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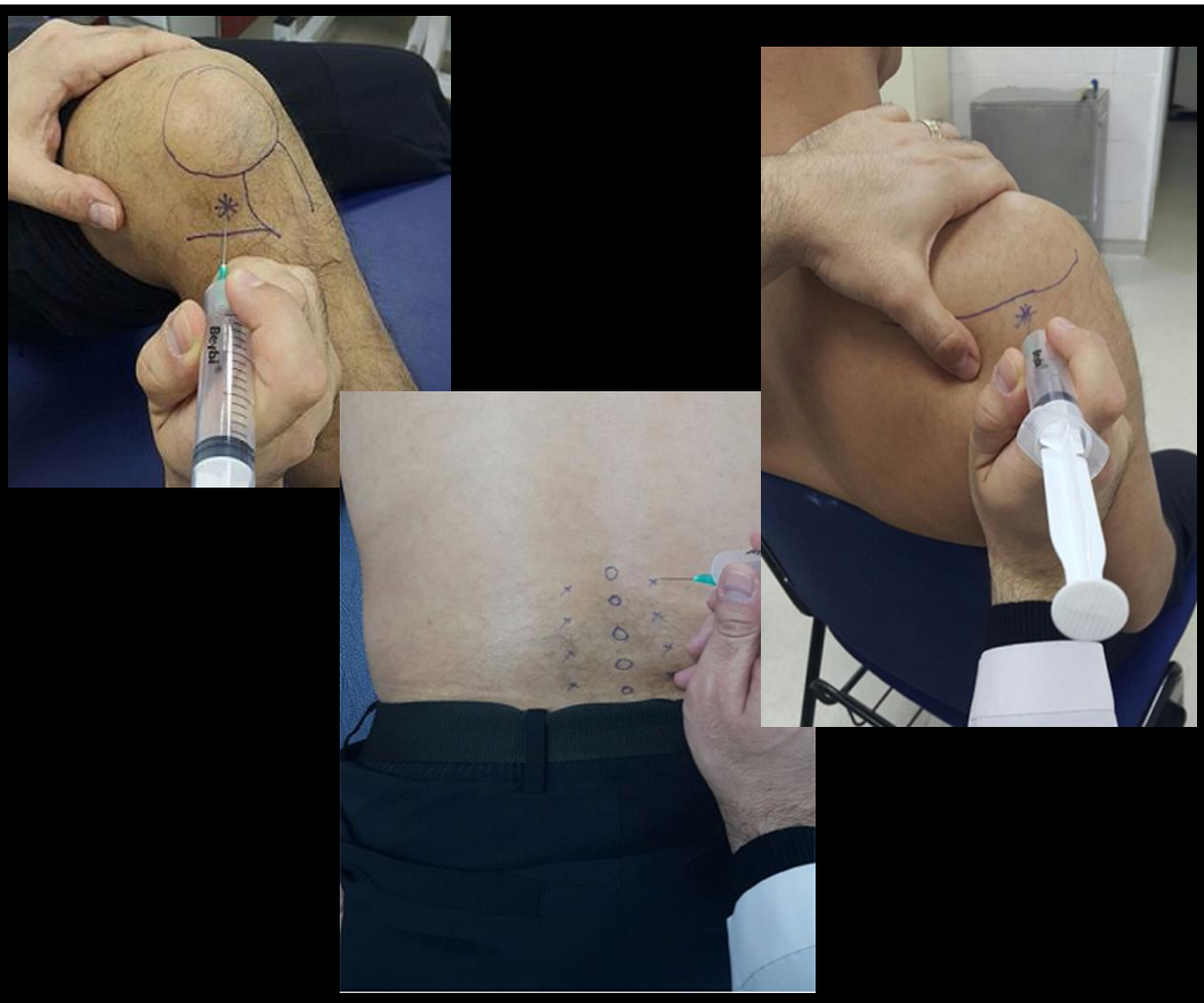

- **A retrospective study for the effect of local ozone injection for treatment of musculoskeletal disorders**
- **The effect of TGN-020 on penicillin induced epileptiform activity in rats**
- **dangerous drug combinations in elderly patients with polypharmacy**
- **Work-related fatigue**

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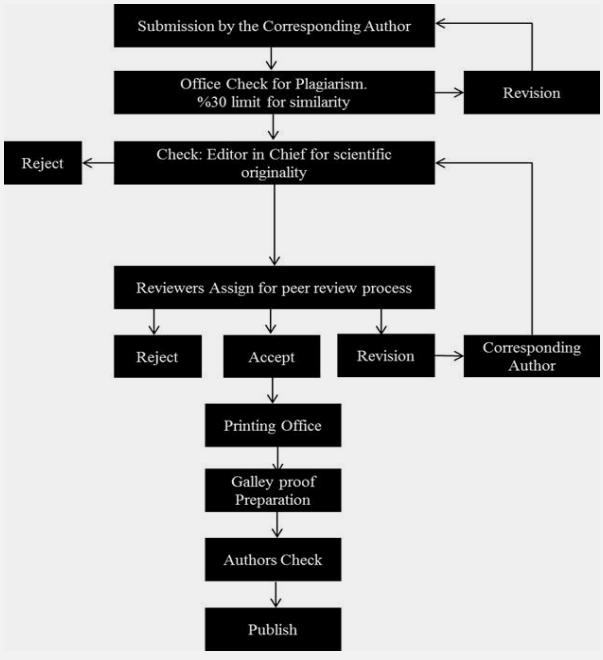

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Work-related fatigue and related factors among nurses working at the Adnan Menderes University Hospital

Safiye Ozvurmaz[1*], Aliye Mandiracioglu[2]

Abstract

Objective: Work-related fatigue is a common health problem among nurses. The objective of this study is to determine the level of fatigue among nurses working at the Adnan Menderes University Hospital in Turkey.

Material and Methods: This cross-sectional study has been carried out in Turkey in 2016. A total of 463 nurses from Adnan Menderes University Hospital in Aydin have agreed and completed the data collection form. They have provided information on sociodemographic characteristics, work place, number of shifts worked, work year and weekly working hours. The visual analogue scale was used to measure levels of energy and fatigue. Linear regression analysis was performed to highlight any significant changes.

Results: In total, 85.3% of the nurses who participated were females. 60.3% were single, 53.6% worked in intensive care units and 26.1% worked only in the day shift, 17.9% worked for more than 45h a week. According to univariate analyses, nurses who were over 40 years of age, married, worked in internal medicine and surgery clinics for over 15 years and constantly worked in the day shift for 40 h a week had lower energy scores and higher fatigue scores. According to the univariate analysis, all the personal and working characteristics are related fatigue and energy subscale scores. Linear regression analysis revealed that age was the only variable affecting energy scores.

Conclusion: It can be stated that factors affecting fatigue among nurses in our study group are associated with age and gender. Prevention and supporting programs may focus on firstly vulnerable nurses (female and older age group).

Keywords: Fatigue; Nurse; Hospital

Introduction

Globalisation demands increased production due to its economic context and imposes rapid changes in work life (1). In modern work life, there are longer working hours, time pressure, reduction in break times, continuous technology processes and work patterns such as shift work have negative effects on employees. One of these negative effects is impairment of the mechanisms of natural biorhythms and fatigue (2,3).

Circadian rhythms are set for high activity during daytime and very low activity during night-time. As temperature and other body rhythms of shift workers do not coincide with the activity patterns of the person, disorientation and fatigue occurs. There is impairment similar to that seen during jet lag (4).

Fatigue is a multidimensional concept. It is a subjective feeling about activity, motivation and concentration. It is a complex perception where somatic and psychological factors play a role (5).

Fatigue is defined as a change in psychophysiological control mechanisms regulating task-related behaviour. Fatigue is not an adverse effect, but a psychophysiological mechanism of compliance or a safety mechanism of an individual faced with a risk of exhaustion and it is a protective response (6). Fatigue is a phenomenon that is affected by individual properties and domestic and social factors such as sex, sleep quality, shift work, psychological state, marital status, employment status, housework, parenting, conflict situations and social support. Work-related fatigue is affected by work environment, work content, work , organisation and policy and poor working conditions (6,7,8,9,10).

As a result of fatigue, performance issues develop at work, poor concentration, attention deficit, problem-solving and decision-making difficulties, memory impairment, delay in response time, professional incompetence and problems in private and social life occur.

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It is a condition that can result in work accidents, presenteeism and other occupational health problems. This condition adversely affects the health of employees and the quality of services provided (4,9,11,12). In modern industrial societies, conditions that negatively affect workers are also experienced in the health sector. Health personnel are expected to work 24/7 under time pressure. For this reason, fatigue is widespread among health personnel, particularly among nurses working in hospitals (5). Fatigue causes an increased number of work accidents and occupational health problems among health personnel. Fatigue can also affect the quality of services provided and may lead to malpractice. The well-being of healthcare workers is important for the health and safety of patients they serve. When the health of medical staff is not considered important, the cost of healthcare services increases (9,13,14). Fatigue in nurses is related to unfavorable hospital working conditions and personal factors. In addition, previous studies emphasized that supernurse values contribute to fatigue. Supernurse barriers for developing a culture of safety are related to nurse fatigue. Supernurse characterizes some values and behaviors defining a superhero that exists within nursing professional culture (15).

The nurse fatigue may have negative consequences for nursing practice and quality outcomes both at the individual and organizational level, the entire health care system (16). It is important to be aware of fatigue and its possible causes in terms of patient safety and employee health (11). Nurse training in Turkey started in 1921 with the establishment of six month voluntary caregiver courses. Later, nursing schools that provided training on a high school level were opened. University level nurse education in Turkey began in 1955. All nurses have the same responsibilities, regardless of where they have graduated from(17).

The hard and exhausting working conditions are well known in Turkey (18). On the other hand, patient rights and patient safety are as important in. The Ministry of Health regards patient safety as an important health system performance indicator. Nurses play an important role in ensuring patient safety and reducing medical errors (19).

In Turkey, the health transformation policy for the development of healthcare services was initiated in 2003 and the Turkish Universal Healthcare system has changed dramatically. The main concept is privatization. This concept is included competitive forces and market-oriented incentives within public institutions. The performance-based payment systems in public health sector has started (20).

This study aimed to determine the level of fatigue and factors affecting fatigue among nurses working at the university hospital.

Materials and Methods

This cross-sectional study was conducted in Adnan Menderes University Faculty of Medicine Hospital in Aydın, Turkey. All registered nurses working at hospital were included 463 (89.1%) and all nurses have completed the data collection form. The inclusion criteria was to provide direct patient care during the data collection periods and willing to participate in the study. Pregnant nurses were excluded from the study.

In 2016, data were collected via an anonymous two-part questionnaire. Information on sociodemographic characteristics, work place, shift work, work year and weekly working hours was obtained. In addition, the visual analogue scale for fatigue (VAS-F), which measures the levels of energy and fatigue, was used. The VAS-F was developed by Lee et al(21) in 1990 and was adapted to Turkish by Yurtsever in 1999(22). It consists of 18 items. The grade scale varied from 1 to 10; the most positive expression in the fatigue subscale is 1 and the most negative expression in the fatigue subscale is 10, whereas the most negative expression in the energy subscale is 1 and the most positive expression in the energy subscale is 10. Items in the fatigue subscale ranged from the most positive to the most negative expression, while items in the energy subscale ranged from the most negative to the most positive expression. A high score in the fatigue subscale was interpreted as high fatigue and a low score in the energy subscale indicate that the severity of low energy levels. This scale is preferred because it is easy to use, brief and clear. Cronbach's α internal consistency coefficient of the fatigue subscale was 0.90 and that of the energy subscale was 0.74. The relationship between fatigue, and participants' characteristics were assessed using the t-test, ANOVA and enter model linear regression analysis. Statistical significance was accepted at $p < 0.05$.

Ethics committee approval was obtained from Adnan Menderes University Medical Faculty Deanery Non-invasive Clinical Research Ethics Committee. The aim and procedures of the study were explained to the nurses and participants were informed that their participation in the study was voluntarily.

Results

The characteristics of the nurses participating in the study are shown in Table 1. In total, 85.3% of the nurses were females, 60.3% were single, 67% were in the age group of 18–29 years and 29.4% had children. Further, 53.6% of the nurses worked in intensive care units and 26.1% worked only in the day shift. Moreover, 60.3% less than 5 years of experience and 17.9% worked for more than 45 h a week. In addition, 47.9% of the nurses stated that they smoked and 8.9% stated that they had at least one chronic illness. The evaluation results of the scores obtained from the fatigue scale according to the sociodemographic characteristics and working conditions of the nurses are shown in Table 2 and 3. The mean score in the energy subscale was 30.59±12.77, and that in the fatigue subscale was 69.18±34.10.

According to univariate analyses, the energy score was lower and the fatigue score was higher among female, over 40 years old, married, vocational high school graduates, worked in internal medicine and surgery clinics, had over 15 years of work experience and always worked in the day shift and for 40 h a week. A linear regression analysis was

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carried out to determine the effect of the variables found to be significantly related in univariate analysis (gender, age, marital status, school graduated, department, work experience years, shift type, weekly study hours) on levels of fatigue. Linear regression analysis revealed that age was the only variable affecting energy scores. Fatigue score was related age and gender (Table 4).

Table 1:. General characteristics of the participant nurses

		N	%
Gender	Female	395	85.3
	Male	68	14.7
Marital status	Married	175	37.8
	Single	279	60.3
	Widow	9	1.9
Age	≥19	20	4.3
	20-29	289	62.7
	30-39	105	22.8
	40-49	47	10.2
Education	High school	127	27.6
	University	289	62.8
	Master degree	44	8.6
Work unit	Internal	121	26.2
	Surgery	93	20.1
	Intensive care	248	53.7
Employment status	Day work	120	25.9
	Shift work	236	51.0
	Night work	103	22.2
Years of experience	0-5	279	60.3
	6-10	68	14.7
	11-14	65	14.0
	15 +	51	11.0
Work hours per week	40	163	35.2
	45	217	46.9
	45 +	83	17.9
Smoking	Yes	222	47.9
	No	207	44.7
	Quit	33	7.1
Chronic Diseases	Yes	41	8.9
	No	420	90.7

Table 2: The energy subscale scores of participant nurses characteristics

Characteristics		**Mean (SD)**	**t-test/ ANOVA**	***p value***
Gender	Female Male	30.03(12.65) 34.26(13.03)	-2.53	0 .012
Age	≤19 20-29 30-39 40-49	42.05(10.65) 32.66(11.49) 26.75(12.72) 21.45(13.79)	21.40	0.000
Marital status	Married Single Widow	26.76(12.99) 33.18(11.92) 28.00(16.32)	14.48	0.000
Education	High school University Master degree	35.62(12.05) 29.33(12.45) 26.56(13.07)	10.04	0.000
Work unit	Internal Surgery Intensive care	26.71(12.90) 27.16(12.06) 33.93(12.14)	18.52	0.000
Years of Experience	0-5 6-10 11-14 15 +	33.95(11.61) 28.35(12.73) 25.32(12.78) 22.44(12.67)	19.67	0.000
Employment status	Day work Shift work Night work	24.73(13.79) 31.85(11.04) 34.38(13.06)	19.64	0.000
Work hours per week	40 45 45 +	25.85(13.15) 34.40(11.50) 30.32(12.15)	22.58	0.000
Smoking	Yes No Quit	31.03(13.01) 31.19(12.53) 24.75(11.85)	3.82	0.023
Chronic diseases	Yes No	29.55(13.04) 30.80(12.77)	-0.59	0.55

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Table 3. The scores of the fatigue subscale by nurses characteristics

Characteristics		Mean(SD)	t-test/ ANOVA	p value
Gender	Female	71.86(33.48)	4.14	0.000
	Male	53.50(33.70)		
Age	≤19	42.70(33.16)	19.79	0.000
	20-29	62.01(30.37)		
	30-39	83.41(32.83)		
	40-49	95.32(33.15)		
Marital status	Married	79.10(34.41)	10.44	0.000
	Single	62.30(32.01)		
	Widow	89.11(39.13)		
Education	High school	66.16(30.37)	9.13	0.000
	University	73.97(32.83)		
	Postgraduate	81.50(33.14)		
Work unit	Internal	77.95(33.71)	15.32	0.000
	Surgery	78.84(32.07)		
	Intensive care	61.20(33.14)		
Years of experience	0-5	58.90(31.11)	28.79	0.000
	6-10	74.75(32.48)		
	11-14	88.66(31.62)		
	15 +	93.26(3093)		
Employment status	Day work	86.84(32.75	23.53	0.000
	shift work	63.88(30.36)		
	Night work	61.62(36.42)		
work hours per week	40	83.90(33.76)	32.22	0.000
	45	57.31(31.79)		
	45 +	71.39(28.76)		
Smoking	Yes	65.20(35.39)	7.19	0.000
	No	70.23(32.44)		
	Quit	88.72(29.04)		
Chronic diseases	Yes	72.47(30.88)	0.64	0.51
	No	68.82(34.34)		

Table 4: Linear regression analysis of the relationship between subscales and independent variables. *Standardized Coefficients

	Fatigue subscale			Energy subscale		
Independent variables	**Beta***	**SE**	**p**	**Beta***	**SE**	**p**
Gender	-0.128	4.238	0.004	0.07	1.63	0.122
Age	0.103	1.564	0.026	-0.097	0.606	0.04
Chronic diseases	0.079	5.262	0.076	-0.064	2.034	0.16
Working unit	-0.092	1.909	0.057	0.132	0.738	0.08
Working years	0.238	3.011	0.012	-0.118	1.164	0.225
Work hours/week	0.066	2.532	0.218	-0.048	0.976	0.383
Education	0.135	4.317	0.145	-0.159	1.669	0.094
Employment status	0.001	2.82	0.982	0.04	1.092	0.501
R^2		0.21			0.25	

Discussion

In the present study, the status of fatigue was determined among nurses. Fatigue is quite a common condition among employees working in the health sector (14,23). Fatigue prevalence was found to be different among studies conducted using different measurement tools. In this study, mean scores energy subscale was 30.59±12.77, and that in the fatigue subscale was 69.18±34.10. Previous study which was carried out on the same scale found that fatigue and energy subscale mean scores were respectively 70.17 (24.66) and 27.5 (9.9) (24). In the present study, according to the univariate analysis results, all of the personal characteristics were associated with fatigue, previous studies reported similar results (25). Most participants are females in this study. In Turkey, the nursing profession is mostly dominated by females. Male nurses generally are a younger age group. Recently, nursing is a profession chosen by men due to easy employment in Turkey.

Fatigue was found to be more prevalent among those who were married. This situation is natural due to the double work load of married nurses; at work and at home. The fatigue scores of women were found to be high and their energy scores were found to be low. Despite social changes in the 21st century, women still take care and manage the house alone child care, child educating, housework and spousal roles (26). Female nurses do their own housekeeping and take care of their children in Turkey.

In our study, employees who were medical vocational high school graduates, worked in the internal medicine and surgery clinics, worked for more than 15 years, only during the day shift and worked for 40 h a week were found to have lower energy scores and higher fatigue scores. All these features are associated with age. In the present study, fatigue was found to be more prevalent among those over 40 years of age. An important proportion of nurses who were over 40 years old in Turkey graduated from medical vocational high schools. Generally, in hospitals, nurses who are senior and over 40 years of age only work in the day shift and in administrative positions and do not stay for the night shift. Although there was a positive discrimination among nurses in this age group in Turkey, fatigue was higher among nurses in this age group than among nurses who were in a younger age group. Age is a factor that negatively affects health. As age increases, adaptation to circadian rhythm decreases and there is more fatigue and energy loss. It has been emphasised that maladaptive fatigue is detected among older nurses (27). Fatigue is reportedly more prevalent among people over 40 years of age (28.29,30). Despite, older nurses' abilities and expertise are valuable. On the other hand, older nurses may be vulnerable (31). Generally, older nurses have greater job responsibility and they work more administrative positions (32). There is a need for more nurses to continue working despite the retirement age and older nurses represent an increasing proportion of the healthcare workforce. Nursing is physically and mentally hard job especially for older nurses due to natural ageing process (31,32).

In this study, no healthy worker effect was determined. Due to the low pension in Turkey, health personnel continue to work even if they are entitled to pension. These people are exempt from working in the night shift in most hospitals. Elderly health personnel experience difficulty in adapting to changing work demands, technology and unstable work environments in cost-focused health institutions (33). Stichler suggested that work stations for older nurses provide a comfortable work environment and that they are motivated to stay healthy, safe and employed and to share their knowledge and skills for the benefit of their younger colleagues and patients (34).

The limitation of this study is that it is a cross-sectional study; therefore, the results are limited in terms of revealing the cause–effect relationship. The results of this study cannot be generalised to all nurses. However, as this is the first work done on this subject in Turkey to the best of our knowledge, it can provide insight into work-related fatigue among nurses.

Conclusion

In conclusion, it can be stated that factors affecting fatigue among nurses in our study group are related to age and gender. Fatigue is an important indicator of opportunities to improve the nursing working systems and to support them. Our suggestion would be to take into consideration age differences in improving of the facilities of nurses and identifying the effects of fatigue on individuals characteristics are when setting fatigue reduction strategies.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: SO, AM: Research concept and design; data collecting, analysis and interpretation of data**. SO:** Preparation of article, and Revisions. All authors approved the final version of the manuscript.

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A retrospective study for the effect of local ozone injection for treatment of musculoskeletal disorders

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Abstract

Objective: Aim of this study is to examine the effect of local ozone application on pain in patients who applied to physical medicine and rehabilitation outpatient clinic with knee, shoulder and back pain problems.

Material and Methods: Records of the patients who were consulted to the Physical Medicine and Rehabilitation outpatient clinic of our hospital with the complaints about knee, shoulder and low-back pain, who met the inclusion criteria and were applied local (intraarticular or intramuscular) ozone as per the routine protocol, was retrospectively investigated The study was conducted with 25 patients. A total of 4 sessions of ozone were administered once weekly. Patients were evaluated using VAS (visual analogue scale) at 3 and 5 weeks.

Results: The mean age of the patients was 57.62 ± 17 (33-97) years. 64% of the patients had knee pain, 20% had shoulder pain and 16% had low back pain. The mean VAS score was 7.44 ± 0.91 before treatment, 5.04 ± 1.45 at 3 weeks, and 3.92 ± 1.57 at 5th week. Statistically significant improvement was observed in VAS scores according to pre-treatment.

Conclusion: Local ozone application reduces the pain level of the patients with knee, shoulder or low-back pain.

Keywords: ozone injection, joint, muscle, pain

Introduction

Musculoskeletal pain usually originate from muscles, bones, ligaments and soft tissues. Such pains may lead to serious workforce losses by affecting motive power of the individual. In general, osteoarthritis, meniscus lesions, impingement syndrome, disc pathologies and painful muscle spasms are diagnosed by physicians in outpatient clinics. Oral and topical analgesics are frequently used to treat the pains of musculoskeletal system. Apart from these drugs, complementary products such as glucosamine and chondroitin are prescribed. Exercises, physiotherapy agents, auxiliary devices and protective measures are applied as non-pharmacological treatment methods as part of this treatment. In case of that all of the treatment methods remain incapable, various surgical treatments are implemented (1). Nowadays, ozone therapy can be applied based on numerous indications. Local ozone injections in the treatment of the inflammatory and degenerative diseases related to musculoskeletal system has increased in the recent years which activates the anti-inflammatory and anti-oxidative capacity. Accordingly, various studies on this subject have been performed (2-5).

The purpose of this study is to investigate the effect of local ozone application on the patients who consulted the Physical Medicine and Rehabilitation outpatient clinic due to knee, shoulder and low-back pain, and who were administered ozone therapy.

Material and Methods

The research was performed by retrospectively investigating records of the patients between January 01, 2018 and March 15, 2018 with the complaints about knee, shoulder and low-back pain, who met the inclusion criteria and were applied local (intraarticular or intramuscular) ozone as per the routine protocol.

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1. Inclusion criteria

- All of the male and female patients aged 18 and older
- The patients with the complaints about knee, shoulder and low-back pain, and were approved for local ozone application in consequence of the examinations and investigations, and who accepted local ozone application as part of the protocol

2. Exclusion Criteria

- The patients who were subjected to local ozone application apart from the protocol determined in terms of dose and number of sessions
- The patients who discontinued the treatment because of a complication developed during the local ozone application, or for another reason
- The patients who have known any coagulation disorder, or who use oral anti-coagulant
- The patients who have known glucose 6-phosphate dehydrogenase deficiency
- Pregnant patients
- The patients aged below 18

The patients subject to study provided their detailed medical history related to their complaints to a certain physician in physiotherapy outpatient clinic before the application of local ozone therapy. The same physician examined them and evaluated their suitability for local ozone therapy. The patients who were deemed suitable for the therapy and accepted the therapy with informed consent form were administered local ozone application as part of a definite protocol.

Protocol

All patients whose data was scanned in the study received four sessions of local ozone application; the application was performed by giving 20 cc every time as follows: 20µg/ml in Session 1, 15µg/ml in Session 2, and 10µg/ml in Sessions 3 and Session 4. Local ozone was applied to knee joint with anterolateral approach as the patient was in supine position and his/her knee flexion angle was 90 (Figure 1). Ozone was administered to shoulder joint with posterior approach as the patient was in sitting position (Figure 2). Ozone was applied to low-back region of the patient who was in prone position, from 2 cm lateral of the inter-spinous regions (Figure 3). All of the ozone applications were performed under sterile conditions. Pain levels of the patients subject to therapy were assessed prior to application and in weeks 3 and 5 after the first application by the same physician who used visual analog scale (VAS). VAS is a one-dimensional measure of pain intensity, which has been widely used in patients for pain with many rheumatic diseases. It is a continuous scale comprised of a vertical line, usually 10 centimeters in length, anchored by 2 verbal descriptors which is most commonly anchored by "no pain" (score of 0) and "worst imaginable pain" (score of 10 [10cm scale]) (6). Data of the patients who discontinued the treatment because of a complication developed during the local ozone application, or for another reason was not used in our study.

SPPS soft-ware 22 was used for the statistical analysis of the data obtained from the records of the patients. The Kolmogorov Smirnov test, Friedman's test, and Wilcoxon signed-rank test were used for statistical analysis. The level of statistical significance was set at $p < 0.05$.

Figure 1: Ozon treatment application for Knee

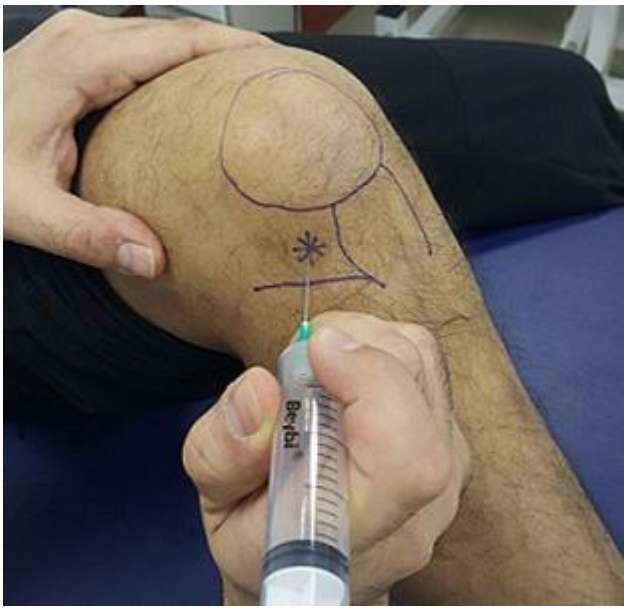

Figure 2: Ozon treatment application for shoulder

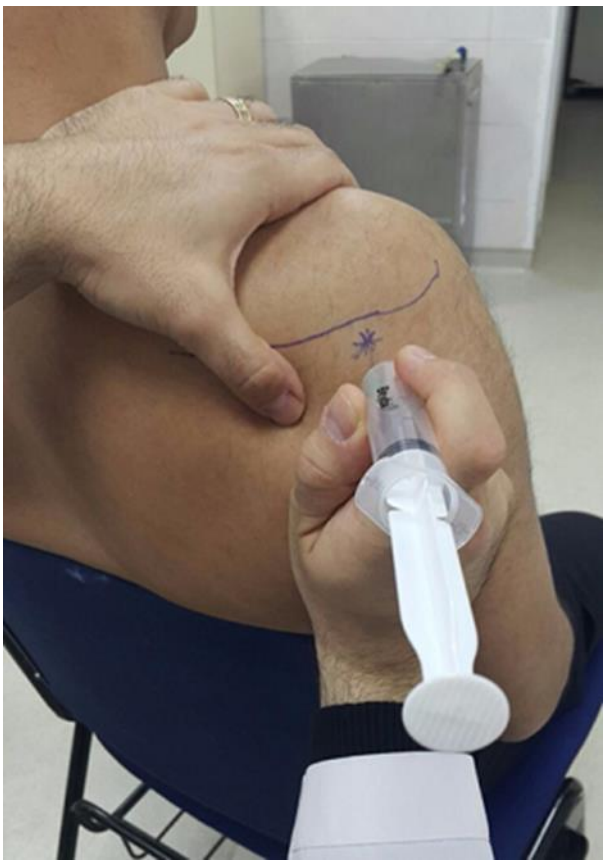

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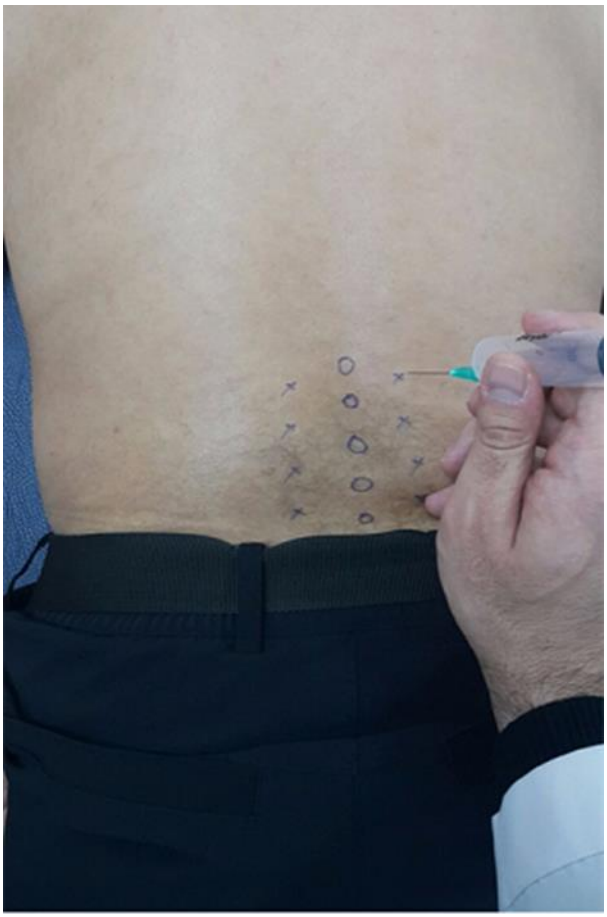
Figure 3: Ozon treatment application for low-back pain



Results

Data of 25 patients who were subject to local ozone therapy in line with the protocol determined was included in our study.

The therapy was applied to knee joints of 16 patients, shoulder joints of five patients and lumbar region of four patients. Demographic data of the patients included in the study is given in Table 1.

When the pain levels measured prior to the therapy, and in weeks 3 and 5 after the therapy were compared, the decrease in the pain levels were found to be statistically significant ($p<0.05$).

All of the VAS parameters improved significantly at 3 weeks post treatment, as compared to baseline, and the observed improvement increased at 5 months post treatment ($p < 0.05$) (Table 2).

The maximum decrease in pain level among all of the painful groups (knee, shoulder and low-back) was seen in Week 5 (Table 2).

Table 1: Demographic distribution of Patients

Age (mean±sd, min-max)	57.62 ± 17 (33-97)
Gender (F/M, %) (n)	60/40 (15/10)
Knee pain (%) (n)	% 64 (n=16)
Shoulder pain (%) (n)	% 20 (n=5)
Low back pain (%) (n)	% 16 (n=4)

Table 2: Pre-treatment Post-treatment Measurements

Measure (VAS)	Pre-treatment	Post-treatment week 3	Post-treatment week 5	P value
Knee	7.62 ± 0.8	5.12 ± 1.62	4.18 ± 1.75	<0.05
Shoulder	6.80 ± 0.83	5.20 ± 0.44	4.00 ± 0.70	<0.05
Low-Back	7.50 ± 1.29	4.50 ± 1.73	2.75 ± 1.25	<0.05
Total	7.44 ± 0.91	5.04 ± 1.45	3.92 ± 1.57	<0.05

P value <0.05 was considered as statistically significant.
Friedman test was used for intergroup comparison
Wilcoxon test was used for post-hoc comparison

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Discussion

In this study, effect of the local ozone application on the pain level was investigated by retrospectively scanning the data of the patients who were administered local ozone based on a definite protocol. It was concluded that this treatment method may give positive results in the pain levels.

Lopes de Jesus et.al (7) compared intra-articular ozone application in osteoarthritis to placebo in their randomized controlled study. They found out that the change in pain level of the group subject to local ozone was more significant. Unlike our study, 20 µg/ml dose of 10 cc ozone was administered in every session in that study, a total of eight sessions each of which was applied weekly were performed and the assessment was made in weeks 4, 8 and 16. Similar to our study, VAS was used for the evaluation of pain. The pain level of the patients decreased gradually with the ozone application just as in our study. Unlike our study, the patients were followed up for a long period and it was reported that analgesic effect of the local ozone application continued until week 16.

Rayegani SM et.al compared the efficacy of intra-articular ozone application in osteoarthritis to that of hyaluronic acid (HA) application (8). Thirty µg/ml dose of 10 cc ozone was administered to patients in ozone group once a week while the patients in HAgroup received 20 mg/2 mL dose of intraarticular injection once a week. Pain scores of the patients in both groups before the treatment and in month 6 after the treatment of 3 sessions were evaluated. Significant improvement in the pain levels of both groups was reported. It can be concluded accordingly that intraarticular ozone application in osteoarthritis can be preferred instead of HA injections due to its cost efficiency. However, similar studies about this subject reported that pain level of the patients subjected to HA injections improved further and the asymptomatic period of such injections was longer than that of the local ozone application during long-term follow-ups (9; 10).

Duymuş et.al made a comparison between intra-articular HA, intra-articular Platelet Rich Plasma (PRP) and intraarticular ozone application in osteoarthritis (9). Unlike our study, 30 µg/mL dose of 15 cc ozone was applied in that study. However, a total of four sessions each of which was performed once a week was applied just line in our study. Duymuş et.al.(9) followed up their patients for 12 months. Although decrease in pain level and clinical effectiveness were seen in three groups at the end of first three months, they were reported to be more significant in PRP and HA groups. At the end of six months, the clinical effectiveness of PRP and HA groups continued, but pain levels of the ozone group got back to initial levels. According to 12-month evaluation, the clinical effectiveness of PRP and HA groups declined. However, the decrease in PRP group was lower. It can be concluded based on the said study that PRP and HA applications are more effective and give more lasting results compared to local ozone application. Further studies are required to arrive at a final decision regarding this subject.

Biazzo et al (5) conducted a study to seek the efficacy of local ozone application in the patients with low-back pain. They administered 27 µg/ml dose of 20 cc ozone to lumbar para-spinal muscles of the patients for 12 weeks, and determined that VAS scores of 79 percent of the patients declined at the end of the treatment. Despite the application of four sessions in our study; pain levels of all patients whose lumbar regions were subjected to local ozone decreased. We believe that the proper dose and number of sessions for low-back region will be clarified further by new future studies.

Conclusion

In consequence, local ozone application reduces the pain level of the patients with knee, shoulder or low-back pain. However, more sophisticated studies which include more participants and focus on the other disorders in physical medicine and rehabilitation are needed.

Limitations: The most important limitations of our study were the absence of control group and small patient group. The other limitations are as follows: Long-term follow-ups of the patients were not performed and evaluation about the other subjects, except pain scale was not made.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: EA: Research concept and design; Patient examinations, Treatment, data collecting, analysis and interpretation of data**.** Preparation of article, and Revisions. All authors approved the final version of the manuscript.

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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An investigation of inappropriate medication use and dangerous drug combinations in elderly patients with polypharmacy

Huseyin Baltacioglu[1], Oguz Eroglu[1*], Figen Coskun[1]

Abstract

Objective: Polypharmacy is common among the elderly patients. The aim of this study was to determine drugs used inappropriately in elderly with polypharmacy or causing a dangerous drug combination (DDC) and to investigate the relation between drug interaction and emergency department (ED) presentation symptoms.

Methods: This prospective study was performed with elderly patients aged over 65. Patients' demographic characteristics, presentation symptoms, comorbid diseases, and the names, numbers, dosages and side-effect of drugs used were recorded.

Results: DDC was present in 94.6% of patients, and 24.3% presented to the ED with drug interaction-related symptoms. The mean age of the patients with DDCs was 72.4±6.7 years, 68.3±5.5 years among those without DDC(p=0.016).The most common comorbid disease was hypertension(75.1%), the most commonly used drug group was anti arrhythmics (15.4%),and the most commonly used medication was aspirin(6.5%). The relation was observed between drug interaction-related presentation symptoms and coronary artery disease (CAD) (p=0.044). Correlation was determined between DDC and the anti-hypertensive drug group (p<0.001).Correlation was determined between drug interaction and subjects with presentation symptoms of dyspnea and bleeding (p<0.001 and p<0.001, respectively). Numbers of drugs used and frequency of presentation to hospital were higher in patients with DDCs (p<0.001; 0.040 respectively).Positive correlation was determined between frequency of presentation and number of drugs used(r: 0.514; p <0.001).

Conclusion: DDCs are more common as age increases in elderly. This situation especially remarkable in subjects with CAD and antihypertensive drugs users. Presentations to hospital caused by drug-drug interactions most commonly involve dyspnea and bleeding, and a higher number of drugs increase rates of DDC development and numbers of hospital presentations.

Key words: Emergency department; elderly; medicine; polypharmacy

Introduction

Old age is defined as a calendar age of 65 or over and is divided into three periods, early (65-75 years), middle (75-85 years) and advanced (>85 years) (1). The average life span in the 20th century, 46.5 years, rose to 66 by the 2000s, and is expected to increase to 76 by 2050. This also means that the proportion of elderly people in the world will grow still further in the years ahead (2, 3). Although old age is a physiological process, for reasons such as impairment in several organs that emerges with aging, a slowing in metabolic rate, and changes in drug pharmacodynamics (decreases in drug absorption, distribution and elimination or impairment of receptor sensitivity), drugs used for therapeutic purposes in elderly patients lead to more serious side-effects and even fatal outcomes at therapeutic doses (4).

Polypharmacy refers to multiple drug use or the combined use of two or more drugs for at least 240 days (5). The prevalence of polypharmacy increases with age, and stands at 35-40% in patients aged over 75 (6, 7).

Polypharmacy increases the incidence of side-effects, drug-drug interactions and dangerous drug combinations (DDCs), and is also a significant cause of mortality and hospitalization in elderly individuals (6-8). There are numerous criteria or classifications concerning the reliability of medication use in elderly patients, the most widely used being Beers criteria. These were first described in 1991, and were updated, assuming their present form, in 2015. Under the Beers criteria, medications used by elderly patients are divided into three categories, 'medications to avoid in older patients', 'medications to avoid in older patients with certain diseases and syndromes', and 'medications to be used with caution in older patients' (9). Nowadays, Beers criteria are frequently used to monitor the quality of life of elderly patients, and their importance is increasing all the time.

The aim of this study was to determine inappropriate drug use or medication combinations constituting a fatal risk in geriatric patients presenting to the emergency department

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(ED), and to investigate the relation between presentation symptoms and medication use.

Materials and Methods

Following receipt of approval from the local ethical committee (No. 2017-05/04), this prospective study was performed with patients aged 65 and over presenting to the Kirikkale University Medical Faculty Hospital ED between 01 January and 30 July, 2017, and using multi-drugs for at least one year. Signed informed consent forms were obtained from patients and/or relatives to confirm that they were participating on a voluntary basis. The study was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Directive.

Patients' age, sex, alcohol use or smoking status, herbal medication use, medications used, comorbid diseases ED presentation symptoms and frequency of presentations were recorded. Relations between DDCs, drug-drug interactions and side-effects and presentation symptoms were checked using Beers criteria and the 'drug interactions checker' from the Medscape web site (9, 10).

Subjects who refused to participate, patients aged under 65, using less than two medications, taking drugs for purposes of suicide, patients with whom clear communication could not be established (with clouded consciousness, impaired cognitive functions, or with speech or hearing disabilities) and trauma patients were excluded.

Statistical Analysis

All data were analyzed on SPSS 23.0 (Statistical Package for the Social Sciences Inc., Chicago, IL, USA) software. Normality of distribution was assessed using the Shapiro-Wilks test. Quantitative data were expressed as median and interquartile range (IQR), and qualitative data as number (n) and frequency (%). Non-parametric data were analyzed using the Mann-Whitney U test and the Kruskal-Wallis test, while categorical data were analyzed using the Chi-square and Fisher Exact tests. Spearman's rho correlation test was used to compare quantitative data. p values <0.05 were regarded as statistically significant.

Results

During the study period, 3313 patients aged 65 or over presented to the ED. Of these, 1029 were excluded because of presenting due to trauma, 1302 due to absence of polypharmacy, 563 because clear communication could not be established, and 119 due to declining to take part in the study, which was eventually completed with 300 patients.

The mean age of the patients was 72.2 ± 6.7 years (range: 65-91), and 54% (n=162) were women. DDCs was determined in 94.6% (n=284) of patients, and 24.3% (n=73) of presentation symptoms were associated with drug interactions. Women constituted 55.3% of the subjects with DDCs and 50.7% of those with presentation symptoms associated with drug interactions. No statistically significant relation was observed between gender and DDCs or presentation symptoms being associated with drug interactions (p=0.061; 0.514, respectively).

The mean age of the patients with DDCs was 72.4 ± 6.7 years, compared to 68.3 ± 5.5 in subjects without DDCs (p=0.016). The mean age of subjects with presentation symptoms associated with drug interactions was 71.2 ± 5.7, compared to 72.5 ± 7.0 in those whose symptoms were not associated with drug interactions (p=0.156) (Table 1).

Mean numbers of medications used per day were 6.8 ± 2.5 in patients with DDCs and 5.5 ± 1.4 in without DDCs (p=0.04). Mean numbers of medications used per day by patients with presentation symptoms associated with drug interactions were 6.7 ± 2.5, compared to 7.0 ± 2.2 in the other patients (p=0.086). Mean numbers of presentations to the ED were 8.3 ± 6.3 /year in patients with DDCs and 1.9 ± 0.4 /year in without DDCs (p<0.001). Mean numbers of presentations to the ED were 9.4 ± 7.1 /year in patients with presentation symptoms associated with drug interactions and 7.1 ± 6.1 /year in the other patients (p=0.045) (Table 1). Positive correlation was determined between frequency of presentations to the EDs among elderly and numbers of medications used (r: 0.514; p<0.001).

Eighteen different drug groups, 192 different pharmacological contents, and 2011 different medications were used. The three most common drug groups were antiarrhythmics (15.4%), antithrombotics and anticoagulants (13.1%) and antihypertensives (10.2%), and the three most commonly used medications were aspirin (6.5%), metoprolol (4.7%) and furosemide (4.7%) (Table 2). The only relation was determined between subjects with DDCs and anti-hypertensive group drugs (p<0.001); no relation was determined between presentation symptoms associated with drug interactions and any drug group (Table 3).

We observed 438 different DDCs in our patients. The most common of these were 'aspirin + anticoagulant' (10.7%), 'aspirin + metoprolol' (9.8%) and 'aspirin + ACE inhibitors' (8.7%) combinations. The most common side-effect resulting from drug interactions in these combinations was 'bleeding' (including gastrointestinal bleeding, intracranial hemorrhage, epistaxis, hematuria, alveolar haemorrhage), which was most prevalent in the "aspirin + anticoagulant" combinations. The next most common side-effect was dyspnea, most prevalent in the 'metoprolol + bronchodilators' combination (Table 4).

The three most common comorbid diseases in elderly patients were hypertension (HT) in 76.1%, diabetes mellitus (DM) in 54.3%, and coronary artery disease at 37.3%. No relation was determined between DDC and comorbid diseases, although a correlation was observed between presentation symptoms and CAD (Table 5).

The three most common symptoms on presentation to the ED were dyspnea (27.7%), chest pain (20.3%), and headache and/or dizziness (13.3%). While no relation was determined between DDCs and presentation symptoms, correlations were observed between dyspnea and bleeding, and drug interaction (p<0.001; <0.001, respectively) (Table 6).

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Examination of the medications used by elderly patients based on Beers criteria revealed that;

- Medications always to be avoided were used at a level of 38.9%,
- Medications to be avoided in elderly patients with certain diseases and syndromes were used at a level of 13.4%,
- Medications to be used with caution in elderly patients were used at a level of 29.4%.

Table 1. Relations between presentation symptoms associated with DDCs and drug interactions and demographic data

	DDCs			Relation between presentation symptoms and drug interaction		
	Yes mean±SD	**No mean±SD**	***P***	**Yes mean±SD**	**No mean±SD**	***P***
Age (year)	72.4±6.7	68.3±5.5	**0.016***	71.2±5.7	72.5±7.0	0.156
Number of drugs (per day)	6.8±2.5	5.5±1.4	**0.040***	6.7±2.5	7.0±2.2	0.086
Number of presentations (year)	8.3±6.3	1.9±0.4	**<0.001***	9.4±7.1	7.1±6.1	**0.045***

DDCs, Dangerous drugs combinations; * Pearson Chi-square test

Table 2. Drug groups and the 10 most commonly used medications in elderly patients

Drug group	**n (%)**
• Antiarrhythmics (Alpha, beta, calcium channel blockers, and others)	310 (15.4)
• Antithrombotics and anticoagulants (Aspirin, UFH, LWMH, warfarin, and others)	263 (13.1)
• Antihypertensives (ACE inhibitors, ARB, Nitrates and others)	206 (10.2)
• NSAIDs (Acetaminophen, and others)	184 (9.1)
• Antidiabetics (Insulin preparations, and oral antidiabetics)	173 (8.7)
• Bronchodilators	162 (8.1)
• Gastrointestinal system drugs (PPI, motility regulators, and others)	159 (7.9)
• Neurology drugs (Antiepileptics, antiparkinsons, and others)	77 (3.8)
• Psychiatric drugs (Antidepressants, antipsychotics and others)	72 (3.6)
• Antilipidemic drugs	64 (3.9)
• Diuretics (Loop, thiazid diuretics, potassium sequestrants, and others)	52 (2.6)
• Hormone preparations (including steroids, thyroid drugs and others)	51 (2.5)
• Antibiotics (Beta lactams, Quinolones and others)	35 (1.7)
• Vitamins and minerals	35 (1.7)
• Genitourinary system drugs	32 (1.6)
• Other drug groups	136 (6.7)
Drug name	
• Aspirin (Acetylsalicylic acid)	131 (6.5)
• Metoprolol	94 (4.7)
• Furosemide	94 (4.7)
• Metformin	62 (3.1)
• Hydrochlorothiazide	61 (3.0)
• Insulin	59 (2.9)
• Clopidogrel	59 (2.9)
• Salbutamol	47 (2.3)
• Amlodipine	46 (2.2)
• Carvedilol	41 (2.0)

UFH, unfractionated heparin; LMWH, low molecular weight heparin; ACE, angiotensin converting enzyme inhibitors; ARB, Angiotensin receptor blockers; NSAID, Non-steroidal anti-inflammatory drugs; PPI, Proton pump inhibitors

Table 3. Relations between DDCs and drug interactions and drug groups

	DDCs			Drug interaction		
Drug groups	**Yes (n=284)**	**No (n=16)**	***P***	**Yes (n=73)**	**No (n=227)**	***P***
Antihypertensives	257	10	**<0.001***	64	203	0.677
Oral antidiabetics	146	7	0.551	41	112	0.310
Antiarrhythmics	111	3	0.103	28	86	0.943
PPI	80	4	>0.999	23	67	0.242
Bronchodilators	69	1	0.130	13	57	0.199
NSAIDs	56	3	>0.999	17	42	0.371
Antibiotics	38	-	0.237	12	26	0.265
Insulin preparations	31	4	0.088	9	26	0.839
Antithrombotics	14	-	>0.999	4	10	0.751
Anticoagulants	19	1	>0.999	6	14	0.590
Antidepressants	26	3	0.193	8	21	0.668

DDCs, Dangerous drugs combinations; NSAID, Non-steroidal anti-inflammatory drugs; PPI, Proton pump inhibitors; * Pearson Chi-square test

Table 4. Effects deriving from DDCs and drug interactions with 10 most commonly used medications in elderly patients

Drug name or group	**Drug name or group**	**Interaction results**	**n (%)**
Aspirin	Anticoagulants (including warfarin)	increase bleeding	47 (10.7)
Aspirin	Metoprolol	increase bleeding	43 (9.8)
Aspirin	ACE inhibitors	increase bleeding	39 (8.7)
Metoprolol	Bronchodilators	dyspnea	36 (8.2)
Metoprolol	Other antiarrhythmics	bradycardia, syncope	23 (5.2)
Furosemide	Metformin	volume retention, hypoglycemia	19 (4.2)
Insulin	ACE inhibitors	hypoglycemia	19 (4.2)
Aspirin	NSAIDs	increase bleeding	15 (3.4)
NSAIDs	ACE inhibitors	renal failure, hypertension	12 (2.6)
Warfarin	NSAIDs	increase bleeding	11 (2.5)

DDCs, Dangerous drugs combinations; ACE, angiotensin converting enzyme; NSAID, Non-steroidal anti-inflammatory drugs

Table 5. Relations between DDCs and drug interactions and chronic or/and comorbid diseases

	DDCs			Relation between presentation symptoms and drug interaction		
Comorbid disease	**Yes (n=284)**	**No (n=16)**	***P***	**Yes (n=73)**	**No (n=227)**	***P***
HT	216	9	0.133	54	171	0.816
DM	153	7	0.430	153	7	0.408
CAD	109	3	0.114	20	92	**0.044***
COPD	99	5	0.768	19	85	0.075
CHF	58	1	0.211	16	43	0.548
PTE	24	1	>0.999	5	20	0.598
Psychiatric disease	22	1	>0.999	4	19	0.419
Acute infection	18	–	0.610	2	16	0.259
Arrhythmia	15	–	>0.999	5	10	0.372
CRF	12	1	0.517	1	12	0.201
Other	107	9	0.147	129	39	0.109

DDCs, Dangerous drugs combinations; HT, Hypertension; DM, Diabetes mellitus; CAD, Coronary artery disease; COPD, Chronic obstructive pulmonary disease; CHF, Congestive heart failure; PE, Pulmonary thromboemboli; CRF, Chronic renal failure; * Pearson Chi-square test

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Table 6. Relations between DDCs and drug interactions and presentation symptoms

Complaint	DDCs			Relation between presentation symptoms and drug interaction		
	Yes (n=284)	No (n=16)	*P*	Yes (n=73)	No (n=227)	*P*
Dyspnea	77	6	0.393	63	7	**<0.001***
Chest pain	60	1	0.208	61	9	0.061
Headache/dizziness	57	3	0.598	60	10	0.916
Nausea and vomiting	36	2	>0.999	38	19	0.072
Abdominal pain	31	2	0.692	33	4	0.083
Urticaria	28	2	0.719	30	6	0.439
Diarrhea	20	5	0.448	25	5	0.601
Bleeding†	15	2	0.227	17	15	**<0.001***
Weakness	13	2	0.364	15	3	0.372
Palpitation	4	–	>0.999	4	1	>0.999
Other‡	5	1	>0.999	9	1	>0.999

DDCs, Dangerous drugs combination; *Pearson Chi-square test; †, Bleeding (including gastrointestinal bleeding, intracranial hemorrhage, epistaxis, hematuria, alveolar haemorrhage); ‡ Other (including constipation, and urinary retention)

Discussion

The prevalence of drug-drug interactions and side-effects is growing because of an increase due to aging in the prevalence of chronic diseases (and thus in the numbers of medication used), the use of herbal therapies, the use of prescribed or over-the-counter medicines, and changes induced by dose repetitions resulting from forgetfulness and in drug metabolism (11, 12). This is related to gender, as well as aging. Studies have shown that since women have longer life expectancies than men, they are also exposed to more chronic disease, and thus to more drug interactions or DDCs development (13, 14). Topbaş et al. reported mean ages of 69.9 years for women and 70.5 for men in elderly patients regularly using medications (15), while Arslan et al. reported mean ages of 77.1 for women and 74.3 for men (16). The mean age of the patients in our study was 72.2, and the majorities were women. Mean age was higher in patients with DDCs than in those without DDCs, and the difference was statistically significant. However, although the number of women was higher among the subjects with DDCs, we determined no correlation between DDC and gender. Although these findings are similar to those of previous studies, DDC development was associated only with increased age, and was independent of gender.

Chronic and/or comorbid diseases are common among elderly individuals (6, 17). Ünsal et al. reported the presence of at least one chronic disease in 82% of elderly patients, the most common being HT, DM and COPD (18). Chiovanda et al. reported a different sequence, in the form of pulmonary system diseases, DM and cardiovascular system diseases (19). Although the sequences vary from study to study, the risk of polypharmacy and associated drug-drug interaction or DDCs due to the presence of chronic diseases or new diseases persists.

Studies on this subject have reported that simultaneous use of anticholinergic drugs, the use of antidiabetic drugs with alcohol, steroids, antihypertensive or antipsychotics, the use of antibacterial medications with mono-amino oxidase inhibitors, and the use of diuretics with antihypertensive all lead to severe drug interactions and DDCs development (20, 21). The most common diseases in our study were HT, diabetes mellitus, and CAD. Moreover, in addition to newly developing infections, musculoskeletal system disorders were also present in some patients. This all indicated that numerous drugs to be used for both new and old diseases will result in new interactions, side-effects and DDCs. However, we determined no correlation between comorbid diseases and DDCs development. Presentation symptoms associated with drug interactions were only correlated with CAD. This may be due to medications from several groups (antiarrhythmic, antihypertensive, diuretic, antithrombotic, anticoagulant, etc.) being used together in CAD. We attributed the lack of any relation between comorbid diseases and DDCs to the absence of combinations that might result in a statistically significant finding due to medications that might cause DDCs being distributed to different patients.

Since multidrug use in elderly patients can give rise to new side-effects, this can suppress the symptoms and findings of existing diseases. Emerging side-effects may also constitute a primary cause of presentation symptoms (22, 23). Cahir et al. reported the most common side effects resulting in presentations to hospital due to drug interactions in elderly patients as bleeding, dyspepsia, dizziness and impairment of the decision-making mechanism. They attributed the emergence of these to warfarin, aspirin, NSAID and psychotropic drugs, respectively (24). Banerjee at al. reported dyspnea and falls as the most commonly seen side-effects (20), Arslan et al. reported abdominal pain and nausea-vomiting (16), while Uz et al. reported constipation as the most common side-effect (25).

The most frequent presentation symptom in our study was dyspnea, and a correlation was determined with drug interaction. Dyspnea is associated with several acute (seasonal conditions and respiratory tract infections) or chronic (COPD, bronchitis, pulmonary embolism, heart failure, kidney failure, etc.) diseases. At the same time, it may also emerge in association with the use of bronchodilator medications and β-blockers, with cardiac dysfunction, or with other drug combinations that reduce the effectiveness of diuretics.

The medications that most commonly cause drug interactions in elderly patients are anticoagulants, NSAIDs, antihypertensives, antiarrhythmics, antidiabetics, diuretics and antibiotics (6, 21, 26). Warfarin is the best known oral anticoagulant and interacts with several medications (27). In addition to reducing the effect of diuretics and β-blockers, NSAIDs also adversely affect gastrointestinal system (GIS) and renal blood supply, but are commonly used in all age groups, not only by the elderly (28). Sari et al. investigated the relation between GIS bleeding and medication use in elderly patients and determined an association with NSAID, aspirin and warfarin use (29). In our study, both aspirin and NSAIDs were frequently used by elderly patients. The most commonly employed combinations were 'aspirin + metoprolol', 'aspirin + furosemide' and 'aspirin + anticoagulant'. In addition, we determined that antithrombotics, anticoagulants, antiarrhythmics, antihypertensives, diuretics, and angiotensin receptor blockers were frequently used together during the treatment of inter-related diseases such as CAD and HT. Although this is essential for the routine treatment of various diseases, it also leads to bleeding associated with drug-drug interactions. Another finding was that the incidence of DDCs was significantly higher in patients using anti-hypertensive medications. We attribute this to monotherapy being insufficient in the treatment of HT, to patients using numerous and various anti-hypertensive medications on the market together under different names (although their contents are the same), or to the addition of further drugs to treat secondary organ damage occurring due to HT.

The number of daily medications used is another cause of interaction-related effects. Studies have reported 2-9 medications a day being used in elderly patients (6, 30, 31). Several studies have shown that an increase in the numbers of drugs used also raises the probability of drug interactions, DDCs and side-effects (11, 12, 32). Field et al. reported that the risk of adverse reactions in multidrug use increased to 13% in patients using two medications, to 58% in those using five, and to 82% in those using seven or more (32). Bjorkman et al. reported a positive correlation between the probability of drug-drug interaction and numbers of medications used (33). In contrast, Pozzi et al. reported that an increase in hospital presentations associated with drug interactions was correlated, not with the numbers of medications used, but with the combined used of potentially inappropriate medications (34). In our study, the prevalence of DDCs increased in subjects using larger daily numbers of medications. Numbers of presentations to hospital were also significantly high in these patients. A higher number of medications facilitates potential side-effects, drug-drug interactions, the emergence of DDCs and an increased number of ED presentations. We think that although more DDCs emerged in patients using higher numbers of medications, numbers of presentations to the ED were not solely associated with the mean number of medications used, and that various combinations may result in hazardous interactions irrespective of medication numbers.

Studies have reported that elderly people constitute 9-23% of patients presenting to the ED, and that polypharmacy causing drug interactions in these patients increases both outpatient treatment and hospitalization (35, 36). Numbers of medications used in elderly individuals are related to drug interactions and numbers of presentations to hospital (37, 38). Sehgal et al. showed a positive correlation between multidrug use and DDCs and numbers of presentations to hospital (39). The level of patients presenting to the ED due to drug interactions in the present study was 24.3%. This was statistically significantly higher than the level in patients presenting with both DDCs and drug interaction. In addition, we also determined positive correlation between numbers of presentations and numbers of medications used. These findings are in agreement with previous studies reporting that increased numbers of medications used in elderly patients also increase DDC- and drug interaction-related hospital presentations.

Conclusion

Numbers of medications used and DDCs increase with age in elderly. This particularly applies to subjects with CAD, and using antihypertensive drugs. An increase in the number of medications used results in an increase in drug interactions and hospital presentations, the main presentation symptoms being dyspnea and bleeding. Under these conditions, dyspnea is generally caused by combined use of bronchodilators and β-blockers, while bleeding is generally caused by combined aspirin and warfarin. Further, wide-ranging studies regarding rational medication use and patient safety in the elderly are now needed.**Limitations:** The most important limitations of our study were the absence of control group and low number of patients. The other limitations are as follows: Long-term follow-ups of the patients were not performed and evaluation about the other subjects, except pain scale was not made.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: HB, OE, FC: Research concept and design; Patient examinations, Treatment, data collecting, analysis and interpretation of data**. OE:** Preparation of article, and Revisions. All authors approved the final version of the manuscript.

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under

the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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The effect of TGN-020 on penicillin induced epileptiform activity in rats

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Abstract

Objective: Aquaporin-4 (AQP-4) is a water channel protein which is the most abundant aquaporin isoform in the brain. Recent studies indicate the relationship between AQP-4 with epileptogenesis. Therefore, we examined the potential effect of the AQP-4 inhibitor TGN-020 on penicillin-induced epileptiform activity in rats.

Material and Method: Epileptiform activity was induced by intracortical (i.c.) administration of penicillin (200 IU, 1 µl). TGN-020, at doses of 25 µg, 50 µg, 100 µg and 200 µg, was administered by intracerebroventricular (i.c.v.) 30 minutes after penicillin injection. The epileptiform activity was verified by electrocorticographic (ECoG) recordings. Twenty four hours later, animals are decapitated for the collection of blood samples and brain tissue.

Results: The dose of 100 µg TGN-020 decreased the mean spike frequency of epileptiform activity in the 30 min after the injection without changing the amplitude ($p < 0.05$). Serum neuropeptide Y level was up-regulated by 25 µg TGN-020 in comparison with the other groups ($p<0.001$). Plasma levels of calcineurin in the 50 µg dose of TGN-020 were lower than 25 µg and 200 µg doses of TGN-020 ($p<0.01$). Enzymatic ativity of glutathione peroxidase (GP_x-1) in brain tissue was higher in the penicillin and 25 µg TGN-020 group compared with the sham group ($p < 0.05$).

Conclusion: Given all these data, the anticonvulsant effect of TGN-020 which is aquaporin-4 water channel inhibitor in the brain has been studied extensively for the first time in an experimental model of epilepsy. Inhibition of AQP-4 might be useful in the treatment of epilepsy in future.

Key words: TGN-020, Aquaporin 4, Epilepsy, EcoG, DMSO, Neuropeptide Y

Introduction

Aquaporin 4 (AQP-4) is a water channel protein, which is primarily abundant aquaporin isoform in the brain (1). This aquaporin is considered to be an important role in the physiopathology of brain disorders including ischemia, epilepsy, traumatic brain disease, tumor-induced brain swelling, infections and hydrocephalus (2-4). These studies indicate that AQP-4 has a crucial role in the movement of water into and out of the brain tissue (5, 6). Seizure susceptibility of Aqp4-/- mice was found to be less than the all wild-type mice in pentylenetetrazol (PTZ) induced epilepsy and the latency to generalized seizures is also reported to be longer in Aqp4-/- mice (7). This reduction in neuronal excitability is thought to be effective in the extracellular fluid ions and osmolarity changes.

Recently, various chemical structures were defined as AQP-4 water channel inhibitors including 2-nicotinamide-1,3,4-thiadiazole (TGN-020) (8).

In vitro studies indicated that TGN-020 was found the most powerful inhibitor of AQP-4 water channel (9). Pretreatment with TGN-020 significantly reduced brain edema in a mouse model of focal cerebral ischemia using 7.0-T magnetic resonance imaging (MRI) (10). Through increasing the osmolarity of extracellular space, water moves into the cell and ionic edema occurs (11). Therefore, AQP-4 inhibition is a new approach to reduce the cerebral edema and is shown to be promising for the development of effective drugs in the clinical treatment (10). Same as in cerebral edema brain excitability is highly sensitive to acute changes in osmolarity (12). Altering the osmolality of the extracellular fluid changed the amplitude and duration of the epileptiform bursts activity in rat dentate gyrus (13). According to this study decreasing the osmolality increased the amplitude of the spikes within the burst and increasing the osmolality decreased the amplitude in the rat hippocampal slices (14).

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These experimental findings are parallel with various clinical situations such as the dialysis disequilibrium syndrome, compulsive polydipsia and the syndrome of inappropriate ADH secretion (15). Consequently, studies on the role of AQP-4 and its inhibitor, TGN-020, in epileptiform activity is quite limited, therefore it is necessary to investigate the effect of TGN-020 in different experimental models of epilepsy to better understand AQP-4 working mechanism.

In vivo experimental models are frequently used to explain the pathophysiological mechanisms of epilepsy. Application of penicillin G intracortically is one of these models (16). Penicillin is structurally a GABA antagonist that is similar to bicuculline eliminates GABA inhibition and causes the induction of epileptic seizures (17) This model is more similar to the human focal epileptic activity (18). The data concerning the effects of TGN-020 on penicillin-induced epileptic activity under the monitoring of electrocorticography (ECoG) is still not sufficiently reported in the currently available literature. In the present study, we used intracortically injected penicillin method to induce epileptiform activity and investigated the effects of TGN-020 on this epilepsy model in rats.

We measured levels of brain nitric oxide (NO), superoxide dismutase [Cu-Zn] (SOD), general malondialdehyde (MDA), glutation peroxidase (GPx-1) and serum S100B protein, neuron-specific enolase (NSE), Neuropeptide Y (NPY) and calcineurin levels in order to show the effects of TGN0-20 on neuronal excitability.

Recent studies have implicated oxidative stress resulting from excessive free-radical release in the initiation and progression of epilepsy, so it is plausible that excessive free-radical may have a functional role in the hyperexcitability characteristic of epilepsy. Rauca and colleges showed that increased reactive oxygen species in the brain accompanies the development of PTZ kindling and is an important pathogenic factor in the PTZ kindling-induced neuronal death (19). Based on these data, free radicals might be affected from convulsion. Hence in the present study, we investigated the effects of TGN-020 on biochemical parameters including NO, SOD [Cu-Zn], MDA and GPx-1 in the brain tissue of penicillin induced epileptic animals.

Various biochemical markers have been investigated in the context of brain damage and epilepsy. However, S100B protein and NSE are the most widely studied biochemical markers of central nervous tissue damage (20, 21). S100B protein is a large subset of calcium-binding proteins (22). This protein stimulates nerve growth and increase the survival of neurons; thus acting as a protective factor (23). In addition, it is reported to be used as a clinical marker to assess prolactin, NSE and S-100B protein levels in the serum of children and adult patients with temporal lobe epilepsy (24, 25). Thus, we investigated a possible link between AQP-4 activity and serum S-100B, NSE, NPY and calcineurin levels in epileptiform activity of rats using specific inhibitors of AQP-4 (TGN-020).

Materials and Methods

Animals

Experiments were carried out on adult male Wistar albino rats weighing 240-300 g. The animal usage protocol was approved by the local ethics committee of the Bezmialem Vakif University. Along all experiments, local guidelines for the care and use of laboratory animals and the guidelines of the European Community Council for experimental animal care were applied. Animals were housed in temperature of 21 ± 2 oC on a 12 h light/dark period. All animal groups were allowed ad libitum food and water except for the short time when animals were removed from their cages to do experiments. The experiments were materialized between 11:00 and 17:00 periods of day time. Animals were divided into six groups: (1) intracortical (i.c.) delivery of 2.5 µl artificial cerebrospinal fluid [aCSF containing (mM): NaCl, 124; KCl, 5; KH2PO4, 1.2; CaCl2, 2.4; MgSO4, 1.3; NaHCO3, 26; glucose, 10; HEPES, 10; pH 7.4 when saturated with 95% O2 and 5% CO2]; (2) 500 units penicillin (2.5 µl, i.c.); (3) 500 IU penicillin (2.5 µl, i.c.) + TGN-020 (25 µg, i.c.v.); (4) 500 IU penicillin (2.5 µl, i.c.) + TGN-020 (50 µg, i.c.v.); (5) 500 IU penicillin (2.5 µl, i.c.) + TGN-020 (100 µg, i.c.v.); (6) 500 IU penicillin (2.5 µl, i.c.) + TGN-020 (200 µg, i.c.v.); (7) 500 IU penicillin (2.5 µl, i.c.)+dimethylsulfoxide (DMSO, i.c.v); (8) 100 µg TGN-020 (i.c.v.). Each animal group was composed of seven rats.

Induction of epileptiform activity

Induction of epileptiform activity was performed as described previously by Kozan & co-workers (26). Briefly, animals were anesthetized with an intraperitoneal injection of urethane (1.25 g/kg). Rectal temperature was maintained between 36.5 and 37.5 oC using a feedback-controlled heating system (Homeothermic Blanket Control Unit, Harvard Apparatus, MA, USA). The left cerebral cortex was carefully exposed by craniotomy (5 mm posterior to bregma and 2 mm lateral to sagittal sutures). Subsequently, incision of the skull, head of the animal was fixed by utilizing standard stereotaxic methods (Harvard Instruments, South Natick, MA, USA). The epileptic focus was produced by 500 international units of penicillin G potassium injection which is acute in an experimental model of focal epilepsy; 1 mm beneath the brain surface by a Hamilton microsyringe type 701RN; infusion rate 0.5 µl/min).

Drugs and drug administration

TGN-020 and DMSO (Sigma Chemical Co., St. Louis, MO, U.S.A.) were used in this study. All of the solutions were prepared just before experiments. TGN-020 was dissolved in DMSO and the requisite doses were administered intracerebroventricularly. Intracerebroventricular injections were administered into the left lateral ventricle of each rat through a stereotaxic apparatus, with the coordinates of 0.8 mm posterior to the bregma, 2.0 mm lateral to the midline and 4.2 mm ventral to the surface of the skull based on the stereotaxic atlas of the rat brain (27).

First of experiment sets, penicillin was prepared in sterile apyrogen distilled water and 500 IU penicillin was administered intracortically in a volume of 2.5 µl into the left cortex. Secondly, aquaporin-4 water channel antagonist TGN-020, at doses 25, 50, 100 and 200 µg, was administered 30 min after penicillin application (28).

Electrocorticography (ECoG) recordings

All EcoG recordings were obtained in urethane anesthesized animals. Two Ag-AgCl ball electrodes were placed over the left cortex and the coordinates of electrode were first electrode, 1.5 mm lateral to sagittal suture and 1 mm anterior to bregma; second electrode, 2 mm lateral to sagittal suture 5 mm posterior to bregma. Recording electrodes were placed on the cortex surface by means of two different electrode holders. The common reference electrode was stabilized on the pinna. The ECoG activity was continuously monitored by using an eight-channel data acquisition system (PowerLab, 8/SP, AD Instruments, Australia) for at least 120 minutes. During the whole ECoG recording four different corners of the scalp were sutured by surgical threads and stretched as to form a liquid vaseline pool at 37 oC that protects brain tissue from water and electrolyte loss. Recordings were stored on computer. Analysis of epileptiform activity was made off-line. Spike frequencies and amplitudes for each animal were automatically calculated and measured by using the LabChart v 7.3 (PowerLab Software, 8/SP, AD Instruments, Australia). In the study, only the number of spikes with amplitudes greater than threefold baseline activity was taken into consideration.

Biochemical analysis

Following that the ECoG records were completed, the scalp of animal was sutured by surgical threads. Twenty four hours later, animal was harvested under light ether anesthesia for the collection of blood samples and brain tissue. Serum was obtained from blood samples and they were stored at -80ºC degrees until the time of study. Serum levels of S- 100B protein biomarker, NSE, NPY and Calcineurin parameters were studied using a commercial rat specific ELISA kits (ElAab Science Co. Ltd) and each assay was applied (21, 29). Calcineurin Subunit B type level was qualified using ELISA, Rat S100B ELISA kit E1323r, Protein S100-B by Rat S100B ELISA kit E0567r, Pro-neuropeptide Y by ELISA kit E0879r and Neuron-Specific Enolase by Rat NSE ELISA kit E0537r.

The cerebral hemispheres, cerebellum and brain stem of the brains that were extracted were allocated. These structures were separated as right and left hemispheres and stored at -80º C until biochemical studies were completed. Brain tissues dissolved during biochemical studies were broken into pieces, were washed with saline fluid, and were dried with blotting paper. After that, the tissue quantity was weighed and recorded then they were homogenized in TRIS Buffer at a speed of 16.000 rev/min by glass teflon homogenizer in 5 min and the homogenate obtained in this way was used in the studies. From brain tissue, oxidant /antioxidant parameters of the malondialdehyde by MDA ELISA kit, E0597Ge, nitric oxide level by total NO detection kit, (Enzo Life Sciences), ADI-917-020 kit, NO2/NO3 rate was studied and superoxide dismutase by Rat SOD ELISA kit, E0596r, glutathione peroxidase by Rat GPx-1 ELISA kit, E0295r activities were determined using a commercial enzyme-linked immunoassay. The minimal measurable concentrations for these detection systems were 0.15 ng/ml for Calcineurin Subunit B, 15.6 pg/ml for S100-B, 31.2 pg/ml for Pro-neuropeptide Y, 1 pg/ml for NSE, 31.2 U/ml for GPx-1, 0.312 nmol/ml for MDA, 1.56 U/ml for SOD and 20.86 µM/l for NO.

Statistical analysis

All statistical procedures were made by using SPSS statistical software (version 22.0). The differences between the groups were analyzed within the one-way ANOVA. Significant differences were further evaluated through multiple comparisons posthoc Scheffe test for the electrophysiological data and Tukey test for the biochemical parameters. Furthermore, repeated ANOVA test is used to determine difference within each group. Data are expressed as the means ± SEM. Statistical significance was set at $p < 0.05$.

Results

Effects of TGN-020 on epileptiform activity

Baseline activities of each animal were recorded before the administration of intracortical penicillin. Along this period, none of the animals showed spontaneous epileptiform activity (Fig 1A). Intracortical injection of 500 IU penicillin induced an epileptiform ECoG activity which began within 2-4 minutes. The frequency and amplitude of epileptiform activity reached at a constant level in 30 minutes and lasted for 2-3 hours. The means of spike frequency and amplitude were 30.3 ± 3 spikes/min, 940.6 ± 88.3 µV, respectively (Fig 1B).

The intracortical injection of aCSF (2.5 µl) and intracerebroventricular injection of TGN-020 or DMSO (1.5 µl) did not cause any change in the frequency or amplitude of ECoG activity with respect to the control base line in the nonpenicillin injected animals.

Figure 2, indicates the effect of single administration of different doses (25, 50, 100 and 200 µg) of aquaporin-4 water channel antagonist TGN-020 on the penicillin-induced epileptiform activity. Repeated Anova test revealed a significant anticonvulsant effect for TGN-020, at a dose of 100 µg with a maximal effect. Application of TGN-020, at a dose of 100 µg, decreased the mean spike frequency of epileptiform activity in the 30 min after the injection without changing the amplitude. The other three doses of TGN-020 (25, 50 and 200 µg) did not significantly change either the frequency or amplitude of the epileptiform activity. The mean spike frequency of epileptiform activity was 29.68 ± 2.1, 29.64 ± 1.9, 15.78 ± 1.2 and 27.81 ± 2.3 spike/min, and the mean amplitude was 1138 ± 140, 717 ± 72, 1182 ± 128 and 791 ± 85 µV after 70 min from TGN-020 injection in response to dose of 25, 50, 100 and 200 µg TGN-020 respectively (Fig.1C-F).

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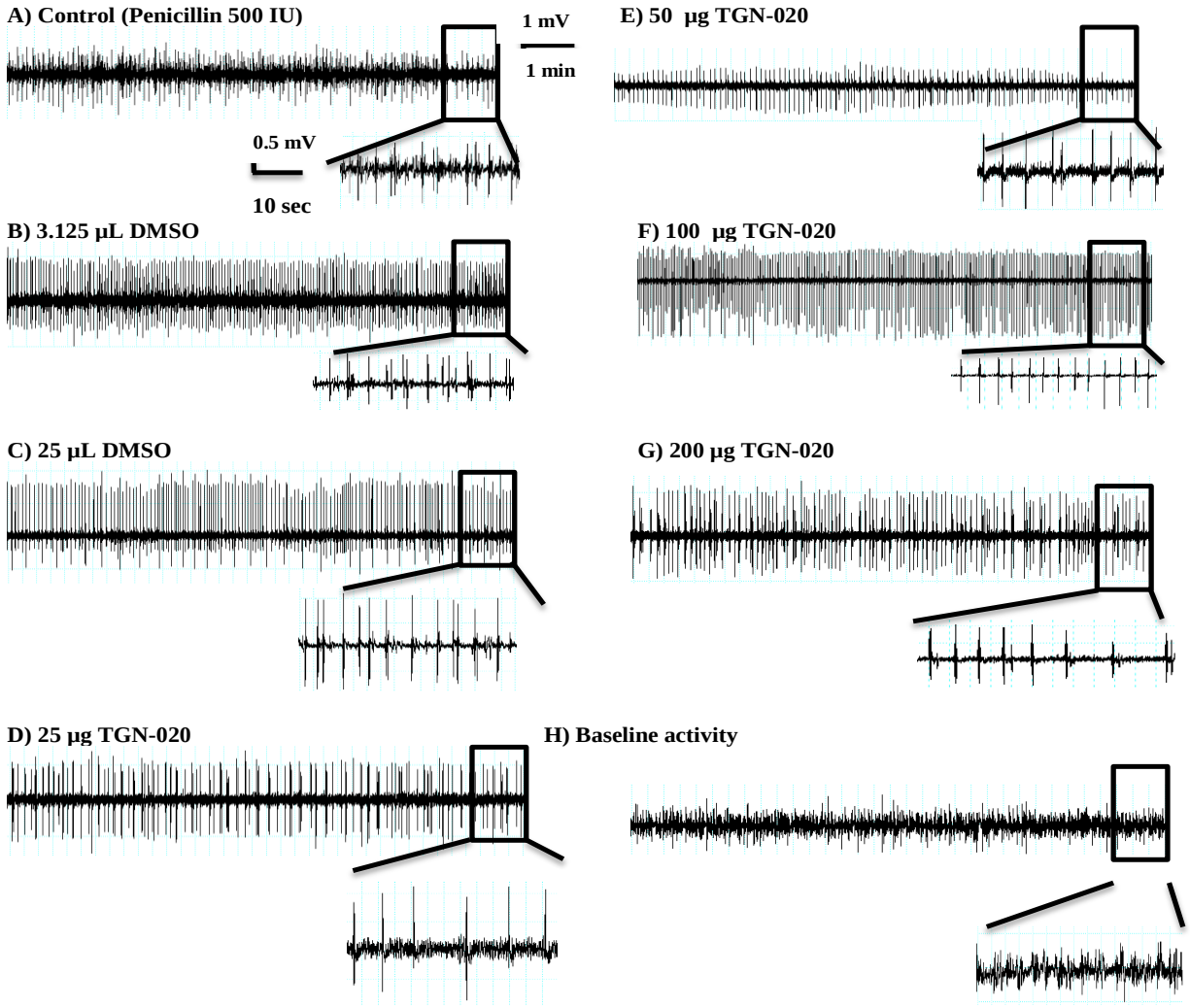
The effects of TGN-020 on biochemical parameters

The intracerebroventricular injection of TGN-020 and DMSO (1.5 µl) did not cause any change in the biochemical parameters of brain tissue and serum levels with respect to the intracortical injection of aCSF (2.5 µl) injected animals.

The serum levels of NSE, S100B protein, NPY and Calcineurin are summarized in Table 1. As shown in Table 1 injection of 25 µg TGN-020 up-regulated significantly serum NPY level in comparison with the penicillin and others doses of TGN-020 groups, respectively ($p<0.001$).

In addition, when compared with the 50 µg dose of TGN-020, plasma levels of calcineurin in 25 and 200 µg doses of TGN-020 were significantly lower ($p<0.01$). There was no significant result in serum Protein S-100B and NSE levels. Table 2 summarizes the activities of brain tissue SOD and GPx-1 enzymes and brain MDA and NO levels in all groups. The activity of SOD and MDA or NO levels did not differ between the groups. However, GPx-1 activity in the brain tissue was higher in the penicillin and penicillin+25 µg TGN-020 groups compared with the sham group ($p < 0.05$).

Figure 1: TGN-020 and DMSO regulates the frequency of epileptiform activity



A) The intracortical injection of penicillin (500 IU) induced epileptiform activity on ECOG. **B)** The intracerebroventricular (i.c.v.) administration of DMSO at a dose of 3.125 µL, significantly decreased the mean frequency of epileptiform activity in the 50 min after DMSO injection without changing the amplitude. **C)** The application of DMSO (i.c.v), at a dose of 25 µL, did not significantly change either the mean frequency or amplitude of penicillin-induced epileptiform activity. **D)** The intracerebroventricular (i.c.v.) administration of aquaporin-4 water channel antagonist TGN-020, at a dose of 25 µg did not significantly change either the mean frequency or amplitude of penicillin-induced epileptiform activity. **E)** The administration of TGN-020, at a dose of 50 µg did not significantly change either the mean frequency or amplitude of penicillin-induced epileptiform activity. **F)** The administration of TGN-020, at a dose of 100 µg with a maximal effect, decreased the mean spike frequency of penicillin-induced epileptiform activity in the 60 min after TGN-020 injection, without changing the amplitude. **G)** The administration of TGN-020, at a dose of 200 µg did not significantly change either the mean frequency or amplitude of penicillin-induced epileptiform activity. **H)** Displays baseline ECoG activity before giving penicillin or the injection of other chemicals.

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Figure 2: The figure 2 is associated with the effects of intracerebroventricular administration of DMSO on the mean spike frequency of penicillin-induced epileptiform activity. DMSO, at a dose of 3.125 µL (i.c.v.), significantly decreased the mean frequency of epileptiform activity in the 10 min after DMSO injection. DMSO, at a dose of 25 µL, did not significantly change the mean frequency of epileptiform activity. *p < 0.05.

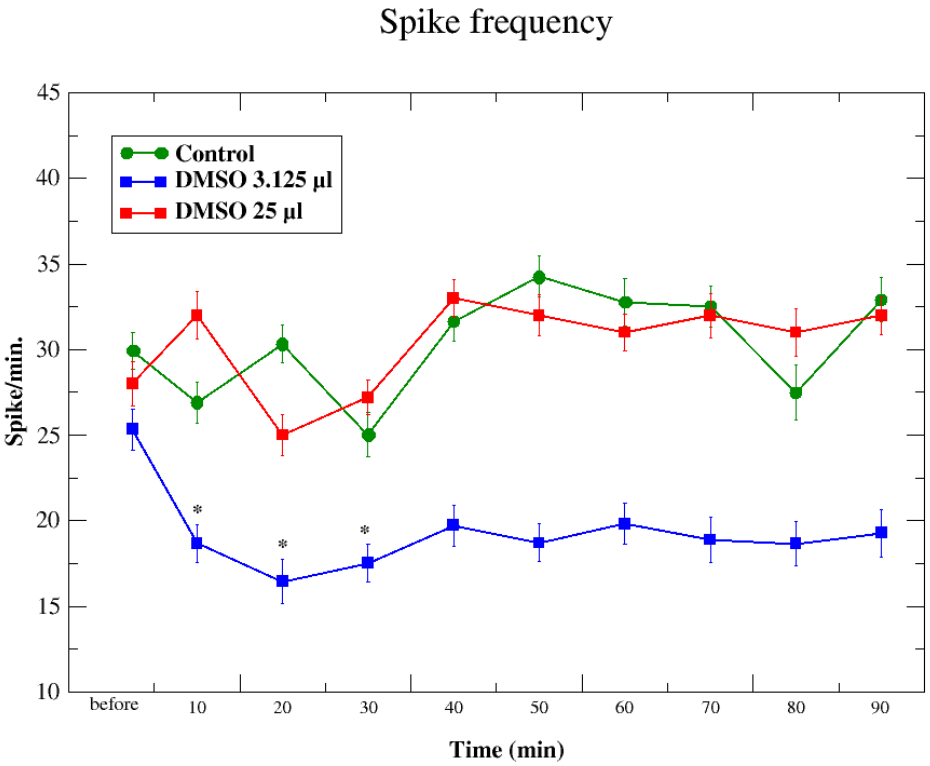

Figure 3: It indicates the effects of intracerebroventricular administration of aquaporin-4 water channel antagonist TGN-020 on the mean spike frequency of penicillin-induced epileptiform activity. TGN-020, at doses of 25, 50 and 200 µg did not change the mean spike frequency. TGN-020, at a dose of 100 µg (i.c.v.), decreased the mean spike frequency of epileptiform activity in the 30 min after TGN-020 injection. The best effect appeared in the 100 µg (i.c.v) administered group. *p < 0.05.

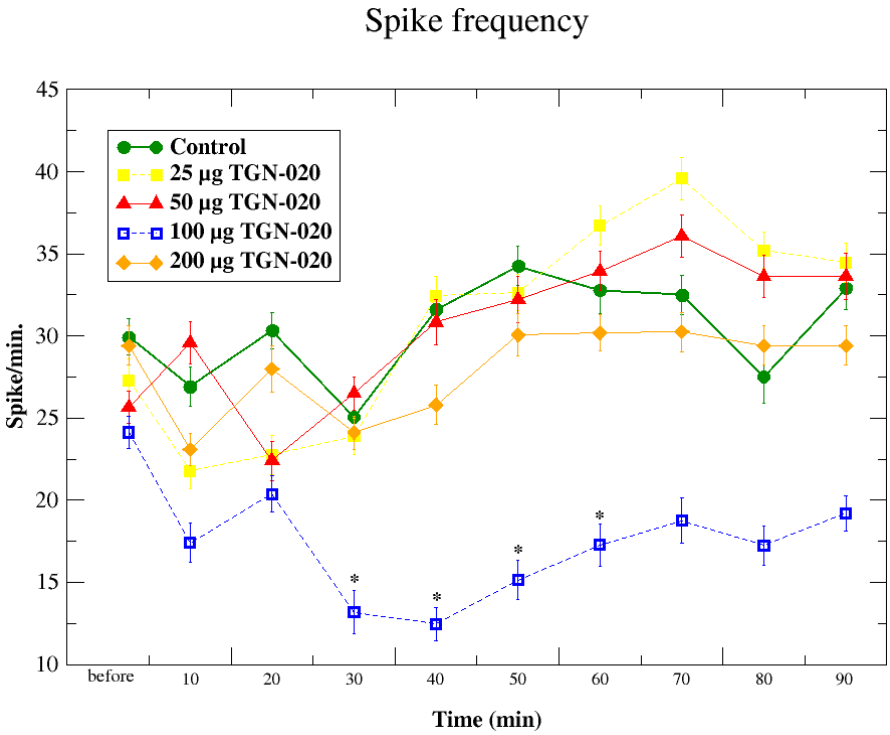

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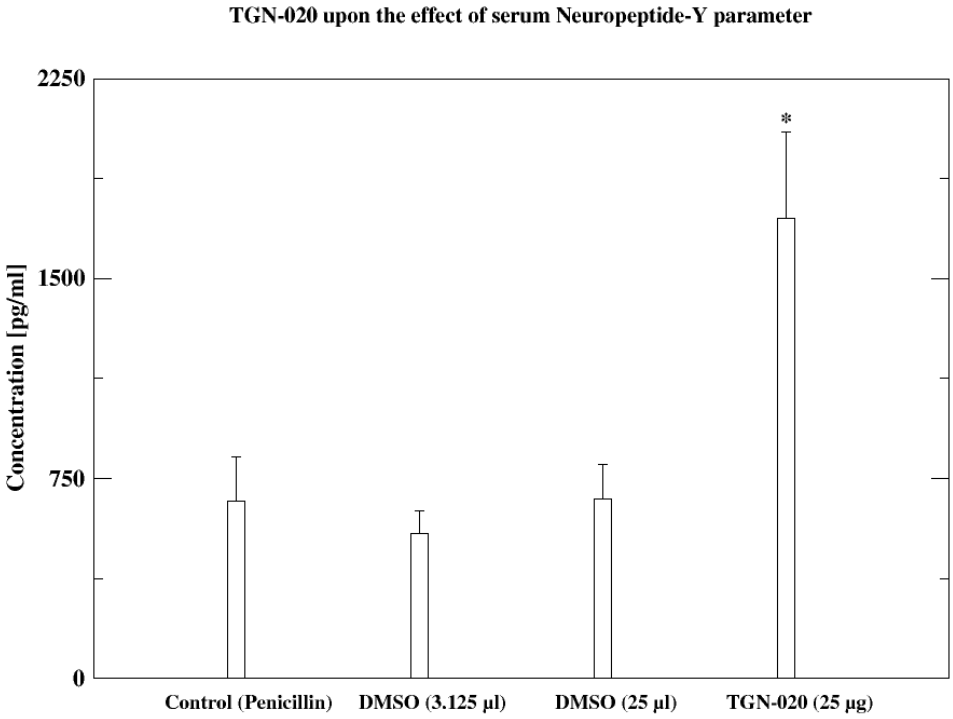
Figure 4: TGN-020 application significantly increased serum neuropeptide Y concentration in comparison with penicillin and both DMSO groups. There were no differences between control and both DMSO groups. *p < 0.05.



Discussion

TGN-020, 2-nicotinamido-1,3,4-thiadiazole, is a potent inhibitor of AQP-4 (9, 28). TGN-020 was identified on the basis of conserved physical and chemical features of several known drugs found to inhibit transport of water channel AQP-4 in vitro. One of the previous studies suggested that, intraperitoneal administration of TGN-020, a dose of 200 mg/kg, significantly reduces ischemic cerebral edema in mice (10). In addition, same workgroup demonstrated that in vivo effect of TGN-020, a dose of 200 mg/kg, on AQP-4 inhibition, namely an increases regional cerebral blood flow in mice (28). On the basis of these studies we used at 25, 50, 100 and 200 µg (i.c.v.) doses of TGN-020 in the current study. TGN-020, a dose of 100 µg, decreased the frequency of penicillin-induced epileptiform activity without changing the amplitude. The remaining doses of TGN-020 (25, 50 and 200 µg) did not affect either the frequency or amplitude of epileptiform activity.

The studies suggest novel roles for water channel AQP-4 in control of seizure susceptibility (30, 31). These suggestions with changes in human epileptic tissues lead to the unifying hypothesis that AQP-4 and its molecular partners may play a functional role in epilepsy (32, 33). K+ accumulates at extracellular space in epilepsy (34). After a certain period with epileptic activity, this accumulation may end up with hyperosmolar state of the extracellular volume.

Hyperosmolar state triggers water imbalance in the brain tissue that is water efflux from the cell. Therefore, we aimed preserve intracellular water by blocking the water channels on the cell membrane. Our data are the first results for the anticonvulsive effects of TNG-020 in experimental model of epilepsy by using electrophysiological method. Intracerebroventricular administration of TGN-020, at a dose of 100 µg, caused significant decrease in mean spike frequency of penicillin-induced epileptiform activity in rat. This finding is in-line with the previous reports on the high expression of AQP-4 in the brain tissue of epileptic rats (35, 36). Song and co-workers (2015) showed that AQP-4 had the high expression in the brain tissue of lithium chloride-pilocarpine epileptic model of rats. Likewise, in another study it was found that AQP-4 expression was higher in 30 minutes of pilocarpine-induced status epilepticus group in comparison with control (35).

It has been reported that Protein S100 B, NSE (21), NPY (37-39) and calcineurin (40) are good markers to be investigated for neuronal damage in epilepsy. The anticonvulsant effect of NPY has been demonstrated in different models of epilepsy (38, 41, 42). NPY prevents seizures by increasing the seizure threshold. NPY occurs this effect by enhancing the GABAergic inhibitory neurotransmission onto pyramidal neurons neocortex and reducing the excitatory neurotransmission (41).

On the other hand, stimulation of metabotropic and ionotropic glutamate receptors induces expression of NPY in hippocampal granule cells (43). Although the main mechanisms affecting of seizure-induced synthesis of NPY are not clearly explained, a gradual increase in NPY was reported up to 7 days after seizure (39). In our study, 24 hours following the epileptiform activity, there was a significant increase in serum levels of NPY in rats treated with TGN-020, a dose of 25 µg. It seemed that low dose of TGN-020 had convulsant effect in terms of the serum NPY level on contrary to anticonvulsant effect of 100 µg TGN-020 on ECoG recordings. There is very limited literature to make further comments on the interaction between NPY and AQP-4 and their roles in neuronal excitability. Only one research was found positive correlations between plasma NPY concentration and gene and protein expression levels of NPY and AQP-4 in the jejunum following traumatic brain injury in rats (44).

Calcineurin is a calcium dependent serine/threonine phosphatase that is widely distributed in the brain with high levels in the hippocampus and caudate putamen (45, 46). It is shown to increase highly expression of calcineurin in epileptic tissue. Consistent with the previous studies (47, 48), we found that the calcineurin level was increased in the plasma of at the 50 µg dose of TGN-020 administration rats compared to 25 µg and 200 µg doses of TGN-020 groups following penicillin induced epileptic rats. Recent data suggest that calcineurin may also involve in the regulation of cAMP mediated PKA-dependent phosphorylation of aquaporin-2 in kidney collecting duct (49). However, there is not yet any data regarding the interaction between calcineurin and AQP-4 and their roles in brain tissue. Thus, our results concerning with the interaction between calcineurin and AQP-4 are the first data from the epilepsy research.

There are numerous evidences linking oxidative stress to the initiation and progression of epilepsy in experimental models and patient with epilepsy (50-52). Oxidative stress is known to be propagating by epileptic seizures. On the contrary, induced severe seizure activity animal models, can lead to neurotoxic effects mediated by oxidative stress. GPx-1 is an endogenous antioxidant enzyme that reacts with the free radicals and prevents the formation of the hydroxyl radical which is the most toxic form of free radicals. We found significantly lower level of GPx-1in the sham group as compared with the penicillin and 25 µg dose of TGN-020 groups. Our findings contradict other reports that PTZ models have oxidant effects (50). However, (53) found no alteration on GPx-1 activity in the hippocampus and cortex of kainic acid induced epileptic animals. The possible explanation could be the diversity in obtaining the results of GPx-1 following epileptic activity, the different strain of animal species, types of the experimental models and difference in duration of chemicals injection.

In conclusion, we showed that TGN-020 which is aquaporin-4 water channel inhibitor in the brain significantly decreased the spike frequency of penicillin-induced epileptiform activity without changing the amplitude. These results firstly indicate the anticonvulsant effect of TGN-020 on an experimental model of epilepsy. In addition, we found a possible link between inhibition of AQP-4 and serum NPY or calcineurin levels and brain GPx-1 activity in penicillin induced epileptic animals.

Conclusion

Given all these data, the anticonvulsive effect of TGN-020 has been studied extensively for the first time in an experimental model of epilepsy. Consequently, present study suggests that inhibition of AQP-4 might be useful in the treatment of epilepsy in the future.

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Author's Contributions: EA, MP, RK, HK: Research concept and design; Animal experiments, data collecting, analysis and interpretation of data**. EA:** Preparation of article, and Revisions. All authors approved the final version of the manuscript.

Ethical issues: All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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