







Sleep quality in people with chronic pain undergoing hemodialysis*

Qualidade do sono em pessoas submetidas à hemodiálise com dor crônica

How to cite this article:

Sousa EVD, Medina LAC, Martins MG, Teixeira CRS, Maniva SJCF, Caetano JA. Sleep quality in people with chronic pain undergoing hemodialysis. Rev Rene. 2023;24:e91962. DOI: <https://doi.org/10.15253/2175-6783.20232491962>

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*Extracted from the Course Conclusion Paper “Avaliação da qualidade do sono em pacientes com doença renal crônica com dor crônica”, Universidade Federal do Ceará, Brasil, 2019.

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Conflict of interest: the authors have declared that there is no conflict of interest.

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ABSTRACT

Objective: to evaluate sleep quality in people with chronic pain undergoing hemodialysis. **Methods:** observational, prospective, and cross-sectional study carried out in two hemodialysis clinics. The sample was formed by 76 people with chronic kidney disease and chronic pain who were undergoing hemodialysis. We used a sociodemographic and clinical form, the visual analogue scale for pain, the McGill questionnaire, and the Pittsburgh Sleep Quality Index. Results were analyzed using descriptive and inferential statistics and correlation tests. **Results:** most participants had very poor sleep quality. There was a correlation between sleep quality and the visual analogue scale for pain ($p=0.027$). There was a negative correlation between McGill pain scale descriptors and sleep quality ($p=0.033$). **Conclusion:** the sleep quality levels of most participants suffered alterations and were classified as poor or very poor. **Contributions to practice:** this study provides data on correlations associated with the sleep quality of patients with chronic pain undergoing hemodialysis. It also gives support for nursing teams to develop interventions to improve the sleep quality of these patients.

Descriptors: Renal Insufficiency, Chronic; Chronic Pain; Renal Dialysis; Sleep Quality.

RESUMO

Objetivo: avaliar a qualidade do sono em pessoas submetidas ao tratamento hemodialítico com dor crônica. **Métodos:** estudo observacional, prospectivo e transversal, realizado em duas clínicas de hemodiálise. A amostra foi composta por 76 pessoas com doença renal crônica em tratamento hemodialítico e dor crônica. Utilizou-se um formulário sociodemográfico e clínico, a escala visual analógica de dor, o questionário McGill e o Índice de Qualidade do Sono de Pittsburgh. Os dados foram analisados utilizando estatística descritiva e inferencial e testes de correlação. **Resultados:** a maioria dos participantes teve uma qualidade de sono muito ruim. Houve correlação entre a qualidade do sono e a escala visual analógica de dor ($p=0,027$). Houve correlação negativa entre os descritores da escala de dor McGill e a qualidade do sono ($p=0,033$). **Conclusão:** o presente estudo mostrou que a maioria dos participantes apresentaram alterações nos níveis de qualidade do sono, que foram classificados como ruins ou muito ruins. **Contribuições para a prática:** o estudo fornece dados sobre a correlação da qualidade do sono em pessoas em hemodiálise com presença de dor crônica, além de fornecer subsídios para o desenvolvimento de intervenções por parte das equipes de enfermagem que ajudem a melhorar a qualidade do sono dessas pessoas.

Descritores: Insuficiência Renal Crônica; Dor Crônica; Diálise Renal; Qualidade do Sono.

Introduction

The etiology of pain in people with Chronic Kidney Disease (CKD) is multi-causal, as it can be a result of other comorbidities, such as diabetes, peripheral vascular disease, or even of hemodialysis treatment procedures⁽⁹⁾.

Pain can cause physical limitations that compromise the performance of daily activities from physical, emotional, and psychological standpoints. This causes the person with CKD to feel insecure, threatened, and frightened, which in turn has consequences such as social distancing, irritability, delayed immune function, depression, and anxiety, in addition to other, negative repercussions on ones quality of life⁽²⁻³⁾.

The prevalence of pain caused by CKD is high. This has been described by literature, which shows that 60 to 90% of people undergoing hemodialysis experience moderate to severe pain⁽⁴⁾. Several factors contribute to the presence of pain in this type of disease, such as: high incidence of bone fragility, progressive loss of muscle mass, vascular diseases, polycystic kidney disease, and treatment consequences. Certain procedures can also lead to pain, such as osteomyelitis in central catheters, ischemic neuropathy of arteriovenous fistulas, ischemic pain, neuropathic pain, and muscle cramps⁽⁵⁻⁶⁾.

Pain, according to the International Association for the Study of Pain (IASP) is an unpleasant physical or emotional sensation, associated with potential or actual harm, which can be acute, subacute, or chronic⁽⁷⁾. NANDA International, Inc., states that acute pain has a sudden or slow onset, with mild to severe intensity, and ends fast or predictably. Chronic pain, on the other hand, has constant or recurring intensity, it does not end at a predictable time, and lasts for more than three months⁽⁸⁾.

It is worth noting that paing can lead to fatigue, changes in appetite, nausea, constipation, difficulty concentrating, impotence, anxiety, and changes in sleep pattern⁽⁹⁻¹⁰⁾. Sleep is a biological need that enables metabolic, hormonal and biochemical changes necessary for the proper functioning of the body. In

people with CKD, sleep-related issues are considered clinically important, as they can lead to stress, to alterations in basic activities of daily living, and even aggravate illnesses such as chronic cardiovascular diseases⁽¹¹⁾.

Therefore, sleep disorders deserve to be emphasized, since literature shows that about 80% of people with CKD have some form of sleep pattern dysfunction, and insomnia is one of the most prevalent diagnosis in this population^(4,12). People with CKD in hemodialysis often present restless legs syndrome, itchy skin, thirst, depression, and other symptoms that lead to sleep disorders⁽¹³⁾. Furthermore, the quality of life of people with CKD is worse, mainly as a result of pain and poor sleep⁽¹⁴⁾.

People with CKD undergoing hemodialysis may trigger factors that cause the appearance of pain, sleep alterations, fatigue, CKD effects, and physical function impairment. Therefore, factors such as pain, sleep, and quality of life are important aspects of people with severe and limiting diseases who undergo long and painful treatments; as a result, these factors must be systematically evaluated⁽¹⁵⁾. Nonetheless, most works in literature are specifically focused on pain, and no study about this population has evaluated sleep quality and chronic pain simultaneously.

This leads to the question: What is the quality of sleep of patients with chronic pain who undergo hemodialysis? Thus, it is necessary to evaluate the sleep quality of people on dialysis, as well as how it relates to chronic pain. The goal of this study was to evaluate sleep quality in people with chronic pain undergoing hemodialysis.

Methods

This is an observational, prospective, and cross-sectional quantitative analysis, following the guidelines of the EQUATOR network through the instrument Strengthening the Reporting of Observational Studies in Epidemiology (STROBE). It was conducted in May 2019, with CKD and chronic pain who were undergoing hemodialysis in the city of Fortaleza, CE, Brazil.

The population was formed by people with CKD and chronic pain undergoing hemodialysis. For sample calculation, we used the formula for cross-sectional studies with finite populations (a) and applied a correction formula for finite populations (b):

$$\text{a) } n_o = \frac{Z\alpha 2.P.(1-P)}{e^2} \quad \text{b) } \frac{n_o}{N} > 0.05n = 1 + \frac{n_o}{N} - 1$$

We aimed for the confidence level of $Z\alpha=1.96$, with a prevalence of the outcome $P=50\%$, and an estimated margin of error of $e=5\%$. The total population of CKD patients and chronic pain in both hemodialysis clinics was $N=94$ patients, resulting in a sample of 384 people. When applying the correlation for infinite populations, we found the value of 6.4. Since this value is higher than 0.05, we considered the population to be finite. Therefore, the population size for the finite population was 69 participants. To avoid potential losses, we added 10% to this value, reaching a final population of 76 participants.

We used non-probabilistic convenience sampling to select participants. Inclusion criteria were: being registered in the clinic, over 18 years old, under treatment for more than three months, and feeling chronic pain for more than three months according to nursing records and a verbal confirmation from the patient. Exclusion criteria were: people with visual and/or hearing disabilities and who reported or presented psychiatric disorders.

Sociodemographic and clinical data were collected using a structure questionnaire including the following information: age, sex, source of income, ethnicity, marital status, education (years of formal study), and follow-up during hemodialysis sessions. The clinical data considered was: comorbidities; duration of dialysis treatment; type of access; presence of pain (in years); pain intensity (zero to ten); most common time of pain.

Pain was evaluated using the Visual Analogue Scale for Pain (VASP) and the McGill Pain Questionnaire (MPQ). The VASP evaluates on dimension of pain, using a line numbered from zero to ten (0-10) where 0

represents no pain, while 10 represents a very intense pain. In addition, the scale is divided into mild pain (0-2), moderate pain (3-7), and severe pain (8-10), with the drawings of faces showing happiness and sadness, and going from blue to red⁽¹⁶⁾. To avoid bias and help the participant understand how to classify their pain, the scale was printed on colored paper sheet.

The MPQ was adapted and validated for the Portuguese language⁽¹⁷⁾. It is a widely used instrument, divided in four groups that allow evaluating the sensory, affective, temporal, and miscellaneous characteristics of the pain. It has 78 words distributed in 4 groups and 20 subgroups. The sensory group has 10 subgroups, with 42 descriptors; the affective group is divided into 5 subgroups with 14 descriptors; the temporal group is divided into 1 subcategory and 5 descriptors; finally, the miscellaneous group has 4 subgroups and 17 descriptors. The participant can choose or not a descriptor from each subgroup. Then, the number of descriptors chosen from each subgroup will be summed, reflecting the total pain index and the number of groups marked. The maximum score is 78 points⁽¹⁷⁾.

To evaluate the sleep of participants we used the Pittsburgh Sleep Quality Index (PSQI), an instrument to collect qualitative and quantitative sleep data from the previous month. It includes 19 self-reported questions divided into 7 components: C1- Subjective sleep quality, C2- Sleep latency, C3- Sleep duration, C4- Habitual sleep efficiency, C5- Sleep disorders, C6- Use of sleep medications, C7- Sleepiness and nighttime dysfunctions. These components are evaluated using Likert-type questions scored from 0 to 3, where 3 is the negative extreme. The sum of the scores can vary from 0 to 21, and there are three cutoff points according to the scores: 0-4 indicates good sleep quality; 5-10, poor sleep quality; and more than 10, very poor sleep quality⁽¹⁸⁾.

For data collection, the nursing team of hemodialysis clinics pointed out their patients who could participate in the research, and the researchers checked the inclusion and exclusion criteria for each patient. We explained the research to participants that

fit the criteria according to the Informed Consent Form, and then asked them to sign two copies of the document. After the participant signed the form, they filled in the sociodemographic and clinical data questionnaire, the VASP, the MPQ, and the PSQI. These instruments were applied during their hemodialysis.

Statistical analyses were performed using the SPSS software, version 2016. We conducted a descriptive analysis of the variables, and prepared tables with the absolute and relative frequencies, means, and standard deviations. Fisher's exact test was used to verify the statistical association between sociodemographic and clinical data, and the level of sleep quality.

We also used Mann-Whitney's U for independent samples to test the equality of the medians of the pain scales and the Pittsburgh Sleep Quality Index. Finally, we used Spearman's rho to evaluate the correlation between the VASP and MPQ scales and the components of the Pittsburgh Sleep Quality Index. We considered the value of 0.05 as having statistical significance.

This investigation complied with the recommendations of Resolution 466/12, which provides for research involving human beings. It was approved by the Research Ethics Committee at the *Universidade Federal do Ceará* under opinion 3,263,780/2019 and Certificate of Submission for Ethical Appreciation 04458418.1.0000.5045.

Results

Most participants were male (43 - 56.6%), brown (46 - 60.5%), married (43 - 56.6%), dependended

on sick pay (48 - 63.2%), had no one accompanying them (42 - 53.3%), had other comorbidities (60 - 78.9%), felt more pain in the morning (43 - 56.6%) and during hemodialysis (14 - 31.8%), and the arteriovenous fistula was the most common type of vascular access (66 - 86.8%).

Sociodemographic and clinical variables of people with chronic kidney disease showed that the mean age of participants was 55.2 (standard deviation (SD)=±14), the mean educational level was 10.3 (SD=±5.5), their time under treatment was 7.8 (SD=±8.2), they had been feeling pain for 6.8 years (SD=±9.9), and the intensity of their pain was 7.8 (SD=±2.3).

Concerning the PSQI evaluation of participants with chronic kidney disease and chronic pain on hemodialysis, the highest mean was related to component 1 (subjective sleep quality), with a value of 2.3 (SD± 0.8) of a maximum of 3. The second greatest mean was in the components: 6 (use of sleep medication) and 7 (Sleepiness and nighttime dysfunctions), both with 1.8, but with SD=±1.0 and SD=±0.9, respectively.

These results show that, at least once a week, some participants needed to resort to sleep medication or had trouble staying awake during daytime activities. The lowest mean was associated with sleep efficiency (0.1 - SD=±0.2), showing that people evaluated had a sleep efficiency > 75%. Regarding the mean of the total score, the result was of SD=±2.5, showing that participants are in the third cutoff point, suggesting a very poor sleep quality (Table 1).

Table 1 – Evaluation of the Pittsburgh Sleep Questionnaire of people with chronic pain undergoing dialysis. Fortaleza, CE, Brazil, 2019

Components	Mean	Standard deviation	Median	Percentile 25	Percentile 75
1) Subjective sleep quality	2.3	0.8	2.0	2.0	3.0
2) Sleep latency	1.6	1.0	2.0	1.0	2.0
3) Sleep duration	1.4	0.9	2.0	1.0	2.0
4) Sleep efficiency	0.1	0.2	0.0	0.0	0.0
5) Sleep disturbances	1.7	0.5	2.0	1.0	2.0
6) Use of sleeping medication	1.8	1.0	2.0	1.0	3.0
7) Sleepiness and nighttime dysfunction	1.8	0.9	2.0	1.0	3.0
Total Scale Score	10.7	2.5	11.0	9.0	13.0

Comparing the mean global score of the Pittsburgh Sleep Quality Index with the mean pain intensity level according to the VASP scale showed that 25 (67.5%) research participants whose pain was classified as severe had poor sleep quality. Additionally, 21 (53.8%) patients who experienced moderate pain presented sleep disturbances (Table 2).

Furthermore, a comparison between the mean VASP scores and the Pittsburgh Sleep Quality Index results showed that patients with poor sleep quality had a higher VASP mean (8.3 - SD±1.9) than those with sleep dysfunctions. Poor sleep quality levels and the total VASP scale had a statistically significant correlation ($p=0.027$).

Table 2 – Comparison of medians of pain intensity and sleep quality of people with chronic pain undergoing dialysis. Fortaleza, CE, Brazil, 2019

Pain intensity (Visual Analogue Scale)	Poor sleep quality (Pittsburgh) (5-10) (n=37)	Very poor sleep quality (Pittsburgh) (>10) (n=39)	p-value*
	n (%)	n (%)	
Mild	0 (0.0)	1 (2.6)	0,050
Moderate	12 (32.4)	21 (53.8)	
Intense	25 (67.6)	17 (43.6)	
Total	37 (100.0)	39 (100.0)	

*Fisher's exact test

Residue analysis showed that pain classified as intense had a statistical difference with a residue of 2.1. Regarding the comparison of the MPQ pain scale with the PSQI, participants with poor and very poor sleep quality were more able to describe their pain using sensory descriptors, with medians of 8.0 and 7.0 (SD±2.1 and ±2.54), and affective descriptors, with medians of 4.0 and 4.0 (SD±1.44 and ±1.5), respectively. Regarding the total number of descriptors, who

se maximum is 20, participants with poor sleep used more (15.0 - SD±4.12) to classify their pain than those with very poor sleep 14.0 (SD±4.48) (Table 3).

The MPQ scale also allowed assessing pain level by summing the value of each descriptor, which resulted in the medians of 33.0 (SD±13.23) and 27.0 (SD±13.01), respectively, in participants with poor and very poor sleep. The maximum result possible was 78.

Table 3 – Comparison between the medians of the McGill Pain Questionnaire descriptors and the quality of sleep of people with chronic pain undergoing dialysis. Fortaleza, CE, Brazil, 2019

No. of descriptors	Poor Sleep Quality (Pittsburgh) (5-10)			Very poor sleep quality (Pittsburgh) (>10)			p-value*
	Median	Percentile 75	Percentile 25	Median	Percentile 75	Percentile 25	
Sensory	8.0	9.0	0.275	7.0	9.0	5.0	0.275
Affective	4.0	5.0	0.859	4.0	5.0	2.0	0.859
Temporal	1.0	1.0	0.947	1.0	1.0	1.0	0.947
Miscellaneous	3.0	3.0	0.361	2.0	3.0	1.0	0.361
Total number of descriptors	15.0	18.0	0.287	14.0	18.0	10.0	0.287
Pain index	33.0	44.0	0.097	27.0	36.0	22.0	0.097

*Mann-Whitney U test for independent samples

Regarding the correlation between the number of descriptors of the MPQ and the PSQI components, there were negative and significant correlations between the number of miscellaneous descriptors and the components No. 2 (sleep latency) and 4 (sleep efficiency), with $p=0.006$ and $p=0.007$, respectively (Table 4).

Additionally, there was a negative correlation between the total number of MPQ descriptors and component 4 (sleep efficiency), showing statistical significance ($p=0.033$). Negative correlations indicate inversely proportional relationships, i.e., when one variable increases, the other tends to decrease. Thus, the highest the number of descriptors, the better/lower is the score of sleep quality components (Table 4).

Table 4 – Correlation of McGill Pain Questionnaire descriptors and the components of the Pittsburgh Sleep Quality Index of people with chronic pain undergoing dialysis. Fortaleza, CE, Brazil, 2019

No. of descriptors	Components						
	1 Subjective sleep quality	2 Sleep latency	3 Sleep duration	4 Sleep efficiency	5 Sleep disturbances	6 Medication	7 Sleepiness and nighttime dysfunctions
Sensory							
r*	-0.109	0.135	0.135	-0.178	-0.770	-0.003	0.120
p	0.347	0.244	0.244	0.124	0.541	0.978	0.300
Affective							
r	0.108	0.126	-0.048	-0.104	-0.077	0.015	-0.196
p	0.353	0.280	0.682	0.370	0.511	0.901	0.091
Temporal							
r	0.128	0.127	-0.04	0.078	0.037	0.015	-0.196
p	0.272	0.276	0.731	0.505	0.130	0.901	0.091
Miscellaneous							
r	-0.017	-0.315	-0.002	-0.306	-0.007	-0.005	-0.189
p	0.886	0.006 [†]	0.986	0.007 [†]	0.953	0.964	0.102
Total							
r	-0.031	-0.207	0.059	-0.245	-0.090	0.001	-0.068
p	0.787	0.72	0.614	0.033 [†]	0.442	0.994	0.562
Pain index							
r	-0.12	-0.129	-0.013	0.155	-0.174	-0.106	-0.185
p	0.300	0.268	0.914	0.181	0.133	0.361	0.109

*Spearman's correlation coefficient; [†]Statistical significance according to Spearman's rho test ($p<0.05$)

Concerning the correspondence between the PSQI and the MPQ pain scale, component 4 (sleep efficiency) presented statistical significance ($p=0.007$ and $p=0.033$) in the correlation with the number of miscellaneous descriptors and with the total number of descriptors, respectively. The total number of miscellaneous descriptors also showed a statistical relationship with component 2 (sleep latency) ($p=0.006$).

Discussion

Regarding sociodemographic characterization, the results show that people with CKD showed many similarities regarding the variables sex, ethnicity, and marital status. There was also a predominance of males with brown skin color, who received sick pay⁽¹⁹⁻²¹⁾.

The mean pain intensity value was 7.8 (SD=

2.8), indicating moderate to severe pain. The number of years with chronic pain was 6.8 (SD= 9.9). Similarly, other research on chronic pain in people with CKD showed that 28% had been feeling pain for more than five years, corroborating the findings of this research⁽²⁰⁾.

Regarding the sleep quality of people with CKD, as evaluated by PSQI, no research participant presented good sleep quality, and the mean total PSQI score was 10.6 (SD= 2.5), indicating poor sleep quality or sleep dysfunctions. Similar results were found in a study⁽²²⁾ where, of 240 participants, 159 (66%) had poor sleep quality. We found a significant difference between the average PSQI scores of people with CKD and people with terminal CKD (9.6 ± 12.4 vs. 11.4 ± 3.9 , respectively).

Nevertheless, these findings are different from those of a study carried out in China, which also evaluated the sleep quality of people with CKD, finding mean PSQI scores of 6.46 in a sample of 132 people and of 5 in a sample of 152 people⁽²³⁻²⁴⁾.

We also found that component 1 (subjective sleep quality) had the highest mean (2.3 - SD= 0.8), showing that, in the previous month, patients' sleep quality was classified as poor (2) or very poor (3). Additionally, this component showed the highest discrepancy in the total PSQI score of study participants with chronic kidney disease. On the other hand, another study, which also used the PSQI⁽²⁵⁾ to evaluate sleep quality in people with CKD, showed that most participants reported having good sleep quality (65.4%). This variation could be related to the different approaches and methodologies used, different sample sizes, populations, ethnicities, and culture.

The means of components 6 (use of sleeping medication) and 7 (Sleepiness and nighttime dysfunction) were, respectively, 1.8 (SD= 1.0) and 1.8 (SD= 0.9). These values indicate that participants needed sleeping medication at least once a week and/or had trouble staying away during the day. This finding is contrary to the evidence available in literature, which showed that 98.5% of participants had not used sleeping medication in the previous month⁽²⁵⁾.

A large portion of people with poor sleep quality seek benzodiazepines, which should only be used in acute crises due to the risk of causing dependence and interfering with alertness during the day, potentially leading to lower concentration levels and risks of falls in people with CKD⁽²⁶⁾. Therefore, it is important to emphasize the need for nurses and their teams to guide and monitor sleep medication use, and to suggest new, non-pharmacological therapies, that can help improve sleep quality.

On the other hand, component 4 (sleep efficiency), had the lowest mean (0.1, SD= 0.2) and presented the best score. This shows that participants, despite reporting a high level of poor sleep quality, seem to have satisfactory sleep efficiency > 75%. In this regard, our results were similar to a study that compared the sleep quality of people with CKD in Pakistan and Malaysia, finding that, in both populations, most participants reported satisfactory sleep efficiency (46.2% and 41.5%, respectively)⁽²⁷⁾.

Regarding pain and sleep quality, this study found that participants classified as having poor sleep quality had a mean pain score of 8.3 on the VASP scale. Patients classified as having sleep disturbances had a mean of 7.4, with statistical significance between the variables ($p=0.027$). This finding is similar to that of a study which evaluated chronic pain in people with CKD, showing a statistical association between the variable "impaired sleep" and the total score of the VASP scale, and inferring that pain interferes with the quality of sleep of people with chronic kidney disease⁽²⁰⁾.

It is common to associate people with chronic pain and poor sleep. Some people with insomnia report pain as a cause of interruption of sleep, since there is a bidirectional relationship between chronic pain and sleep disturbances. However, studies indicate that sleep disturbances favor the development of chronic pain, not the other way around. A study in Minas Gerais did not find a significant correlation between sleep and pain, but showed that pain had a greater impact on quality of life and mental health, factors that reverberate in insufficient sleep⁽²⁸⁾.

Sleep quality may be subjective and more influenced by factors related to kidney function than by psychological factors, such as pain. In this regard, a subjective and qualitative characterization of pain is also relevant to investigate its relationship with the quality of sleep of people with CKD⁽²⁹⁾.

The presence of pain in people with CKD interferes in sleep quality. Thus, nephrologist nurses must, together with the multidisciplinary team, improve their knowledge about chronic pain, and the team must manage and document pain better, in order to prevent it from impairing patients' sleep quality.

Study limitations

This study has a set of limitations, one of which is inherent to its cross-sectional approach, which makes it impossible to present information regarding potential future changes; the sample size and the voluntary recruitment of participants is another limitation. Additionally, there is a possibility of bias in the classification participants give to their own pain, when it is evaluated as moderate or intense at time of data collection.

Contributions to practice

This study provides data on the correlations between sleep quality in people with chronic kidney disease and chronic pain undergoing hemodialysis. Furthermore, it gives support for nursing teams to evaluate the development of interventions to improve the quality of sleep of these persons. Also, it highlights the relevance of further research emphasizing the monitoring of the disease, its treatment, and interventions or alternative therapies that help improve chronic pain and sleep quality in people on hemodialysis.

Conclusion

Most participants with chronic pain undergoing hemodialysis had alterations in sleep quality levels,

which were classified as poor or very poor. We found statistical evidence showing that subjective characteristics and pain intensity are associated with sleep outcomes. However, the statistical tests used only allow us to reach conclusions about the association between variables and the difference between score medians.

We found that participants with very poor sleep quality feel, for the most part, moderate to intense pain. Also, participants with very poor sleep quality used fewer descriptors to classify their pain when compared to participants who had poor sleep quality. Similarly, we found that the highest the number of descriptors used in the pain scale, the poorer the sleep efficiency of study participants, the higher the intake of sleep-inducing drugs, and the greater the difficulty in performing daytime activities.

Authors' contributions

Study and project design and data collection: Sousa EVD, Martins MG, Caetano JA.

Statistical analysis and data interpretation: Medina LAC, Teixeira CRS, Maniva SJCF.

Writing of the article, relevant critical review of the intellectual content; final approval of the version to be published and agreement about all parts of the manuscript: Sousa EVD, Medina LAC, Martins MG, Teixeira CRS, Maniva SJCF, Caetano JA.

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