encouraging and boosting of self-esteem, educating on self-care and helping in everyday activities, using different aids or demonstrating to the patients how to do a certain activity in a simpler way. Intuitive understanding of patients and their family members is a concept that nurses have recognized as a road towards better outcomes (38). Family members feel fulfilled and invest great effort in order for their loved-one to get the best possible care. Patients sometimes start feeling as if they are dependent on nurses, whose role is not sufficiently recognized. In order to achieve greater independence and progress, the method of providing rehabilitation is equally important as all the other interventions and procedures. Research has shown that some family members perceive a nurse based on their own experiences and stereotypes, and not based on the current situation, which does not contribute to the welfare of the patient and his/her motivation.

Conclusion

Nurses should invest more effort in order for the partner relationship to become better integrated in the standards of healthcare provision. The prospect of family participation in the decision-making process depends on the organizational politics in the healthcare system. Genuine

cooperation with patients and their families should be integrated in the organizational culture and more effort should be invested in cooperation with the family in order to further develop and change the rules of practice and encourage the initiative of the patient and family members to become included in the decision-making process. Nurses' dedication and their encouragement for a change to happen within their organizational units can result in change of the entire healthcare system

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