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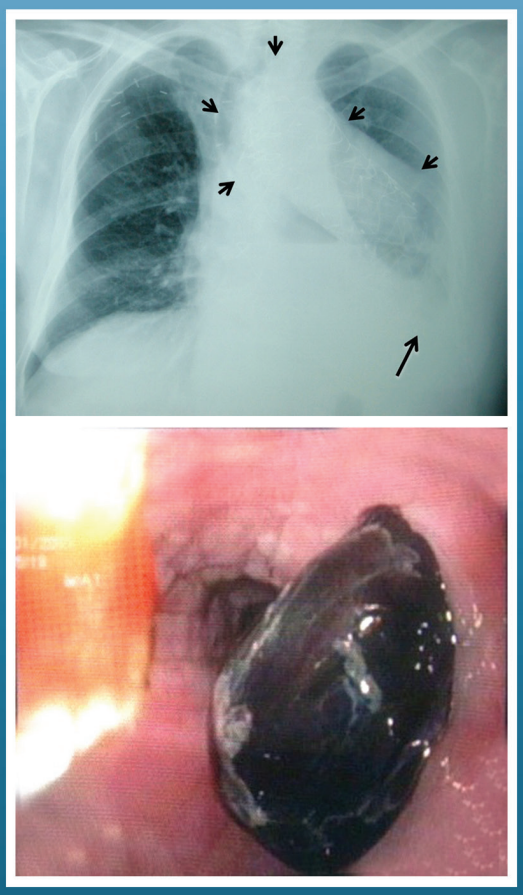

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Review

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Examinations and curriculum in medical education and learning-assessment relations

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ABSTRACT

In recent times, the increasingly growing developments contribute to be configured to curriculum and examinations. Even though the curriculum planning and development are extremely important: it should be noted that students' examination anxiety. We may consider it as the relationship among the student-tutorials-education program.

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1. Introduction

The recent development in data processing in medical education, continuously developing, evolving and increasing knowledge, learning methods and reflections of technology on teaching methods contribute to determine educational targets and to structure curriculum and examinations. Although determination and development of curriculum in medical schools is very important; it should be noted that students mainly focus on the subjects that are asked in examinations; because they are mainly concerned about examination results. The process of creating expected changes in the behavior of the learners constitutes one of the integral parts of education. We can consider them as the relationship between the student- instructor and the educational program. The instructors should do educational planning activities in order to provide targeted behaviors, knowledge, skills and attitude changes which are aimed to be gained by medical students in order to be used in their professions.

2. The curriculum development in medical education

In the World Congress on Medical Education which was held on 2002, it was emphasized the importance of assessment of clinical skills at the end of medical education by measurement-assessment systems (World Federation for Medical Education, 2003). It was especially recommended that; the educational methods with small groups should cover one-third of the curriculum and it was targeted that the elective courses should consist approximately 20% of total courses (World Federation for Medical Education, 2003).The curriculum of medical faculties should be prepared in such system that it would make the students to attain knowledge and skills which would be useful during their lives. It is expected that; a good educational curriculum should be systematic, student-centered, problem-based, community-based, elective, and integrated (Harden et al., 1984). Harden's medical educational curriculum provides precisely these principles. Harden examined curriculums in four different groups. These are science-based curriculum, organ systems-

based curriculum, problem-based learning and oriented, non-oriented curriculum (Harden, 2009). According to Kern, curriculum development process can be assessed by a six-step approach; these can be listed as identifying the problem, determining the student's needs, planning objectives and measurable targets, developing training methods and strategies, using effectively to the assessment methods and evaluating feedback. The assessment either can be in developmental and formative structure which is supported by feedback (formative) or can have ratable and decisive nature (summative) (Thomas and Kern, 2004).

3. The historical overview of medical education applications

The teachings of Hippocrates and practices of medicine has formed the future of medicine in Europe (Georgantopoulou, 2009). Anatomy and physiology began to take a part of medical education especially in the period of the Greeks (Elçin, 2010). Galen brought another approach to medical education when he said that knowledge of doctors should be assessed (Okka and Demirci, 2012). During the medieval period, in the Islamic cultural geography, the professional competency of doctors was measured and assessed for the first time in this sense (Elçin, 2010). During the 1800's, the medical school students in France were allowed to work in any field of medicine they preferred after passing written and oral exams at the last year of the school. At the beginning of the 19th century, the General Medical Council in England emphasized that professional competency examinations should have been implemented and this change was quickly spread in all over Europe (Humphrey et al., 2011). The search for standardization of medical education was started with the publication of "the Flexner Report'' in 1910 in the United States and then, the importance of creating a curriculum was emphasized and this influence brought on the understanding of organ-system-based training within a short time in Europe in the beginning of the 20th century (Weatherall, 2006). The first medical education unit was established at Case Western Reserve University in the same century (Al Shawwa, 2012). During the development process, the instructor-student relationship of the educational aspects of medical education was persisted; however, the idea of developing educational models not only in terms of quantity but also in terms of quality with the measurement-assessment process created for the students became stronger.

4. The relationship between curriculum and assessment methods of examinations

World Federation for Medical Education (WFME) defines the purpose of medical education as to improve the human health. Globalization, doctors' regional mobility, the increasing number of health care institutions, the dynamic nature of medical science and continuous updating of educational standards brought on the agenda of establishing standards for learning and assessment in medical education. As a result of the studies in order to make medical education more qualified and more effective in Turkey, it was planned to reorganize and standardize the medical education in accordance with "The World Federation for Medical Education'', The European Specifications for the Global Standards of Medical Education. These standards are determined as undergraduate medical education, graduate medical education and a dynamic process which includes continuous professional development. It was taken very important steps in the international arena about medical education as a result of the 'Edinburgh Declaration' which was adopted by the World Health Assembly (World Federation for Medical Education, 2003). The education programs established with this declaration should reflect national health issues; therefore they should not only include the participation of hospitals; but they should also include the active use of all healthcare facilities and the basic education method should be developed around problem-oriented solution. Life-time planning of professional education, the active learning methods such as studying with independent and small groups are among the targets determined to be used in the standardization phase of medical education. The lecturers in medical education should not be only the best experts in their fields, but also they should be educated as good instructors. Although there is large freedom in terms of measurement and assessment methods in medical schools; it has been experienced significant difficulties in terms of the implementation and development of such methods and the assessment of the new designs (Frenk et al., 2010).

The design process of a "Good'' test does not only include the assignment of tasks, but it also requires effective assessment in terms of the content and adequacy of tests, definition of the grading system, examination security, the statistical interpretation of tests, analyzing and reporting the results (Möltner et al., 2010). Most of the faculties prefer to apply multiple-choice questions in the assessment of examinations; because the orientation through more innovative question types depends on effective use of time and innovative thinking (Hochlehnert et al., 2012). Similarly, some faculties experience difficulties on the assessment procedure due to resource constraints. Automatic test correction, scoring systems, classification of exam grade, statistical analysis, document reading systems, assessment programs or the use of computer-supported applications provide savings in the use of resources (Jünger et al., 2010).

The general increase of medical care necessities in the United States also increased the number of medical students and required to adopt a model as basis which was developed by the accreditation council for graduate medical education and can provide comparative standardization. This model aims to provide knowledge, skills and attitude changes such as medical knowledge related to each other, patient care, professionalism, communication, practice-based learning and development (Batalden et al., 2002). Proficiency in medical education should be regarded as a life-long learning habit for the doctors in terms of self-assessments and determining of their learning necessities. Proficiency is also a conceptual context which reflects the relations between a person's personal skills and which is expected to complete some specific professional tasks in life (Frank et al., 2010). Contextuality often includes the local prevalence of a disease, the natural forms of symptoms, the educational levels of the doctor and the patients and the other demographic characteristics. The students will gain mental, behavioral and professional development through practice-based applications and teaching methods which will reflect to the experiences and the effective assessment mechanisms

for existing development process. Over the last decade, the faculties have shown extensive efforts in order to measure and evaluate the students' medical development timely and effectively through accurate and reliable methods; some of them are determining the skills of students and instructors through future learning targets, providing formal services for advanced training of the students creating learning and implementing targets against the public health damages might occur due to improper medical practices and using effectively the measurement and evaluation methods (Cox and Irby, 2007).

5. The formative and summative assessment

Different assessment methods are used in medical education since 1950. Until recently, the clinical skills and medical information of doctors was often assessed by written and oral examinations. Oral examinations were typically performed by students' gathering information at patient's bedside, getting patient's history and evaluating treatment options with physical examination findings and the students' performances were assessed according to their answers to the questions of the instructors (Norcini, 2005). In the past, the decisions regarding the methods of the exams were taken primarily according to validity and reliability. Validity was considered as an assessment which was based on the accuracy of the assessment points of medical proficiency (White and van den Broek, 2004). The assessment method can be formative and summative. Although it was aimed to provide professionalism, professional proficiency and responsibility by summative assessments, it was also provided that they could constitute barrier on development of education (Schuwirth and van der Vleuten, 2004). It is one of the commonly used forms of assessment in education and it determines the success level by taking decisions such as "sufficient, passed, failed, successful or unsuccessful". The midterms and final exams at the end of the terms can be done in this way. In formative assessment, it is dealt with learning difficulties and all factors which contribute to complete learning of the learners while it is determined how much they gained the targeted behaviors in learning process (Ben-David, 2000). Generally the education results are monitored by summative assessment. Cowie and Bell define summative assessment as the interactive assessment of student development and determination of student necessities and re-organization of the teaching accordingly (Cowie and Beverley, 1999). Nicol and Macfarlane-Dick was determined the role of students in formal education and emphasized the importance of dealing with the content and performance details of qualitative feedback created for students and instructors sake. According to the result of another study made by Crooks; summative assessments have less effect to education than the formative assessments (Nicol and Dick, 2006). A meta-analysis of the studies on formative assessment showed that there are important teaching gains in all content areas, knowledge and skill types and the level of education (Black and Wiliam, 1998).

6. The assessment methods in medical education, and an overview to examinations

Medical educators from all over the world have used many different oral and written methods in order to assess their students; and multiple choice and oral exams were the basis of medical education for a long time. Written exams are made on a pre-determined date and time period and they are still used in some disciplines of science. The written exam questions typically are prepared as open-ended or multiple-choice in a rich or poor content. It does not provide adequacy in student's education and may direct students to superficial learning because most times it is not given feedback after the examination. GE Miller considers that human behavior is so complex that it cannot be measured or summarized by only one score or one observation (Miller, 1990). The oral exams and multiple-choice exams which have been used for many years were created during the educational process. The authenticity of exams makes oral examination as an effective and valid method which compares the written exam success level with the performance. Although unmeasured skills in written exams are assessed in oral exams; the less content of knowledge in comparison with written exams, less feasibility of application for large groups and providing security criteria overshadow the effectiveness and qualitative characteristics of oral examinations. The fairness, being comparable and improvable of the assessment methods is important in terms of determination the proficiency of medical students. In this sense, the classic oral exams and multiple-choice examinations have left their position to the new assessment means such as clinical simulation, objectively structured clinical examinations (Objective Structured Clinical Examinations-OSCEs), clinical reasoning tests, question banks creation and student report concepts (Norcini and McKinley, 2007).

7. The clinical simulation, the use of simulated/ standardized patients, and objectively structured clinical examinations

The term of clinical simulation can be summarized as creation of co-conditions with a behavior, situation or process in order to use especially in medical education. Thanks to the use of simulated patients; the use of real patients has been reduced, the patients have been protected against incorrect applications of inexperienced students, the improperness of ambient conditions and impossibility of gaining knowledge and skills of all the students at once have been prevented (Yelland, 1998). Simulated patients were used for the first time in 1964 by Barrows and they were defined as real patients or volunteers who were educated for providing clinical case (Turan et al., 2010). Simulated patient use is very useful as it provides to overcome the reservations of students-which they might have when they examine the pelvic and reproductive system and therefore it provides them to demonstrate professional attitude. Simulated patients are widely used in measuring the clinical skills of students as a part of the OSCEs'. The use of simulated patient has important contributions because of standardizing learning objectives and educational programs, providing the proper use of time, planning difficulty levels of cases, providing high efficiency and security. Although creating proper clinical environments in educational program with simulated patient seems disadvantages in terms of the cost and the application process; the studies performed have proven that clinical simulation is a valid, reliable, cost-effective method in medical education (Norcini and Boulet, 2003).

OSCEs are modern type of examinations which are commonly used in the health sciences. Harden defines OSCE as a method whose clinical proficiency components are well-planned and it is carefully implemented in terms of structure (Hodges, 2003). It is an examination which is designed to test the adequacy of many clinical skills performances such communication, clinical examination, medical procedures, prescriptions, joint mobilization and evaluation of radiological imaging results; and it is a kind of examination which assesses the knowledge, skills and behaviors with objective criteria (Harden, 1988). Students are assessed in a large number of stations. The parts of communication, physical examination, medical intervention and laboratory are applied in processing stations; and the clinical reasoning step is applied in question station. Using simulated patients, the clinical knowledge and skills of students are assessed at the each station within 3-30 minutes. Among the negativities of OSCE's which have highly efficient structure in order to develop knowledge, skills and attitude in comparison with classical test methods are negative cost-effectiveness, lack of confidence due to shortening of the time allocated for the students, lack of diversity of clinical scenarios are counted, some studies argue the incompetency of the OSCE's for assessing attitudes and behaviors according to the learners' cognitive evaluations (Özdemir, 2005). The reliability of a test is a measure of the repeatability and accuracy of that test. OSCE's are generally considered as a reliable form of assessment. There are many features which contribute to the reliability. The assessor consistency is very high. As a result, especially multiple cases are some of the important features which contribute to enough test time OSCE's reliability (Swanson, 1987). The empirical results of a systematic evaluation which will be made in literature show that the reliable assessment of communication skills is more effective with clinic skills among stations. Use of more stations in examinations shows that assigning two observers in the examination instead of one has tendency of showing higher reliability (Brannick et al., 2011).

8. Conclusion

In this sense, revising the issues such as clinical environment, number of lecturers, variety of clinical scenarios, the standardization of assessment time would be very efficient in terms of the medical education.

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Review

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What is new in hypertension guidelines?

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ABSTRACT

In recent years, trials were published and a series of new evidences were presented in relation to hypertension (HT), requiring changes in guidelines. In the light of these studies, the European Society of Hypertension (ESH)/European Society of Cardiology (ESC) HT guideline which was published in 2007 and reviewed in 2009 was revised. The new ESH/ESC HT 2013 guideline announced at the European Hypertension Congress in Milan was published in both Journal of Hypertension and European Heart Journal, and put at the disposal of doctors. In December 2013, the eight Joint National Committee (JNC 8) HT treatment guideline which had been expected for a long time and published online on the Journal of the American Medical Association was issued. Unlike the previous JNC guidelines and ESH/ESC HT guideline, it was a brief guideline containing only recommendations and explaining the grounds of the recommendations. A day before the publication of JNC 8 hypertension treatment guideline, American Society of Hypertension/International Society of Hypertension (ASH/ISH) HT treatment guideline was published. This guideline was also kept short like JNC 8, but contained more details. In this review, we summarized and compared the changes made in the new guidelines.

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1. Introduction

Significant changes were made in the European Society of Hypertension (ESH)/European Society of Cardiology (ESC) Hypertension (HT) 2013 guideline (Mancia et al., 2013). For the first time in the guideline, all the data were rated according to ü other guidelines. Unlike the previous Joint National Committee (JNC) guidelines and ESH/ESC HT guideline, JNC 8 (James et al., 2013) is a brief guideline containing only recommendations and explaining the grounds of the recommendations. American Society of Hypertension/ International Society of Hypertension (ASH/ISH) guideline (Weber et al., 2014) was also kept short like JNC 8, but contained more details.

European Society of Hypertension (ESH)/European Society of Cardiology (ESC) hypertension guideline

The previous ESH/ESC HT classification was not changed (Mancia et al., 2007; Mancia et al., 2013). Blood pressure values were classified as optimum (systolic blood pressure=(SBP)<120 mmHg and diastolic blood pressure=(DBP)<80 mmHg), normal (SBP 120-129 mmHg and/or DBP 80-84 mmHg), high normal (SBP 130-139 mmHg and/or DBP 85-89 mmHg), grade 1 hypertension (SBP 140-159 mmHg and/or DBP 90-99 mmHg), grade 2 hypertension (SBP 160-179 mmHg and/or DBP 100-109 mmHg), grade 3 hypertension (SBP≥180 mmHg and/or DBP≥110 mmHg) and isolated systolic hypertension (SBP≥140 mmHg and DBP<90 mmHg) (Mancia et al., 2013).

Using different types of measurements, minor changes were made in blood pressure (BP) threshold values for the definition of HT; the limits in the previous guidelines were further clarified. Threshold values were defined as SBP≥140 mmHg and/or DBP≥90 mmHg for office BP, SBP≥135 mmHg and/or DBP≥85 mmHg for ambulatory daytime BP, SBP≥120 mmHg and/or DBP≥70 mmHg for ambulatory nighttime BP, SBP≥130 mmHg and/or DBP≥80 mmHg for ambulatory 24

hour BP, and SBP≥135 mmHg and/or DBP≥85 mmHg for home BP (Mancia et al., 2013).

Emphasis was placed on the importance of diagnosing HT and it was recommended that HT diagnosis should be verified with two measurements each time in a patient who was examined no less than twice. It was emphasized that office BP measurement was the gold standard for the screening, diagnosis and treatment of hypertension. Cut-off values for out-of-office BP (ambulatory and home) measurement were 130/80 mmHg, 135/85 mmHg, and 120/70 mmHg for 24 hour ambulatory BP, home and ambulatory daytime BP, and ambulatory nighttime BP, respectively. It was stressed that, for out-of-office BP measurement, major indications were suspicion of white-coat HT, masked or nocturnal HT and hypotensive episodes, and resistance to drug therapy (Mancia et al., 2013).

Starting from 2003 ESH/ESC guideline (Guidelines Committee, 2003), the guidelines pointed out that diagnosis and treatment of HT should be accompanied by measurement of total cardiovascular risk. In other words, diagnosis and treatment should rely on total cardiovascular risk assessment in addition to appropriate BP measurement. Total cardiovascular risk is stratified into four risk categories (low, moderate, high, very high) according to presence or absence of risk factors such as smoking and dyslipidemia; presence or absence of asymptomatic organ damage; diabetes; the stage of chronic kidney disease and symptomatic cardiovascular disease. Some changes were made in the cardiovascular risk chart according to hypertension and risk factor/target organ damage categories. In the new guideline (Mancia et al., 2013), presence of three or more risk factors in the absence of target organ damage was defined as a separate category. Thus, the number of risk factor target organ damage categories were raised from four to five. No risk was assigned any longer to the normal blood pressure category among the blood pressure categories. Therefore, the number of columns was reduced by one (Table 1).

In all hypertensive patients, the target BP level is <140/90 mmHg including low, moderate and high risk groups. In line with the changes to total cardiovascular risk classification, treatment recommendations for the normal blood pressure group were removed. No drug treatment other than a change of lifestyle was recommended for the patients in the high normal class (130-139 mmHg systolic, 85-90 mmHg diastolic) irrespective of the risk factor. For the high or very high risk class, it was recommended that antihypertensive drugs be initiated immediately together with lifestyle measures. For the low and medium risk, it was recommended that antihypertensive therapy be initiated if BP proves to be above 140/90 mmHg after lifestyle changes for a few months or weeks. Drug treatment was not recommended for young patients with high normal BP and isolated systolic hypertension (Mancia et al., 2013).

In ESC/ESH 2013 guideline (Mancia et al., 2013), BP targets were <140 mmHg, save a few exceptions. For elderly hypertensive patients under 80 years of age, HT treatment initiation threshold was ≥160 mm Hg and target systolic blood pressure was 140-150 mmHg. It was emphasized that target SBP value for the elderly patients aged above eighty was 140-150 mm Hg. It was recommended that SBP <140 mmHg could be targeted in the fit elderly under eighty years of age. DBP target was recommended as <90 mmHg except for diabetic patients. DBP target for diabetics was expressed as <85 mmHg.

Special attention is accorded to lifestyle changes in treatment, and salt reduction, regular appropriate exercise, weight control, reduction of alcohol intake, giving up smoking, and diet are recommended. Where salt reduction is concerned, the previous guideline (Mancia et al., 2007) recommends <5 grams, while the new guideline (Mancia et al., 2013) recommends between 5-6 grams.

It was stated that the real benefit of hypertensive treatment was due to the fall in BP and therefore all the drugs in the five main classes [thiazide-type diuretics, calcium channel blockers (CCB), angiotensin-converting-enzyme-inhibitors (ACEI), angiotensin receptor blockers (ARB) and beta blockers] could be used as a mono-therapy or a combination therapy in any sequence when initiating the treatment (Mancia et al., 2013). Appropriate combinations of these drugs other than ACEI + ARB combination and beta blockers are recommended. Combination therapy was recommended to be initiated immediately in high risk patients and Grade

Table 1. Comparison of total cardiovascular risk in different hypertension guidelines

Other risk factors, asymptomatic organ damage or disease		Blood pressure									
		Normal		High normal		Grade 1 HT		Grade 2 HT		Grade 3 HT	
ESH/ESC 2007 HT guidelines	ESH/ESC 2013 HT guidelines	2007	2013	2007	2013	2007	2013	2007	2013	2007	2013
No other RF	No other RF	Average risk		Average risk		Low added risk	Low risk	Moderate added risk	Moderate risk	High added risk	High risk
1-2 RF	1-2 RF	Low added risk		Low added risk	Low risk	Moderate added risk	Moderate risk	Moderate added risk	Moderate to high risk	Very high added risk	High risk
≥3 RF, MS, OD or Diabetes	≥3 RF	Moderate added risk		High added risk	Low to moderate risk	High added risk	Moderate to high risk	High added risk	High risk	Very high added risk	High risk
Established CVOD, CKD stage 3 or renal disease	diabetes	Very high added risk		Very high added risk	Moderate to high risk	Very high added risk	High risk	Very high added risk	High risk	Very high added risk	High to very high risk
	Symptomatic CVD, CKD stage ≥4 or diabetes with OD/RFs				Very high risk		Very high risk		Very high risk		Very high risk

CKD: Chronic kidney disease; **CVD**: Cardiovascular disease; **OD:** Organ damage; **RF**: Risk factor; **MS**: Metabolic syndrome; **HT**: Hypertension; Adapted from European Society of Hypertension (ESH)/ European Society of Cardiology (ESC) hypertension 2013 and ESH/ESC hypertension 2007 guidelines.

2 and 3 patients. It was reported that combination therapy induced faster response, less side effects, better tolerance, higher physiological and pharmacological synergy, higher possibility to reach the target blood pressure, and higher patient adherence, and if applicable, administration of a fixed dose with a single tablet was recommended. In addition, the therapy was recommended to be initiated with an optimal dose, i.e. full dose (Mancia et al., 2013).

A minor change was made in the section dealing with antihypertensive therapies, which should be preferred in special cases. While only calcium antagonist was recommended for the peripheral arterial disease in the previous guideline (Mancia et al., 2007). ACEI was added to calcium antagonist in the new guideline (Mancia et al., 2013). Aortic aneurysm was also included as a manifestation, wherefore the use of a beta blocker was proposed (Mancia et al., 2013). In the previous guideline (Mancia et al., 2007) atrial fibrillation classified recurrent and permanent categories, and an ARB or ACEI was recommended for the former, and a beta blocker or a non-dihydropridin calcium antagonist was recommended for the latter. In the new guideline atrial fibrillation treatment was split into prevention and ventricular ratecontrol categories; an ARB, ACEI, beta-blocker or mineralocorticoid receptor antagonist was recommended for the former, whereas a beta blocker or a non-dihydropridin calcium antagonist was recommended for the latter. While the previous guideline recommended ACEI, ARB, and loop diuretic for end-stage kidney disease/proteinuria, the new guideline removed loop diuretic (Mancia et al., 2007; Mancia et al., 2013).

In ESC/ESH 2013 guideline (Mancia et al., 2013) BP target for diabetics was <140/85 mmHg. In the presence of microalbuminuria and proteinuria, renin-angiotensin system (RAS) blockers should be preferred as they are more effective in reducing proteinuria than other antihypertensive agents, but dual blockage of RAS should be avoided (Mancia et al., 2013). In the presence of nephropathy, initiation of the drug was recommended if SBP≥140 mmHg. Target SBP is <140 mmHg. While the target was 120/80 mmHg for overt proteinuria in the previous guideline, SBP<130 mmHg was recommended in the new guideline. Aldosterone receptor antagonist was not recommended in chronic kidney disease especially in combination with a RAS blocker because of the risk of excessive reduction in renal function and of hyperkalemia (Mancia et al., 2013).

In ESC/ESH 2013 guideline (Mancia et al., 2013), resistant HT was defined as HT that cannot be controlled with a treatment strategy that includes appropriate lifestyle measures plus minimum three drugs, including a diuretic. In resistant individuals, it was recommended to include mineralocorticoid receptor antagonist, amiloride and/or alpha 1 blocker doxazosin in the treatment strategy. If drug therapy fails, invasive procedures such as renal denervation (denervation of renal sympathetic nerves with radio frequency ablation) or baroreceptor stimulation (a baroreceptor activation device comprised of an implantable pulse generator that can activate the carotid sinus with electric signals) were recommended. However, it was stated that further procedures were required to be performed at experienced centers since these procedures were yet at investigation stage and results were insufficient (Mancia et al., 2013).

There were minor changes in the recommendation of drug therapy in pregnancy. In the previous guideline (Mancia et al., 2007), the BP levels for the initiation of drug treatment were SBP ≥150 mmHg or DBP ≥95 mmHg for pregnants and >140/90 mmHg for gestational HT (with or without proteinuria). SBP≥170 mmHg or DBP≥110 mmHg were regarded as emergency cases that require urgent hospitalization (Mancia et al., 2007). In the new guideline class I drug treatment for SBP>160 mmHg or DBP>110 mmHg was recommended. For SBP≥150 mmHg or DBP ≥95 mmHg, it was stated that drug treatment (Class IIb) may be considered. Similar to the previous guideline, the threshold value for drug treatment was 140/90 mmHg for women with gestational HT (with or without proteinuria), preexisting hypertension with the superimposition of gestational hypertension or hypertension with asymptomatic organ damage or symptoms at any time during pregnancy. Metildopa, labetalol and nifedipine are the recommended antihypertensive agents. Intravenous labetolol and nitroprusside infusion are recommended as preferable antihypertensive agents in emergency cases (preeclampsia) (Mancia et al., 2013).

While BP target in the previous guideline was 130/80 mmHg for hypertensive patients with a history of stroke and transient ischemic attack, the target was lowered below 140 mmHg in the new guideline. No treatment strategy to lower BP is recommended in the first week after acute stroke. Similarly, in coronary heart disease, antihypertensive treatment was recommended to be initiated for SBP>140 mmHg, and target SBP was assigned as <140 mmHg (Mancia et al., 2007; Mancia et al., 2013).

Joint National Committee (JNC) 8 and American Society of Hypertension/International Society of Hypertension (ASH/ISH) hypertension guidelines

Unlike the previous JNC guidelines and ESH/ESC HT guideline, JNC 8 (James et al., 2013) is a brief guideline containing only recommendations and explaining the grounds of the recommendations. JNC 7 (Chobanian et al., 2003) published in 2003 was about 47 pages whereas JNC 8 is a guideline of 14 pages. The authors presented 9 recommendations substantiated by strong evidences. Randomized controlled trials (RCTs) were taken as the gold standard for the evidences. JNC 7 defined prehypertension, whereas the new guideline defined threshold values for pharmacological treatment (Chobanian et al., 2003; James et al., 2013) JNC 7 addressed several issues such as blood pressure methods, secondary hypertension, resistant hypertension, and HT in a special population based on expert opinions and literature reviews (Chobanian et al., 2003). The new guideline assessed a limited number of issues because RCTs were given higher priority (James et al., 2013).

Recommendation 1 (James et al., 2013): In the general population aged < 60 years, target BP should be below 150/90 mmHg. Pharmacologic treatment should be initiated in BP ≥150/90 (Strong Recommendation, Grade A). In this age group, there is no need to change treatment if the treatment is well tolerated and there are no side effects even if SBP falls below the target values (for example <140 mmHg) (Expert Opinion-Grade E).

Recommendation 2 (James et al., 2013): In the general population aged <60 years, the target should be DBP <90 mmHg and treatment should be initiated above this value

(For ages 30-59, Strong Recommendation Grade A; For ages 18-29, Expert Opinion-Grade E). This recommendation depends on 5 DBP trials conducted on hypertensive patients between 30-69 years of age (Effects of treatment on morbidity in hypertension, II: Results in patients with diastolic blood pressure averaging 90 through 114 mmHg, 1970; Hypertension-Stroke Cooperative Study Group, 1974; Hypertension Detection and Follow-up Program Cooperative Group, 1979; Report by the Management Committee, 1980; Hypertension Detection and Follow-up Program Cooperative Group, 1982; Medical Research Council Working Party, 1985). The reason why the recommendation remains as an expert opinion for individuals <30 years is lack of sufficient RCT on this issue.

Recommendation 3 (James et al., 2013): In patients aged <60 years, the threshold to initiate treatment is SBP≥140 mmHg. Target SBP<140 mm Hg (Expert Opinion-Grade E)

Recommendation 4 (James et al., 2013): In patients aged >18 years with chronic kidney disease (CKD), treatment should be initiated at SBP≥140 or DBP≥90 mmHg. The treatment target is <140/90 mmHg (Expert Opinion-Grade E)

Recommendation 5 (James et al., 2013): In patients aged ≥18 years with diabetes, treatment should start at SBP ≥140 mmHg or DBP ≥90 mmHg. The treatment target is <140/90 mmHg (Expert Opinion-Grade E).

In JNC 7, the target BP was <130/80 mmHg for patients with diabetes or chronic kidney disease (Chobanian et al., 2003). ACCORD trial was a study that investigated the effect of intensive BP control on cardiovascular events in diabetic patients with high cardiovascular risk (Cushman et al., 2010). According to the results of ACCORD-BP trial, no difference was identified in diabetic patients in terms of primary end-points (cardiovascular death, non-fatal stroke, myocardial infarction) between a strategy that targeted a SBP of <120 mmHg and a strategy that targeted a SBP<140 mmHg (Cushman et al., 2010). The panel made this recommendation as a result of these data.

Recommendation 6 (James et al., 2013): In the general non-black population, including those with diabetes, initial hypertensive treatment should include a thiazide-type diuretic, CCB, ACEI or ARB (Moderate Recommendation-Grade B). Since higher primary endpoints (cardiovascular mortality, myocardial infarction and stroke) were seen in beta-blockers in a randomized controlled trial (LIFE) making a comparison with ARB, beta blockers were not recommended as the choice of initial treatment.

In ALLHAT trial (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial Colaborative Research Group, 2003), alpha blockers were not recommended as the choice of initial treatment as they exhibited worse cardiovascular outcome compared with diuretic treatment.

Due to lack of sufficient RCTs of appropriate quality, the use of carvedilol, nebivolol, clonidine, hydralazine, spironolactone, reserpine and furosemide were not recommended as an initial treatment. Due to lack of sufficient RCTs of appropriate quality, dual $\alpha_1\beta$-blocker agents (carvedilol), vasodilator β-blockers (nebivolol), central α_2–adrenergic agonists (clonidine), direct vasodilators (hydralazine), aldosteron receptor antagonists (spironolactone), peripheral-acting adrenergic antagonists (reserpine) and loop diuretics (e.g. furosemide) were not recommended as first-line treatment (James et al., 2013).

Recommendation 7 (James et al., 2013): In the general black population, including those with diabetes, initial antihypertensive treatment should include thiazide-type diuretic or CCB. (For general black population: Moderate Recommendation-Grade B; for black patients with diabetes: Weak Recommendation-Grade C).

Recommendation 8 (James et al., 2013): In the population aged ≥18 with chronic kidney disease (CKD), initial or add-on antihypertensive treatment should include an ACEI or ARB to improve kidney outcomes (Moderate Recommendation-Grade B). This recommendation applies to all chronic kidney patients, independently of race and diabetic condition.

Recommendation 9 (James et al., 2013): The primary purpose of HT treatment is to reach and maintain target blood pressure. If BP treatment target has not been reached in the first month, the dose of the initial drug should be increased or a second drug added. The add-on drug should be one of the drugs listed in Recommendation 6 (Thiazide, CCB, ACEI or ARB). If target blood pressure cannot be reached with two drugs after a reasonable period of time, a third drug should be considered. ACEI and ARB should not be used together. If target blood pressure cannot be reached using the drugs in recommendation 6 because of a contraindication or the need to use more than three drugs, antihypertensive drugs from other classes can be used. Referring complicated patients with whom the target blood pressure cannot be reached using the above strategy and who require additional clinical consultation to a hypertension specialist may be indicated (Expert Opinion-Grade E).

When the results of ALLHAT trial (Antihypertensiveand Lipid-Lowering Treatment to Prevent Heart Attack Trial Collaborative Research Group, 2003) showed that thiazide diuretics were effective drugs in BP control as a first-line and combination treatment, JNC 7 guideline recommended the use of diuretics in the first-line therapy (Chobanian et al., 2003). Although five drug classes were suitable for initial treatment in JNC 7, thiazide diuretics were recommended as initial treatment unless there were indications requiring the use of another class (Chobanian et al., 2003). JNC 8 stated that any one of thiazide diuretic, CCB, ACEI or ARB could be used as initial treatment. Beta blocker was not included in the drugs of first choice unlike ESH/ESC and JNC 7. In diabetic patients and patients with CKD, treatment initiation threshold and the target blood pressure to be reached were revised as 140/90 mmHg. In JNC 7 guideline (Chobanian et al., 2003), the recommended level was 130/80 mmHg for these patients. First of all, treatment initiation threshold was revised as ≥150/90 mmHg and target blood pressure as <150/90 mmHg for patients aged 60 and above (James et al., 2013). These figures were ≥140/90 mmHg and <140/90 mmHg, respectively, in JNC 7 (Chobanian et al., 2003).

In JNC 8, instead of the word “elderly”, a clear statement such as ≥60 years was included. However, 150 mmHg was accepted instead of 160 mm Hg in ESH/ESC (James et al., 2013; Mancia et al., 2013). While ESC/ESH retained beta blockers among the primary drug group to be preferred first, JNC 8 excluded beta blockers (James et al., 2013; Mancia et al., 2013). ESC/ESH revised target blood pressure to 140/85 mmHg in patients with diabetes and patients with kidney dysfunction, and recommended a systolic blood pressure of

<130 mmHg for overt proteinuria. JNC 8 suggested 140/90 mmHg as a target for patients with diabetes and CKD. It did not make a special emphasis on overt proteinuria. Both guidelines recommended to avoid combined use of ACEIs ve ARBs (James et al., 2013; Mancia et al., 2013).

Some confusing differences are noted in JNC 8 (James et al., 2013) and American Society of Hypertension/International Society of Hypertension (ASH/ISH) (Weber et al., 2014). While the same target BP of <140/90 is recommended for DM and CKD patients, there are differences in other aspects (James et al., 2013; Weber et al., 2014). The initial treatment threshold value, i.e. 150/90 mmHg which applies to patients aged 60 and above in JNC 8 applies to patients aged 80 and above in ASH/ISH guideline (James et al., 2013; Weber et al., 2014). In ASH/ISH guideline, ≥140/90 mmHg was recommended for the diagnosis of HT in adult patients under 80 years of age. ASH/ISH guideline recommends different drugs than those recommended by JNC 8 as initial treatment subject to race, age and blood pressure level of patients. While JNC 8 states an ACEI, ARB, CCB or thiazide diuretic as the initial choice for the white race, ASH/ISH guideline recommends an ACEI or ARB for the white race under 60 years of age and a CCB or thiazide for the white race aged 60 and above. Similar to JNC 8, CCB or thiazide is recommended for the black population. (James et al., 2013; Weber et al., 2014). In HT classification, the definitions of prehypertension (SBP 120-139 and/or DBP 80-89 mmHg), stage 1 (SBP 140-159 mmHg and/or DBP 90-99 mmHg) and stage 2 (SBP≥160 mmHg and/or DBP≥100) which were given in JNC 7 were preserved (Chobanian et al., 2007; James et al., 2013; Weber et al., 2014).

Table 2. Hypertension guidelines comparisons of goal blood pressure and initial drug therapy

Guideline	Population	Goal BP,mmHg	Initial drug treatment options
JNC 8	aged≥60	<150/90	
	aged<60	<140/90	
	Diabetes	<140/90	
	CKD	<140/90	
ESH/ESC 2013	nonelderly	<140/90	ACEI, ARB, β-Blocker,diuretic or CCB
	elderly aged<80y	<150/90	
	aged ≥80 y	<150/90	
	Diabetes	<140/85	ACEI or ARB
	CKD no proteinuria	<140/90	ACEI or ARB
	CKD+proteinuria	<130/90	
ASH/ISH	aged ≥80	<150/90	White and other non-black patients: Aged <60 ACEI or ARB, White and other non-black patients: Aged >60 years CCB or thiazide-type diüretic, black patients all ages CCB or thiazide-type diüretic
	aged<80	<140/90	
	Diabetes	<140/90	ARB or ACE inhibitor Note: in black patients, it is acceptable to start with CCB or thiazide
	CKD	<140/90	ARB or ACE inhibitor Note: in black patients, good evidence for renal protective effects of ACE inhibitors

*__ESH:__ European Society of Hypertension; **ESC**: European Society of Cardiology; **JNC:** Joint National Committee; **ASH**: American Society of Hypertension; **ISH**: International Society of Hypertension; **CKD**: Chronic kıdney disease; **ACE**: Angiotensin-converting enzyme; **ARB**: Angiotensin receptor blocker; **CCB**: Calcium channel blocker.

2. Conclusion

In this review, the changes in new hypertension guidelines were summarized and compared. Significant differences between the guidelines with respect to blood pressure values that may be used to define hypertension and that may be targeted are summarized in Table 2.

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Nutritional habits and obesity: Primary school students in Sinop, the northernmost point of Turkey

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ABSTRACT

The conditions in which energy intake is more than energy consumption and the clinical conditions which arise with the increase in fat tissue is generally called obesity. The World Health Organization (WHO) defines obesity as the abnormal or excessive accumulation of fat in the body such that it endangers health. A combination of environmental and genetic factors rather than an organic illness underlie most cases of pediatric obesity. The aim of this research is to examine the nutritional habits and obesity conditions of primary school students. This descriptive study was carried out on all students in primary schools affiliated to the Sinop Provincial Directorate of National Education, from May 10th, 2010 to May 10th, 2011, after gathering all necessary permissions. The research comprises 3352 students who agreed to take part in the research; there was no sub-selection within the sample. After examining the percentile assessment of the participating children, the following categorization has been made: 7% (under five) underweight, 78.4% (between 5-85) normal, 8.7% (between 85-95) overweight and 6% (95 and over) obese. Examining the children's BMI according to gender found that 9.6% of boys and 7.6% of girls were overweight, and 7.6% of the boys and 4.3% of the girls were obese, a higher rate of excessive weight in boys than in girls. As a result, it is suggested that nurses should continue an effective counseling service to enable children's sufficient and balanced nutrition, that they cooperate with the families while carrying out this service, and consider social factors that increase tendencies to obesity. It is also suggested that further research should be done on the relationship of parents and children.

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1. Introduction

Definition, diagnosis and etiology

The conditions in which energy intake is more than energy consumption and the clinical condition, which arises with the resultant increase in fat tissue, is generally called obesity. The World Health Organization (WHO) defines obesity as the abnormal or excessive accumulation of fat in the body such that it endangers health (Ergül and Kalkım, 2011). A combination of environmental and genetic factors, rather than organic illnesses, usually underlies most cases of pediatric obesity (Rosenthal and Gitelman, 2003). Some studies show the hereditary factors in the etiology of obesity. For example, children with an obese mother show an increase in obesity risk of 40%, while an obese father increases a child's risk to 80%. As well, children with obese parents have a lower metabolisc rate (Günöz, 2002). On the other hand, because it is impossible to explain the worldwide excessive increase of childhood obesity by hereditary means alone, the Public Health Institution of Turkey considers environmental factors to have a prominent role in this phenomenon (PHIT, 2012a).

Risks

Obesity is a very important risk factor for coronary heart disease, hypertension, paralysis, type 2 diabetes, cancers of the uterus, breast, prostate, and colon, osteoarthritis, varicosis, sleep apnea syndrome, birth difficulties, ovarian cysts, and

depression. Today, obesity has been accepted as a disease, which needs treatment (Tiryakioğlu, 2012).

With the increase in the frequency of the obesity during childhood period and in the length of time people remain obese, morbidity and mortality rates will inevitably rise. Examining research on the matter shows that 40% of those who are obese in their childhood, and 75-80% of those who are obese during puberty are also obese in adulthood. Meanwhile, the longer the time one spends obese, overall lifespan and quality of life will decrease (Aycan, 2012).

Extent of obesity in Turkey and in the world

Obesity has become an important public health problem as it continually increases in developed and developing countries. The MONICA study, which WHO carried out in six different regions of Asia, Africa and Europe over 12 years, discovered a 10-30% increase in the prevalence of obesity (PHIT, 2012b). In the United States, the most recent national research comparing BMI with older data shows a 50-60% increase in childhood and adolescent obesity over a single generation (Schwarz, 2012). Spain and Portugal have the highest prevalence of overweight school children across both genders: 35% of 6-9-year-old Spaniards, 32% of 7-9-year-old Portuguese. The countries with the lowest rates are Slovakia at 15% of 7-9-year-olds, France at 18% of 7-9-year-olds, and Switzerland and Iceland each with an obesity rate 18% of 6-9-year-olds (Ergül and Kalkım, 2011). Obesity frequency in both children and adults has rapidly increased particularly in the last 20 years, and the trend is estimated to continue into the 21st century. In the USA, 430,000 obesity-related deaths are reported each year, although after excluding other reasons, the number of deaths caused only by obesity is 120,000. It is estimated that in the 21st century, the expected lifespan of obese American children will grow shorter than that of their parents (Aycan, 2012).

The Turkish Obesity Research Association (TOAD) studied 1821 children of the 12-15 age group in Istanbul's Şişli district. The study found that the percentage of children with a BMI of 18-25 kg/m^2 (overweight) is 9.9%, while 6.2% have a BMI of greater than 30 kg/m^2 (obese) (PHIT, 2012c). In Kayseri, a study of 1032 children from ages 6-10 and 2671 children from ages 11-17 age group, a total of 3703 children from 6-17 age group, found that 10.6% of the children are overweight and 1.6% of the children obese (PHIT, 2012c). Research carried out in Istanbul, Ankara and Izmir on 1044 adolescents from ages 12-13, found that 12% of the children are underweight, another 12% are overweight and 2% are obese. Another study in those three major cities on 1014 adolescents from 12-13, found a total obesity prevalence of 15.1% for boys and 13.3% for girls. A Yeditepe University nutrition study on 1669 wealthy children from 20 private kindergartens, primary schools, and high schools found that one child out of six is on the verge of obesity, the obesity rate for girls is 16.7% and 25% for boys, while 34.4% of boys from age 10-12 is under great threat of obesity. Away from these major cities, a study in Muğla of 4260 children from 6-15 years found that 7.6% of girls and 9.1% of boys are obese (PHIT, 2012c).

The aim of this research is to examine the nutritional habits and obesity conditions of primary school students.

2. Method

This descriptive research was carried out on all primary school students affiliated to the Sinop Provincial Directorate of National Education, between May 10th 2010 and May 10th 2011 after all the necessary permits had been taken. 3352 students took part in the research, and there was no further selection within the sample

Research data was obtained selection has been carried out through an information form with 19 questions regarding the students' nutritional habits. The form also includes questions about age, gender, height and weight, whether they had breakfast regularly, their frequency of eating fast food, snacking on junk food between meals, and what foods are consumed most often. These factors are thought to affect body mass index.

After explaining what was to be done, the questionnaires are given, and the participants filled them at the same time.

The BMI evaluation was done according to the following criteria: a score under five is underweight, between 5-85 is normal, between 85-95 is overweight, 95 and over is obese (Arı and Süzer, 2008; Neyzi et al., 2008; Güler et al., 2009). The chi square test and Anova test in SPSS for Windows 13.0 were used to calculate and evaluate data.

Regarding ethics. Participation was secured only after taking necessary verbal and written permissions according to the voluntary participation principle. Students gave verbal permission, while written permission was secured from the Directorate of Education.

3. Results

The gender breakdown of the study's participants was 52.4% male and 47.6% female; 58.3% are ages 7-11, and 41.7% are ages 12-16 (Table 1).

Table 1. Distribution of the student groups according to the age and gender

	Categories	n	%	Total
Age	Age 7-11	1954	58.3	3352
	Age 12-16	1398	41.7	
Gender	Girl	1597	47.6	3352
	Boy	1755	52.4	

Examining the education levels of the mothers of participating children 8.5% of mothers for the underweight group of students had graduated from primary school only, 81.6% of mothers for students of normal weight had graduated secondary school, while the maternal education levels of overweight and obese students were 11.3% and 11.4% respectively. Only 8.6% of the children participating in the study consume fast foods such as hamburger and pizza very often, while 91.4% do not consume this type of food very often (Table 2).

Correlating the children's frequency of meal times with their body mass index, 67.8% of obese and 78.2% of overweight students eat a full meal three times each day (Table 3). However, a statistically reasonable correlation ($p>0.05$) could not be found between BMI and the times of day that children ate (Table 4).

When children are sad, excited or angry, 27.4% of the children reported feeling a need to eat, while 72.6% of them did not.

Regarding nutritional habits, results show that 94.4% of participating children have breakfast, while 5.6% do not. Re-

Table 2. Distribution of the student groups according to the education level of the mother

	Categories									
	Underweight		Normal		Overweight		Obese		Total	
Education of the mother	Number	%	Number	%	Number	%	Number	%	Number	%
Illiterate	6	6.8	68	77.3	4	4.5	10	11.4	88	100
Literate	22	6.5	245	72.7	38	11.3	32	9.5	337	100
Primary school	117	8.5	1070	77.6	112	8.1	79	5.7	1378	100
Secondary school	49	5.4	734	81.6	76	8.4	41	4.6	900	100
University	39	6.0	510	78.6	60	9.2	40	6.2	649	100

Table 3. Distribution of the student groups according to the number of meals a day

Categories	2 meals		3 meals		4 or more meals		Total	
	n	%	n	%	n	%	n	%
Underweight	13	5.5	181	77.6	39	16.9	233	100
Normal	193	7.4	2066	78.6	368	14.0	2627	100
Overweight	27	9.3	227	78.2	36	12.5	290	100
Obese	29	14.4	137	67.8	36	17.8	202	100

Table 4. The relationship between the number of meals and BMI

Categories	BMI			
	n	Mean	Std. Deviation	Statistics
The number of meals				
2 times	262	1850.90	410.85	f=1.593 P=0.204
3 times	2611	1821.67	321.27	
4 or more	479	1804.55	381.12	
Total	3352	1821.51	338.18	

*f: One Way ANOVA test (p>0.05).

Table 5. Distribution of the nutritional behaviors of the students

Categories	n	%
Those with the habit of having breakfast	3165	94.4
Those with no habit of having breakfast	187	5.6
Total	**3352**	**100**
Those having lunch at school	232	6.9
Those having lunch at home	2949	88.0
Those having lunch at other places	171	5.1
Total	**3352**	**100**
Those having dinner at a diner/restaurant	42	1.3
Those having dinner at home	3262	97.3
Those having dinner at other places	48	1.4
Total	**3352**	**100**
Those with the habit of drinking acid drinks during the meal	1256	37.5
Those with no habit of drinking acid drinks during the meal	2096	62.5
Total	**3352**	**100**
Those who consume foods such as hamburger, pizza, very often	288	8.6
Those who do not consume foods such as hamburger, pizza, very often	3064	91.4
Total	**3352**	**100**
Those who are insisted by their families to finish their plate	2855	85.2
Those who are not insisted by their families to finish their plate	497	14.8
Total	**3352**	**100**
Those who feel a need to eat under great sadness, anger or excitement	920	27.4
Those who do not feel a need to eat under great sadness, anger or excitement	2432	72.6
Total	**3352**	**100**

garding where children eat lunch and dinner, 6.9% have their lunch at school, 88% of them at home, and 5.1% of them in other places. As for dinner, 1.3% of the children have dinner at a restaurant, 97.3% of them at home, and 1.4% somewhere else. The study found that 37.5% of the children drink soda with their meals and 62.5% of them abstain from acidic carbonated beverages. Only 8.6% of the children participating in the study consume fast foods such as hamburger and pizza very often, while 91.4% do not consume this type of food very often (Table 5).

When children who participated in the research is examined according to junk-food eating between meals; 17.2% of them eats junk-food and 82.8% doesn't. It's seen vegetable dishes are most liked meals by 32.6% and most eaten meals by 44.7% (Table 6).

Besides, when junk-food eating habits of the children between meals is examined, it's seen all groups answer "No" (Table 7).

According to the information intended for how these children relieve their hunger in school; God decides the situation of donut-pastry type is seen at highest level in all groups as 68.2% in underweight group, 66.2% in the normal group, 59.7% in overweight group and 51.5% in the obese group (Table 8).

The gender breakdown of the children's BMI shows a

Table 6. Distribution of the students according to nutritional habits and choices

Categories	n	%
Those who prefer to eat bagel-pastry when hungry at school	2175	64.9
Those who prefer to eat biscuit when hungry at school	296	8.8
Those who prefer to eat the food in their lunch box when hungry at school	881	26.3
Total	**3352**	**100**
Those who eat 2 meals a day	262	7.8
Those who eat 3 meals a day	2611	77.9
Those who eat 4 or more meals a day	479	14.3
Total	**3352**	**100**
Those who eat junk food between meals	577	17.2
Those who do not eat junk food between meals	2775	82.8
Total	**3352**	**100**
Those who prefer to eat at the table	3110	92.8
Those who prefer to eat in front of the television	242	7.2
Total	**3352**	**100**
Those whose favorite foods are cookie and pastry	518	15.5
Those whose favorite foods are milk, yogurt and ayran	809	24.1
Those whose favorite foods are chips, cola and chocolate	485	14.5
Those whose favorite foods are vegetable dishes	1093	32.6
Those whose favorite foods are other than those	447	13.3
Total	**3352**	**100**
Those whos consume most cookie and pastry	231	6.9
Those whose consume most milk, yogurt and ayran	760	22.7
Those who consume most chips, cola and chocolate	306	9.1
Those who consume most vegetable dishes	1499	44.7
Those whose consume most are other than those	556	16.6
Total	**3352**	**100**

higher rate of obesity and being overweight in males than females; 9.6% of males and 7.6% of females are overweight, while 7.6% of males and 4.3% of females are obese.

Comparison across age-groups finds a statistically meaningful difference in BMI between the age ranges of 7-11 and 12-16, with the younger children being heavier (Table 9).

Table 7. Distribution of student groups junk-food eating habits

Categories	Underweight		Normal		Overweight		Obese	
Junk-food eating between meals	n	%	n	%	n	%	n	%
Yes	51	21.9	458	17.5	37	12.8	31	15.4
No	182	78.1	2169	82.5	253	87.2	171	84.6
Total	233	100	2627	100	290	100	202	100

Table 8. Distribution of BMI by school students hunger reduction behaviors

Categories	Underweight		Normal		Overweight		Obese	
	n	%	n	%	n	%	n	%
Bagel-Pastry	159	68.2	1739	66.2	173	59.7	104	51.5
Biscuit	19	8.2	242	9.2	18	6.2	17	8.4
Food in their lunch box	55	23.6	646	24.6	99	34.1	81	40.1
Total	233	100	2627	100	290	100	202	100

Table 9. Student groups by age and sex distribution of BMI

Categories	Underweight		Normal		Overweight		Obese		Total	
Gender	n	%	n	%	n	%	n	%	n	%
Girls	139	8.7	1267	79.4	122	7.6	69	4.3	1597	100
Boys	94	5.4	1360	77.4	168	9.6	133	7.6	1755	100
Age	n	%	n	%	n	%	n	%	n	%
7-11	108	5.5	1472	75.2	211	10.8	163	8.5	1954	100
12-16	125	8.9	1155	82.6	79	5.7	39	2.8	1398	100

4. Discussion

The weight classes of participating children broke down as follows: 7% were underweight (BMI under 5), 78.4 were normal (between 5-85), 8.7% were overweight (between 85-95), and 6% were obese (95 and over). This is a higher rate of obesity than was discovered in the Istanbul, Ankara and Izmir survey of 1044 adolescents from ages 12-13, in which 12% of the children are underweight, 12% overweight and only 2% obese (PHIT, 2012a).

According to the information intended for how these children relieve their hunger in school; God decides the situation of donut-pastry type is seen at highest level in all groups as 68.2% in underweight group, 66.2% in the normal group, 59.7% in overweight group and 51.5% in the obese group (Table 8).

The gender breakdown of the children's BMI shows a higher rate of obesity and being overweight in males than females; 9.6% of males and 7.6% of females are overweight, while 7.6% of males and 4.3% of females are obese. However, all our research samples are taken from urban areas, and obesity rates in cities are much higher compared to rural areas (Parlak and Çetinkaya, 2007; TOMKP, 2009). Research carried out in Poland found that obesity is twice as common among men living in urban areas than those living in rural areas (Aneta et al, 2012), which may clarify why obesity rates in our research turned out high. Furthermore, a 2012 survey in Kayseri found a prevalence of overweight children of 12.4% and an obesity prevalence of 6.5%, similar to our work (Öztürk and Aktürk, 2011). BMI data for the Kayseri survey show a similar gender disparity with 9.6% of boys and 7.6% of girls being overweight, and 7.6% of boys and 4.3% of girls being obese. However, among the 7-11 age group, girls have a higher BMI.

A different survey of 1014 adolescents from ages 12-13 in Istanbul, Ankara and Izmir discovered a total obesity prevalence rate of 15.1% for boys and 13.3% for girls (PHIT, 2012a). Research on 4260 children from ages 6-15 in Muğla found that 7.6% of girls and 9.1% of boys are obese (PHIT, 2012c), although being overweight tends to be more common among girls during primary school and puberty compared to boys (Spinu, 2012). Those results show parallelism with our own.

Schools can play a pivotal role in preventing childhood obesity. Institutions offering full-time schooling have control over the content of student lunches (Öztora, 2005). Foods available to children in their schools are often the donut-pastry type, no matter their weight class: 68.2% in the underweight group, 66.2% for the normal group, 59.7% of overweight children and 51.5% of the obese. These foods are part of our culture, and children and adults prefer them because they are easily accessible, fast, comfortable and satisfying foods. They can become more healthy meals when supplemented with additional nutrients such cheese and olives.

When a child's obesity situation is correlated with the education level of their mothers, children of university graduates mothers have healthier weights than children of non-literate mothers, indicating that obesity decreases as a family's education level increases. It also parallels Chinese research results that specify high levels of maternal education as a protective factor against obesity (Xiaoqing et al., 2012). The rate at which children eat three meals per day is high across all weight groups, while the obese group exceeds this limit most frequently, eating four or more meals each day. However, the gap between them is not statistically meaningful ($p>0.05$) (Öztürk and Aktürk, 2011).

The content of the meal is as important as the frequency of eating. Therefore, the best solution is to provide children with a nutrition program that will maintain a diet in accordance with the requirements of their bodies. For this reason, it is

important to work with the school nurses and pediatric nurses to prepare school programs for supplying children the energy they need. Furthermore, nurses can only carry out this role in cooperation with the students' family (Güler et al., 2009).

Even though obesity rates are rising across Turkey, only 17.2% of participating children eat junk-food regularly while 82.8% do not; in fact, vegetable dishes are the most liked meals of 32.6% of children and are the most frequently eaten type of meal for 44.7% (Table 6). Virtually no respondents eat junk food between meals (Table 7). Press publications, schools and parents express that eating junk foods negatively affect health; children cannot escape exposure. However, attractive junk-food advertisements also appear frequently across media, affecting the eating habits of adults as well as children.

Meta-analysis on interventions to prevent childhood obesity includes 37 studies on a total of 27.947 children found evidence supporting the beneficial effects of programs similar to our recommendations, especially programs targeting the 6-12 age group. Examining evidence-based programs shows that developing a school curriculum including quality of food available at school, increasing physical activity during school time, and encouraging body image confidence work best in collaboration with parents (Waters et al, 2011). This conclusion is corroborated by a study of 64 randomized controlled trials focused on treatment programs including interventions in lifestyle, physical activity, and diet. (Oude Luttikhuis et al., 2009). An Australian study, discovered that expert advice physical education helps to regulate students' percentage of body fat across age groups and contributes to academic improvement (Telford et al., 2012).

Poor mother-child relationships in early life is a closely related to obesity. Therefore, it is suggested to examine potential mechanisms including stress response and emotion regulation to assess the effects of mother-child relationships in cases of excessive childhood weight gain (Anderson et al., 2012).

In conclusion, in the light of this information, it is suggested that nurses continue maintaining effective counseling services to enable sufficient and balanced nutrition in every area in which children are offered service, that they cooperate with the families during their duties, to consider social tendencies, and carrying out research on parent-child relationships.

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Long-term effects of pentoxifylline in heart failure therapy

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ABSTRACT

The aim of the present study is to investigate the effects of pentoxifylline on left ventricular ejection fractions (EF) and volumes, New York Heart Association (NYHA) functional class, left ventricular diastolic parameters and hospitalization for heart failure in patients with ischemic or non-ischemic cardiomyopathy. A total of 60 patients were randomised to either peroral 1200 mg/day pentoxifylline or control group. All patients were on optimal heart failure therapy and their EF was <40% by transthoracic echocardiography. The patients were followed up for 12 months. Twenty-one patients (70%) in pentoxifylline group and 20 (66.7%) in control group completed the study. Baseline and 12 months' end-diastolic volume, end-systolic volume, EF and NYHA class were as follows in pentoxifylline group; 160.5±51.3 mL vs 156.6±43.1 mL, 109.1±40 mL vs 106.1±33.4 mL, 32.4±5.7% vs 33.2±5.2%, 2.4±0.5 vs 2.2±0.5, p=0.5411, 0.5257, 0.4099 and 0.1037; respectively. There were also no difference in baseline and follow-up diastolic parameters. Mean hospitalization numbers for heart failure were similar between groups (1.26±0.71 vs 1.60±1.04, p=0.1717). Contrary to previous reports, no beneficial effect of pentoxifylline was observed on clinical or echocardiographic parameters.

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1. Introduction

Congestive heart failure with the features of high frequency, prevelance, morbidity and mortality is one of the most important health problems (Del Carlo and O'Connor, 1999). Most common cause of hospitalization over sixty five years old patients is congestive heart failure (Narula et al., 2001). Although incidence of numerous cardiovascular diseases decreased in the last 20 years, the incidence of heart failure has increased (O'Connell and Bristow, 1994). While one year-mortality in mild and moderate heart failure is 15-25%, mortality in severe heart failure is between 40-50%. Heart failure caused by myocardial, valvular, pericardial and non-cardiac pathologies affects various organs such as renal, pulmonary, endocrinological and musculoskeletal systems. Pathophysiology and medical treatment of heart failure is well known (Silistreli and Oto, 1999). Current medical treatment modalities (digoxin, diuretics, angiotensin converting enzyme (ACE) inhibitors, beta blockers and phosphodiesterase inhibitors) aim reducing afterload and increasing contractility (Durdu et al., 2003).

Pentoxifylline, a derivative of metilxanthine, inhibits phosphodiesterase enzyme and shows haemorrhagical and antiinflammatory effects. Pentoxifylline reduces the viscosity of blood, decreases aggregation of thrombocytes and improves tissue oxygenation. Hypoxia and ischemia are important in the occurence of inflammation. It has been speculated that pentoxifylline can reduce inflammation by enhancing tissue oxygenation. It was shown that pentoxifylline inhibits tumor necrosis factor (TNF) and interleukin-2 (IL-2) production which are derived from monocytes and T-cells via polysaccharide signalling and supresses cytokine replication (Ward and Clissold, 1987; Sullivan et al., 1988; Edwars et

Table 1. Baseline clinical characteristics

	Pentoxifylline group (n=30)	Control group (n=30)	p
Age (year)	60.8±11.7	62.9±10.7	0.4910
Sex			
Female	13 (43.3%)	7 (23.3%)	0.1709
Male	17 (56.7%)	23 (76.7%)	
Averaged NYHA class	2.7±0.7	2.8±0.6	0.3079
NYHA class			
2	13 (43.3%)	8 (26.7%)	
3	14 (46.7%)	19 (63.3%)	0.2998
4	3 (10%)	3 (10%)	
Hypertension			
Present	12 (40%)	18 (60%)	0.164
None	28 (60%)	12 (40%)	
Diabetes Mellitus			
Present	10 (33.3%)	12 (40%)	0.035
None	20 (66.7%)	18 (60%)	
Ischemic CMP			
Present	13 (43.3%)	15 (50%)	0.7958
None	17 (56.7%)	15 (50%)	
Smoking			
Smoker	3 (10%)	9 (30%)	0.1277
Non-smoker	18 (60%)	11 (36.7%)	
Ex-smoker	9 (30%)	10 (33.3%)	
SBP (mmHg)	131.6±21.4	121.4±25.4	0.1095
DBP (mmHg)	79.5±13.1	76.6±14.8	0.4402
Rythm			
Sinus	27 (90%)	24 (80%)	0.4696
AF	3 (10%)	6 (20%)	

NYHA: New York Heart Association; **CMP:** Cardiomyopathy; **SBP:** Systolic blood pressure; **DBP:** Diastolic blood pressure; **AF:** Atrial fibrillation.

al., 1991; Schandene et al., 1992). In the treatment of heart failure immunological mechanisms are suggested to be substantial determinants and resistance to treatment is still an essential problem. In this paper we investigated the efficacy of a new hemorheological and immunomodulator agent, pentoxifylline.

2. Materials and methods

Study protocol

This is a single centered, randomised and prospective study. Inclusion criteria are: Left ventricular ejection fraction less than 40% via echocardiographic assesment, presence of New York Heart Association (NYHA) functional class II-IV heart failure symptoms, having a high quality echocardiographic view and having optimal medical treatment for heart failure. Severe liver disease is defined as elevated liver enzymes twice or more, serum creatinine level more than 2.5, conditions that may effect serum cytokine levels except cardiomyopathy (sepsis, rheumatoid arthritis, acquired immunodeficiency syndrome) and age less than 18 are considered as exclusion criteria. The study protocol was approved by Ankara Numune Education and Research Hospital (ANEAH) ethical committee and all patients signed the informed consent before the study. Between September 2010 April and 2011, 60 heart failure patients who were under optimal medical treatment adjusted according to heart rate and blood pressure measurements were randomised to study and control groups consisting of 30 patients. Assessment of NYHA functional capacity of patients initially and after follow up was performed by a physician unaware of the study protocol (Chacko, 1995).

Pentoxifylline (400 mg three times a day) was added to routine treatment protocol in the study group while control group continued to take existing medical therapy. Baseline characteristics, functional capacity and echocardiographic parameters were recorded in the beginning and after 12 months follow up, they were re-evaluated. The primary endpoint of the study is to investigate the affects of pentoxifylline treatment in 12 months period on left ventricular ejection fraction, end-diastolic and end-systolic volumes and NYHA functional class in patients with heart failure. The secondary endpoints are comparison of two groups about hospitalization for heart failure and diastolic parameters.

Echocardiographic assesment

The echocardiographic assessments of all patients initially and at the end of the study were performed by an experienced physician unaware of study groups using Vivid 7 echocardiography device (GE Ultrasound, Horten, Norway) equipped with a 1.5 MHz-3.3 MHz probe.

Measurements were taken from parasternal long axis, parasternal short axis, apical four chamber, apical two chamber views in the left lateral decubitis position and from subcostal view in supine position in the guidance of American Echocardiography Association guideline (Sahn et al., 1978). Modified Simpson method was used to calculate ejection fraction, and left ventricular volumes and diastolic mitral flow parameters were acquired by pulsed-wave (PW) Doppler from apical four chamber view. Isovolumetric relaxation time was obtained by measuring time from closure of aortic valve to opening of mitral valve with placement of PW Doppler on the point of mitral and aortic flows. Peak systolic pulmonary pressure was calculated using modified Bernoulli equation from tricuspid regurgitant flow. Three measurements in patients with sinus rhythm and ten in patients with atrial fibrillation were averaged.

Table 2. Baseline echocardiographic parameters

	Pentoxifylline group (n=30)	Control group (n=30)	p
EDV (ml)	165.1±57.8	186.4±65.1	0.1906
ESV (ml)	115.2±45.6	132.9±54.5	0.1811
EF (%)	31.2±6.3	29.7±6.5	0.3801
EDD (mm)	63±8.5	63.7±8.9	0.7745
ESD (mm)	54.1±7.2	53.2±10.5	0.8030
FS (%)	15.9±4.1	16.9±4.9	0.5625
Left atrium (mm)	50.1±7.5	48.9±6.3	0.5341
Right ventricle (mm)	33.1±7.7	31.8±6.2	0.5278
Mitral E (m/s)	0.78±0.23	0.79±0.25	0.8004
Mitral A (m/s)	0.67±0.23	0.75±0.18	0.2401
E/A ratio	1.25±0.63	1.12±0.48	0.4806
EDT (ms)	169.2±53.2	193.3±61.4	0.1642
IVRT (ms)	94.1±24.6	95.5±22.6	0.8861
PAP (mmHg)	38.1±15	37.1±12.7	0.8067

EDV: Left ventricle end diastolic volume; **ESV:** Left ventricle end systolic volume; **EF:** Left ventricle ejection fraction; **EDD:** Left ventricle end diastolic diameter; **ESD:** Left ventricle end systolic diameter; **FS:** Fractional shortening; **EDT:** E deceleration time; **IVRT:** Isovolumetric relaxation time; **PAP:** Peak systolic pulmonary artery pressure.

Table 3. Comparison of findings in pentoxifylline and control groups initially and at the end of 12 months follow-up

	Pentoxifylline group (n=21)			Control group (n=20)		
	Baseline	**1. year**	**p**	**Baseline**	**1. year**	**p**
NYHA class	2.4±0.5	2.2±0.5	0.1037	2.8±0.6	2.4±0.5	0.0541
EDV (ml)	160.5±51.3	156.6±43.1	0.5411	194.6±83.3	202.9±79.7	0.1615
ESV (ml)	109.1±40	106.1±33.4	0.5257	140.1±68.8	144.0±67.9	0.3086
EF (%)	32.4±5.7	33.2±5.2	0.4099	29.1±7.3	30.7±6.2	0.1418
EDD (mm)	61.3±8.4	62.4±9.2	0.0904	63.0±10.6	62.9±9.4	0.9344
ESD (mm)	51.2±5.2	54.2±5.1	0.0066	56.3±19.1	56.3±17.5	1.0000
FS (%)	17.3±3.9	15.7±2.1	0.2014	16.0±8.2	17.0±7.2	0.2254
Left atrium diameter (mm)	49.8±8.2	50.2±7.3	0.6580	48.5±6.6	49.3±6.4	0.5372
Right ventricle diameter (mm)	33.4±7.4	31.1±6.8	0.1809	30.8±6.8	29.1±3.8	0.2271
Mitral E (m/s)	0.73±0.24	0.77±0.22	0.1963	0.67±0.19	0.75±0.25	0.2262
Mitral A (m/s)	0.69±0.25	0.71±0.19	0.7468	0.76±0.17	0.79±0.19	0.6143
E/A ratio	1.99±0.71	1.11±0.43	0.4519	0.90±0.28	1.00±0.49	0.3932
EDT (ms)	188.6±59.2	200.7±47.3	0.3138	193±51.2	197±28.7	0.7699
IVRT (ms)	97.0±23.9	117.4±16.6	0.1613	95.0±14.1	104.5±5.5	0.5000
PAP (mmHg)	39.2±17.0	41.9±20.9	0.4548	35.3±15.5	38.2±22.6	0.6228

NYHA: New York Heart Association; **EDV:** Left ventricle end diastolic volume; **ESV:** Left ventricle end systolic volume; **EF:** Left ventricle ejection fraction; **EDD:** Left ventricle end diastolic diameter; **ESD:** Left ventricle end systolic diameter; **FS:** Fractional Shortening; **EDT:** E deceleration time; **IVRT:** Isovolumetric relaxation time; **PAP:** Peak systolic pulmonary artery pressure.

Statistical Analyses

Analyses were performed, using SPSS 11.0 (SPSS Inc., Chicago, IL, USA) package program. The continuous data were expressed as the mean±standard deviation (SD) while non-continuous variables were described as percentages (%). Variables were tested for normal distribution using the Kolmogorov-Smirnov test. Comparisons between patients were made using Student's independent t-test for normally distributed data. Difference between categorical variables was assessed using Chi-square or Fisher's exact test The results were regarded as significant when $p<0.05$.

3. Results

Baseline clinical characteristics and echocardiographic parameters of 60 patients evaluated in the study were given in Tables 1 and 2. There is no difference between two groups except diabetes mellitus frequency and diabetes mellitus is a little bit more in the control group ($p=0.035$). At the end of 12 months period, 9 patients in pentoxifylline group (death 4 patients, biventricular pacemaker placement 2 patients, leaving controls 3 patient) and 10 patients in control group (death 6 patients, biventricular pacemaker placement 2 patients, leaving controls 2 patients) could not complete the study and statistical analysis of variables were performed in the remaining 21 patients (mean age: 64±12.7), in pentoxifylline group and 20 patients (mean age: 64.9±8.9) in the control group ($p=0.4185$).

Consequently, there was no difference between two groups in the primary endpoints consisting of ejection fraction, left ventricular end-diastolic and end-systolic volumes and NYHA class that were evaluated in the begining and at the end of 12 months period (Table 3). At the end of 12 months, there was no significant difference between two groups in terms of left ventricular end-diastolic and end-systolic diameters, left atrial and right ventricular diameters, peak systolic pulmonary artery pressure, diastolic mitral flow velocities, E deceleration time and isovolumetric relaxation time.

Hospitalization for heart failure, the second endpoint of the study, also showed no significant difference between pentoxifylline and control groups (1.26±0.71 vs 1.60±1.04, $p=0.1717$).

4. Discussion

This paper revealed that pentoxifylline treatment had no effect on left ventricular ejection fraction, left ventricular diastolic and systolic volumes, NYHA class, left ventricular diastolic parameters and hospitalization in patients with heart failure.

Immune activation is one of the potential processes which is emphasised on the pathophysiology of heart failure. This was hypothesized after it was noticed in the observational studies that immune system activity is increased in patients with heart failure. Proinflammatory cytokines, especially TNF-alpha levels are elevated in patients with heart failure (Levine et al., 1990). In the light of these data, increased plasma proinflammatory cytokines are believed to be the result of immune process that is responsible for the progression of heart failure. Immunomodulatory agents, especially TNF-alpha, were investigated in order to prevent this process (Shaw et al, 2009). Randomized Etanercept Worldwide Evaluation (RENEWAL) study which was done with etanercept, a recombinant TNF receptor and inactivator of circulating TNF were early terminated due to adverse events (Mann et al., 2004). Similar results were acquired in the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) study which used infliximab, again a TNF-alpha monoclonal antibody (Chung et al., 2003).

These studies about immunomodulatory agents in heart failure therapy revealed unexpected outcomes (Levine et al., 1990; Shaw et al., 2009; Mann et al., 2004; Chung et al., 2003). Pentoxifylline is a peripheric vasodilator agent which reduces the viscosity of blood and it is in routine use in the treatment of peripheric vascular disease. Pentoxifylline became the focus of interest with its TNF-alpha inhibition effect in immunomodulation area. There are several small-scale randomised studies on this issue. In a study which was pre-

sented by Sliwa et al. (1998) pentoxifylline was applied to 28 idiopathic dilated cardiomyopathy patients with NYHA class II-III symptoms. Although there was no statistically significant difference between study and control groups, tendency to improvement in the functional capacity of study population was observed. When the number of patients was increased and 39 patients were evaluated, it was noticed that improvement in functional capacity in pentoxifylline group reached statistical significance. Improvement of ejection fraction was also remarkable in this study (Skudicky et al., 2000).

Another study evaluated 18 idiopathic dilated cardiomyopathy patients with NYHA class IV symptoms and revealed improvements in ejection fraction and functional capacity (Sliwa et al., 2002). Additionally 38 patients with ischemic heart disease and NYHA class I-IV symptoms had improvements in functional capacity and ejection fraction significantly with addition of pentoxifylline to routine heart failure treatment (Sliwa et al., 2004). On the other hand outcomes about mortality were not in the same direction with other parameters. Counterwise data was obtained in a different study Bahrmann et al. (2004) presented and pentoxifylline treatment derived no additional benefit on any clinical variable when used in 47 congestive heart failure patients. In this paper we aimed to investigate the effects of pentoxifylline on left ventricular ejection fraction, left ventricular volumes, NYHA functional class, left ventricular functional parameters and frequency of hospitalization. As in the previous studies we added 1200 mg/day pentoxifylline to routine optimal heart failure treatment protocol.

This study presented that addition of pentoxifylline to routine treatment protocol had no significant effect on clinical and echocardiographic parameters. While previous studies evaluated patients after 6 months follow up, in present study we planned a long term follow up to observe late period effects of pentoxifylline. Only patients with sinus rhythm were reviewed in the previous studies but we enrolled three patients with atrial fibrillation in pentoxifylline group and six patients in control group. Additionally a more extensive assessment including diastolic parameters and pulmonary artery pressure was performed not to overlook any significant variance. Another feature of this study that it lets us to make interpretation about frequency of hospitalization, which was not evaluated in the previous studies.

Beside these, our study has some limitations. The number of patients evaluated in the study is insufficient. Furthermore, levels of TNF-alpha and other cytokines that are asserted to be influenced by pentoxifylline could be investigated. In this study, we hypothesized that inhibition of TNF-alpha may provide improvement in heart failure because TNF-alpha levels are elevated in these patients. Because TNF-alpha contributes to progression of heart failure by accelerating apoptosis.

Significance of TNF-alpha in pathophysiology of heart failure is not well defined. Moreover, in the recent studies, although pentoxifylline treatment improved symptoms and ejection fraction, a notable reduction was not seen in the serum levels of TNF-alpha. Therefore it can be speculated that potential effects reported in the previous studies can act via different mechanisms.

Consequently, no additional benefit was observed in clinical and echocardiographic parameters with addition of pentoxifylline to standart treatment protocol in patients with heart failure. Despite existence of studies resulted positively, the potantial role of pentoxifylline in heart failure therapy is still controversial. As a result, more extensive clinical researches with larger study populations are needed.

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A new review of seizure types in psychogenic non-epileptic status

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ABSTRACT

While the incidence of non-epileptic psychogenic seizures (PS) is 1.5-3/100000 in community, the prevalence of PS varies between 2-33/100000. The ratio of PS was reported 5-20% for epileptic patients in community and it was reported 10-40% in epileptic centers. Approximately 20% of patients, who applied to epilepsy centers because of drug resistance seizures, were diagnosed and reported as a PS. In 2005, 2009 and, as the last time, 2010, International League Against Epilepsy (ILAE) divided the status seizure types into two main groups as generalized onset and focal onset. It was categorized by subgroups as generalize convulsive status (tonic-clonic, tonic, clonic and myoclonic), generalize non-convulsive (absence) status, partial status and complex partial status by considering both EEG and clinical observations. In this study, we wanted to discuss the psychogenic non-epileptic status (PNE-status) types in our cases which are not elaborated in literature.

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1. Introduction

Non-epileptic psychogenic seizures (PS) appear similar to epileptic seizures. However, ictal or postictal electroencephalography (EEG) differences do not accompany PS, and this type of seizure is related to emotional periods (Bora et al., 2011).

While the incidence rate of PS is 1.5-3/100000 in the community, the prevalence of PS varies from 2-33/100000. The ratio of PS was reported as 5-20% for epileptic patients in the community and 10-40% in epilepsy centers. Approximately 20% of the patients who applied to epilepsy centers due to drug resistance seizures were diagnosed and reported as having PS (Benbadis and Hauser 2000; Szaflarski et al., 2000; Bora et al., 2011).

Psychogenic non-epileptic status (PNE-status) is characterized as continuously or frequently recurrent epileptic activation and long-lasting behaviors that have no relationship to ictal or postictal EEG changes. A common view of PNE-status duration does not exist. Some studies found that PS continued for at least 20 minutes, and other studies found a duration of 30 minutes. Dworetzky et al. (2010) conducted a survey of adult and pediatric epileptologists in 19 epilepsy centers. In this survey, the epileptologists agreed on at least 20 minutes of prolonged PS duration but emphasized that some seizures lasting between 10-20 minutes should be considered PS. Overall, 27-78% of PS patients also experience PNE-status (Dworetzky et al., 2010).

Status Epilepticus (SE) is characterized as more than 30 minutes of seizure duration or a recurrence of seizures in 30 minutes while unconsciousness occurs (Bradley et al., 1996). SE has recently been characterized as more than five minutes of generalized and convulsive seizures or as abnormal mind activity in patients after two or more exacerbations (Lowenstein et al., 1999). The incidence of SE varies between 60000 and 160000 in the USA (Hesdorffr et al., 1998).

Several classifications for SE classification exist in the literature. SE was first classified in 1980 at the Santa Monica International SE symposium as follows (Wasterlain, 1997):

1) Primary generalized convulsive status (tonic-clonic, myoclonic, clonic-tonic-clonic),

2) Secondary generalized convulsive status (partial onset tonic-clonic, tonic),

3) Basic partial status (partial motor, partial sensorial, affective, autonomus, partial with vegetative symptom)

4) Non-convulsive status (Absence, complex partial)

In 2005, 2009 and 2010, the International Committee of Epilepsy (ILAE) divided the status seizure types into two main groups, separating them by generalized or focal onset. These groups were further categorized into subgroups as generalized convulsive status (tonic-clonic, tonic, clonic and myoclonic), generalized non-convulsive (absence) status, partial status and complex partial status based on EEG and clinical observations (Berg et al., 2010).

In this study, we will discuss the psychogenic non-epileptic status (PNE-status) types observed in our cases that have not been elaborated in the literature.

2. Materials and Methods

In this study, 1548 seizures in 840 patients who were hospitalized for epilepsy surgery assessment were analyzed retrospectively by Video-EEG imaging in the Neurology Department clinics at Medical Faculty of Uludağ University, between 2001 and 2012. The patients who participated in this study were placed in the video-EEG unit, and their seizures and EEG were monitored. Cranial magnetic resonance imaging (MRI), neuropsychometric test (NPT) and psychiatric consultations were performed in the other unit, and the patients were diagnosed as having PNE-status.

3. Results

In total, 20% of the seizures (305/1548) were observed in 10.8% of the patients (91/840) with PS. Of these 91 patients, nine had PNE-status, and 39 PNE-status seizures were completely observed. The SE ratio was 10.5% (88/840).

In total, nine patients (9/840) were followed up for PNE-status. Of those patients, five were women, and the other four were men. The mean age at seizure onset was 21.7 (8-35). All PNE-status patients were used antiepileptic drugs, and the mean antiepileptic drug number was 2.2 (1-4). The mean seizure frequency was 6.1(1-15) per month. Complex partial seizures (CPS)-like status seizure was observed in two patients (Video-1), generalized tonic-clonic (GTC)-like status seizure was observed in three patients (Video-2), secondary generalized status-like seizure was observed in one patient (Video-3) and focal motor status-like seizure was observed in two patients (Video-4) who were monitored in the Video-EEG monitoring (VEM). Of the nine patients, six had a prior hospitalization history for SE. One of the nine patients was operated previously for a left parietal mass and had an abnormal cranial MRI as a complication of the operation. Patients were monitored with the video-EEG unit for four days on average (2-6 days). Demographic and seizure information about the SE and PNE-status of the patients is presented in Table 1.

Eight patients with PNE-status had seizures for 30 minutes, and one patient with PNE-status had seizures for 45 minutes. Pathology was detected in the interictal EEG of three of nine patients. Three of the nine patients had prior epileptic seizures. Neuropsychometric tests of the patients with PNE-status were generally concluded as frontal disorder or normal test results with some inattentiveness. The prolactin levels were measured three times in the postictal period and were normal. Psychiatric examination of the patients showed that two patients had conversion disorder; one patient had somatization disorder; one patient had dysthymia; one patient had major depression; one patient had borderline personality disorder; one patient had dependent personality and major depression; one patient had immature personality disorder and anxiety disorder; and one patient had conversion disorder and major depression.

In total, 88 patients (88/840) were followed up for SE. Of those patients, 51 were women, and 37 were men. The mean age at seizure onset was 6.6 (2-71) years. Of these 88 patients, 71 had previously used antiepileptic drugs, and the mean antiepileptic drug number was 3.7 (2-6). The mean seizure frequency was 4.8 (3-15) per month generalized tonic-clonic (GTC) and secondary.

GTC status-like seizure was observed in 43 patients, and focal motor status-like seizure was observed in 13 patients monitored in the VEM. Of the 88 patients, 28 had a history of prior hospitalization for SE. Of the 88 patients, 56 had an abnormal cranial MRI (e.g., focal critical dysplasia, mesial temporal sclerosis, tumor, schizencephaly, hemimegalencephaly) (Table 2).

Table 1. Demographic and seizure information about SE and PNE-status patients

	PNE-STATUS (n=9)	SE (n=88)
The mean age	30.7(18-59)	42.1 (18-71)
Gender (M/F)	5/4	51/37
The age at onset of epilepsy	21.7(8-35) age	16.6 (2-43) age
The used of AEDs/number/average	9/2.2	(71/88)/3.7
Seizure frequently/mounth/time/average	6.1	4.8
The VEM observed seizure type	CPS-like status=2	Primary GTC=43
	Primary GTC like=3	Secondary GTC=32
	Secondary jen. Status-like=1	Focal motor status=13
	Focal motor status=2	
SE with a history of hospitalization	6/9	28/88
Abnormal cranial MR	1/9	56/88
Compatable with the SE ictal EEG	No	88/88

GTC: Generalized tonic-clonic; **CPS:** Complex partial seizures; **AED:** Antiepileptic drug; **SE:** Status Epilepticus; **VEM:** Video-EEG monitoring

Table 2. Demographic, seizure characteristics and laboratory findings of psychogenic non-epileptic status-status patients.

	Case-1	Case-2	Case-3	Case-4	Case-5	Case-6	Case-7	Case-8	Case-9
Gender/age	M/20	M/24	F/26	M/35	M/32	F/35	F/18	F/28	F/59
Occupation/ Marital status	Worker/single	Worker/married	House wives/ single	Unemployed/ married	Worker/ married	Housewife/ married	Housewife/ single	Secretary/ single	Housewife/ married
The age at onset of epilepsy	14 age	23 age	8 age	34 age	15 age	25 age	19 age	20 age	38 age
Seizure frequency/ month/time	1-2/m/10-15 minute	15/m/45minute	1.seizure: 1-2/ m/20 minute 2.seizure: 3-4/ m/2 minute	8-10/m/ 30minute	1.seizure: 3-4/m/2 minute 2. seizure1/m/ 30 minute	10/m/30 minute	15/m/ 15minute	1-2/m/ 10-15 minute	10/m/ 30minute
Seizure type described	The left arm focal motor	Frontal and GTC	CPS, secondary generalized	Starting from left to secondary generalized	GTC	Focal motor in both arms	Right focal status	The left arm focal motor	Frontal and GTC
SE with a history of hospitalization	2 times Phenytoin, diazepam, intubated	1 times Intubated	3 times Diazepam, phenytoin, Valproate infusion	1 times Diazepam, phenytoin	2 times Intubated	No	No	1 times Diazepam, phenytoin	No
The use of AEDs	CBZ, VPA, LEV	LEV, PHT	CBZ, LEV	PHT, CBZ, TPM	VPA, CBZ, TPM	CBZ, FB	LEV, CBZ, TPM	CBZ, VPA	PHT, VPA
Interictal EEG	Normal	Normal	Left frontotemporal SA	Normal	Generalized SA	SWA left parietal	Normal	Normal	Normal
Ictal EEG	Normal	Normal	Normal and left frontotemporal SWA	Normal	Normal and left frontal SWA	Normal	Normal	Normal	Normal
VEM number of seizures	6 times PNE-Status	4 times PNE-Status	3 times PNE-status, 2 times CPS	6 times PNE-Status	5 times PNE-Status, 3 times seconder generalzie	15 PNES times	4 PNE-Status	3 PNE-Status	3 PNE-Status
VEM type of seizures	CPS like status	Frontal and seconder GTC like	CPS like status	GTC like	GTC	Focal motor like Status	Focal motor like status	Focal motor like Status	GTC like
Cranial MRI	Syringomyelia	Normal	Normal	Normal	Mild cortical atrophy	Encephalomalacia in the left parietal	Normal	Normal	Normal
NPT	Attention deficit	Normal	Frontal challenges	Attention deficit	Limited mental capacity MMSE:22/30	Attention deficit	Normal	Attention deficit	Attention deficit
Psychiatric examination	Somatic disorder	Borderline Personality Disorder	Dependent personality, major depression	Conversion disorder	Immature, people, anxiety disorder	Conversion disorder and major depression	Conversion disorder	Dysthymia	Major depression
Other history	Uncle epileptic	Aunt reluctant marriage with his daughter	Febrile Convulsion (+) Mixed Seizure	Unemployed and his wife had abandoned	The patient has an epileptic brother	Operated for the left parietal glial tumor	His father was separated from home	No	No
PRL levels	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

GTC: Generalized tonic-clonic; **CPS:** Complex partial seizures; **AED:** Antiepileptic drug; **SE:** Status Epilepticus; **MMSE:** Mini mental state examination; **PRL:**Prolactin; **NPT:** Neuropsychometric tested; **SWA:** Slow wave activity; **SA:** Spike activity; **CBZ:** Carbamazepine; **VPA:** Valproic acid; **LEV:** Levetiracetam; **PHT:** Phenytoin; **TPM:** Topiramate; **FB:** Phenobarbital, **M:** Male; **F:** Female; **PNE:** Psychogenic non-epileptic status; **VEM:** Video-EEG monitoring.

4. Discussion

PNE-status, also termed psychogenic status, pseudo status, psychogenic status epilepticus and prolonged psychogenic status, has no relationship to ictal or postictal EEG changes. PNE-status is characterized by continuous or frequently recurrent psychogenic originating episodes that last more than 30 minutes. It is important that the episodes of PNE-status look similar to seizure-related SE (Reuber et al., 2003).

SE occurs if any type of epileptic seizures is of long enough duration or if seizures occur so frequently that the neurologic condition does not improve between two seizures. It is reported that 1.3-1.6% of chronic epileptic patients can have SE. Mortality depends on various factors, including age and duration, and is between the ratio of 8-32% at status epilepticus (Sepkuty, 2006). GTC, secondary GTC and focal motor status, which we diagnosed as SE, were observed in 43, 32 and 13 patients, respectively.

Reuber et al. (2000) published five postoperative pseudostatus cases in 2000. They observed patients who had suicide attempts, psychiatric history and tonic-clonic-like contractions after minor operations. The authors indicated that the patients did not respond to stimulus while their eyes were open. The patients were intubated, and intravenous antiepileptic drugs and anesthesia were given to the patients. The postictal prolactin and the EEG recorded during the episode were observed as normal. The postictal prolactin levels of our nine PNE-status patients were normal, in accordance with the literature (Reuber et al., 2003).

In the literature, there is a case about the 3rd trimester of pregnancy, in which the patient did not respond to the first step treatment. The patient was intubated with thiopental after using diazepam and phenytoin for recurrent generalized seizures during treatment in the epilepsy center. This was not a case of eclampsia of pregnancy. All tests, including cranial imaging and lumbar puncture, were normal, and pseudostatus was diagnosed after a five day observation (Peters et al., 2007). In our cases, three patients had GTC-like seizures, and one patient had secondary generalized status-like seizures. It attracted our attention that these patients were diagnosed with status epilepticus, and the intravenous antiepileptic treatment protocol was applied by our clinicians.

Venous catheter placement had to be performed 24 times for a 20-year-old patient in Sri Lanka due to resistant seizures. Non-generalized tonic-clonic contractions were observed at the time of the patient's hospitalization; these contractions continued for seven hours and could not be controlled with diazepam. The patient took organophosphates for the purpose of suicide (Gunatilake et al., 1997). In our cases, nine patients applied several times to the emergency clinic, six of whom were intubated for SE. Many tests were performed, and these patients were hospitalized 20 days in total, including their emergency clinic stays. This finding indicates that the incorrect diagnosis may cause the unnecessary hospitalization of patients.

There is a report of a pediatric PNE-status in the literature. A nine -year-old patient with epilepsy and mental retardation had decreasing contractions in some seizures that differed from his other seizures, and he had a pedaling sign at his legs. His seizures lasted for long periods, and he did not respond to diazepam, phenytoin or phenobarbital. The case was acknowledged as PNE-status and was psychiatrically treated as conversion disorder (Tuxhorn and Fishbach, 2002). In our cases, we had no pediatric patients, but three of the nine cases had epileptic seizures in addition to PNE-status. It is reported that 1.3-1.6% of patients with chronic epilepsy can have PNE-status at any time during the disease process. SE can develop in epileptic patients. For patients with psychiatric disorders, it should be remembered that PNE-status and psychogenic epileptic seizures can develop on a psychiatric basis.

Cases reflecting the psychiatric symptoms of PNE-status, such as a suicide attempt by organophosphate ingestion, were present in the literature. Additionally, psychotherapy was used in a case of a mentally retarded pediatric patient, in whom significant improvement was observed. In our cases, two patients had conversion disorder; one patient had somatization disorder; one patient had dysthymia; one patient had major depression; one patient had borderline personality disorder; one patient had depended personality and major depression; one patient had immature personality and anxiety disorder; and one patient had conversion disorder and major depression. Although the published series contain few PNE-status cases, these series provide researchers with very important information. These series contain assessment information about the cases and the applied treatment methods. They also attract our attention to the psychiatric predisposition for PNE-status (Gunatilake et al., 1997).

The acute and dramatic process of PNE-status patients (although some are epileptic) can lead us to the SE diagnosis. If these patients do not respond to conventional SE treatment and have a prior hospitalization history with previously diagnosed SE, we should consider a PNE-status diagnosis for these patients.

According to our findings, the incidence rate of PNE-status cases has recently increased because of physicians, especially emergency clinicians, who are inexperienced with epilepsy and seizure semiology, a lack of centers where video-EEG imaging is available, and numerous wrong assessments of EEG by inexperienced clinicians.

Video-1: The seizure began with the right deviation of the eyes. The patient was looking with empty eyes, and the patient's face was fearful. The patient did not answer questions. The patient began to gulp and take deep breaths. A dystonic posture that looked like a tonic contraction was observed in the left wrist. Some movements that looked like automatisms were observed when the patient moved his right arm to a flexion posture. The patient's seizure continued for approximately 30 minutes. In that time, he moved his neck to a flexion posture and began to breathe wheezily. It was observed that patient had cyanosis.

Video-2: The patient began to contract his teeth and move his legs continuously. The patient had a tonic-clonic seizure during 30 minutes in the left lateral lying posture. The patient's eyes were closed, and the patient did not talk during seizure. The patient did not answer questions during the seizure and did not remember keywords that were given to him after the seizure. The light reflex could not be taken in the neurologic examination. There were no pathologic reflexes.

Video-3: The patient began to breathe deeply. The patient's eyes deviated to the right. Contractions were observed in his legs and arms. The patient's face was fearful. His head and

eyes deviated to right. He did not understand spoken words and did not answer questions. The patient moved his neck to a flexion posture. The patient began to foam at the mouth, and stronger contractions were observed. The patient seized for approximately 35 minutes.

Video-4: The seizure began with a gulp movement. The patient's eyes were open slightly. The patient had rhythmic high amplitude contractions in the right arm and began to swing his arm in the air. The patient's seizure continued for 45 minutes in this way. The patient also moved his left arm similarly.

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Anti-John Cunningham Virus antibody prevalence in multiple sclerosis patients in Turkey

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ABSTRACT

Exposure to John Cunningham Virus (JCV) has been associated with Progressive Multifocal Leukoencephalopathy (PML) in Multiple Sclerosis (MS) patients under specific treatments. In order to assess the identifiable risks of Turkish relapsing-remitting multiple sclerosis (RRMS) patients, the seroprevalence rate of JCV antibodies was investigated and compared with demographic and therapy related factors. Serum samples were collected from 308 RRMS patients at 24 centers, and tested by STRATIFY JCV assay for the presence of anti-JCV antibodies. Also, demographic, disease and treatment data were collected at the same time and were used for analysis of associations with seropositivity. The prevalence of anti-JCV antibodies was observed to be 68.2%, which is higher than most countries. There was a trend in terms of higher seroprevalence of antibodies in older patients, and male patients. There was no association with previous natalizumab use, but there was a significantly higher seroprevalence in patients who used immunosuppressant therapies ($p<0.05$). JCV prevalence rate is observed to be high in Turkish MS patients, therefore it would be beneficial to use this marker as a risk stratification tool in clinical decision making.

Keywords:
John Cunningham Virus
Multiple Sclerosis
Natalizumab
Turkey

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1. Introduction

In recent years, there has been an increase in the number of pharmaceuticals used in treatment of Multiple Sclerosis (MS), made available by new high technology developments. Although these new treatments are becoming more and more effective in the way we manage this debilitating disease, they may come with a burden. MS disease effects over one million people around the world, and it is mediated by the patient's own immune response targeting the central nervous system (Steinman, 2012). It is not surprising that when the immune systems of MS patients are modified in order to treat the disease, opportunistic infections kept under control by immunity may start to cause unwanted side effects. Weighing the benefits and risks of any given treatment in daily clinical practice and using all the available tools to determine which is the most benefit for the patients is crucial. One of the most efficient therapies for MS, the monoclonal antibody natalizumab, has been associated with Progressive Multifocal Leukoencephalopathy (PML), a rare but potentially fatal disease (Clifford et al., 2010; Hellwig and Gold, 2011; Vermersch et al., 2011). Typically, PML is caused by opportunistic John Cunningham Virus (JCV), in patients with immunocomprimising diseases, or under immunosuppressive/ immunomodulatory therapies (Major, 2010). JCV exposure among different populations was reported to be 33%-91%, in various geographies and using different methods (Sorensen et al., 2012).

JCV infection is asymptomatic in healthy people; the virus needs to gain pathogenicity and some additional factors play role in development of PML (Sunyaev et al., 2009). Data from multinational studies show approximately 50-69.5% prevalence in various populations, by testing JCV specific antibodies from serum samples by specifically developed assays, optimized to a false negative rate of 2.5-2.7% (Gorelik et al., 2010; Bozic et al., 2011; Bozic et al., 2012, ECTRIMS). As a guide for clinical decision making, risk stratification algorithms are developed, for which the JCV antibody assay plays a central role. In patients exposed to JCV, prior immunusuppressant treatments and duration of natalizumab treatment are shown to effect the risk of development of PML (Sorensen et al., 2012). The estimated incidence of PML is still quite low in patients who have been exposed to JCV and immunosuppressants during the first two years of natalizumab treatment: 1.6/1,000 (Bloomgren et al., 2012). The mortality rate in natalizumab treatment related PML is 22% (Foley et al., 2011). Thus, it is recommended that patients who are currently being treated or candidates for natalizumab treatment are tested for JCV antibody status and therapeutic decision is made considering the likely benefit/ risk. Serum antibody testing was recently optimized using a 2-step ELISA (Gorelik et al., 2010).

Since there have been many reports with different seroprevalence rates from various countries, we have started a programme in Turkey to identify patients who will benefit more from a highly effective therapy, in order to give the clinician a more informed decision making during everyday practice. As the first step, we tested 308 relapsing-remitting multiple sclerosis (RRMS) patients for anti-JCV antibodies, from various geographical regions, in order to determine the seroprevalence in Turkey.

2. Experimental procedure

Study patients and sample collection

This observational study was approved by the Ethical Committee and Ministry of Health before patient enrollment. For the collection of patient samples and demographic data, 24 study centers were included in the study. These centers represent the most experienced clinical care and high patient rate MS clinics from various geographical regions of Turkey. Serum samples were collected from 308 adult male and female RRMS patients, over 10 months. Patients with a diagnosis of RRMS according to McDonald criteria, admitted to the clinics for routine exams and using various therapies were included. Written consents were taken from patients, in accordance with the Declaration of Helsinki and Turkish laws and legislations on clinical studies.

All serum samples were tested for anti-JCV antibody positivity for a single time point. Serum samples were prepared from peripheral blood according to protocol (Focus Diagnostics, Cypress, CA) and tested by 2-step ELISA method specially developed for JCV antibody detection.

Demographic data and disease history

Each patient was coded in order to protect privacy. At the time of blood withdrawal, demographic data and disease characteristics were collected from the patients; gender, age, time when first symptoms started and time of diagnosis. Treatment history was recorded in detail including previous immunomodulatory and immunosuppressant treatments, natalizumab treatments as well as the duration of each treatment. Clinical disease characteristics included number of relapses in last 2 years, Expanded Disability Status Scale (EDSS) scores, magnetic resonance imaging (MRI) status of gadolinium-enhanced lesions, and presence of any other diseases.

Anti-JCV antibody determination

After collection, serum samples were stored in -20°C until they were shipped to Focus Diagnostics, Cypress, CA, USA. Samples were tested by STRATIFY JCV™ , a 2- step ELISA validated for JCV antibody detection. Serum antibody status was dichotomous, being positive or negative for each patient.

Data analysis

Primary goal of this study was to determine the prevalence of anti-JCV antibodies in Turkish population. Additionally, clinical data like disease duration, relapse rates, EDSS score, treatment and MRI lesion status are defined in detail in this cohort.

The demographic data and the anti-JCV antibody prevalence were described using descriptive statistics (minimum, maximum, mean, median and standard deviation values for the continuous variables, and frequency and percentage values for the categorical variables). For specific data like disease and treatment history, some patient data were missing. No substitution was done for the missing values, these were excluded from the statistical analysis. Anti-JCV antibody prevalence was defined as the percentage of patients with positive anti-JCV antibodies. Annualized relapse rate was defined by dividing the number of relapses in last two years by 2.

Demographics (age and gender), disease and treatment history were analyzed for association to seropositivity. For the analysis of association with age, patients were separated into several age groups and the p-value across age categories was determined using Fisher's Exact Test because of the small sample size. For the analysis of association of anti-JCV antibody with gender, immunosuppressant use, and natalizumab use, Pearson Chi-Square Test was used. Results at $p<0.05$ level were considered statistically significant.

Study data were analyzed by an independent statistician. Statistical analyses were performed using IBM SPSS 20 software.

3. Results

Demographic information was available from 307 patients, summarized in Table 1. Mean age of the patients that were enrolled in the study was 32.93 (±9.05) years. Among 307 patients, 223 (72.6%) were female. Mean total duration of MS disease was 8.8 (±5.68) years from the start of first symptoms. From the diagnosis of RRMS, a mean of 7.4 (±4.92) years had passed.

Disease characteristics are listed in Table 2. Relapse history in the 2 years prior to sample collection were available from 304 patients. Most of the patients had 2 or more relapses, with a mean annualized relapse rate of 1.2 (±0.90). EDSS scores were available at the time of sample collection from 296 patients. Median EDSS was 3 and most of the patients had EDSS scores between 2 and 3. Only EDSS scores of 10.5% patients were above 5. According to the MRI results in the past 1 year, 175 of 278 patients (62.9%) had gadolinium-enhanced lesions.

Table 1. Demographic data and background disease characteristics

	(N=308)
Age (yrs.)	
n	307
Mean (SD)	32.93 (9.05)
Median	32.0
Min, max	16.0, 63.0
Gender	
Male	84/307 (27.4%)
Female	223/307 (72.6%)
MS disease duration (yrs.)	
n	303
Mean (SD)	8.8 (5.68)
Median	7.7
Min, max	0.2, 36.0
MS diagnostic duration (yrs.)	
n	307
Mean (SD)	7.4 (4.92)
Median	6.5
Min, Max	0.0, 25.0

Table 2. Summary of relapses, EDSS, and contrast lesion status

Relapses in last 2 years	
0	41/304 (13.5%)
1	56/304 (18.4%)
2	83/304 (27.3%)
3	49/304 (16.1%)
4	37/304 (12.2%)
5	16/304 (5.3%)
6	14/304 (4.6%)
>= 7	8/304 (2.6%)
Annualized relapse rate	
n	304
Mean (SD)	1.2 (0.90)
Median	1.0
Min, max	0.0, 4.5
Last EDSS	
n	296
Mean (SD)	3.05 (1.62)
Median	3.0
Min, Max	0.0, 7.5
Last EDSS	
>=0 and <=1	47/296 (15.9%)
>1 and <=2	55/296 (18.6%)
>2 and <=3	71/296 (24.0%)
>3 and <=4	55/296 (18.6%)
>4 and <=5	37/296 (12.5%)
>5 and <=6	21/296 (7.1%)
>6	10/296 (3.4%)
Contrast lesion in last 1 year	
Yes	175/278 (62.9%)
No	103/278 (37.1%)

Prior treatments are listed in Table 3. A total of 99/307 (32.2%) patients were using/had used natalizumab, and 47/307 (15.3%) patients were exposed to immunosuppressant treatment previously with nine receiving mitoxantrone. Almost all patients had received one of four Disease Modifying Treatments (DMTs): Interferon beta-1a sc, interferon beta-1a im, interferon beta-1b, glatiramer acetate. Most received only one type of DMT (56.2%), while 33.0% switched to a second DMT. Mean durations of treatment were 15.35, 8.63, 15.55 and 9.95 months for IF1a sc, IF1a im, IF1b, GA, respectively.

The number of the patients who tested positive for anti-JCV antibodies was 210 of 308 patients (68.2%). For the analysis of association of anti-JCV antibody positivity with age, the patients were separated into age groups (<30, 30-39, 40-49, 50-59, ≥60) (Fig. 1). When the patients under 30 years old and above 50 years old were compared, there was a higher seropositivity rate in the older group (64.7% and 86.7%, respectively). However, when all age groups were compared, no significant difference was shown (p=0.218). There was a significant difference between women and men

Table 3. Treatment history

Prior Tysabri Use	
Yes	99/307 (32.2%)
No	208/307 (67.8%)
Prior IS Use	
Yes	47/307 (15.3%)
No	260/307 (84.7%)
Prior DMT use	
0	11/306 (3.6%)
1	172/306 (56.2%)
2	101/306 (33.0%)
3	21/306 (6.9%)
>= 4	1/306 (0.3%)
IF1 a sc	
n	304
Mean duration of use (months) (SD)	15.35 (27.84)
Min, max	0.0, 168
IF1 a im	
n	305
Mean duration of use (months) (SD)	8.63 (19.97)
Min, max	0.0, 144
IF1b	
n	299
Mean duration of use (months) (SD)	15.55 (29.55)
Min, max	0.0, 180
GA	
n	299
Mean duration of use (months) (SD)	9.95 (18.80)
Min, max	0.0, 108

(64.1% vs 78.6%, respectively p<0.05) (Fig. 2). Looking at the effects of treatment on anti-JCV antibody prevalence, there was no significant difference between the patients who have been treated by natalizumab and the patients who have never used natalizumab, p=0.155 (Fig. 3). Patients who were exposed to immunosuppressant therapies had a seropositivity of 83.0%, significantly different from patients who did not use immunosuppressants, 65.4% (p<0.05) (Fig. 4).

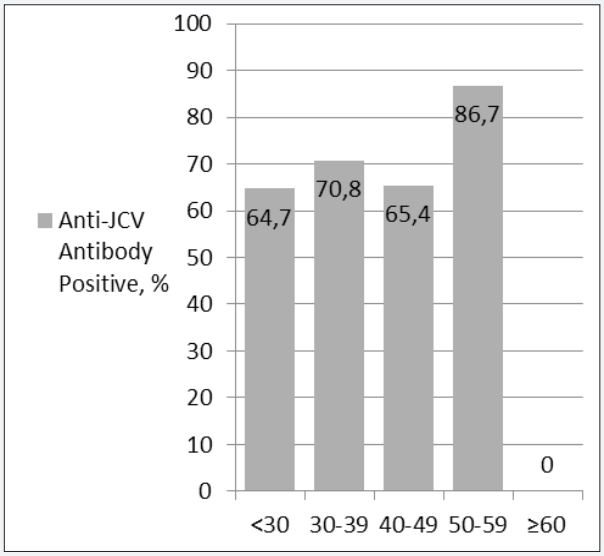

Fig. 1. Seroprevalance among the age groups. p value across age categories was determined by Fisher's Exact Test (p=0.218).

4. Discussion

There has been a growing interest in JCV epidemiology since the emergence of PML cases associated with the use of monoclonal antibodies for the treatment of MS. Various national and multinational studies have reported the prevalence of JCV antibodies to be around 50-60%, assessed by STRATIFY JCV assay (Bozic et al., 2011; Bozic et al., 2012; Trampe et al., 2012). By determining the serostatus of patients, it is possible to make more informed decisions about treatment options, minimising risks and giving the patients best possible care. Natalizumab is a powerful MS treatment, especially in patients with highly active disease, and it is warranted to assess JCV status of patients using or thinking of using natalizumab (Polman et al., 2006; Rudick et al., 2006; Sorensen et al., 2012). This study revealed the seroprevalence of anti-JCV antibodies in Turkey. RRMS patients came from various geographical regions of Turkey, without any selection of demographics or disease/treatment history. Analysis of JCV antibodies in serum samples with the STRATIFY JCV assay resulted in 68.2% prevalence rate in Turkish patients. This represents one of the highest reported rate. To date, the highest seropositivity was reported for Portugal (69.5%) (Bozic et al., 2012). JCV is a ubiquitous virus usually transmitted in childhood, but the main route of transmission is not clearly defined (Matos et al., 2012).

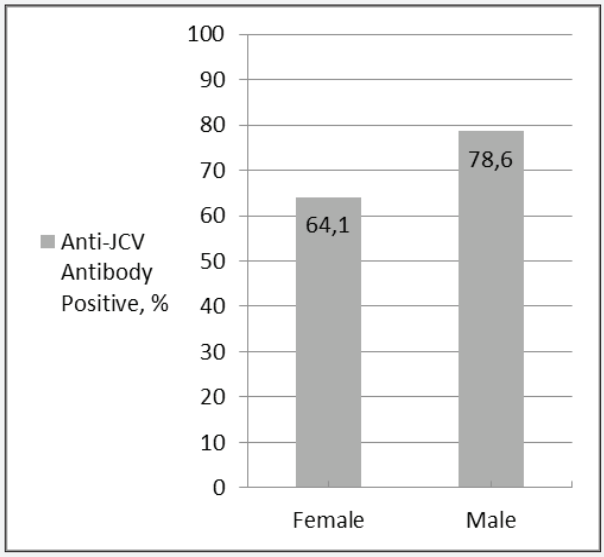

Fig. 2. John Cunningham Virus antibody positivity is higher in men than in women (p<0.05).

It may be speculated that the social mode of interaction, close family relations and cultural habits are factors affecting the prevalence. Also, yet undiscovered genetic factors might play a role in susceptibility to infection.

Evaluation of demographic factors in the present patient population showed age, gender and disease histories to be similar to other studies conducted in various countries (mean patient age 32.93± 9.05, 72.6% women, mean disease duration 7.4±4.92 years, median EDSS 3.0). Thus, this group reflects general MS population in terms of disease characteristics. In previous reports, older age was correlated with an increase in seropositivity, maybe as a result of more exposure in years (Bozic et al., 2011; Outteryck et al., 2012; Trampe et al., 2012). In the present study, seropositivity was higher in the

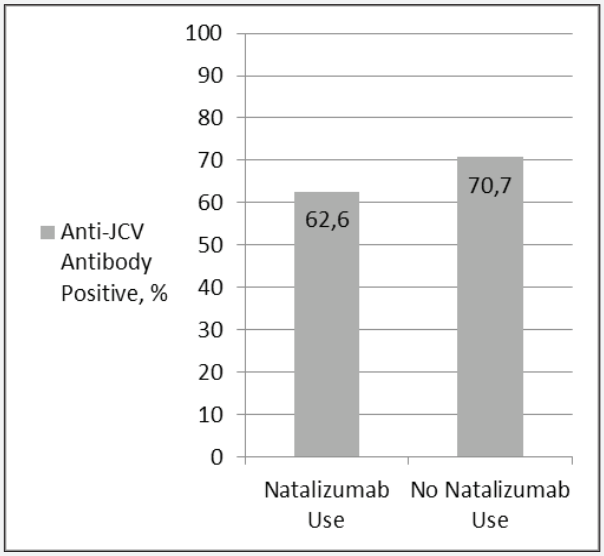

Fig. 3. Prevalence between the patients exposed to natalizumab (p=0.155).

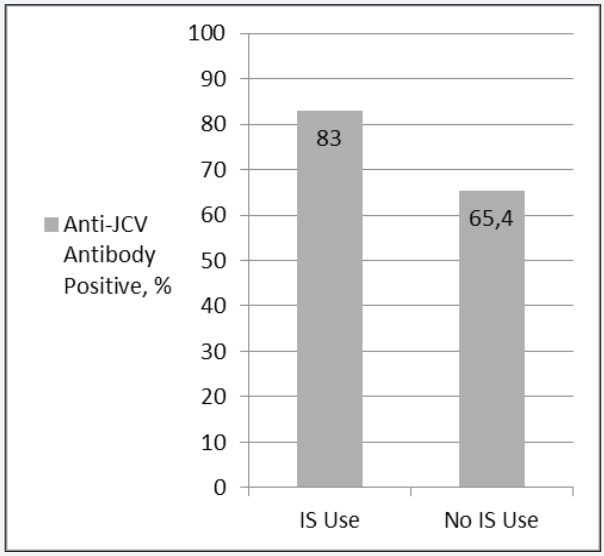

Fig. 4. Prevalence of anti-JCV antibodies is higher in the patients who have used immunosuppressant treatments (p<0.05).

older group; however the 40-49 age group does not reflect the expected rate of positivity, so there was no statistically significant difference among groups. Seropositivity was higher in males, as previously reported although the reason behind this remains unknown (Gorelik et al., 2010; Outteryck et al., 2012; Trampe et al., 2012). Exposure to natalizumab was not found to be correlated with seropositivity, as reported in the earlier studies (Bozic et al., 2011; Trampe et al., 2012).

However, there was a significant correlation between prior immunosuppressant use and JCV antibody positivity (83.0% in users, 65.4% in non-users). In previous studies, no significant correlation was found, although there was a a tendency towards more positivity in immunosuppressant users groups in some cohorts (Bozic et al., 2011; Olsson T. ECTRIMS 2011, P338; Trampe et al., 2012; Outteryck et al., 2012). Our findings may be spurious and may have resulted because of the overall high seropositivity rate while the number of patients using immunosuppressants was low (15.3%).

Given the fact that JCV-antibody seropositivity is high in our population, this study was a major step forward in risk stratification for daily clinical practice in Turkey. Although a majority of the patients may test positive for this marker, this does not necessarily mean that they will develop PML if they are treated with natalizumab and immunosuppressants. The benefits and risks of treatment need to be assessed in such cases. Risk assessments for various conditions have been determined quite clearly and guidelines are available for clinicians to aid in decision making (O' Connor, 2012; Sorensen et al., 2012). It should be kept in mind that the estimated false negative rate of the test is 2.5-2.7% (Gorelik et al., 2010; Bozic et al., 2011). If a patient is negative for the anti-JCV antibody, relative PML risk is estimated to be equal or less than 1 in 10,000 (Bloomgren et al., 2012; Sorensen et al., 2012). It is recommended that these patients are tested periodically as about 5-10% are shown to have a serostatus change to positivity over time (Trampe et al., 2012). In positive patients, estimated incidence differs according to treatment history. It is still 0.56/1000, in positive patients who have not used immunosuppressants and had up to 2 years of natalizumab treatment (Bloomgren et al., 2012). After 2 years of treatment, it becomes 4.6/1000 (Bloomgren et al., 2012). If the patient has used immunosuppressants and up to 2 years of natalizumab treatment, estimated incidence of PML is 1.6/1000 and 11.1/1000 after 2 years. This information can be used for risk assessment in clinical decision making. Some patients with very active disease who benefit from natalizumab treatment may prefer to continue after 2 years, accepting the risks, if they are fully aware of them. The risks and benefits of treatment according to patient status should be discussed. It should also be kept in mind that not much is known about long-term use associated with PML risks. Nevertheless, patients suffering from debilitating effects of MS can still be offered potent therapies following recommendations published for early recognition and treatment of PML (Bloomgren et al., 2012; O' Connor, 2012; Sorensen et al., 2012).

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Evaluation of the combined treatment with cisplatin and melatonin on neuroblastoma cell viability and antioxidant capacity

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ABSTRACT

In this study, we investigated the cell viability and antioxidant effects of melatonin both with and without cisplatin (CDDP) on the cultured neuroblastoma cancer cell line. Neuroblastoma cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) at 37°C with 5% CO_2 to examine the cytotoxic effect of melatonin; cells were cultured both CDDP with and without melatonin and thereafter counted in a 48-well microplate. To examine the effect of melatonin and CDDP, cells were divided into ten groups (control, vehicle, melatonin-5nM and10nM, CDDP-50μM and 100μM, melatonin-5nM+CDDP-50μM, melatonin-5nM+CDDP-100μM, melatonin-10nm+CDDP-50μM and melatonin-10nM+CDDP-100μM) and thereafter cell viability was determined in a 48-well microplate using 3-(4,5-dimetiltriazol-2-il)-2,5- difeniltetrazolium bromid (MTT) assay. In different series, cells were cultured and treated with ethanol, melatonin, CDDP, and combination of melatonin and CDDP. After harvest, TAS and TOS were measured via Elisa assay kits. Melatonin and combination of melatonin and CCDP produced no cytotoxic effect on neuroblastoma cancer cells after 24 hours, but a decrease in the cell viability after 48 hours. Furthermore, CDDP treatment significantly decreased the cell viability both after 24 and 48 hours periods compared to untreated controls. Melatonin enhanced the cytotoxic effects of CDDP after 48 hours neuroblastoma cell lines. Therefore, melatonin may be used as an adjunctive therapy agent to both improve neuroblastoma cancer chemotherapy and depression and for inhibition of CDDP side effects.

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1. Introduction

Cisplatin (CDDP) is a chemotherapeutic agent, which is used for the treatment of human malignancies such as neuroblastoma and osteosarcoma (Karadeniz et al., 2011). However, this drug has many side effects due to severe cytotoxicity against normal cells and tissues. A number of studies have suggested that using a combination of chemotherapeutic agents and natural antioxidant products is a beneficial therapeutic strategy to overcome its cytotoxic effects (Liu et al., 2008; Reiter et al., 2009).

Melatonin, as a hormone, is produced in the pineal gland and several organs, which orchestrate the numerous biological activities in the body such as the modulation of circadian rhythms and regulation of immune systems (Brzezinski, 1997; Dubocovich et al., 2010). It also plays a major role in some psychiatric disorder such as some sleep disorders and insomnias (Bartsch et al., 1989; Colleoni et al., 2000).

Several researchers reported the potential effects of melatonin as a potent combination therapeutic agent to support the efficacy of chemotherapeutic agents and also decrease their side effects in vitro (Futagami et al., 2001) and in vivo (Şener et al., 2000; Parlakpinar et al., 2002). Also, melatonin and melatonin agonists are antidepressant agents, which can be used for clinical treatment of depression

Table 1. Results of MTT (cell viability) analysis after CDDP and/or melatonin treatment for 24- and 48- hour incubation period

Groups	Cell Viability (24 hours)	Cell viability (48 hours)
Control	0.1366±0.023[a]	0.0823±0.016[a]
Vehicle	0.1320±0.035[a]	0.0826±0.032[a]
Mel-5nM	0.1398±0.032[a]	0.0835±0.010[a]
Mel-10nM	0.1412±0.025[a]	0.0880±0.014[a]
CDDP-50μM	0.1119±0.017[b]	0.0700±0.009[b]
CDDP-100μM	0.1068±0.014[b]	0.0690±0.005[b]
Mel-5nM+CDDP-50μM	0.1398±0.013[a]	0.0679±0.003[b]
Mel-10nM+CDDP-50μM	0.1336±0.045[a]	0.0638±0.004[bc]
Mel-5nM+CDDP-100μM	0.1322±0.021[a]	0.0699±0.003[b]
Mel-10nM+CDDP-100μM	0.1395±0.042[a]	0.0589±0.005[c]

Values are expressed as mean ± standard deviation. abc The footnote letters in the same column indicate significant differences between groups (n = 6); P<0.05. For statistical analysis, differences between the groups were tested by analysis of variance followed by the Duncan post hoc test (ANOVA).

(Colleoni et al., 2000; Courtet, 2012). Depression incidence in patients with cancer has been reported as 22-24% (American Psychiatric Association, 2000) Therefore, many neoplastic patients need psychological support, especially after surgery. An interaction between psychological health and anti-cancer therapy has been explained with a possible assumed tendency of depressed patients to be less eager in obtaining health care (Watson et al., 1999). Therefore, treatment of depression might be essential for tailoring the treatment of malignancies.

Reactive Oxygen Species (ROS) are highly reactive molecules with unpaired electrons such as hydrogen peroxide, hydroxyl radicals, and superoxide anion. They are produced in normal physiological processes such as aerobic metabolism or inflammation. The excessive ROS production shows detrimental effects on cells or tissues, which are inhibited by the antioxidant defence system in the cellular defence system. Chemotherapeutic agents can cause a disproportional increase in intracellular ROS, cell cycle arrest and apoptosis activation via death signalling pathways. The advantage of this therapeutic strategy is that normal cells are not affected by the chemotherapeutics. However, if a threshold of toxicity in these cancer cells is reached and an additional increase in the ROS level occurs these can cause more mutations and invasion in cancer cells (Storz et al., 2009; Trachootham et al., 2009). Therefore, a combination of chemotherapeutics with antioxidant substances increases the ROS level in cancer cells and protects normal cells from cytotoxic effects of chemotherapeutics (Trachootham et al., 2009). Nevertheless, ROS mediated signalling pathways selectively killing cancer cells and overcoming drug resistance are unknown.

Understanding the detailed effects on living cells during cisplatin treatment would aid to the development of new treatment strategies to improve the therapeutic roles. The first aim of the study was to investigate the cytotoxic effect of melatonin alone on the neuroblastoma cell line. The second aim was to evaluate the interaction between melatonin and cisplatin treatment on the neuroblastoma cell line. The third purpose was to investigate the antioxidant capacity when neuroblastoma cell line was treated with melatonin, CDDP, or melatonin plus CDDP.

2. Materials and methods

Chemicals

Melatonin was purchased from Sigma Chemical Company (St. Louis, MO) and CDDP were purchased from Koçak Farma, Turkey. Melatonin was dissolved in ethanol to a concentration of 0.1M. The final concentration of solvents in the culture was less than 0.1%, such a concentration that exhibits no effect on cell growth and viability, as was experimentally confirmed.

Cell line

The neuroblastoma cell line used was NA/An1 (mouse neuroblastoma cancer), which was obtained from Sap Institute, Turkey. The cell line was subcultured once a week at 37°C atmosphere of 5% CO_2 and 100% relative humidity, and maintained at a low passage number (Hrushesky, 1985; Lissoni et al., 1997). The culture medium was Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% foetal bovine serum, 2mM L-glutamineand 1mM penicillin–streptomycin-amphotericin (Sigma Chemical Company).

Cell growth and viability assays

Adherent cells at a logarithmic growth phase were detached by the addition of 3mL of a trypsin-EDTA mixture (Gibco-BRL) and incubated for 5 minutes at 37°C. Cells were plated (200mL per well) in 48-well micro-plates at a density of 5,000 cells per well. Cells were left for 24 hours at 37°C to resume exponential growth. Six replicate wells for each concentration were used. Cell growth was measured 24 and 48 hours later with MTT assays. All experimental measurements were performed at least twice.

Table 2. Total antioxidant status (TAS) and total oxidant (TOS) status analysis results after CDDP and/or melatonin treatment for 24- and 48- hour incubation period

Groups	TAS 24h	TAS 48h	TOS 24h	TOS 48h
Control	0.2810±0.168[a]	0.3472±0.117[a]	2.9038±0.080[a]	2.8606±0.048[a]
Vehicle	0.2781±0.103[a]	0.3384±0.094[a]	2.9241±0.105[a]	2.8732±0.124[a]
Mel-5nM	0.2957±0.069[a]	0.4012±0.024[b]	2.9567±0.182[a]	3.0385±0.221[a]
Mel-10nM	0.3207±0.033[b]	0.5747±0.065[c]	2.8269±0.145[a]	3.0769±0.117[a]
CDDP-50μM	0.1572±0.083[c]	0.2775±0.131[c]	2.8654±0.142[a]	3.0769±0.135[a]
CDDP-100μM	0.2470±0.149[c]	0.2292±0.091[c]	2.9038±0.219[a]	3.0192±0.160[a]
Mel-5nM+CDDP-50μM	0.1665±0.038[c]	0.2552±0.099[c]	2.8846±0.135[a]	3.0000±0.291[a]
Mel-10nM+CDDP-50μM	0.1792±0.021[c]	0.2860±0.084[c]	3.2308±0.602[b]	3.0962±0.299[a]
Mel-5nM+CDDP-100μM	0.1774±0.086[c]	0.2216±0.084[c]	3.0385±0.174[a]	2.8269±0.109[a]
Mel-10nM+CDDP-100μM	0.1798±0.082[c]	0.2644±0.040[c]	2.9808±0.152[a]	2.6923±0.067[b]

Values are expressed as means ± standard deviation. abc The footnote letters in the same column indicate significant differences between groups (n=6); P<0.05. For statistical analysis, differences between the groups were tested by analysis of variance followed by the Duncan post hoc test (ANOVA).

Effects of CDDP and/or melatonin on cell viability

The experiment was designed to examine the effects of melatonin (5 and 10nM) (Kim et al., 2012) and/or CDDP (50 and 100μM) (Spano et al., 2008). The concentration of melatonin and CDDP was selected in accordance with the IC50 of the neuroblastoma cancer cell line used and previous studies (Spano et al., 2008; Kim et al., 2012). Test substances (CDDP and melatonin) were placed in tubes filled with 10mL of the medium, and 100mL of that medium was used for experiments. Cells were plated (200mL per well) in 48-well micro-plates and left for 24 hours. The control, melatonin and/or CDDP combination groups were divided into ten subgroups:

Control, in which the medium was replaced with a fresh medium and incubated for 24 and 48 hours;

Vehicle, in which the medium was replaced with a vehicle solution (ethanol) including the medium and incubated for 24 and 48 hours.

Mel-5nM, in which the medium was replaced with a 5nM concentration of melatonin including the medium and incubated for 24 and 48 hours.

Mel-10nM, in which the medium was replaced with a 10nM concentration of melatonin including the medium and incubated for 24 and 48 hours.

CDDP-50μM, in which the medium was replaced with a 50μM concentration of CDDP including the medium and incubated for 24 and 48 hours.

CDDP-100μM, in which the medium was replaced with a 100μM concentration of CDDP including the medium and incubated for 24 and 48 hours.

Mel-5nM+CDDP-50μM, in which the medium was replaced with a 5nM concentration of melatonin 50μM concentration of CDDP including the medium and incubated for 24 and 48 hours.

Mel-10nM+CDDP-50μM, in which the medium was replaced with a 10nM concentration of melatonin 50μM concentration of CDDP including the medium and incubated for 24 and 48 hours.

Mel-5nM+CDDP-100μM, in which the medium was replaced with a 5nM concentration of melatonin 100μM concentration of CDDP including the medium and incubated for 24 and 48 hours.

Mel-10nM+CDDP-100μM, in which the medium was replaced with a 10nM concentration of melatonin 100μM concentration of CDDP including the medium and incubated for 24 and 48 hours.

Cell viability assay

The cytotoxic effects of melatonin and/or CDDP on neuroblastoma cell line (NA/An1-mouse neuroblastoma) were assessed using an MTT cell proliferation kit (Sigma Chemical Company) according to the manufacturer's instructions. Briefly, 5,000 cells/well in a 200mL medium were seeded to attach type 48-well plates and after overnight incubation at 37°C media were changed with melatonin and CDDP containing media. The first and sixth rows of a plate were designated as the medium control that contained the medium only and cell control that included cells in the medium, respectively. Starting from the second row, serial dilutions of melatonin and CDDP containing medium were added to wells at a 2:3 dilution ratio up to the last row. Four identical plates were prepared, and they were incubated at 37°C for 24 and 48 hours. After 24- and 48- hour incubation periods, an MTT reaction solution containing an activation reagent (2%) was added to all of the wells containing the medium. Following three hours of incubation at 37°C, the absorbance was measured with an ELISA plate reader (μ-Quant, BioTek Instrument, USA) at 570nm. The Microsoft Excel programme was utilised to generate the percentage of cell viability versus dose curves.

Determination of TAS and TOS

Measurement of the Total Antioxidant Status (TAS): TAS concentrations were measured using an ELISA kit TAS assays Kits (Rel Assay Diagnostics, Turkey) according to the manufacturer's protocols. TAS levels of the cell medium for all groups was determined using a novel automated measurement method developed by Erel (Erel, 2004). The total antioxidant capacity method is based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenz-thiazo-line-6-sulfonic acid) (ABTS) radical cation by antioxidants. The medium samples were measured with an ELISA plate reader (μ-Quant, BioTek Instrument, USA) at 660nm and the results were expressed as mmol Trolox Equiv/L.

Measurement of the Total Oxidant Status (TOS): TOS concentrations were measured using an ELISA kit TAS assays Kits (Rel Assay Diagnostics) according to the manufacturer's protocols. TOS levels were determined using a novel automated measurement method developed by Erel (Erel, 2005). The total oxidant status method is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange. The medium samples were read with an Elisa plate reader (μ-Quant, BioTek Instrument) at 560nm. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micro-molar hydrogen peroxide equivalent per litre (μmol H_2O_2 Equiv./L).

3. Results

Cell viability results

An MTT assay was used to investigate whether or not melatonin has cytotoxic effects on neuroblastoma cell line both with and without CDDP treatment. Cells were treated with melatonin (5 and 10nM) and/or CDDP (50 and 100μM) for 24- and 48-hour periods.

For the 24-hour period, while melatonin treatment did not show a significant effect on the cell viability, CDDP treatment significantly reduced the cell viability compared to the control groups ($P<0.05$). In addition, melatonin and CDDP treatments had no significant effect on the viability, even at the higher concentration of CDDP compared with the untreated control ($P<0.05$) (Table 1).

For the 48- hour period, CDDP reduced the cell proliferation or viability, but melatonin did not affect the cell proliferation or viability. Conversely and interestingly, combined treatment with cisplatin and melatonin significantly suppressed the cell viability compared with the untreated control ($P<0.05$). These results were confirmed by morphological analysis under an inverted microscope (Fig. 1, 2) (Table 2).

TAS and TOS analysis results

In analysis of TAS levels, after the 24- and 48- hour periods, TAS levels were increased with the melatonin treatment, but decreased with CDDP and a combination of CDDP with melatonin treatment compared to the untreated and vehicle treated controls ($P<0.05$).

Analysis of TOS levels revealed that in group 7 after 24 hours the TOS level was significantly increased, while in group 10 after 48 hours TOS level was significantly decreased when compared with other groups ($P<0.05$). However, there were no significant differences between the remaining groups after 24 and 48 hours ($P>0.05$).

4. Discussion

Neuroblastoma is the most common cancer type and has shown a higher mortality rate. For the treatment of neuroblastoma cancer, surgical and chemotherapeutic applications were used for neoplastic patients. However, a surgery and platinum-based chemotherapy combination were recommended for advanced neuroblastoma cancer at stage IIb–IV (Evans et al., 1971). CDDP is a platinum-based chemotherapy agent, although it has shown limited therapeutic efficacy due to serious side effects. Several studies have reported that combined chemotherapy of CDDP and natural products may be useful for the treatment of many cancer types with improved therapeutic efficacy and ameliorated side effects (Choi et al., 2011; Su et al., 2011; Yunos et al., 2011). Therefore, in this study, we focused on the cell viability and antioxidant effects of melatonin both with and without CDDP treatment.

The present studies demonstrated that melatonin showed the synergetic cytotoxic effects with CDDP treatment on the neuroblastoma cell line. Contrary to CDDP, melatonin treatment induced the cell growth in 24 hours and a higher dose of melatonin (10nM) showed the synergic effect on cisplatin-induced cell death for 48 hours. Furthermore, previous studies have suggested the cytotoxic effect of melatonin on many cancer types of human (Futagami et al., 2001).

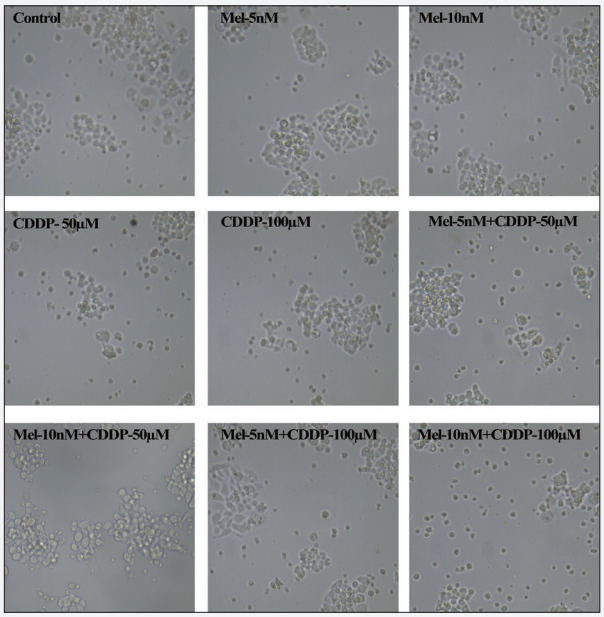

Fig. 1. Morphologic analysis of cell proliferation under inverted microscope for 24-hour incubation period groups

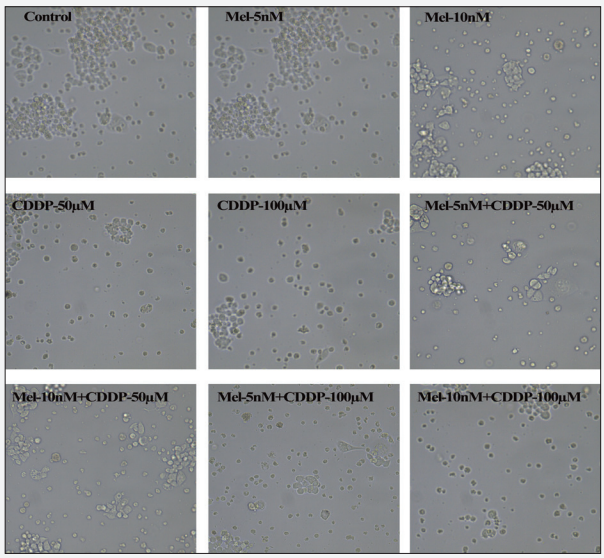

Fig. 2. Morphologic analysis of cell proliferation under inverted microscope for 48- hour incubation period

Under normal conditions, the physiological levels of ROS protect the cells from death. However, in cancer cell lines, growth factors or cytokines stimulate the production of ROS to induce cell death (Lissoni et al., 1999; Shiu et al., 2010). Therefore, melatonin can enhance the production of ROS and accelerate the cell death in neuroblastoma cell line, which is consistent with other studies (Hara et al., 2001; Reiter et al., 2002).

In an attempt to protect body function from CP toxicity several antioxidants have been analysed (Karadeniz et al., 2011, Kim et al., 2012). Melatonin is a powerful antioxidant agent that potentially attenuates the cytotoxicity of CDDP and inhibits the cell growth and proliferation (Lissoni et al., 1997; Kim et al., 2012). Also, pro-apoptotic, anti-tumour, and anti-angiogenetic effects of melatonin were reported by some researches (Hermann et al., 2002, Wenzel et al., 2005). However, many scientists argue the relationship between the plasma concentration and anti-tumour effect of melatonin (Blask et al., 1999; Hill et al., 2009). Actually, inhibition of cell proliferation by high concentration of melatonin is strongly associated with the antioxidant properties (Garcia-Navarro et al., 2007; Hill et al., 2009). Also, high plasma level of melatonin can promote the effect of chemotherapeutic drugs, suggesting that melatonin can be cytotoxic for malignant cells in particular conditions.

In the biochemical analysis, the total antioxidant and oxidants capacities were changed during melatonin and/or CDDP treatment owing to an increase in the antioxidant and a decrease in the oxidant status levels. In the proliferation of cancer cells, activation of the MAPK (mitogen-activated protein kinase)/Erk1/2 (extracellular-regulated kinase 1/2) pathways due to the effect of ROS inhibitors functionally regulates the cancer cell survival (Liou and Storz, 2010). In cancer treatment strategies, many different chemotherapeutic agents are designed to increase cellular ROS levels to induce cell damage, resulting in tumour cell apoptosis (Trachootham et al., 2009), dependent on the tumour type (Bairati et al., 2005; Alexandre et al., 2006; Llobet et al., 2008). Furthermore, contrary to cancer cells, which have high ROS levels during all malignancy stages, cancer stem cells have a

higher antioxidant capacity (Trachootham et al., 2009). This suggests that the antioxidant and oxidants' capacity may be changed by melatonin and/or CDDP treatment depending on cell proliferation activity.

In conclusion, treatment with melatonin may be attenuated to the toxic effects of ROS in neoplastic patients with CDDP treatment and can be useful in a combination with chemotherapeutic agents to improve the therapeutic effects.

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Behavioural responses in rats; modulation with beta-lactam antibiotics and antioxidants

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ABSTRACT

N-Acetylcysteine (NAC) has been in clinical practice for several decades as a mucolytic agent and has been used also for the treatment of paracetamol intoxication, doxorubicin-induced cardiotoxicity, stable angina pectoris, ischemia-reperfusion cardiac injury, acute respiratory distress syndrome bronchitis, chemotherapy-induced toxicity, HIV/AIDS, radio-contrast induced nephropathy, heavymetal toxicity and psychiatric disorders including schizophrenia, bipolar disorder and addiction. It has been recently shown that NAC modulates the glutamatergic system through the system xc (Cystine-Glutamate Antiporter): Antiporter cysteine/glutamate. Ceftriaxone (CTX), a β-lactam antibiotic, is also shown to led to an increase of excitatory amino-acid transporter 2 (EAAT2) expression and glutamate transport activity in the brain in animal studies. It has been demonstrated that CTX has neuroprotective effects in both in vitro and in vivo models based on its ability to inhibit neuronal cell death by preventing glutamate excitotoxicity. The aim of the present study was to investigate the neurobehavioural effects of acute administration of NAC and CTX alone and in combination in open field and elevated plus maze tests. For this aim, three different doses (50, 100 and 200 mg/kg, i.p.) of CTX and NAC alone in the first part and their combination in the second part of the experiments and two different doses of diazepam were evaluated in open field and elevated plus maze tests. 200 mg/kg of NAC revealed anxiolytic-like behaviours in both tests while CTX 200 mg/kg failed to produce. Further investigations need to be conducted to rule out the involvement of system xc- on anxiety related behaviours. Increased system xc may represent an effective therapeutic endpoint.

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1. Introduction

Anxiety disorders (ADs) are the most common psychiatric conditions encountered by doctors in general. The range of lifetime-prevalence data of different ADs are as follows: specific phobia 1.5-12%; social phobia 0.2-9.4%; obsessive-compulsive disorder (OCD) 0.1-3%; generalized anxiety disorder (GAD) 0.1-6.9%; panic disorder 0.2-5%; post-traumatic stress disorder (PTSD) from 1 to over 10% (the wide range of prevalence data mirrors that these are derived from populations of different countries). Despite the variety of anxiolytic agents available over the last decades, not only the adverse effects causing poor treatment or complete abandonment of therapy or insufficient targeting the pathophysiology of the disease and patients characterizing resistance to the treatment, requires new anxiolytic agents, not only to reduce acute symptoms but also to prevent relapses in the long term. These unmet needs have stimulated the introduction of new pharmacological approaches for the treatment of anxiety in monotherapy or in augmentation to standard treatments (Faludi et al., 2012). The alteration of the relation between oxidative stress and neurotransmission in several psychiatric conditions has been previously demonstrated. N-acetylcysteine (NAC), the acetylated precursor of the amino acid L-cysteine, is a cysteine prodrug. It has been used as a mucolytic agent and for the treatment of paracetamol intoxication, doxorubicin-induced cardiotoxicity, stable angina pectoris, ischemia-reperfusion cardiac injury, acute respiratory distress syndrome bronchitis, chemotherapy-induced toxicity, HIV/AIDS, radio-contrast induced nephropathy, heavymetal toxicity and psychiatric disorders including schizophrenia, bipolar disorder and addiction. (Samuni et al., 2013). NAC

Table 1. Statistical analysis of behavioural performance of first and second part in elevated plus maze (EPM)

Treatment (mg/kg)	Open arm enteries (%) med (min-max)	Open arm time (%) med (min-max)	Closed arm enteries (%) med (minmax)	Total arm enteries (n) med (minmax)
1st Part				
Control(saline)	13.8 (11.1-20)	5.1 (1.5-15.7)	8 (4-13)	9 (5-15)
CTX 50	17.1 (17.1-33.3)	3.3 (2.5-7.7)	6 (2-13)	7 (3-14)
CTX 100	20 (10-33.3)	9.4 (3.3-15.9)	8 (4-11)	10 (6-13)
CTX 200	23.6 (20-33.3)*1	12.5 (8.8-18.4)	7 (2-9)	9 (3-13)
NAC 50	11.8 (10-40)	4.2 (1.3-16.6)	8 (3-9)	9 (5-12)
NAC 100	17.1 (11.1-20)	2.7 (2.2-6.9)	6 (4-8)	7 (5-10)
NAC 200	26.8 (12.5-37.5)*2	15.9 (15.2-17.1)*1	6 (5-11)	8 (7-14)
Diazepam 1	26.4 (11.1-42.8)	14.2 (9.2-24.7)	6 (4-8)	7 (7-13)
Diazepam 2	48.1 (41.7-80)*1	48.8 (20.6-82.89)*1	5 (1-8)	9 (5-16)
2nd Part				
Control (saline+saline)	11.1 (8.3-14.3)	7.5 (2.5-19.5)	8 (6-11)	9 (7-12)
Diazepam 2+saline	40.8 (33.3-45.5)*1	67.9 (45.6-82.8)*1	6 (6-8)	11 (10-12)
CTX200+saline	26.1 (11.1-50)*2	13.0 (5.1-47.9)	6 (1-8)	8.5(2-10)
NAC200+saline	22.2 (16.7-25)*1	19.0 (15.5-22.3)*3	7 (3-10)	9 (4-12)
NAC200+CTX200	15.5 (10-30)	19.5 (4.1-45.0)	7 (5-10)	9.5 (6-12)

n=8-8 per group; **CTX50:** Ceftriaxone 50 mg/kg; **CTX 100:** Ceftriaxone 100 mg/kg; **CTX 200:** Ceftriaxone 200 mg/kg; **NAC 50:** N-acetylcysteine 50 mg/kg; **NAC 100:** N-acetylcysteine 100 mg/kg; **NAC 200:** N-acetylcysteine 200 mg/kg; Diazepam 1 mg/kg, Diazepam 2 mg/kg. Comparisons were done between control and other groups. (Mann-Whitney U test, The Bonferroni correction was used to adjust the P value for each hypothesis Values of p<0.006 (for first part) and p<0.010 (for second part) were considered statistically.)
*1: p<0.001 *2: p=0.003 *3: p=0.007 *4: p=0.021

is widely used antioxidant that acts not only as a direct free radical scavenger, but also promotes production of glutathione (GSH) by furnishing its limiting precursor l-cysteine (Chakraborti et al., 2008). It has been also shown in the recent literature that NAC modulates the glutamatergic system through the astrocytic antiporter cysteine/glutamate (system xc) that results in the activation of NMDA receptors and in the stimulation of metabotropic glutamate receptors, reducing synaptic glutamate release. The potential usefulness of NAC in the treatment of various psychiatric disorders is due to its antioxidant effect and glutamate modulation. In recent literature, NAC has been demostrated as an useful add-on medication for treating Parkinson's disease, schizophrenia and the depressive symptoms in bipolar patients in several clinical trials and to decrease the immobility time in the forced swimming test (a well accepted model with a predictive value for antidepressants) in animal studies (Linck et al., 2012). Glutamate is the major excitatory neurotransmitter responsible for excitatory neurotransmission in the brain, as well as a potent neurotoxin that may lead to excitotoxicity of nerves. Glutamate released from glutamatergic nerve endings participates in the signaling process through different types of glutamatergic receptors and then must be cleared from the synaptic cleft by glutamate transporters. Five subtypes of glutamate transporters have been identified to date. Three of these glutamate transporters were identified in rat brain: Named as GLAST, GLT-1 and EAAC1 with their human homologues which are: EAAT1, EAAT2 and EAAT3, respectively. The two remaining human and rodent subtypes, EAAT4 and EAAT5 share common nomenclature. It is noteworthy to highlight that the impaired glutamate transport by EAATs is common in many central nervous system (CNS) disorders. In the literature it has been shown that many β-lactam antibiotics are transcriptional activators of EAAT2 resulting in increased EAAT2 protein levels. Ceftriaxone (CTX), a β-lactam antibiotic, is also known to led to an increase of EAAT2 expression and glutamate transport activity in the brain in animal studies. It has been demonstrated that CTX has neuroprotective effects in both in vitro and in vivo models based on its ability to inhibit neuronal cell death by preventing glutamate excitotoxicity (Kim et al., 2011).

The elevated plus-maze (EPM) test, which is rapid and sensitive to the effects of both anxiolytic and anxiogenic agent, is based on the natural aversion of rodents from open spaces (Holmes et al., 2000). Percent time spent on open arms is a classical parameter usually measured for evaluating changes in the anxiety state. The EPM test has become a convenient procedure to measure not only the anxiety like behaviours but also sedation and activity. Total arms entry, it is mostly an indicator of motor activity, closed arm entries has already been defined as a pure index of locomotor activity. Open arm entries, considered as a motor activity pattern by some authors, was not found to reflect general activity of the mouse, in accordance to (File, 1992; Cruz et al., 1994; Hogg, 1996; Fernandes and File, 1996; Espejo, 1997). Open field test's (OFT) procedure is based on the involvement of forced confrontation of a rodent with the situation. The animal is placed in the center or close to the walls of the apparatus and the following behavioural items are recorded for a period ranging from 2 to 20 min (usually 5 min): Horizontal locomotion (number of squares passed on the marked flor), number of rearings or leaning (sometimes termed as vertical activity) (Prut and Belzung, 2003). The open field test provides to assess novel environment exploration, general locomotor activity and anxiety-related behaviour in rodents. Rodents tend to spent a significantly greater amount of time exploring the periphery of the arena, usually in contact with the walls (that is called thigmotaxis), than the center area. (Bailey and Crawley, 2009). Increase of time spent in the central part as well as of the ratio central/total locomotion or decrease of the latency to enter the central part are indications of anxiolysis (Walsh and Cummins, 1976; Prut and Belzung, 2003). The aim of the present study was to evaluate the neurobehavioural

Table 2. Statistical analysis of behavioural performance of first and second part in OFT

Treatment (mg/kg)	Time spent in center(s) med (min-max)	Squares passed (n) med (min-max)	Center crossings (n) med (min-max)	Rearings (n) med (minmax)
1st Part				
Control(saline)	5 (1-17)	35 (7-84)	2 (2-6)	21 (13-35)
CTX 50	1 (1-29)	12 (3-40)	2 (2-4)	14 (10-19)
CTX 100	5 (1-11)	51 (6-78)	2 (2-4)	18 (9-30)
CTX 200	1 (1-23)	46 (36-78)	2 (2-12)	23 (13-32)
NAC 50	4 (1-10)	57 (29-90)	4 (2-10)	23 (17-31)
NAC 100	4 (1-13)	37 (3-61)	2 (2-4)	19 (5-24)
NAC 200	16 (15-19)*1	37 (16-51)	6 (4-8)*1	34 (33-37)*1
Diazepam 1	7 (1-16)	27 (31-67)	4 (2-6)	14 (12-21)
Diazepam 2	19 (17-35)*2	26 (17-79)	6 (4-8)*1	13 (11-28)
2nd Part				
Control(saline+saline)	4 (1-6)	57.5 (45-75)	3 (2-4)	20.5 (12-30)
Diazepam 2+saline	9 (7-15)*2	43.5 (42-60)	6 (4-6) *4	14.5 (12-20)
CTX200+saline	4 (1-13)	49.5 (20-90)	2 (2-4)	20 (15-30)
NAC200+saline	5.5 (5-7)*3	45.5 (6-68)	6 (4-6) *5	28 (27-34)*4
NAC200+CTX200	3.5 (1-13)	47.5 (26-68)	2 (2-6)	17.5 (12-22)

n=8-8 per group; **CTX50:** Ceftriaxone 50 mg/kg; **CTX 100:** Ceftriaxone 100 mg/kg; **CTX 200:** Ceftriaxone 200 mg/kg; **NAC 50:** N-acetylcysteine 50 mg/kg; **NAC 100:** N-acetylcysteine 100 mg/kg; **NAC 200:** N-acetylcysteine 200 mg/kg, Diazepam 1mg/kg, Diazepam 2 mg/kg. Comparisons were done between control and other groups. (Mann-Whitney U test, The Bonferroni correction was used to adjust the P value for each hypothesis Values of p<0.006 (for first part) and p<0.010 (for second part) were considered statistically.)
*1p=0.003, *2p<0.001, *3 p=0.007, *4p=0.005, *5p=0.002

effects of acute administration of NAC and CTX over anxiety using open field and elevated plus maze tests.

2. Material and methods

Animals

Experimentally naive, one hundred and twelve, adult, male, Wistar albino rats (290-320 g each), obtained from Kobay Laboratories (No. 003679) were allocated to fourteen groups (n=8 per group). Rats were maintained for at least 2 weeks before the experiments. The rats were housed in standard plastic cages (4 animals per cage), maintained under standardized conditions of light 12-h light/dark cycle, room temperature (22±2°C) and humidity (60%), with free access to food (standard chowpellets) and tap water in Dollvet Laboratories animal facility.

Ethics

This study was approved by the Animal Experiments Local Ethics Committee of Dollvet Laboratories (Approval number:13-08). All the experiments were carried out in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals (USA, NIH).

Behavioural tests

Elevated plus-maze test

The elevated plus maze is a pharmacologically validated model for assessment of anxiety state in rodents. The plus-maze consisted of two open arms (50x10cm), facing each other, and two closed arms (50x50x40 cm) with an open roof and was elevated to a height of 50cm and placed in a quiet dimly lit room. The following measures were taken by an observer during a 5 min test period after the rat had been placed on the centre of the maze facing an open arm: The number of entries into (with the criterion for an arm entry defined as, all four paws in an arm of the maze) and the time spent in each of the two types of arm. Distribution of behaviour (arm entries and time spent) on the maze was additionally calculated as 'percent total' both for frequency and duration measures. After each subject completes its test session, fecal boli and urine are removed, surfaces are wiped with 70% ethanol and the test chamber is allowed to dry completely before starting another subject and rats were returned to their home cages.

Open field test

The open field apparatus consisted of a square arena 100cm × 100cm with 40cm walls. The floor was subdivided in a centre and margin compartment with 16 squares. Rats were placed singly in one corner of open field, following measures were observed during a 5-min exposure period; rearings (vertical locomotion), center crossings, squares passed (horizontal locomotion) and time spent in the periphery and center of the arena. At the end of each trial, mice were returned to their home cages, and test box was carefully cleaned with %70 ethyl alcohol and permitted to dry between tests. It is a validated test that benzodiazepines increase the amount of time a rodent will spend in the center of the arena (Bailey and Crawley, 2009).

Drugs and experimental groups

The drugs administered were; CTX sodium was a gift from Deva Pharmaceuticals (50, 100 and 200 mg/kg, i.p.), NAC was a gift from Bilim Pharmaceuticals (50, 100 and 200 mg/kg, i.p.) and diazepam (1 and 2 mg/kg, i.p.) (Sigma-Aldrich). All drugs were dissolved in physiological saline, freshly prepared on the days of the test and administered i.p. in a volume of 1 ml/kg. Control animals received physiological saline. Testing was conducted between 13.00 pm and 18.00 pm. and rats acclimated to the testing room for a minimum of 45 min prior to testing for all tests. First, open field (OF) behaviour was measured as part of a locomotor testing paradigm. The plus-maze procedure was validated pharmacologically in rats by systemic treatment with a benzodiazepine, diazepam.

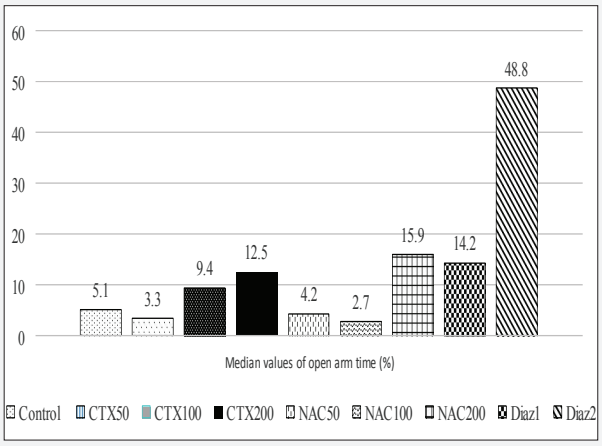

Fig. 1. EPM-1st Part

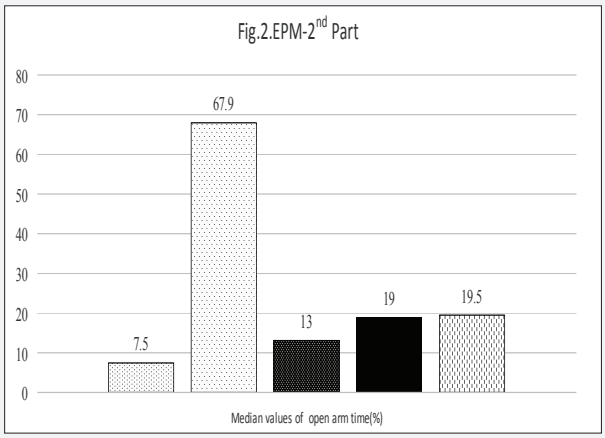

Fig. 2. EPM-2nd Part

Diazepam (2 mg/kg) and in the second part diazepam 2 mg/kg+saline caused a significant increase in the percentage of open arm entries and percentage of open arm time when compared with controls, applied 30 min before testing without significantly changing locomotor activity. Treatments were given 65 min before the elevated plus maze testing because of the pharmacokinetics of NAC and CTX (Rebuelto et al., 2003, Linck et al., 2012). The doses of diazepam, NAC and CTX were selected on the basis of those reported in the literature (Kurt et al., 2003; Chakraborti et al., 2008; Karaman et al., 2013). In the first part of the experiments, three different doses of each drugs were administered and in the second part, the combination of statistically significant doses of NAC and CTX were evaluated.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 software. All numerical data are expressed as median values (minimum-maximum). For each continuous variable, normality was checked by Kolmogorov Smirnov and Shapiro-Wilk tests and by histograms. Comparisons between groups were applied using Kruskal Wallis test were used for the data not normally distrubuted. Since analysis of variance was significant, comparisons were applied using Mann-Whitney U test. Pre-post measures data were analysed using Wilcoxon test. The Bonferroni correction to adjust the P value for each hypothesis to 0.0055 and 0.010. Values of $p<0.006$ and $p<0.010$ were considered statistically.

3. Results

There were no significant differences in the body weights when compared before and after the injections in the statistical analysis ($p>0.050$).

Elevated plus-maze test

The results of the first part of EPM test are presented in Table 1. The analysis of elevated plus maze test revealed significant differences in the percentage of open arm entries and time spent in open arms ($H=52.38$, $H=34.33$ respectively, $p<0.050$) but there were no significant difference between groups for the number of total arm and close arm entries ($H=11.35$, $H=8.53$, $p>0.050$) those can be implemented as predictors of locomotor activity. The post-hoc test showed that the percentage of time spent in the open arms was increased only by diazepam 2 mg/kg and NAC 200 mg/kg ($p<0.0055$, $U=0.00$, $U=2.00$ respectively) versus control and no significant differences were detected among remaining groups when compared to the control (Fig. 1). The analysis of the percentage of open arm entries revealed a significant effect of treatment with 2 mg/kg of diazepam ($p<0.0055$ $U=0.00$) and 200 mg/kg CTX ($p<0.0055$, $U=1.50$) 200 mg/kg NAC ($p<0.0055$, $U=5.00$) groups when compared to the control group (Table1). No significant differences were found in the remaining groups. It should be mentioned that determined elevation in the percentages of time spent in open arm and open arm entries of aforementioned drugs doses were not due to an increase in locomotor activity because the analysis of total arms and close arms entries data of these groups, did not revealed a significant difference when compared to control group. In EPM, a slight elevation was evaluated with CTX200 mg/kg that were not significant but a significant difference is determined with CTX 200mg/kg in the percentage of open arm enteries that may be implemented in favour of anxiolytic-like behaviour. 200 mg/kg of CTX and NAC, 2 mg/kg of diazepam is selected for the second part of experiments.

The data of the EPM test in the second part of the experiments, is shown in Table 1. The non-parametric-Kruskal Wallis test showed significant differences in the percentage of time spent in the open arms ($H=23.36$, $p<0.050$). Intergroup post-hoc comparisons showed that this parameter was significantly increased by the groups treated with 2 mg/kg of diazepam+saline ($U=0.00$, $p<0.010$) and 200 mg/kg NAC+saline ($U=7.00$, $p<0.010$) versus control group (saline+saline) (Fig. 2). The analysis of the percentage of open arm entries showed significant difference among treatments ($H=25.85$, $p<0.050$), this effect is augmented in 2 mg/kg of diazepam +saline ($U=0.00$, $p<0.010$), 200 mg/kg NAC+saline ($U=0.00$, $p<0.010$) and 200 mg/kg CTX+saline groups($U=5.00$, $p<0.010$) respect to control group (Table 1). The anxiolytic-like behavioural change seen with these drugs may be implemented as their changes were not due to a non-specific affect of these drugs on locomotor behaviour because the data analysis of these treatments, number of closed arm entries and total arm entries, did not revealed a significant difference when they were both compared to control. In addition to this, in Kruskal Wallis analysis, the number of closed arm entries showed no significant differences among groups ($H=9.06$, $p>0.050$), but there were significant

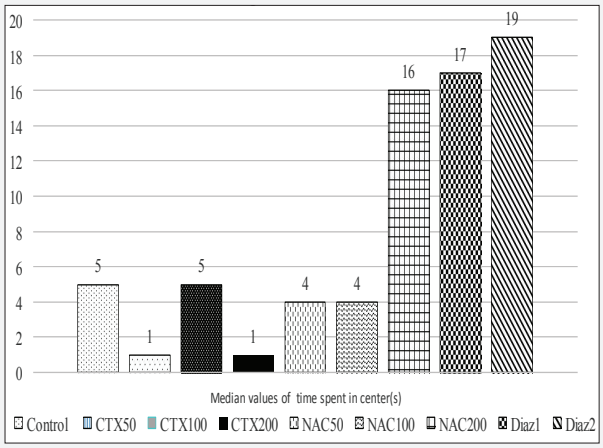

Fig. 3. OFT-1st Part

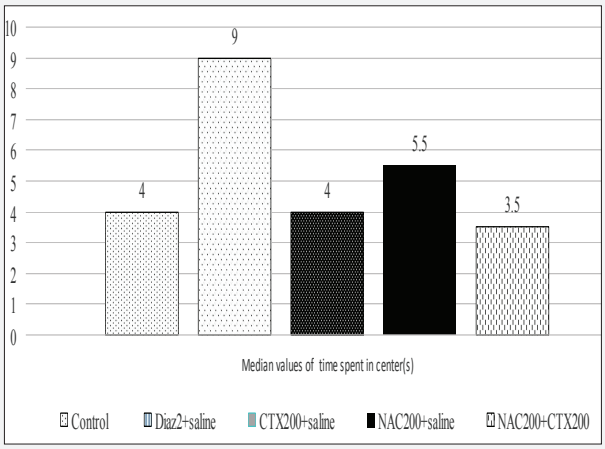

Fig. 4. OFT-2nd Part

difference in the number of total arm enteries among groups (H=11.04, p<0.050), intergroup post-hoc analysis is made to show the differential groups, there were no significant difference between all groups when compared to control but only significant difference was determined between 2 mg/kg Diazepam+saline group other and 200 mg/kg CTX+saline group when they were compared to each other. The post-hoc comparision of CTX 200 mg/kg+saline and CTX combination with NAC did not revealed any significant difference in EPM when compared to each other. Post-hoc comparision of NAC 200 mg/kg+saline and NAC combination with CTX did not presented statistically significant difference in EPM when compared to each other.

Open field test

The effect of the treatments administered in the first part of the study on time spent in the centre, number of squares passed, center crossing and rearings in the OFT is presented in Table 2. The analysis of non-parametric-Kruskal Wallis test showed significant differences in those aforementioned measures (H=34.81, H=17.17, H=28.59, H=35.21, p<0.050, respectively). Post hoc analysis of the time spent in the centre presented that doses of NAC and Diazepam increased dose-dependently, but it was significantly increased with only diazepam 2 mg/kg (p<0.0055, U=1.50) and NAC 200 mg/kg (p<0.0055, U=5.00) versus control and no significant differences were found in the remaining groups when compared to control (Fig. 3). Number of squares passed, did not show any significant difference between groups (with 200 mg/kg NAC, p>0.0055, U=31.00 and 2 mg/kg Diazepam, p>0.0055 U=29.50) when they were compared to control, so the anxiolytic like effets of NAC and Diazepam may be implemented as they were not due to an increase in locomotor activity. Post-hoc analysis for center crossing showed significant increase with only diazepam 2 mg/kg (p<0.0055, U=5.00) and NAC 200 mg/kg (p<0.0055 U=5.50) versus control and no significant differences were found in the remaining groups when compared to control (Table 2). For rearings measurements, post-hoc analysis presented significant difference only for 200mg/kg NAC dose when compared to control (p<0.005, U=5.00), there were no differences in other groups when they compared to control group(Table2). The CTX 200 mg/kg presented a slight elevation in time spent in center parameter when CTX 100 mg/kg slightly decreased but those were not significant. The elevation seen in squares passed with CTX100 mg/kg was not significant, either. 2 mg/kg diazepam, 200mg/kg CTX and NAC were selected for the second part of experiments.

The effect of administered treatments on time spent in the centre, number of squares passed, center crossings and rearings in the open field in the second part of the study is presented in Table 2. The analysis of the non-parametric test revealed significant differences in time spent in the center, center crossings and rearings (H=14.44, H=25.12, H=21.44 respectively, p<0.050). Post-hoc comparisons showed that time spent in centre and centre crossings significantly augmented in 2 mg/kg Diazepam+saline (U=0.00, U=6.00, p<0.010) and 200 mg/kg NAC+saline(U=7.50, U=4.00, U=6.50, p<0.010) (Fig. 4). In addition to these, rearing was also augmented significantly with 200 mg/kg NAC+saline group (U=6.5, p<0.010) when compared with control group (saline+saline) (Table2). Post-hoc comparision of NAC 200 mg/kg+saline and NAC combination with CTX revealed that there were significant decrease in center crossings and rearings in combination (U=6.00, U=0.00, respectively, p<0.010). The post-hoc comparision of CTX 200 mg/kg+saline and CTX combination with NAC did not revealed any statistically significant difference in OFT when compared to each other.

4. Discussion

Stress is considered as the response of an organism to environmental stimuli (stressors) that threaten its internal equilibrium, also called homeostasis (Ramos and Mormede, 1998). Stress causes changes in anxiety-like states by effecting emotional behaviour in regulation of stress responses and the central nervous system plays a crucial role. Stressor factors effects the neurobehavioural profile of an organism and can cause an anxiety-like syndrome and behavioural factors such as emotionality that are the valid predictors of stress susceptibility. This roles consist of complex neurochemical pathways (Chakraborti et al., 2008). The anxiolytic-like baseline behaviour is determined in the open field test if a rodent spends significantly more time exploring the center area. The two factors have been suggested to trigger the anxiety-like behaviour in this test: individual testing (Social isolation, a result of the physical separation from cage mates when performing the test) and the second one is: the stress created by the brightly lit, unprotected, novel test

environment, agoraphobia as the arena is very large relative to the animal's breeding or natural environment. The elevated plus maze test that also takes the same advantage of the natural tendency of rodents to explore novel environments. Rodents tend to avoid the open areas, favoring darker, more enclosed spaces (Hogg,1996; Bailey and Crawley, 2009). In the present study, we explored the locomotor activity and anxiolytic-like effects of three different doses (50, 100 and 200 mg/kg, i.p.) of NAC and CTX, and also their combination in open field and elevated plus maze tests, respectively. The results of the study showed that highest dose of NAC produced anxiolytic-like effects without changing the locomotor activity in both open field and elevated plus maze tests. Our results are in agreement with Chakraborti et al. (2008), who has evaluated acute administration of the doses of 100 and 200 mg/kg NAC on restraint wistar rats and reported the anxiolytic like effects with 200 mg/kg NAC in elevated plus maze and open field tests. In mice study of Bernabucci et al. (2012), study, it is shown that a single injection of NAC (100 mg/kg, i.p.; 30 min before the test) causes analgesia in the second phase of the formalin test by the activation of system xc. The System xc-is a membrane antiporter that mediates the chloride-dependent, sodium-independent, 1:1 exchange of extracellular L-cystine and intracellular L-glutamate, and provides the intracellular L-cysteine required for the synthesis of glutathioe (GSH) and oxidative protection. The exchange-mediated export of L-glutamate represents a non-vesicular route of glutamate release through which it can participate in either neuronal signalling or excitotoxicity pathology. In the present study, behavioural changes produced by the highest dose of NAC may be a result of increased GSH and glutamate. Free radicals play important role in health and disease because of their being highly reactive moieties. The central nervous system is especially vulnerable to free radical damage because of brain's high oxygen consumption, abundant lipid content and relative paucity of antioxidant enzymes. For this reason cell damage formed by the oxidative stress has been suggested to lead to several CNS disorders (Chakraborti et al., 2008). In the study of Chakraborti et al. (2008), it is suggested that therapeutic effects shown with NAC may be provided as a result of elevation of GSH levels as NAC is likely to stimulate the L-cystine/L-glutamate membrane exchanger System xc that mediates non-vesicular release of glutamate from astrocytes and microglia, thereby also promotes glutamate release into the extrasynaptic compartment, resulting in the stimulation of presynaptic mGlu2/3 receptors. Lutgen (2009), have reported that acute inhibition of system xc with sulphasalazine was anxiogenic in both elevated plus maze and open field paradigms. Additionally, the effects of sulphasalazine have been completely reversed with administration of NAC after sulphasalazine treatment in the EPM and OFT. Nonvesicular glutamate released during cystine-glutamate exchange activates extrasynaptic glutamate receptors by stimulating extrasynaptic group II mGluRs without exerting postsynaptic effects. This extrasynaptic glutamate seems to inhibit synaptic glutamate release, resulting a protection from excitotoxicity (Bridges et al., 2012). This factors may have limited the extreme behaviour responses in ambulation and helps the interpretation of anxiolytic like behaviour without any change in locomotion. The stimulated system xc may have given rise to an apparent hyperglutamatergic state largely involving either increased glutamate release into the extrasynaptic compartment or enhanced GSH levels after increased cystine transport. In contrast to the extrasynaptic compartment, stimulated system xc activity may lead to a hypoglutamatergic state within the synapse. This may have been occured as a result of increased activation of group II mGlu Rs which have been shown (Bridges et al., 2012) to inhibit synaptic release of glutamate and dopamine. In Baumann et al. (2008) study, it has been shown that dopamine (DA) nerve terminals in the nucleus accumbens are important mediators of amphetamine-induced locomotor activity, especially ambulation (horizontal locomotion). Destruction of DA nerve terminals in the nucleus accumbens markedly inhibited ambulation produced by systemically injected 3,4-methylenedioxy-N-methylamphetamine (MDMA). Moreover, microinjection of (+)- MDMA into the accumbens stimulated ambulation, and this effect has been shown to involve DA but not 5-hydroxytryptamine (5-HT). According to Baumann et al. (2008), study such findings implicate n.accumbens DA in the mechanism of MDMA's locomotor actions. Additionally, pretreatment with D1 or D2 receptor antagonists can reduce ambulation produced by i.p. administered MDMA, suggesting both receptor subtypes are involved (Baumann et al., 2008). In conclusion, while the beneficial effects of mGluR2/3 agonists in clinical trials are inconclusive, the results in preclinical and clinical studies suggest that stimulation of the mGluR2/3 receptor may lead to improvements in schizophrenic symptoms as would increased extrasynaptic glutamate potentially through system xc (Bridges et al., 2012). There is a growing amount of preclinical and clinical reports representing therapeutic potential of administration of NAC for psychiatric diseases. Clinically, NAC has been used as an adjunct therapy in the treatment of schizophrenia and shown beneficial effects and improvements in overall symptom severity, mismatch negativity and improved prefrontal cortex synchronization using EEG recordings (Chakraborti et al., 2008). Multiple nonvesicular release mechanisms may contribute to extracellular levels of glutamate, nonvesicular release of glutamate into the extrasynaptic compartment is incapable of stimulating high-affinity glutamate receptors in the synapse unless EAAT function is compromised. EAATs have been shown to clear glutamate released by system xc, supporting the idea that these transporters function to compartmentalize extracellular glutamate into multiple domains (e.g., synaptic, extrasynaptic) (Bridges et al., 2012). In addition to these, it has been also shown to be a potent activator of system xc. The beta-lactam family of antibiotics are shown to increase the expression and function of GLT-1 in vitro and in vivo. GLT-1 upregulation is attributable to an increase in gene transcription through the nuclear factor signaling cascade. Genetic deletion of glial GLT-1 produced elevated extracellular glutamate levels (Trantham-Davidson et al., 2012). In the rat study of Verma et al., 2010, presented that a single dose of 100 mg/kg i.v CTX was given 2 h after the reperfusion in cerebral ischemia/reperfusion injury also resulted in upregulation of GLT-1 protein. And also in the study of Maculoso et al. (2013), it is shown that a single preoperative dose of ceftriaxone (200 mg/kg) caused analgesia in humans and also performed animal

studies to examine whether a single dose of ceftriaxone (200 mg/kg) was sufficient to induce analgesia. A single intraperitoneal injection of ceftriaxone could also caused analgesia in mouse models of inflammatory or postsurgical pain, and upregulated GLT-1 in the spinal cord. Ceftriaxone is also a transcriptional regulator of xCT, Nrf2, and thereby increases system xc activity (Lewerenz et al., 2009). In the present study, acute administration of three different doses of CTX was insufficient to produce anxiolytic like effects. The dose 200 mg/kg CTX increased only the percentage of open arm entries in EPM. The combination of acute administration of NAC 200 mg/kg and CTX 200 mg/kg fail to produce anxiolytic like effects when they were administered in combination in both tests in the second part of the experiments. Post-hoc comparision of NAC 200 mg/kg+saline and NAC 200 mg/kg combination with CTX 200 mg/kg revealed a significant decrease in center crossings and rearings in OFT. A decrease is also seen in the compared varibles of EPM in the analysis of post-hoc comparision of NAC and its combination with CTX but it was not significant. It should be kept in mind that system xc and EAATs are different from each other. The system xc activity was chloride-dependent, sodium-independent, and electroneutral, properties that clearly differentiate it from the sodium-dependent, electrogenic EAATs. It is noteworthy that the EAATs have been implicated in the transport of both cystine and cysteine GSH, also serves to modulate the synaptic transmission. System xc is capable of contributing to the antioxidant capacity of a cell through the maintenance of glutathione levels and to glutamate homeostasis. The dual nature of system xc may be advantageous because it permits increased antioxidant capacity via cystine uptake to buffer the potential toxic effects of glutamate release (Bridges et al., 2012). This effect may have served as an advantage of NAC when used alone. The results produced by CTX in two parts of the study may indicate that anxiolytic-like behavioural changes is not directly modulated by GLT-1 or perhaps a higher dose or more likely a sub or chronic treatment regimen of CTX could produce alterations in these tests. When they are used in combination NAC and CTX, glutamate changes may not be stabile enough to produce significant changes or both activators may be insufficient to balance the homestasis. It is noteworthy that in general, a neuroprotective role has been clearly established for glutathione and NAC. The precise role of glutathione balance in anxiolytic-like behavioural changes needs to be conducted with further studies. In clinical approach for the bacterial infections, 1 or 2 g for adult and 50 to 100 mg/kg for pediatric patients for CTX administrations is given once daily. The dose used in this study (200 mg/kg per day) is relatively high as compared with clinical practice but normal antimicrobial CTX doses are likely to be sufficient to induce GLT-1 upregulation in humans (Chu et al., 2007). However, chronic use of CTX, a parenteral antibiotic, is not practical for routine treatment of behavioural disorders and also may change the microflora of the gut in favour of a superinfection and in addition to this, concern of antibiotic resistance with the repeated doses of the drug should be kept in mind. Given the role of EAATs to compartmentalize glutamate into functionally distinct pools (e.g., synaptic, extrasynaptic), it is possible that receptors located in the synapse or in the extrasynaptic compartment are being stimulated by glutamate diffusing across microdomains (Bridges et al., 2012). It may be a further investigation area for us to demonstrate whether the possible interaction of glutamate regulation and anxiety behaviour may arise from a loss of signal integrity instead of, or in addition to, abnormal levels of receptor activation. Glutamate homeostatic control mechanism, includes two main pathways: the cystine/glutamate exchanger system xc and the glial glutamate transporter EAAT2/GLT-1. Changes in the balance between synaptic and extrasynaptic glutamate levels in turn influence signaling through pre and postsynaptic glutamate receptors, and thus affect synaptic plasticity and circuit-level activity. Synchronization of cortical activity is regulated by complex inter-neuronal connections (Reissner and Kalivas, 2010).

Research literature indicating the role of glutamate pathways of these drugs is becoming increasingly important to reveal the relation between glutamate and psychiatric conditions. There is a growing body of literature of potential benefit of NAC and CTX in a wide range of neuropsychiatric disorders (Linck et al., 2012; Alajaji et al., 2013). Further preclinical efforts to define their neuropsychopharmacological activity profile could be useful for more precisely defining and understanding the entire therapeutic potential of these drugs either used as monotherapy or in combination with presently used drugs. The results of the present study suggest that system xc- contributes to states of anxiety, increased system xc - may represent an effective therapeutic endpoint; however the specific brain region and neurotransmitter system mediating these behaviours will require further investigations.

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A rare upper gastrointestinal system bleeding case: Aortoesophageal fistula

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ABSTRACT

Aortoesophageal fistula is a rare condition with fatal prognosis. It is one of the life-threatining causes of massive upper gastrointestinal bleeding. With this case report, we will discuss an instance of a fatal aortoesophageal fistula in a patient to whom was implanted a stent due to an aorta aneurysm. In endoscopic examination blood clot on the mouth of the fistula was visualized.

J. Exp. Clin. Med., 2014; 31:51-53

1. Introduction

Gastrointestinal system (GIS) bleedings related to aorto-esophageal fistulas (AEF) are seen quite rarely and their mortality rate is reported as high. AEF was first described in a soldier who swallowed a beef with bone and died in 1818 (Dubrueil, 1818). Thoracic aort aneurysm is the most common cause of the AEF. Other important factors causing AEFs are foreign body aspiration and advanced esophageal cancer (Ghosh et al., 2011). Sepsis, hemorrhage, and infection that spreads to the surrounding are the most important problems encountered in the treatment of these patients. Chest radiography, computerized tomography (CT), CT aortography, and endoscopy are the most important diagnostic tools in the AEF (Amin et al., 1998; Flores et al., 2004).

Endoscopy in the diagnosis of the AEF has high sensitivity and specificity. Open surgical and endovascular treatments are the most preferred choices among the limited treatment options (Flores et al., 2004). With this presentation we examine a case of upper GIS bleeding related to AEF.

2. Case

A 69-year-old man patient who had black coloured stool for the last two days admits to the emergency department with the complaint of bloody vomiting. He had two stent placements due to aortic aneurysm and he was using aspirin. During the physical examination he was conscious and cooperative. Blood pressure: 110/80 mm-Hg, pulse: 118/min. On the left lung lower zones respiratory sound was not heard. Laboratory tests revealed an urea of 80 mg/dL, creatinine of 1.3 mg/dL, albumine of 2.1g/dL, white blood cell of 13000/µL, haemoglobin (Hgb) of 6.29 g/dL, platelets of 179000/µL, sedimentation rate of 30 mm/h, prothrombin time of 15.5 seconds and an INR of 1.23. Hepatitis markers were negative.

The patient was hospitalized with a preliminary diagnosis of an aspirin related GIS bleeding. Medical treatment has been started. With erythrocyte transfusion, Hb was elevated over 10 g/dL. On the posterio-anterior (PA) chest X-ray; there were blunt image of left pleura, thoracic aorta with aneurysmatic condition and enlarged mediastinum (Fig. 1). The general state of the patient was rapidly recovered with

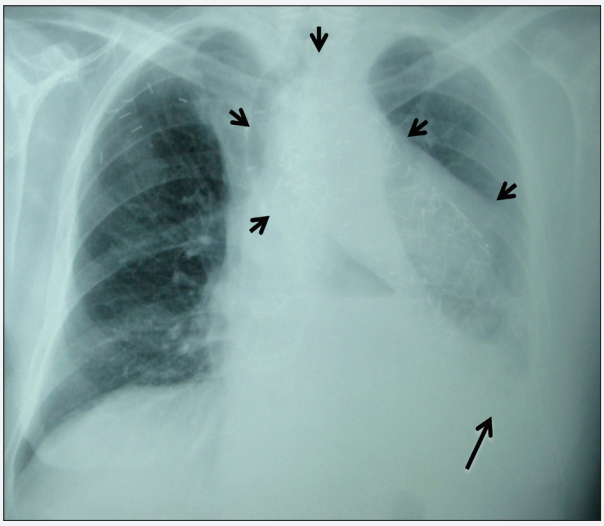

Fig. 1. The posterior-anterior (PA) chest X-ray showing the blunt image of the left pleura (large black arrow) and aneurysmatic thoracic aorta with stent that enlarges the mediastinum, displaces the trachea to the right and superimposed on the left lung (short black arrows).

the treatment of the huge clot (Fig. 2) that fills the half of the esophagus lumen. An oval defect giving oscillation on the middle esophagus, 30 cm from the incisor teeth was seen during the endoscopy performed two days after his arrival. Because AEF is suspected no further actions are taken and the procedure was terminated. The department of cardiovascular surgery was urgently consulted. During the thoracic computerized tomography (Fig. 3 a, b, c) AEF was confirmed.

There were two options to close the aortoesophageal fistula: Surgery and endovascular stent placement. Because of high mortality risk the surgery was not performed. Endovascular stent placement was also not applicable because diameter of the available stent was smaller than the stent already placed. Following a sudden nausea vomiting a massive bleeding began. The patient was developed respiratory and cardiac arrest. He was intubated. An attempt to adjust his hemodynamics was made but the patient could not be recovered and died on the third day of his arrival.

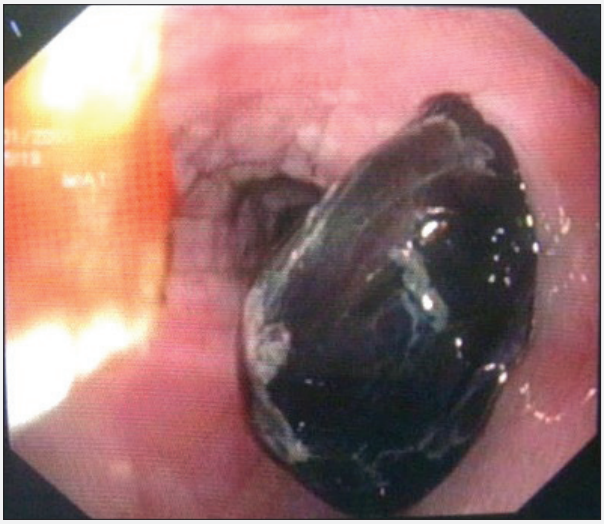

Fig. 2. Clot in the aortoesophageal fistula region owerflowing into the esophagus lumen.

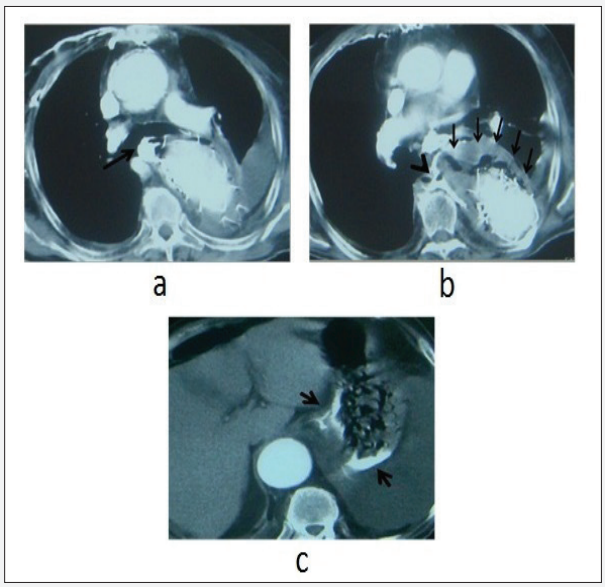

Fig. 3. CT images taken on the arterial phase after giving only intravenous contrast agent; a) Contrast agent nearly filling the whole esophagus location at the level of trachea bifurcation (black arrow). b) Collection of air and fluid (sequential black arrows) in the periaortic area together with the extravasated aortic contrast agent (black arrow head). c) Gastric mucosa coated with the contrast agent (small black arrows).

3. Discussion

There are two types of AEF. In the primary AEF thoracic aortic aneurysm, swallowing foreign objects, esophageal malignancy and traumatic aorta injuries are the main reasons; its incidence is 0.04-0.007% (Voorhoeve et al., 1996; Saers and Scheltinga, 2005). In the secondary AEF stents used during the repair of aortic aneurysms, aortic surgery and esophageal surgery are the main reasons (Grundy and Glees, 1997; Kieffer et al., 2003; Okita et al., 2005). Its incidence is 0.7-1.7% (Hollander and Quick, 1991; Okita et al., 2005). Endoscopy, CTscan and aortography are used to diagnosis. Classical symptoms are dysphagia, mid-thoracic pain, sentinel arterial hemorrhage and bleedings then a asymptomatic period (Chiari's triad). Also in our case a slow course without pain, a short asymptomatic period and later an abundant bleeding were seen. The appearance of the clot that gives oscillation and overflowing into the esophagus lumen when entering first with the endoscope was visually stunning. It is reported that in these cases endovascular repair is the best choice, the morbidity and mortality rate of the surgical approach is recommended high (Hance et al., 2003; Flores et al., 2004). The proposed treatment approach is placement of an endovascular stent to gain time for surgical treatment of the esophagus. Otherwise the risk of mediastinitis and stent infection is increasing (Metz et al., 2006).

In these cases, it is important that the clot should not be removed. In summary, in AEF cases early diagnosis, the placement of endovascular stents if applicable and surgical approach are vitally important. Physicians should consider aortoesophageal fistula in cases of massive gastrointestinal bleedings.

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Thyrotropin-secreting pituitary macroadenoma presented with pulmonary embolism

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ABSTRACT

Although studies have indicated that hyperthyroidism is associated with pulmonary embolism, an association with thyroid stimulating hormone-producing pituitary adenoma (TSHoma) and pulmonary embolism has not been reported so far. A 43 year-old man hospitalized in the pulmonary diseases department with the diagnosis of pulmonary embolism. Thyroid function tests revealed increased thyroid hormone concentrations in the presence of inappropriately elevated serum TSH levels. Pituitary magnetic resonance imaging showed a 15 mm macroadenoma. Serum concentrations of the alpha-subunit of TSH (αTSH) and the αTSH/TSH molar ratio were 2.7 IU/L and 2.7, respectively. He underwent transsphenoidal adenomectomy with the diagnosis of TSHoma. Histopathological staining confirmed the diagnosis of TSHoma. We report an unusual case of pulmonary embolism accompanying TSHoma. TSHoma, like other causes of hyperthyroidism, may be associated with pulmonary embolism.

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1. Introduction

Thyroid hormones are important mediators of some systems such as metabolism, embryonal development, cellular differentiation, and coagulation systems. Hyperthyroidism is a common endocrine disorder and various changes in the coagulation-fibrinolytic system have been described in patients with excess thyroid hormones. Hyperthyroid patients show tendency toward thromboembolic complications (Hofbauer and Heufelder, 1997). Patients with overt hyperthyroidism have prothrombotic abnormalities leading to increased risk of both arterial and venous thrombotic complications. It is showed increased risk of pulmonary embolism in hyperthyroidism (Lin et al., 2010).

Thyroid stimulating hormone-producing pituitary adenomas (TSHoma) are rare causes of hyperthyroidism. They account for less than one percent of all functioning pituitary tumors and much less than one percent of all cases of hyperthyroidism (Socin et al., 2003). We present a case with pulmonary embolism accompanying TSHoma.

2. Case

A 43-year old man admitted to pulmonary diseases department with chest pain, dyspnea and hemoptysis. He had no significant past medical history. The family history was also unremarkable for any medical problems, including thrombophilia. On physical examination, blood pressure was 110/64 mmHg, pulse was regular at 78 beats/min and respiratory rate was 20 breaths/min. There was no goitre on palpation and chest auscultation was normal. The oxygen saturation was 83% at room air. Electrocardiogram showed normal sinus rhythm. Chest computed tomography (CT) scan showed right pulmonary basal posterior and, left pulmonary basal lateral consolidation and infarct formation. In pulmonary CT angiography, embolism was detected in the branches of both pulmonary arteries. Transthoracic echocardiography was normal. Lower extremity Doppler ultrasonography did not reveal any venous thrombosis. After initial heparinization, treatment was continued with warfarin. On further questioning, he complained of unintentional weight loss, palpitations and heat in-

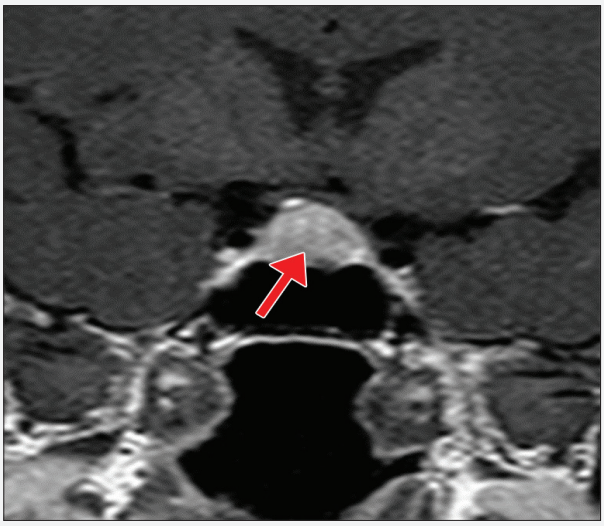

Fig. 1. Magnetic resonance imaging (MRI) showing a 15 mm pituitary macroadenoma.

tolerance which were present for several years. Laboratory measurements revealed high thyroid hormone concentrations [free T3: 5.7 pg/mL (normal range 2.0-4.4), free T4: 2.6 ng/dL (normal range 0.9-1.7 ng/dL)] in the presence of inappropriately elevated levels of serum TSH [TSH: 10.6 μIU/mL (normal range 0.27-4.2 μIU/mL)]. Anti-thyroglobulin and anti-thyroidperoxidase antibodies were below detection levels. Serum concentrations of the α-subunit of TSH (αTSH) and the αTSH/TSH molar ratio were 2.7 IU/L and 2.7 respectively. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), adrenocorticotropic hormone (ACTH), prolactin, testosterone, cortisol and insulin-like growth factor-1 (IGF-1) were within the normal range. Magnetic resonance imaging (MRI) showed a 15 mm pituitary macroadenoma with suprasellar extension but without cavernous sinus invasion (Fig. 1). Visual field examination was normal. Based on these findings, a diagnosis of TSHoma was made. Preoperatively two doses of somatostatin analogues (octreotide LAR 30 mg intramuscular) monthly was administered. Two months later thyroid function tests normalized (TSH: 3 μIU/mL, free T3: 1.5 pg/mL, free T4: 0.9 ng/dL). Six months later, after the resolution of pulmonary embolism transsphenoidal surgery was performed. Histopathological examination revealed a TSHoma with strong positive TSH staining. Two months after the surgery, TSH, free T3 and free T4 were 7.3 μIU/mL, 5.9 pg/mL and 2.3 ng/dL, respectively. Postoperative pituitary MRI showed a six mm left sided residual adenoma. After initiation of somatostatin analogue, the patient remained euthyroid and asymptomatic.

3. Discussion

The presence of high serum T4 and T3 concentrations and normal or high serum TSH concentrations in the presence of anatomic evidence of a pituitary tumor by MRI constitute very strong evidence that the patient has a TSHoma. Patients with TSHomas and hyperthyroidism must be distinguished from those with the syndrome of resistance to thyroid hormone (RTH) (Paolo et al., 2009). In addition to the described findings, high molar ratio of serum alpha-subunit to TSH, strongly positive TSH staining of the tumor and a good response to octreotide indicate presence of TSHoma in this patient.

It is well known that excess or deficit of thyroid hormones interact with coagulation-fibrinolytic system causing a hypercoagulability in the state of hyperthyroidism and vice versa (Squizzato et al., 2007). TSH-secreting adenomas secrete biologically active thyrotropin in an autonomous fashion. Most patients have typical symptoms of hyperthyroidism. Various factors contribute to hypercoagulability in hyperthyroidism such as increased hepatic protein synthesis, acute phase reactants, plasma thrombin and plasmin activity and increased tissue factor, one of the major triggers for the extrinsic pathway of coagulation (Erem, 2001). A systematic analysis supports an increased risk of venous thrombotic complications, including cerebral venous thrombosis, deep vein thrombosis and pulmonary embolism in patients with hyperthyroidism (Franchini et al., 2011). In a retrospective case-controlled study pulmonary embolism was found 2.3 times greater for hyperthyroid patients than control group after adjustment for the risk factors which could be interfering with thrombosis (Lin et al., 2010).

Hypercoagulation, endothelial dysfunction and hemodynamic changes are the important pathophysiological factors for thrombosis.It is demonstrated shortened activated thromboplastin time and higher fibrinogen levels in hyperthyroid patients when compared with euthyroid patients (Lippi et al., 2009). In our patient, activated thromboplastin time was found to be normal. In one study including 41 patients with overt hyperthyroidism compared with euthyroid controls, patients with hyperthyroidism had increased levels of plasma fibrinogen, factor IX, von Willebrand factor (VWF), antithrombin and plasminogen activator inhibitor (PAI-1), along with decreased levels of factor X and tissue plasminogen activator (t-PA), thus suggesting a globally reduced plasma fibrinolytic activity (Erem et al., 2002). These parameters were not investigated in our patient since anticoagulation therapy was already started.

It has been suggested that thrombophilia screening is controversial, except in patients with first venous thrombosis at young age and/or a strong family history of venous thrombosis (Lijfering et al., 2009). In our patient, personal and family medical history were unremarkable. In addition, no other risk factors for thrombosis were detected.

Octretoide is an effective treatment option for TSHomas, especially for inoperable and incompletely removed tumors (Socin et al., 2003). The excellent response to somatostatin analogues in TSHomas may also prevent the risk of pulmonary embolism in such patients.

To our knowledge this is the first reported case of pulmonary embolism accompanying TSHoma. TSHoma, like other causes of hyperthyroidism, may be associated with pulmonary embolism.

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Late diagnosis of trans-scaphoid perilunate dislocation: A case report

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ABSTRACT

Perilunate fracture dislocations are rarely seen severe injuries among wrist traumas. Trans-scaphoid perilunate fracture dislocation and dorsal perilunate dislocation are the most commonly encountered types of these injuries. In addition, other pathologies may also accompany in the same extremity. As they are rarely seen, they may be diagnosed late. When these cases are overlooked and not treated in the early stages, there is a high possibility of the development of limited wrist movements and chronic pain. In this respect, the importance of early diagnosis and treatment is evident. With accompanying literature, we present a case of 41-years old male operated for a diagnosis of perilunate dislocation due to falling down.

J. Exp. Clin. Med., 2014; 31:59-61

1. Introduction

Carpal dislocations and fracture dislocations are rare injuries which occur after high-energy traumas with the weight on the wrist in hyperextension (Witvoet and Allieu, 1973). Apart from children and the elders, it is seen frequently in young males in their twenties (Ball et al., 2012). Trans-scaphoid perilunate fracture dislocations and dorsal perilunate dislocations are the most frequently seen types of these injuries (Bal et al., 2011). With similar findings in the physical examination of wrist sprain and fractures which do not require emergency surgery, varying degrees of swelling, widespread sensitivity, deformity and restricted wrist movements may be seen (Weil et al., 2006).

In radiological imaging, there should be anteroposterior and lateral wrist direct radiographs and if there are clinical indications of other special imaging methods such as scaphoid radiographs should be selected. On the lateral radiograph, the appearance of the volar angle lunatum as 'spilled tea glass' is a diagnostic sign (Gilula et al., 1984). In addition, computerized tomography (CT) and magnetic resonance imaging (MRI) examinations give detailed information about bone and soft tissue. An emergency method for carpal dislocation is closed reduction of the dislocation under regional block or general anaesthesia (Ball et al., 2012). Although good functional results are obtained from early diagnosis and closed treatment, degenerative changes are often seen. For better functional results, bone fixation with open reduction and soft tissue repair with a dorsal or volar approach is recommended, although the situation requires serious anatomic information together with clinical and surgical experience (Fonseca et al., 2012).

2. Case presentation

A 41-year old male was evaluated in the emergency department with complaints of pain on the left wrist. From the patient's history, it was learned that he had fallen from a height of approximately two meters on his left wrist. At the emergency department of another centre, a short arm brace had

Journal of Experimental and Clinical Medicine 31 (2014) 59-61

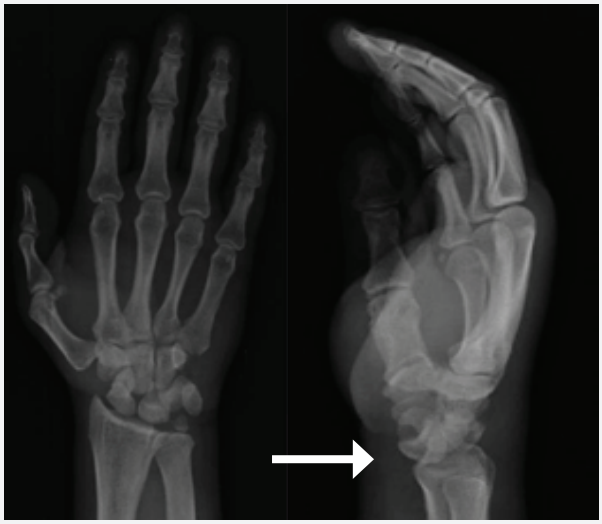

Fig. 1. Preoperative wrist anteroposterior and lateral direct radiographs (Arrow shows 'spilled tea glass' sign)

been applied and he had been referred to the orthopedics clinic. With an initial diagnosis of perilunate fracture dislocation the patient had been referred to our hospital. In the physical examination, there were swelling, sensitivity and pain on movement of the left wrist. Neuromotor and vascular examination results were normal. The patient had no additional diseases. In the two-direction direct radiograph, dislocation to the volar in the lunatum and a suspected fracture line in the scaphoid were observed (Fig. 1). On CT, dislocation to volar in the lunatum and that the scaphoid had changed rotational place were observed. As two days had passed, closed reduction was not attempted. Open reduction with a volar approach was applied and by placing a kirschner wire (K-wire) in the direction of radius-lunatum-hamatum, fixation was achieved. Following soft tissue repair, the incision was closed and a scaphoid plaster cast was applied (Fig. 2). After four weeks, the plaster cast and the K-wire were removed and exercises were started. In the eighth month of follow-up the patient was healthy with pain-free but restricted wrist movements and continued his normal daily life.

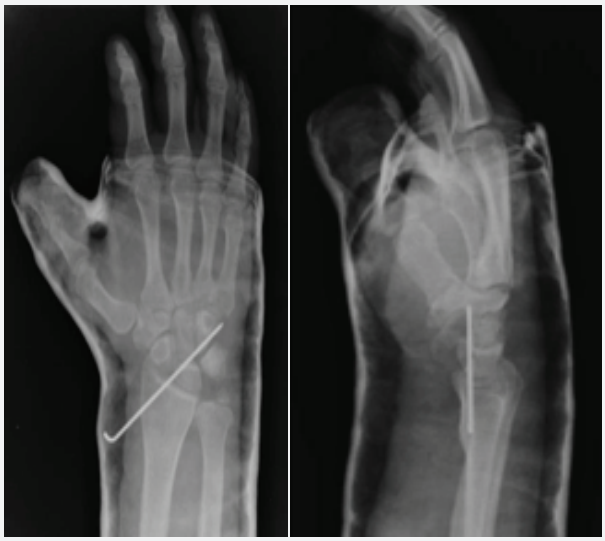

Fig. 2. Postoperative wrist anteroposterior and lateral direct radiographs inside the plaster cast.

3. Discussion

The position of the lunatum is defined by several ligaments such as the radiolunate, scapholunate, radioscapholunate and lunotriquetral. These ligaments provide stability between the radioulnar joint and the carpal joints. As the lunatum has this degree of stability, a high energy trauma is required for injury to occur. Lunatum dislocation is generally a part of a perilunate carpal dislocation. It mostly dislocates in a dorsal direction. After impairment of the stabilising ligaments, the dorsal dislocation locates behind the lunate fossa of the lunatum (Mayfield, 1980).

Incorrect alignment of the ligaments stabilising the lunatum may result in semi-dislocation or full dislocation. Ligament injuries were defined by Mayfield (1980) in four stages. The first stage shows scaphoid dislocation/instability with scapholunate and radioscaphoid ligament injury. The second stage has capitatum dislocation including impairment of the 'space of poirier'. In the third stage, there is radiotriquetal ligament rupture and triquetrum dislocation. In the final stage, full failure of the radiocapitate, radiotriquetal and dorsal radiocarpal ligaments occurs. The Mayfield classification is used to understand the mechanism of ligament damage in the planning of repair and that the weakness in the ligaments may cause dislocation in other carpal bones such as the lunatum and the triquetrum (Mayfield, 1980). It has been reported that entrapment neuropathies occur from affected ulnar and median nerves following volar lunatum dislocation. Carpal tunnel syndrome is generally seen together with late diagnosed volar lunatum dislocation. For those with median nerve indications, emergency carpal tunnel decompression may be necessary in acute situations (Shariff et al., 2009). Herzberg and Forissier (2002) showed acute median nerve dysfunction in 4 of 23 patients with carpal dislocation. While evaluating the location of the carpal bones on direct radiographs, it is useful to start from the Gilula arcs. Gilula (1979) established three plain radiographic arc models by comparing anteroposterior direct radiographs of 90 patients showing dislocation together with carpal fracture, with normal direct radiographs. It was reported that the impairment of the arc shape of one or more carpal bones may not always be pathological, but may occur as a result of incorrect alignment or taking the wrong radiograph. When evaluating the patients in that study, it was shown that in cases where the three arcs model was impaired, after surgery the three arcs were within normal limits and the joint had been restored. In a study by Kömürcü et al. (2008) in which lunatum dislocation and scapholunate ligament damage were defined together with scaphoid dislocation, open reduction was seen to be appropriate for patients with ligament injury together with fracture and dislocation. It was also shown that for a fall on an open hand to result in a fracture in the scaphoid and damage to surrounding ligament structures, a high energy trauma would be required.

The mean carpal height can be estimated using the technique described by Youm et al. (1978) in that the carpal height of the distal radius joint surface is proportional to the length of the third metacarpal and this rate is 0.5 (0.54 +/- 0.03). Loss of carpal height shows a decrease in this rate. Bilos and Hui (1981) reported the case of a boxer with dorsal dislocation of the lunatum, who was treated with open reduction with K-wire fixation and multiple ligament repair followed by two months follow-up in a plaster cast (Bilos and Hui,

1981). Seidenstein (1956) reported a case where 75% of the lunatum bone was dislocated following a truck accident and the lunatum had to be excised. Several cases have been reported of lunatum dislocation together with another bone and more than one ligament injury (Bjerregaard and Holst-Nielsen, 1988; Schwartz et al., 1990; Neavin et al., 2009). A case was presented by Bjerregaard and Holst-Nielsen (1988) of lunatum dislocation together with radius styloid fracture following a truck and train collision. Neavin et al. (2009) reported a case of 5th metacarpal fracture and radius styloid fracture extending intra-articularly following a truck accident.

Early mobilisation of wrist injuries has been shown to be useful by reducing swelling, pain and stiffness to a minimum and thus accelerating functional recovery. In patients with a minimal level of soft tissue damage, after closed reduction and fixation with K-wire, the short-arm brace was removed after only three weeks and thus early return to work was achieved (Dias et al., 1987; McAuliffe et al., 1987). In a case with an old dorsal lunatum dislocation, multiple tendon transfer and proximal row carpectomy was performed (Schwartz et al., 1990). Viegas et al. (1987) applied open reduction and osteosynthesis with Herbert screws to five dorsal trans-scaphoid fracture dislocations and the results obtained from Green and O'Brien clinical and radiological scoring were three excellent, one good and one fair. Using the same scoring system, Fernandez and Ghillani (1987) obtained scores of seven fair and three poor after having applied open reduction and K-wire osteosynthesis to 10 cases of perilunate fracture dislocation. In the case presented here, the lunatum was observed to have dislocated towards the volar on direct radiograph and on CT, the rotational place of the scaphoid was seen to have changed together with the dislocation. As the diagnosis was made two days later, closed reduction was not attempted. Open reduction with a volar approach was applied and fixation was achieved with one K-wire placed in the direction of radius-lunatum-hamatum. Following soft tissue repair, the incision was closed and a scaphoid plaster cast was applied. At the end of four weeks, the plaster cast and the K-wire were removed and exercises were started. The patient's rehabilitation continued through eight months of follow-up with pain-free but limited wrist movement.

Perilunate fracture dislocations are rarely seen, in severe wrist traumas and are often diagnosed late. Careful physical and radiological examination are important in the diagnosis. It should certainly be suspected and ruled out which is the purpose of the text. Even if closed reduction is successful in cases diagnosed early, subsequent collapse and arthritis are often seen. Therefore, it can be considered that it is necessary to select treatment of open reduction with fixation and soft tissue repair.

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Case Report

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An important topic in medical education: Multidisciplinary approach to a sexual assault case in the emergency clinic

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ABSTRACT

Alcohol related sexual violence is an important public health problem. However, there is a few research conducted on this problem in Turkey. The purpose of this case report is to compose a scientific approach in the view of literature. We report an alcohol related sexual assault case for presenting adequate approach to these cases according to current guidelines. The victim was 17 years old female adolescent diagnosed and treated in the emergency service. The patient was hospitalized in the Child and Adolescent Psychiatry Service after emergency treatment in the Pediatric Emergency Medicine and Intensive Care Unit. A review and discussion of alcohol related sexual assault case is presented, and also multidisciplinary diagnosis and treatment approach is offered for this entity. The blood alcohol level of the patient was 410 mg/dL, Glasgow coma scale (GCS) was seven and body temperature was 35°C in the emergency service. Whole body examination findings revealed physical assault and also genital examination findings confirmed sexual assault. It should be remembered that delay in diagnosis and treatment of cases may lead to significant health problems in the victims of sexual assault. Also assessing sexual assault cases, especially in emergency services needs to integrate assessments of different social and medical disciplines. Thus, latest guidelines for the evaluation of sexual abuse of children in emergency services must be learned by medical students and physicians.

*Study results were presented at VIII. National Pediatric Emergency Medicine and Critical Care Congress in 03-06 April 2011 in Izmir, Turkey.

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1. Introduction

Rape is defined as engaging vaginal, anal or oral sexual intercourse acquired through force without a person's consent or in situations where that person is unable to give consent (for reasons of mental disease, intoxication, age, etc.) (Abbey, 2002). Studies in the literature show a correlation between cases of sexual assault and alcohol use, a positive correlation between sexual assault and alcohol use by the aggressor, victim or both (Abbey et al., 1996; Ullman et al., 1999; Abbey, 2002). In one study of 391 cases subjected to sexual assault in Great Britain, 81% of victims were reported to have taken alcohol, and 60% had blood alcohol levels above 150 mg/dL during the incident (Scottham and Burton, 2006). Additionally, the aggressor having an irascible character and using alcohol has been determined to increase the degree of the victim's physical injuries (Ullman and Brecklin, 2000). The purpose of this study is to describe the adequate medical approach steps in a case attending the emergency department with suspected sexual assault and to discuss importance of topic in medical education.

2. Case

A 17 years old female patient had been found in an unconscious state in an empty area and brought to pediatric emergency department. In physical examination: general condition was poor; she was unconscious, exhibited involuntary retching and Glasgow Coma Scale (GCS) score was 6-7. Blood pressure was 80/50 mmHg, heart rate was 78/min and body temperature was 35°C (hypothermia). The appropriate first emergency interventions were performed, and heat was applied to the trunk for warming. Laboratory tests showed a blood alcohol level of 410 mg/dL. The child psychiatry, pediatric surgery, gynecology and forensic medicine departments were consulted. Blood samples, vaginal and anal smear specimens were sent to the judicial authorities in line with procedures for the appropriate investigations. At consultation in the emergency department with the child psychiatry department on the day of presentation, no psychological examination could be performed as the patient was still unconscious. At forensic medicine consultation which held in the pediatric emergency department, the patient was conscious, but not oriented and cooperated. Examination revealed superficial, red skin abrasions measuring 3x0.1 cm, 4x0.1 cm and 2x0.1 cm beneath the umbilicus on the right side of the abdomen, a superficial red skin abrasion measuring 3x0.2 cm 10 cm beneath the umbilicus on the middle of abdomen, a 3x1 cm red skin abrasion 3 cm beneath the left iliac crest bone, purple ecchymosis approximately 4x15 cm in size on the back region near the inferior part of the left rib cage and two superficial skin abrasions 1 cm in diameter to the right and left of the midline in the vaginal region. No significant pathology was determined at anal examination. Since the patient was unconscious and in a recumbent position, not moving voluntarily and hypothermic, and since emergency medical interventions were still being performed, the internal genital region could not be accurately assessed. Gynecological and obstetric examination was therefore advised. Two vaginal and one anal smear specimens were provided for the emergency clinic. No intra-oral smear specimen could be collected as the patient was vomiting and a breathing tube was attached to the mouth. Examination at the gynecology and obstetric department revealed an annular-type hymen. Two new abrasions were identified, one extending to the floor in a 7 o'clock direction and one not extending to the floor in a 5 o'clock direction. The pediatric surgery department report described that a 15x4 cm ecchymosed area on the left side of the lumbar region and a 3 cm skin abrasion on the left of the pelvis. No ecchymosis or hematoma was observed at anal examination. No findings suggestive of fissure or abrasion were determined in the four quadrants, sphincter tonus could not be clearly assessed at rectal palpation, and the patient was transferred to the pediatric intensive care unit for maintenance of existing treatment. O_2 blood saturation was between 92% and 96%. The pupils were mydriatic, the extremities were cold, spontaneous respiration was present (20/min) and no deep tendon reflexes were elicited. The pediatric intensive care report dated the following day stated that she had been transferred to the pediatric intensive care with pre-diagnosis of alcohol intoxication and sexual abuse, that a toxicologist had been contacted and his advice sought, that the patient's blood pressure had fallen and she had been loaded with saline solution one time, that blood alcohol level measured in the evening was 332 mg/dL, that her GCS rose under observation, that the blood alcohol level decreased to 65 mg/dL, that at 24-h observation vital signs were stable, she was conscious and her general condition had improved.

She was recommended to be transferred to the general pediatric unit and from there to the child psychiatry department for psychiatric monitoring and assessment. History taken by a doctor from forensic medicine specialist accompanied by a child psychiatry department revealed that she had gone for a walk with a male friend studying at the same high school, that the friend forced her to drink alcohol, that she did not fully recall the course of events and that menstrual bleeding has taken place on the day of the incident, that the patient also stated that the pain in the back region still persisted, and pulmonary imaging revealed normal thoracic bone structures and surrounding soft tissues. The patient was examined after a year to determine the effects of the incident on the victim's mental and physical health, no sexually transmitted disease was found. Inspections and control examinations of the case was performed in a child and adolescent psychiatry clinic and necessary treatment approach was implemented.

3. Discussion

High concentrations of blood alcohol have been reported to lead to vomiting, disorientation and memory loss (Knight, 1997). Alcohol is also known to compromise individuals' decision making ability and adversely affect the capacity to decide on any course of action (Finch and Munro, 2003). In high doses it can lead to loss of memory and consciousness (Scottham and Burton, 2006). During sexual assault, these states will clearly compromise the individual's ability to resist and make self-defense impossible. Our patient was a high school student. We encountered no studies regarding the prevalence of sexual assault at secondary school and university years in Turkey. Studies performed abroad, particularly among college students, have reported that incidents of sexual assault are frequently encountered and can have a severe impact on victims' health (Abbey, 1991; Abbey et al., 1996). Careful investigation of the causes of sexual assault and the development of preventive public health programs aimed at the causes can prevent such incidents and/or reduce their negative impacts on health. Additionally, we think that it will be useful to train doctors and assistant health personnel in emergency departments in Turkey on the subject of an up-to-date standard algorithmic approach to the identification and treatment of individuals injured as a result of sexual assault.

Sexual assault victim frequently apply to hospital emergency departments and are always medicolegal in character. Appropriate diagnostic and therapeutic approaches will save a patient's live. With an appropriate forensic medical approach, medical evidence can be obtained, the guilty party or parties can be identified, and the legally appropriate sentence can be handed down. With the identification and treatment of sexually transmitted infectious diseases, the health of the individual can be protected and serious infections (such as hepatitis B, HIV etc.) that might affect results of forensic evaluation can be identified. Psychiatric treatment, rehabilitation and evaluation by social services will guarantee that the patient can continue living a healthy life. To provide adequate treatment, multi-dimensional medicolegal approach must be educated before graduation in medical school.

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