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OUR DATA AND LITERATURE DATA ON THE PHARMACOTHERAPY OF OBESITY

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НАШИ И ЛИТЕРАТУРНИ ДАННИ ВЪРХУ ФАРМАКОТЕРАПИЯТА НА ЗАТЛЪСТЯВАНЕТО

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РЕЗЮМЕ

Затлъстяването е хронично заболяване, което се характеризира с прекомерна натрупване на мастна тъкан и се свързва с повишен риск от развитие на сърдечно-съдови заболявания и смъртност. Загубата на тегло от 5–10% намалява риска от усложнения и подобрява качеството на живот на хората. Съвременното лечение на затлъстяването включва диетичен режим, увеличена физическа активност и терапия с лекарствени средства. По настоящем единственият одобрен медикамент в Европейския съюз за лечение на затлъстяването е Орлистат. Тази статия разглежда последните научни изследвания в тази област, като фокусът е върху повлияването чрез лекарствени средства на различните

ABSTRACT

Obesity is a chronic disease, characterized by an accumulation of excess adipose tissue and associated with an increased risk for cardio-vascular diseases and mortality. Weight loss of a 5–10% prevents or tempers the severity of many obesity-related morbidities. The current therapy of obesity comprises diet, increased physical activity, and pharmacotherapy. At the moment, the only approved drug for treatment of obesity in the European Union is Orlistat. This paper covers the latest research in the field with emphasis on the modulation of the different physiological systems and peptides in the regulation of appetite and metabolism through drugs as well as new drugs to treat diabetes type 2 and obesity. Furthermore, the authors show own data and

физиологични системи и пептиди, които регулират апетита и метаболизма, както и нови медикаменти за терапия на захарен диабет тип 2 и затлъстяване. В допълнение, авторите посочват собствени данни и резултати за фармакотерапията на затлъстяването при експериментални модели и хора.

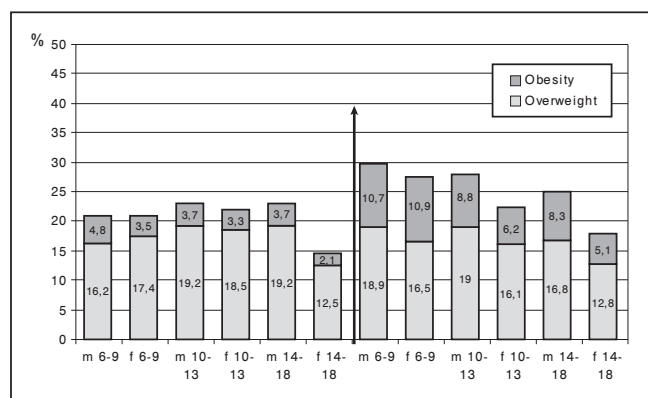
Ключови думи: затлъстяване, лечение, лекарства, хранителни добавки

results on the treatment of obesity in animals and humans.

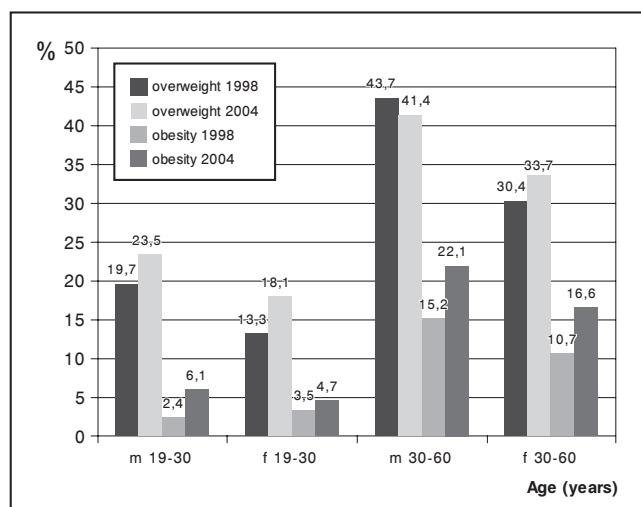
Key words: obesity, treatment, drugs, food supplements

INTRODUCTION

Overweight and obesity are very common diseases around the globe, and prevalence continues to increase in most countries. In Bulgaria about 58% (Obesity – Perception and Policy; EASO 2014, p.51) of the population above 18 years of age is either overweight or obese, and this proportion is still increasing. The below-presented Table 1 and table 2 (Global Obesity Forum 2012) demonstrate the increase in the proportion of obesity in Bulgaria during recent years.



Tabl. 1. Overweight and obesity prevalence in schoolchildren (National survey on nutrition and nutritional status of schoolchildren in Bulgaria – 1998, 2010–2011)



Tabl. 2. Prevalence of Overweight and Obesity among adults (19–60 years old) National Monitoring of Dietary Intake and Nutritional Status, 1998, 2004

DRUGS THAT HAVE

PERIPHERAL MECHANISM OF ACTION

Orlistat inhibits gastric and pancreatic lipases [15, 23, 26] and is the only drug available in Europe [43]. Hence the drug reduces the hydrolysis of lipids and fatty acids and the amount of fat which is absorbed in the gut [23]. Our studies show the role of Orlistat in the balancing of diet especially with respect to fat consumption was

pointed out. Forty-three women with obesity and dislipidemias were treated for the period of 3 months with Orlistat. Anthropometric and biochemical laboratory measurements were applied. Patients kept diet for five months parallel with Orlistat therapy during the first three months. The obtained results showed gradually reduction of body mass, BMI, body fat mass, waist to hip ratio, total cholesterol, LDL-choles-

terol and triglycerides. These our studies were confirmed from Yumuk [43] which emphasises that the antiobesity agents can be used as an adjunct to lifestyle enforcement therapy.

DRUGS THAT ACT ON THE ADRENERGIC SYSTEM

Among centrally-acting catecholamine-interfering drugs phentermine is a drug that suppresses appetite and is used in short-term treatments [22]. It increases the release of noradrenalin centrally and this is thought to be its main mechanism of action to reduce the appetite [11]. Recently a combination of phentermine with topiramate has been designed and showed good results from Phase III clinical trials [2, 11, 13, 40]. Topiramate is an antiepilepsy drug with not entirely clear mechanism of action but thought to act synergistically with phentermine [27]. Data showed lower blood pressure, triglycerides, and glucose levels in the patient on phentermine/topiramate therapy compared with the placebo group and more than 50% of the treated obese people lost $\geq 10\%$ of their body mass [11, 13].

Lorcaserin is a selective 5-hydroxytryptamine (5-HT, serotonin) 2C receptor agonist and also showed good results in trials for sustained weight loss [30]. Some of the drugs act as noradrenalin releasers (diethylpropion) or to increase catecholamine action through various mechanisms including amphetamine-like action (Fenproporex) [32]. The study was conducted in women and lasted one year and the drugs were administered along with lifestyle changes such as increased physical activity and reduced food intake. The results indicated a significant weight loss compared to placebo of all groups except women who were on fluoxetine. The biggest weight loss were with diethylpropion (mean weight loss = 10kg) followed by sibutramine (mean weight loss = 9.5kg).

THE EFFECT OF BEE PRODUCTS ON WEIGHT AND METABOLISM

The food supplements (Biocorect + and Biocorect Universal) have been tested among 302 in-

dividuals diagnosed with obesity (171 – females and 131 – males). The mean age was 46.3 years and mean body mass index (BMI) 32.8 kg/M². After allergy examination the food /Biocorect universal/ was prescribed as follows: 5 days – 3x40 drops 15 minutes before eating; 2 days- 2x60 drops /at 10a.m. and at 4p.m. in a cup of tea/. At the beginning and at the end of the first and second months anthropometric indexes – height, weight, BMI, waist circumference, fat mass, lean body mass, lean body mass/lean mass ratio, visceral body fat – were examined. The anthropometric measurements are completed with impedance apparatus “Tanita 420”. Sagittal diameter was also measured using a caliper. From the markers of the lipid exchange is tested – total cholesterol (mmol/l), LDL-cholesterol (mmol/l) and triglycerides (mmol/l). All tests were made at the beginning and in the end of the research. We have also tested systolic blood pressure (SBP) and diastolic blood pressure (DBP). The results are statistically processed and for a statistical significance is considered $p < 0.05$.

After two months a significant reduction of mean weight and waist circumference was observed– weight by 9 kg, waist circumference by 13 cm, $p < 0.01$. FM decreases by 4.2% and LBM increases by 4.2% and resp. the ration LBM/FM increases. All these results emphasize the favorable effect of the tested supplement on body composition of the patients. It should also be taken into account that the decrease of the VBM, W, the ration W/H and SD shows a lowered risk for cardiovascular diseases.

THE EFFECT OF ALGINATES ON APPETITE AND WEIGHT (EXPERIMENTAL STUDY)

Male Wistar rats ($n = 30$) were randomly assigned to two different groups: 1st group – control (on a chow food) and 2nd group – experimental (on a high-fat diet). The nutritional period consisted of 2 months. The high-fat group increased their body weight in comparison with the control group. Then, the obese rats ($n = 15$) were put either on a food supplement with alginate acid or only on a high-fat diet. At the end

of the study, rats were anaesthetized and killed. Blood specimens were taken for further biochemical analyses.

High-fat diet led to the development of obesity in male Wistar rats. The group treated with alginate acid showed a significant reduction of body weight and adiposity. Moreover, there were changes in the blood levels of ghrelin and leptin between those treated with/ without alginates. Thus, the beneficial effect of alginates on ghrelin could be possibly explained by its mechanical effect of the stomach mucosa, and thus on the ghrelin secretion [17].

Our studies on the effects of the alginate acid (Algigracil-instant) on the parameters of obesity show a significant reduction of body weight with 27.5% and decrease in plasma levels of glucose and triglycerides in our patients with overweight and obesity [36].

GHRELIN-A POTENT ANTI-OBESITY VACCINE

Experiments with vaccination against another gastrointestinal peptide have been carried out [10]. Gastric inhibitory peptide (GIP) is an incretin released from the small intestine which has been shown to have effects on glucose and lipid metabolism [10]. Since antagonists to its receptor and knockout studies appeared to be effective in reduction of weight gain Fulurija [10] vaccinated mice by attaching GIP to virus-like particles which induced an immune response and protected against endogenous GIP. They showed a significant difference in vaccinated versus control mice weight 70 days after start of treatment and the fat tissue of vaccinated mice at day 142 was reduced significantly. Essential part of their experiments were the results showing that GIP does not impair normal glucose tolerance and metabolism which might have been expected as a side effect of such a therapy [10].

Our data shows the effect of Orlistat on take ghrelin secretion by the stomach mucosa in rats [15]. In our study we have demonstrated that Orlistat group has

significantly lower plasma levels of ghrelin, compared to the control group. Most changes in ghrelin secretions could be as a result of interaction between the Orlistat molecules and the ghrelin receptors in the stomach. Further investigations are needed to examine the mechanism of actions between Orlistat and ghrelin. Handjieva-Darlenska et al. [10] have demonstrated data for beneficial lifestyle changes (resp. reduced fat intake) in obese patients after 3-month treatment of Orlistat.

PEPTIDES THAT DECREASE APPETITE

GLP-1 is another peptide which is secreted from the gut and have anorexigenic and incretin properties [35, 42]. It acts via G-protein coupled receptors in the gastrointestinal tract and in the brain (mainly hypothalamus) to decrease gut motility and appetite [35]. Similarly to the other peptides it has a short half-life in blood being degraded by DPP4 enzyme and therefore GLP-1 is not useful as a drug itself [35, 42]. To overcome these problems drugs like exenatide were developed [24]. Being a homolog of the human GLP-1 it acts on the same receptor but it is more potent and in addition to that it is not subject to DPP-4 degradation [24]. This appeared to be a good approach as many clinical studies showed reductions in glucose and body weight of subjects on exenatide [24]. Lixisenatide is a derivative of the exenatide and have also been approved for human use [5].

The search for a GLP-1 analogue that would be even more stable in plasma but that would still act on its receptor continues. Liraglutide has been designed which is very similar to GLP-1 but it is conjugated to a fatty acid chain which allows it to bind to plasma albumin and therefor achieve a longer half-life [35]. Phase III clinical trial assessed the ability of liraglutide to sustain weight loss [36]. This was achieved by liraglutide treatment and even additional weight loss has been observed [36]. Novel delivering technologies are also under investigation. This include slow-release formulation of exenatide or

an osmotic pump device to deliver the same drug in a continuous manner subcutaneously [35].

Therapies exploiting other anorexigenic hormone pathways are also under development such exploiting systems of oxyntomodulin [19, 34, 35] and pancreatic [3, 34, 35]. None of them have reached market yet but some substances can be regarded as potential drug candidates.

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IS THERE A CORRELATION BETWEEN SPINAL CANAL COMPROMISE IN THORACIC AND LUMBAR BURST FRACTURES AND NEUROLOGICAL DEFICIT?

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ИМА ЛИ ЗАВИСИМОСТ МЕЖДУ СТЕСНЕНИЕТО НА ГРЪБНАЧНИЯ КАНАЛ ПРИ ВЗРИВНИ ТОРАКАЛНИ И ЛУМБАЛНИ ФРАКТУРИ И ТЕЖЕСТТА НА НЕВРОЛОГИЧНИЯ ДЕФИЦИТ?

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РЕЗЮМЕ

ДИЗАЙН НА ПРОУЧВАНЕТО

Извършено е ретроспективно клинично и рентгенологично проучване, за да се установи има ли зависимост между степента на стеноза на гръбначния канал при гръдни и лумбални взривни фрактури и тежестта на неврологичния дефицит.

ЦЕЛ

Да се определи дали е налице корелация между тежестта на неврологичния дефицит и стенозата на гръбначния канал.

ВЪВЕДЕНИЕ

В съвременната спинална литература зависи-
мостта между степента на оклузия на гръбнач-

ABSTRACT

STUDY DESIGN

A retrospective clinical and radiographic study was performed, to review the correlation between the traumatic spinal canal stenosis in thoracic and lumbar fractures and neurological deficit

OBJECTIVES

To determine whether there is a correlation between the degree of neurological deficit and the stenosis of the spinal canal.

BACKGROUND DATA

In modern spinal literature, the correlation between the degree of protrusion of bone fragments into the spinal canal and the severity of neurological deficits

ния канал от костни фрагменти при тораколумбални (ТЛ) взривни фрактури и тежестта на неврологичния дефицит не е категорично установена и е обект на много спорове.

МЕТОДИ

За период от 2008 до 2013 г., общо $n = 205$ пациенти с различни видове торакални и лумбални фрактури, класифицирани по AO/Magerl са оперирани в нашата клиника. От тях $n = 109$ (53,17%) са с взривни фрактури. На всички е извършено измерване на степента на стеснение на гръбначния канал. Техниката на измерване включва съотношението на сагитален диаметър на гръбначния канал на нивото на увреда, към диаметъра на съседно ниво на аксиални КТ срезове, което се сравнява с тежестта на неврологичния дефицит, класифициран по Frankel/ASIA.

РЕЗУЛТАТИ

Средното стеснение на спиналния канал е 35.47% (6/97%) сравнено с калибъра на съседно неувредено ниво. Разпределението на фрактурите по сегменти е както следва: торакални $n = 14$ (12.84%), ТЛ $n = 73$ (66.97%) и лумбални $n = 22$ (20.19%). Разпределението на неврологичния дефицит е съответно: Frankel/ASIA A - торакални $n = 4$; ТЛ $n = 7$; лумбални $n = 1$. Frankel/ASIA B-D - торакални $n = 4$; ТЛ $n = 13$; лумбални $n = 2$. Frankel/ASIA E - торакални $n = 6$; ТЛ $n = 53$; лумбални $n = 19$.

ЗАКЛЮЧЕНИЕ

Степента на стеснение на гръбначния канал при взривни тораколумбални и лумбални фрактури показва положителна корелация и е прогностичен фактор за тежестта на непълния неврологичния дефицит (Frankel/ASIA - B, C и D). Пациентите с пълен неврологичен дефицит (Frankel/ASIA - A) не показват такава корелация.

Ключови думи: гръбначни фрактури, торакални вертебрални фрактури, лумбални фрактури, неврологичен дефицит, гръбначна травма, стеноза на спиналния канал.

in thoracolumbar and lumbar burst fractures is not well established and is the subject of much controversy.

METHODS

From 2008 to 2013 a total of $n = 205$ patients with different types of thoracic and lumbar fractures classified by AO/Magerl were admitted in our service. Of these, $n = 109$ (53,17%) were with burst fractures. On these patients was performed measurement of the degree of narrowing of the spinal canal. The measurement technique included the ratio of sagittal diameter of spinal canal at the injury level to that at the adjacent level on axial CT scans and it was compared with the severity of neurologic deficit, classified according to Frankel/ASIA.

RESULTS

The average narrowing of the spinal canal is 35.47% (6/97%) compared to the caliber of the undamaged canal adjacent to the injury level. The distribution of fracture segments is $n = 14$ (12.84%) thoracic, $n = 73$ (66.97%) TL and $n = 22$ (20.19%) lumbar. The distribution of neurological deficit is: Frankel/ASIA A - thoracic $n = 4$; TL $n = 7$; lumbar $n = 1$. Frankel/ASIA B-D - thoracic $n = 4$; TL $n = 13$; lumbar $n = 2$. Frankel/ASIA E - thoracic $n = 6$; TL $n = 53$; lumbar $n = 19$.

CONCLUSION

The narrowing of the spinal canal in thoracolumbar and lumbar burst fractures show a positive correlation between the narrowing of the spinal canal and the severity of the incomplete neurological deficit by the Frankel/ASIA classification. Patients with complete spinal cord injuries (Frankel/ASIA A) did not show this correlation.

Key words: spinal fractures, thoracic vertebrae/injuries, lumbar vertebrae/injuries, neurological deficit, spine trauma, spinal canal stenosis.

INTRODUCTION

Burst fractures AO/Magerl type A3 are the most common fractures in the thoracolumbar and lumbar segments of the spinal column.¹

² They are usually a result of extensive axial load, and are characterized by a loss of height of the anterior column of the body of the vertebrae and the destruction of the posterior wall, with retropulsed bone fragments in the spinal canal.^{1,3,4,5} The most common mechanism of this type of injury is fall from a height and road traffic accidents.^{1, 2,,5} The neurological deficits in these fractures is common, and many authors try to correlate the degree of stenosis of the spinal canal with the severity of the neurological deficit.^{1,5-8} Despite these attempts the results are still controversial and contradictory. Other authors believe that the degree of narrowing of the spinal canal is not a determinant of severity of the neurological deficit, especially in younger patients and at the lower lumbar segments.^{9,10} Furthermore it is shown that the decompression of the neural structures is not always followed by improvement of neurological function. Hence, apart from the common X-Rays such patients does not require further investigations with CT and does not need the removal of the fragments from the spinal canal. However, it is generally accepted that the decision to treat this type of fractures is based on measurements of the stenosis of the spinal canal. Severe stenosis is considered as crucial and surgical treatment is recommended for these patients.⁴ The benefits of surgery are that apart from the decompression of the neural elements, the surgery provides correction of the deformity, stabilization of the segment, early mobilization and faster recovery.^{3,9,11-13}

Radiological investigations and measurements play a crucial role in the diagnosis and treatment of the spinal injuries and especially for the burst thoracic and lumbar fractures.¹⁴ The various measured parameters are used for the uniformity of the severity evaluation of the

stenosis in a variety of traumatic clinical conditions.¹⁵ Usually in the literature in the measurement of the traumatic spinal canal stenosis the ratio of the sagittal diameter or the area of the damaged segment to the diameter of the adjacent intact level is used.^{2,4,15}

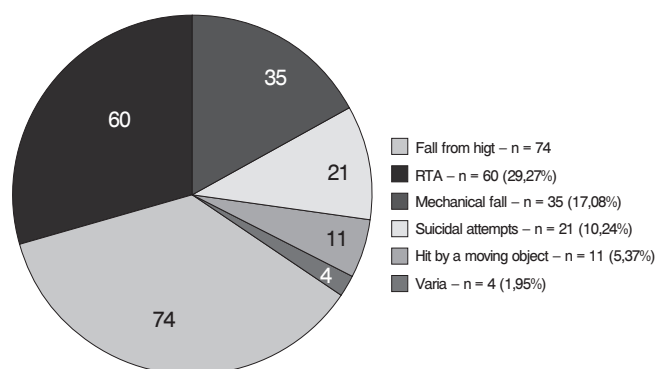
In the present retrospective study we set our goal, based on $n = 109$ operated patients with traumatic stenosis of the canal with burst thoracic and lumbar fractures, to determine if there is a correlation between the severity of the occlusion of the canal and the severity of the neurological deficit.

MATERIAL AND METHOD

STUDY DESIGN

The investigation is based on $n = 205$ spinal trauma patients with different types of thoracic and lumbar trauma graded according to AO/Magerl. The patients have been treated in our unit for the period of 6 years (2008–2013). Among these $n = 143$ are men, with mean age 43,05 years and $n = 62$ are women with mean age of 44,22 years. The overall mean age of the group is 43,4 (14/82). From epidemiological point of view, the most common cause for spinal trauma is fall from height $n = 74$ (36,09%), followed by road traffic accidents – $n = 60$ (29,27%), mechanical falls – $n = 35$ (17,08%), suicidal attempts – $n = 21$, hit by a moving object – $n = 11$ (5,37%), varia – $n = 4$ (1,95%). (Scheme 1)

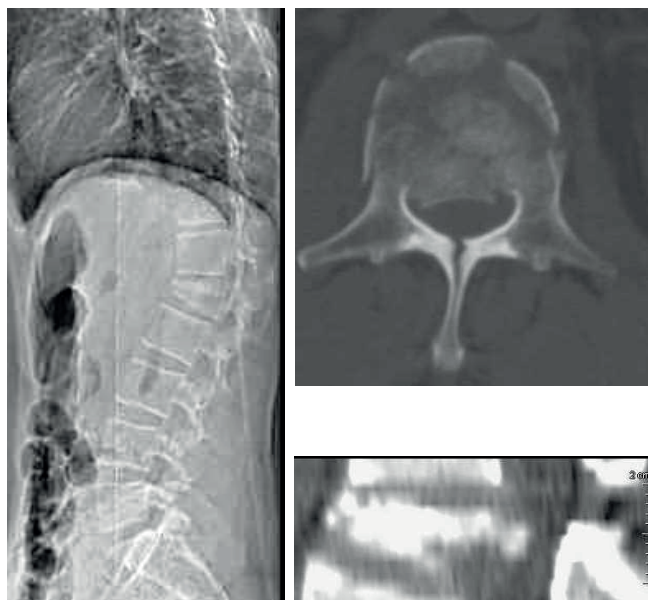
Scheme 1. Patient distribution respecting the cause



Of all patients with spinal trauma, 109 patients (53,17%) have an acute (less than 10 days) burst A3 and B1 type AO/Magerl fractures, that are within the scope of the present study.

The initial neurological deficit was evaluated after the initial spinal shock is passed. The evaluation is done with Frankel/ASIA scales: A – complete loss of motor and sensory function below the level of the lesion; B – complete loss of motor and sensory function below the level of the lesion with preserved some sensitivity; C – preserved minimal movements, gait impossible; D – impaired motor functions, but possible independent gait; E – normal function with minimal or no neurological deficit.

Loss of spinal calibre is measured at the level of the fracture on AP and profile radiographs and axial CT slices (3–5 mm) and compared with the calibre of the canal of the adjacent intact level. The percentage ratio is given by $a = (1 - x/y \times 100)$ wherein “a” is the rate of narrowing of the canal, “x” is the sagittal diameter at the level of the fracture and “y” is the sagittal diameter of the canal at the adjacent undamaged level.^{10, 13, 16} (Fig. 1)



(Fig. 1)

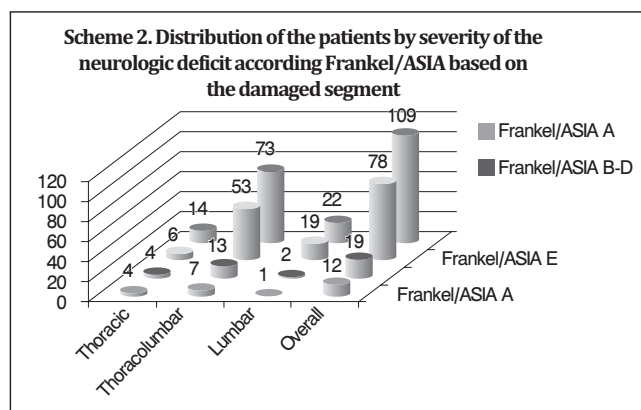
Statistical data analysis is done using correlation analysis by calculating the Pearson coef-

ficient, and comparing the values with standard criteria of t – Student – Fisher where $P \leq 0,05$ is reported as significant.

Fractures are subdivided based on the segment and the severity of neurological deficits as follows: thoracic – T1-T10; thorakolumbar T11-L1; lumbar L2 – L5. (Table 1) (Scheme 2)

Table 1. Distribution of the patients by severity of the neurologic deficit according Frankel/ASIA based on the damaged segment.

Segment	Frankel/ASIA A	Frankel/ASIA B-D	Frankel/ASIA E	Overall
Thoracic	4	4	6	14
Thoraco-lumbar	7	13	53	73
Lumbar	1	2	19	22
Overall	12	19	78	109

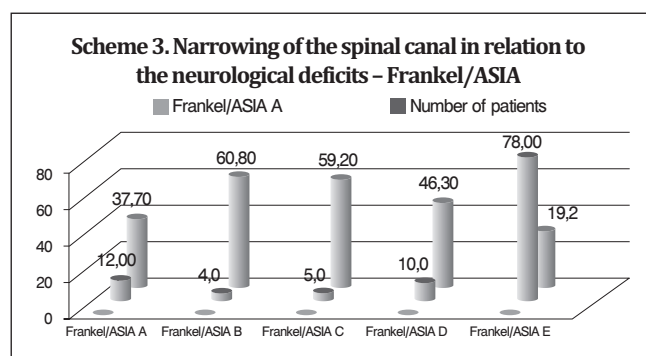


RESULTS

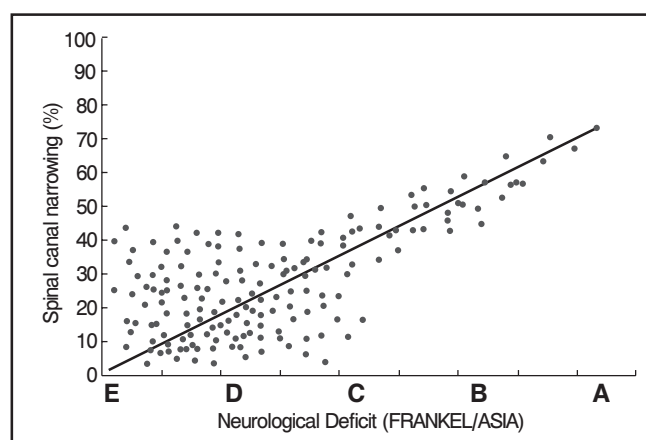
Relative narrowing of the spinal canal is measured in percent with CT in $n = 109$ out of 205 patients (53.17%). Of these, $n = 14$ are in the thoracic region, $n = 73$ are in the thoracolumbar and $n = 22$ in lumbar region. Average narrowing of the spinal canal is 35.47% (6/97%) compared to the calibre of the intact canal adjacent to the injury level. The average narrowing of the canal in the different segments is as follows: thoracic segment – 37.2% (18/42%), TL junction – 34.5% (7/80%) and lumbar segment 43.5% (6/97%). (Table 2) (Scheme 3)

Table 2. Narrowing of the spinal canal in relation to the neurological deficits – Frankel/ASIA.

Type	Number patients	Average narrowing of the spinal canal (%)
Frankel/ASIA A	12	37,7
Frankel/ASIA B	4	60,8
Frankel/ASIA C	5	59,2
Frankel/ASIA D	10	46,3
Frankel/ASIA E	78	19,2
Overall	109	35,47



Narrowing of the spinal canal on admission significantly correlated with neurological deficit ($R = 0,82$, $p < 0.05$). (**Scheme 4**)



Scheme 4. A scatter diagram illustrating the correlation between the degree of narrowing of the spinal canal in% and the severity of the neurological deficit – Frankel/ASIA

Of the patients with fractures and narrowing of the canal, $n = 8$ out of 14 (57,14%) in the thoracic region had neurological deficit. In the TL junction $n = 20$ out of 73 patients (27.40%)

had neurological deficit. In the lumbar segment $n = 3/22$ (13.64%) patients had been found to be neurologically impaired.

Complete neurological deficit on admission (Frankel/ASIA A) was found in $n = 12$ patients with an average 37.7% narrowing of the canal (thoracic segment $n = 4$ patients – 30.5%, $n = 7$ patients in TL segment – 39.5%, $n = 1$ patient in the lumbar segment – 54%). Incomplete neurological deficit Frankel/ASIA B-D was observed in $n = 19$ patients with an average of 52.75% narrowing of the canal (thoracic segment $n = 4$ patients – 37.6%, TL $n = 13$ patients – 56.5% and lumbar $n = 2$ patients – 58.6%). Patients with complete neurological deficit (Frankel/ASIA – A) did not show such a correlation ($R = -0,1$) ($P < 0,05$).

In $n = 78$ patients without neurological deficit, the average narrowing of the canal is 19.2% (thoracic segment $n = 6$ patients – 8.6%, $n = 53$ in TL junction – 18.1%, $n = 19$ patients in the lumbar region – 25.6%).

Patients with a narrowed spinal canal have 3.5 fold higher relative risk (RR) for neurological deficit Frankel/ASIA A-D, if compared with the patients without damage to the canal ($p < 0.05$).

DISCUSSION

In general, TL injuries are followed by neurological deficit in 22–45% of the cases, and the severity of the deficiency has significant impact on the overall prognosis for the outcome.^{4, 5, 17} The majority of the burst fracture in our study are localized in the TL segment 66,97% $n = 73/109$, which corresponds with the incidence of fractures in this area reported by the different authors.^{1, 6, 8} The average narrowing of the canal has the greatest extent in the lumbar region (43.5%) compared with the thoracic region and the TL (37.2% and 34.5% respectively). This trend was also found in studies of Hashimoto et al.⁶, Meves et al.⁵, Mohanty et al.¹⁰ The investigation of the patients with axial radiographs and CT are routine and safe methods in

which the measurement of stenosis of the canal is comparable and statistically reliable.⁴

The relationship between the degree of narrowing of the canal, measured with CT and MRI and the severity of the neurological deficit is widely debated in literature.^{1, 5 - 7, 17} Some authors argue that the presence of bone fragments into the spinal canal cause the neurological injury and are responsible for the persistence of neurological deficit.⁶ Others believe that there is no such a relationship.⁷ Some surgeons like Kostuik JP et al.¹⁸ operate the patients with stenosis of the canal on the CT with more than 40–50%, but this criterion is not established in controlled clinical trials. In laboratory tests it has been shown that the damage to neural structures, and neurological deficit occurs at the time of injury and they do not significantly get influenced by the position of the fragments on the following images. Furthermore, at the higher levels of spinal canal compromise, there is not always an established correlation between the level of highest degree of occlusion of the canal and the neurological damage due to the effects of high pressure created during the momentary high pressure over the spinal cord.^{17, 19}

Therefore it cannot always be demonstrated a high occlusion of the canal and severe neurological deficit and vice versa. In our series, a patient has more than 60% narrowing of the canal at the level of T12 without deficit and a patient with 30% narrowing at the level of L1 with complete neurological deficit.

Burst fractures type A3 and B1 are roughly equal in number in our study, but with neurological deficit were 39.74% of the patients $n = 31/78$, which correspond to the frequency that the contemporary authors report in their studies. In the literature there are no specific recommendations on indications for decompression of the canal, penetrated by bone fragments. Therefore many authors do not associate the degree of narrowing with the severity of neurological deficit especially in young patients.⁹ Mohanty et al.¹⁰ investigate a series of $n = 105$ patients with burst thoracolumbar and lumbar

fractures. They found no significant correlation between the type of the burst fracture and the neurologic deficit. In their series the average channel stenosis among patients with neurological deficit is 50%, and the average stenosis in patients with no deficit is 36%. Upon closer analysis, the authors found same correlation for levels T11 and T12, but not for level L1. This fact they attributed to the different location and sensitivity of the spinal cord, conus medullaris and cauda equina.

Other authors^{1, 5 - 7} try to elaborate on this relationship, although the final conclusions still remain controversial. Using CT authors demonstrate a direct correlation between the degree of the canal narrowing due to fragments retropulsion and the severity of the neurological deficit. In their study Hashimoto et al.⁶ analyzed $n = 112$ consecutive patients with burst TL fractures and found, that the narrowing of the canal $\geq 35\%$ at the level of the epiconus (T11-T12), also $\geq 45\%$ at the level of conus medullaris and $\geq 55\%$ at the cauda equina level, are significant factors contributing for the neurological damage. Furthermore, the authors found that 68% of cases with ruptured posterior ligaments have neurological deficit, while cases with neurological deficit with intact posterior ligaments are only 32%. Meves and Avanzi⁵ studied $n = 198$ patients with burst TL and lumbar fractures and found a positive correlation between the narrowing of the spinal canal and the severity of neurological deficit classified with the Frankel scale. In a retrospective study of series of $n = 216$ patients with burst TL and lumbar fractures Yugue et al.⁸ found that stenosis of the spinal canal and rupture of the posterior ligament complex are statistically significant risk factors for severe neurological deficit. In their systematic review Keynan et al.¹⁵ include measuring the spinal canal percentage of occlusion in trauma patients, as one of the routine radiographic evaluation indicators for severity of spinal fractures. Yang H, et al.⁶ used the percentage of the narrowing pre-and postoperatively as a parameter for evaluation of indirect reduction and

pedicular fixation, without fusion of the studied patients in their series. Same parameter take into account Reinhold et al.² in their study of n = 733 patients with TL fractures, also Katonis et al.²⁰ in the follow – up of n = 30 surgical patients with unstable TL fractures.

In our study, the relative narrowing of the spinal canal is measured in percent (%) based on CT imaging for n = 109 patients. The mean stenosis was 35.47% (6/97%) compared to the calibre of the undamaged channel adjacent to the injury level. We find that the narrowing of the spinal canal on admission significantly correlates with neurological deficit $R = 0,82$, ($P < 0,05$), and patients with a narrowed spinal canal have 3.5 fold higher relative risk (RR) for neurologic deficit (Frankel/ASIA A-D) if compared to patients without impairment of the canal ($p < 0.05$). Patients with complete neurological deficit (Frankel/ASIA – A) did not show such a correlation ($R = - 0,1$) ($P < 0,05$).

CONCLUSION

Our study shows that the degree of the canal narrowing due to burst thoracolumbar and lumbar fractures has a positive correlation and predicts the severity of incomplete neurological deficit (Frankel/ASIA – B, C and D), $R = 0,82$ ($P < 0.05$). Patients with complete neurological deficit (Frankel/ASIA – A) did not show such a correlation ($R = - 0,1$) ($P < 0,05$).

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COMPARISON OF THE RADIATION PROTECTION ACTIVITY IN EXPERIMENTAL MODEL OF EXAMINATION TO BOTH NATIVE METABOLITES TRIMETHYLGLYCINE (BETAINE) AND N-ACETYL-L-CYSTEINE, BY USING OF INNOVATIVE MOLECULAR BIOLOGY TECHNIQUES AND DOSIMETRY METHODS OF ANALYSIS.

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СРАВНЯВАНЕ НА РАДИОПРОТЕКТИВНАТА СПОСОБНОСТ, В ЕКСПЕРИМЕНТАЛЕН МОДЕЛ НА ИЗСЛЕДВАНЕ, НА ДВАТА ПРИРОДНИ МЕТАБОЛИТА ТРИМЕТИЛГЛИЦИН (БЕТАИН) И N-АЦЕТИЛ-L-ЦИСТЕИН, ЧРЕЗ ИЗПОЛЗВАНЕ НА СЪВРЕМЕННИ МОЛЕКУЛЯРНО-БИОЛОГИЧНИ И ДОЗИМЕТРИЧНИ МЕТОДИ ЗА АНАЛИЗ.

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РЕЗЮМЕ

Една от основните цели, които си поставят радиобиолозите е откриване на съвременни, нетоксични радиопротектори, които при облъчване с йонизираща радиация ефективно да намаляват тежките стохастични и нестохастични ефекти върху организма. Откриването на

ABSTRACT

One of the main targets in front of the radiobiologists is determine of modern, non-toxic radioprotectors that effectively reduce the stochastic and non-stochastic effects on the organism. Finding of appropriate radioprotectors is the base of the radiation protection of medical personnel and patients in

подходящи радиопротектори стои в основата на предпазване на медицински персонал и пациенти подложени на облъчване с йонизираща радиация. През последното десетилетие беше установено, че природни продукти с различен химичен състав, структура и функционална активност, могат да имат потенциална радиопротективна способност. Детайлни изследвания на двата природни метаболита N-ацетил-L-цистеин ($C_5H_9NO_3S$) и триметилглицин (бетаин, $(CH_3)_3N^+CH_2CO_2^-$) показаха, че имат ясно изразена антиоксидантна и потенциална радиопротективна способност. Целта на изследването беше да се определи и сравни потенциалната радиопротективна способност на двете аминокиселини, прилагани превантивно на експериментален модел от облъчени, чрез ^{137}Cs -источник с 1 Gy и 3 Gy погълната доза, първични, човешки, лимфоцитни клетъчни култури. За осъществяване на целта на изследването бяха използвани иновативни молекулярно-биологични и дозиметрични методи за анализ. Бяха изследвани пет основни експериментални групи за всяка доза на облъчване. Резултатите от изследването показаха добър, относително еднакъв радиопротективен ефект и на двата нативни метаболита. Получените резултати, корелират с възможността на двете аминокиселини да намаляват вредното въздействие на активните форми на кислорода в клетката и добрата им антиоксидантна активност.

Ключови думи: йонизираща радиация, N-ацетил-L-цистеин, триметилглицин, лимфоцити, радиопротектор;

an environment with higher levels of ionizing radiation. During the past decade it was found that natural products with different chemical composition, structure and functional activity could have potential radioprotective activity. Detailed studies of both natural metabolites N-acetyl-L-cysteine ($C_5H_9NO_3S$) and trimethylglycine (betaine, $(CH_3)_3N^+CH_2CO_2^-$) showed to have excellent antioxidant and potential radioprotective ability. The purpose of that work was to determine and compare the potential radioprotective activity of both amino acids applied preventively to experimental model of irradiated, by ^{137}Cs -unit with 1 Gy and 3 Gy absorbed dose, primary, human, lymphocyte cell cultures. To achieve the objective of the study were used innovative molecular biology techniques and dosimetry methods of analysis. Five basic experimental groups for each dose of irradiation have been tested. The survey results showed significant, relatively equal radioprotective effect of both metabolites. These results correlate with the ability of both amino acids to reduce the harmful effects of the reactive oxygen species (ROS) and their antioxidant activity.

Key words: ionizing radiation, N-acetyl-L-cysteine, trimethylglycine, lymphocytes, radioprotector;

INTRODUCTION

One of the main targets in front of the radiobiologists is determine of modern, non-toxic radioprotectors that effectively reduce the stochastic and non-stochastic effects of ionizing radiation on the organism. ⁽¹⁾ Finding of appropriate radioprotectors is the base of the radiation protection of medical personnel and patients in an environment with higher levels of ionizing radiation. During the past decade it was found that natural products with different chemical

composition, structure and functional activity could have potential radioprotective ability. ⁽²⁾

The biological effects of irradiation are the last consequence of serial process and reactions induced by the action of radiation. Ionizing radiation is radiation, either particulate or electromagnetic, which sufficient energy leads to eject one or more electrons from the atoms or molecules with which it interacts and caused the atom to be ionized. The ionized atoms or molecules (reactive oxygen species, ROS) are chemically reactive and lead to another ioniza-

tion of atoms or molecules. When the energy loss of high-energy electrons is absorbed by water, approximately 100 eV of energy is deposited per ionization event to generate reactive oxygen species and an electron (radiolysis of water).⁽³⁾ The ionization effect of radiolysis of water in the biological environment causes structural changes of the polymer molecules, breaking of chemical bonds, formation of new covalent bonds, degradation of chemical structures and formation of abnormal molecules. The harmful effect of radiation could lead to cellular DNA damages, which are the most important radiation injuries leading to cell death.⁽⁴⁾ Such damages result in base substitutions, insertions and deletions, which might conduce to point mutations of DNA.⁽⁵⁾ These mutations play central roles in carcinogenesis.⁽⁶⁾ Detailed studies of both natural metabolites N-acetyl-L-cysteine ($C_5H_9NO_3S$) and trimethylglycine (betaine, $CH_3)_3N^+CH_2CO_2^-$) showed to have excellent antioxidant and potential radioprotective activity. Previous research studies reported that beer reduces radiation-induced chromosome aberrations in human lymphocytes.⁽⁷⁾ Trimethylglycine (betaine) is a beer component and native cellular metabolite that could have potential radioprotective effect. It is an N-trimethylated amino acid ($CH_3)_3N^+CH_2CO_2^-$ that is biosynthesized by oxidation of choline (trimethylaminoethanol) and is a component of another branch of homocysteine metabolic pathway and participates in the transformation of homocysteine to methionine. The second metabolite N-acetyl-L-cysteine is acetylated amino acid $C_5H_9NO_3S$ that is metabolized to cysteine and glutathione. Conversion of cysteine to glutathione is a part of homocysteine metabolism. N-acetyl-L-cysteine is a powerful antioxidant and has proven radiation protection activity. It has an ability to protect against γ -induced single or double DNA strand breaks (DSBs) and/or DNA deletions in yeast and mammals.⁽⁸⁾ Double DSBs are the most significant cause of the cell death and targeting of the cell directly to apoptosis.

PURPOSE

The purpose of that work was to determine and compare the potential radioprotective activity of two amino acids N-acetyl-L-cysteine (NAC) and trimethylglycine (betaine, TMG) applied preventively to experimental model.

MATERIALS AND METHODS

To achieve the objective of the study had been used innovative molecular biology techniques and dosimetry methods of analysis such as MTT-test for cytotoxicity, DNA extraction and DNA ladder assay.

MTT-assay (MTT-test for cytotoxicity)

Test group of primary, human lymphocytes isolated by ficoll method were tested with different concentrations in range of 50 μ g/ml – 500 μ g/ml of trimethylglycine (betaine, Merck, Germany) and N-acetyl-L-cysteine (Merck, Germany) for cytotoxicity. The lymphocytes were cultured in 96 well plates in a density 1.10^5 cells/ml and treated with different concentrations of both amino acids. The cells were incubated in incubator Heraeus B5060 EK/ CO_2 at 37°C, 5% CO_2 and a humidified atmosphere. Cytotoxicity was measured on 24, 48 and 72 hour after treatment. The MTT-assay is a mitochondrial, colorimetric test for assessing cell viability or loss of cell viability (cytotoxicity). That method is based on the reduction of yellow tetrazole MTT salt (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) to insoluble, purple formazan crystals in living cells. The formazan crystals were dissolved by isopropyl alcohol into a colored solution. The absorbance of this colored solution was quantified by measuring at a wavelength 550/630nm by ELISA reader (Bio-Tek Instruments Inc., USA). The degree of light absorption depended on the solvent and was correlated to the percentage living cells. The cytotoxicity effect was presented as a percentage of surviving cells as calculated against the non-treated control group cells.

When the tested concentration was defined the experiment continued to the next step –

sampling and culturing of the primary, human lymphocytes.

Sampling and culturing

Human peripheral blood was collected from 20 donors (aged 18–60 years), by the staff of the department of blood transfusion hematology of Military Medical Academy-Sofia with the official agreement of the donors. 5 to 10 ml peripheral blood from each donor was collected into a plastic tube BD Vacutainer K2E with EDTA. 0, 5 ml blood of the blood sampling was culturing in 4, 5 ml RPMI-1640 culture medium (Bio-Whittaker, Lonza) supplemented with 20% FBS (PAA Laboratories GmbH, Austria), 2 Mmol L-glutamine (Sigma-Aldrich Co.), 100UI/ml penicillin (Sigma-Aldrich Co.) and 0.1 mg/ml streptomycin (Sigma-Aldrich Co.). 50 µl 2% PHA (Sigma-Aldrich Co.) was added to every plastic tube to stimulate the lymphocytes proliferation. The blood has been cultured in tissue blood plastic tubes at 37°C, 5% CO₂ and a humidified atmosphere in a cell culturing incubator Heraeus B5060 EK/CO₂. Has been used GOU-3M ¹³⁷Cs-source unit (a property of Institute of plants physiology and genetics, Sofia, Bulgarian Academy of Science). The samples were irradiated with 1 Gy and 3 Gy radiation absorbed dose. Have been defined four groups of samples for each radiation dose:

- 1) Control group of non-treated and non-irradiated lymphocytes.
- 2) Control group of non-treated, irradiated lymphocytes.
- 3) Lymphocytes treated with 200 µg/ml TMG two hours before irradiation
- 4) Lymphocytes treated with 200 µg/ml NAC two hours before irradiation
- 5) The blood cultures were culturing 24 hours and 48 hours before DNA extraction. When the fixed time of culturing passed followed the DNA extraction of the samples.

DNA extraction (DNA isolation) for DNA ladder assay

The contents of the culturing plastic tubes were transferred to 15 ml centrifuge tubes. The DNA isolation followed the protocol described below:

- 1) Centrifuged the samples 1300 rpm for 8 minutes, discarded the supernatant and left 0.5 ml cell pellet of mixed blood cells. Brought volume 4.5 ml 1 x RBC Lysis Buffer (800 ml ddH₂O, 8.9 g NH₄Cl, 1.0 g KHCO₃, 2.0 ml 0.5 M EDTA) to 0.5 ml cell pellet at a ratio of 9:1. The RBC (red blood cells) Buffer lysed the erythrocytes and left the lymphocyte fraction. The samples had been left at room temperature for 10–15 minutes to become full lysis of the erythrocytes.
- 2) Centrifuged the samples and discarded the supernatant. Milky white pellet was formed.
- 3) To the pellet was added 500 µl Elution Buffer (109.5 g sucrose, 10 ml 1 M Tris HCl (pH 7.6), 5 ml 1 M, MgCl₂, 1% Triton-X-100), resuspended with the pipette and kept on ice for 30 minutes. The lysate was transferred to 2 ml eppendorf tubes and centrifuged for 5 minutes at 12,000 rpm/min. The supernatant was carefully transferred to another clean 2 ml eppendorf tube.
- 4) To the supernatant was added 10 µl in concentration 20 mg/ml Ribonuclease A (from bovine pancreas, Sigma-Aldrich).
- 5) The samples were incubated for 60 minutes on 37°C by use of wash bath.
- 6) To every sample was added of 10 µl Proteinase K in concentration 20 mg/ml (from Tritirachium album, Sigma-Aldrich).
- 7) The samples were incubated for 60 minutes on 50°C by use of wash bath.
- 8) To every sample of was added 60 µl 3 M CH₃COONa (sodium acetate).
- 9) The DNA was precipitated by mixing supernatant in equal volume of cold isopropyl alcohol (2-propanol, C₃H₇OH) and invert to mix gently. The samples were incubated for 2 minutes. Then were centrifuged on 12000 rpm/min for 7 minutes and the supernatant was discarded.
- 10) The pellet in every sample contained the precipitated DNA. To every sample was added 500 µl 70% ethanol to wash the DNA. Centrifuged the samples on 12000 rpm/min for 7 minutes and discarded the supernatant.
- 11) The washing step repeated with addition of 500 µl absolute ethanol. Centrifuged the samples on 12000 rpm/min for 7 minutes and discarded the supernatant.
- 12) Using a pipettor was carefully removed all of the remaining liquid in the bottom of the tube.

- 13) The DNA-pellets dried for 5 to 20 minutes to remove any remaining ethanol and to every sample was added 10 µl TE-Buffer.
- 14) The samples were stored to -20°C before use.
- 15) For the DNA ladder assay was determine DNA concentration (Abs 260–280 nm) by spectrophotometer NanoDrop 2000 (Thermo Scientific). The concentration of DNA should be in the range of 100–300 ng/µl.
- 16) The sample was ready to be loaded on 1% agarose gel for electrophoresis. The agarose gel electrophoresis was performed by Mini-Sub Cell GT system (Bio-Rad) equipment. The gel was prepared by dissolving agarose powder in TAE Buffer. First 5 µl of DNA loading buffer was added and load DNA samples into dry wells of a 1% agarose gel in TAE Buffer. For size reference and ladder analysis, was loaded a 100-bp size ladder pre-mixed with loading dye (Ez-Way™ DNA 100bp Ladder, Komabiotech). DNA of every sample was visualized by staining the gel after electrophoresis in 1 µg/ml ethidium bromide-containing TAE buffer for 1 hour and washed in H₂O. The gel was running at a low voltage, which improves resolution of DNA fragments (i.e., 35 V for ~4 hours or until loading dye has run two-third of the way down the gel).
- 17) DNA ladders were finally visualized by a UV light source and documented by photography.
- 18) *Statistical analyses*

Descriptive, parametric, non-parametric, correlation, Mann-Whitney test and ANOVA statistical analyses were made by SPSS software (version 22). Graphic analysis of data was performed using MS Office Excel 2013. The standard error of the mean (S.E.M) should be $p < 0.05$ to be considered as statistically significant.

RESULTS AND DISCUSSIONS

MTT assay result

The first part of the study included determination of the cytotoxicity of NAC and TMG in concentration range of 50 µg/ml – 500 µg/ml. According to the cytotoxicity effect was chosen 200 µg/ml concentration for both

tested amino acids. It was chosen concentration the highest concentration that presented excellent survival (non-toxicity) to all tested cultures for both amino acids NAC and TMG. The data analyzing was performed by Mann-Whitney test and ANOVA statistical analyses were made by SPSS software (version 22) and S.E.M. was $p < 0.05$.

DNA LADDER FORMATION

The nucleosome is the fundamental unit of chromatin in eukaryotes, consisting of DNA wrapped around an octamer of two pairs each of H2A, H2B, H3 and H4. Histone H1 is a linker histone that compresses linear nucleosome arrays into a 30 nm chromatin fiber. Some reports have shown that some of the histone variants have specialized biological functions such as DNA repair and regulation of the expression.⁽⁹⁾ Functional isoforms of histones are randomly distributed throughout the mammalian and human genome.⁽¹⁰⁾ The histones comprise the nucleosome core, from which the histone tails are visible and could provide potential sites for histone modifications phosphorylation and acetylation, which are very important for the physiology processes of the cell (replication and transcription of DNA). H2AX is a member of the histone H2A family.⁽¹¹⁾ The sequence that differ H2AX from the other members of H2A family is a located in the C-terminal motif Amino acid Ser139 in this special motif is the place of γ -induced phosphorylation, mediated by an unknown signaling pathway. Phosphorylated H2AX (γ H2AX) forms nuclear foci at the sites of γ -induced double DNA strand breaks (DSBs) and could play an important role in the DNA repair after the irradiation induced DNA damage.⁽¹²⁾ If the reparation process is unsuccessful the cell is going to apoptosis. The γ H2AX foci formation doesn't mean that obviously are formed DNA double strand breaks but correlate with irradiation-induced DNA damage and is used by radiobiologists as a sufficient dosimetry biological marker. In previous studies was examined that DNA ladder formation

requires H2AX phosphorylation and correlate with γ H2AX foci formation. UV-visualized gel of DNA ladder assay could show different color bands on the agarose gel between genomic DNA and nucleosomal DNA fragments, which include histones and γ -induced phosphorylated H2AX molecules. The DNA ladder bands correlate with the quantity of apoptotic cells damaged DNA and γ -induced phosphorylated H2AX. The principle of DNA-ladder method define that apoptotic cells will form a distinct DNA ladder with clear visible bands. The genomic DNA from viable cells will stay on the top of the gel as a highest molecular-weight band.

As it is visible on the Figure 1: A) & B) for the first period of culturing (24 hours), both examined amino acids had significant radiation protection activity. The DNA samples of the group treated with NAC two hours before irradiation (for both absorbed doses) did not show any laddering that correlated with the whole, undamaged genomic DNA. The same result was observed for the DNA samples of the group treated with TMG two hours before irradiation

(for both absorbed dose). The DNA sample of non-treated, irradiated control group presented radiation-induced ladders formation. The non-treated, non-irradiated group did not show any laddering and confirmed the accuracy of the results.

The results are different for the second period of culturing (48 hours) as it is visible on Figure 2: A) & B). The DNA samples of the group treated with NAC two hours before irradiation with 1 Gy absorbed dose did not show any laddering that correlated with the whole, undamaged genomic DNA. Some of the samples of the group treated with TMG two hours before irradiation with 1 Gy absorbed dose showed ladders formation that correlated with DNA damage and apoptosis. The DNA sample of non-treated, irradiated control group presented radiation-induced ladders formation. The non-treated, non-irradiated group did not show any laddering and confirmed the accuracy of the results. (Figure 2: A) The DNA samples of the groups treated with NAC and TMG two hours before irradiation with 3 Gy absorbed dose showed laddering formation to the half of tested group, which

Figure 1: DNA ladder formation for culturing period of 24 hours:

A) Samples treated with 1 Gy absorbed dose; B) Samples treated with 3 Gy absorbed dose.

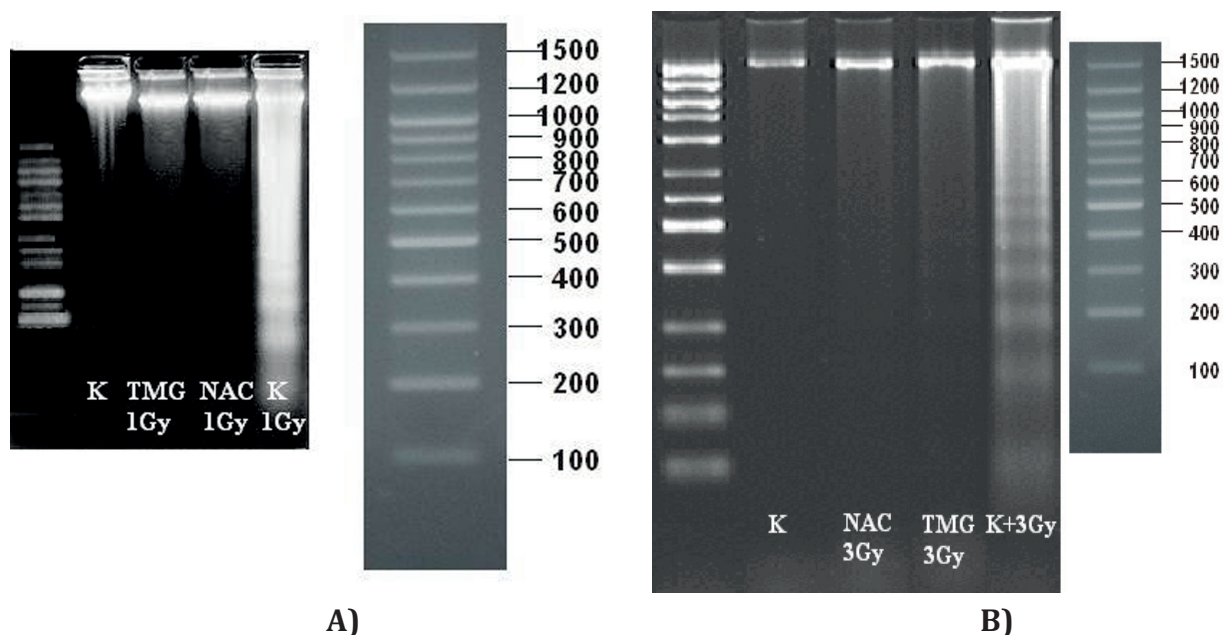
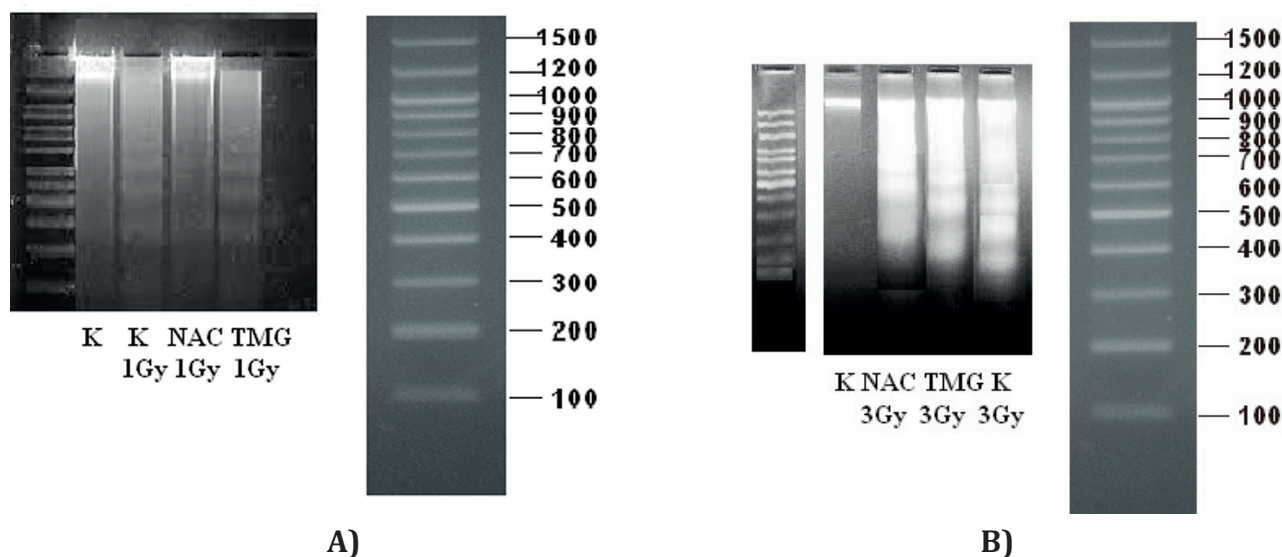


Figure 2: DNA ladder formation for culturing period of 48 hours:
A) Samples treated with 1 Gy absorbed dose; B) Samples treated with 3 Gy absorbed dose.



correlate with DNA damage and cell apoptosis. The radiation protection activity of both amino acids decrease with culture time and increasing of absorbed dose. Their radioprotection ability cannot compensate such serious radiation injuries.

The quantification of the results is shown on the Figure 3.

Both of the amino acids NAC and TMG showed significant radiation protection activity to primary, human lymphocytes irradiated with 1 Gy and 2 Gy absorbed doses. The results of DNA ladder formation assay showed that NAC performance is relatively higher than that of TMG.

CONCLUSION

The results of the presented study showed significant radiation protection activity against DNA damage (chromosome damage and DNA strand breaks) that correlated with their ability to decrease the intracellular reactive oxygen species. The comparison between both amino acid radioprotective ability showed that NAC presented better protection, which correlated with its excellent antioxidant properties.

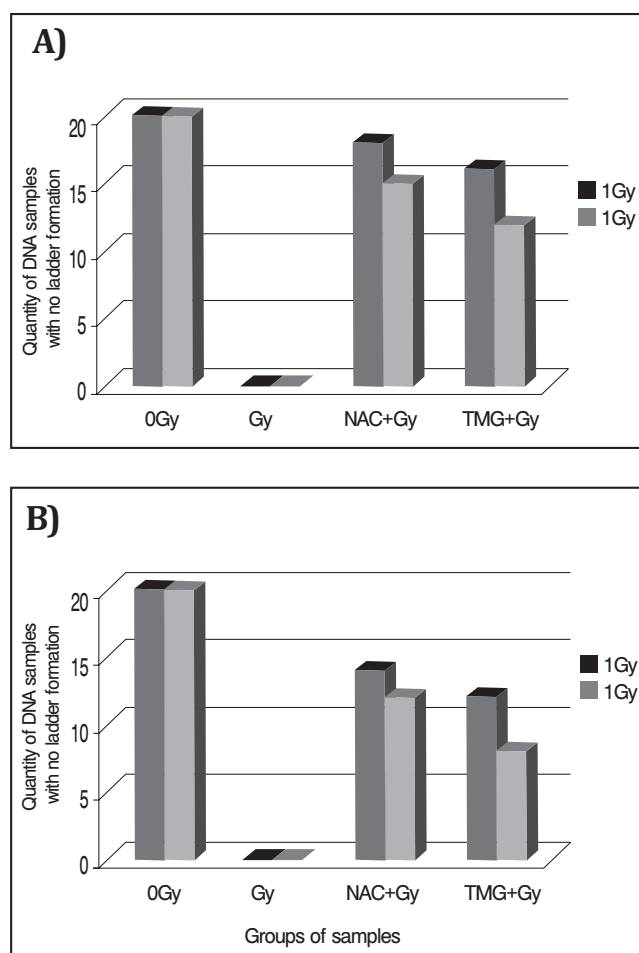


Figure 3: Quantification of the results for 1 Gy and 3 Gy absorbed doses for A) 24 hours cultures; B) 48 hours cultures.

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UP TO DATE PROBLEMS IN EARLY DIAGNOSIS AND REVEALING OF CHRONIC INTOXICATIONS WITH PESTICIDES

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АКТУАЛНИ ПРОБЛЕМИ В РАННАТА ДИАГНОСТИКА И РАЗКРИВАЕМОСТ НА ХРОНИЧНИТЕ ИНТОКСИКАЦИИ С ПЕСТИЦИДИ

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РЕЗЮМЕ

Пестицидите са разнородна в химическо отношение група включваща много на брой широко използвани в селското стопанство препарати за агрохимическа защита.

За разлика от острите отравяния, чиято етиология, патогенеза, клинична картина, диагностика и лечение са известни, проблемът с хроничните интоксикации все още е обект на проучване в специализираната токсикологична литература.

Хроничните интоксикации с пестициди се регистрират по-рядко от острите и причините за това са много на брой. Една от тях е в особеностите на клиничното протичане на острите интоксикации и спецификата на органотропната токсичност присъща за отделните групи препарати. А еднократната експозиция на токсични дози от препарата обуславя остроото начало и бързо развитие на клиничната картина, което улеснява диагностиката на отравянето.

Хроничните интоксикации с пестициди са предимно професионални. Този факт се свързва

ABSTRACT

Pesticides are a various in their chemical characteristics group including a lot of broadly used in agriculture preparations for agrochemical protection. Unlike acute poisonings, whose etiology, pathogenesis, clinical presentation, diagnosis and treatment are familiar, the problem with chronic intoxications is still an object of study in specialised toxicologic literature.

Chronic intoxications with pesticides are registered more rarely than the acute ones and the reasons are numerous. One of them is the characteristic feature of clinical manifestations of acute poisonings and the specific organic toxicity of different groups of preparations. The single use of toxic dosage of the preparation determines the acute onset and fast development of the symptoms, that makes the diagnosis of the poisoning easier.

Chronic intoxications with pesticides are usually occupationally determined. That fact is related to the circumstance that occupational exposure is maintained over a longer amount of time and in

с обстоятелството, че професионалната експозиция се осъществява продължително време в по-ниски концентрации, обикновено на открито, със сезонни прекъсвания и в комбинация с други пестициди.

По-пълнен и точен анализ на този тип патология в национален мащаб може да се направи при наличие на данни от регистъра на професионалните заболявания. Предоставянето на такива, обаче, е регламентиран процес, а и тези данни представят предимно трудово-експертната част на проблема. От друга страна, анализът на този тип патология съотнесен към други страни от Европа и света се затруднява от неограничения брой проучвания в тази област.

Поради факта, че остри интоксикации с пестициди често се явяват битов проблем като израз на суицидни намерения, тяхната регистрация се осъществява в специализираните токсикологични центрове за лечение на остри отравяния, където пациентите се хоспитализират за диагностика и лечение.

Не така, обаче, стои въпросът с хроничните интоксикации, чието диагностициране и регистриране са силно затруднени.

Така се пораждат основателни подозрения за съществуването на този тип патология подобно на „айсберг“ – феномен характерен и за други групи заболявания.

Настоящото проучване се опитва да „повдигне завесата“ за наличието на такъв тип „скрита“ заболяемост, да установи факторите и причините, които допринасят за недостатъчната разкриваемост на хроничните отравяния с пестициди.

Ключови думи : професионална експозиция, пестициди, хронични интоксикации

lower concentrations, it is usually in open areas with seasonal breaks and in combination with other pesticides.

A fuller and more accurate analysis of this type of pathology on a national level could be conducted if data from the register of occupational diseases was available. However this is a question of the occupational and expert part of the problem. On the other hand the analysis of this type pathology done for other European countries and countries from all over the world is difficult because of the few conducted studies.

Due to the fact that acute intoxications with pesticides are often an everyday problem and could be a form of committing suicides their registration is conducted in specialised toxicologic centres for treatment of acute poisonings, where patients are hospitalized for treatment and diagnosis.

The problem with chronic intoxications is different though, their diagnosis and registration are very much hindered.

This is how reasonable suspicions of the existence of that kind of pathology resembling an iceberg are born – phenomenon characteristic for other diseases as well.

The current study is trying to open the curtain for such a hidden type of morbidity, to set the factors and reasons that contribute to the insufficient diagnosis of chronic pesticide poisonings.

Key words: professional exposure, pesticides, chronic intoxications

INTRODUCTION

According to their chemical content pesticides are divided into:

- Phosphorus containing compounds (PCC)
- Chloro – organic compounds (COC)
- Carbamates and dithiocarbamates
- Nitrophenols
- Pyrethroids
- Dipyrindyles
- Thiazide derivatives

- Coumarin compounds
- Mercury containing pesticides
- Cuprum compounds
- Sulphur containing compounds
- Arsenic compounds
- Cyanogenic compounds

The possible ways of pesticides getting in human organism regardless if it's a long-term or short-term exposure, are inhalatory, oral, through the skin and mucosa.

Toxicokinetics and pathogenesis of chronic intoxications with different types of pesticides are different. Those differences come mainly due to different physico-chemical properties, way of influence, duration of contact, rhythm and dosage of exposure, volatility, organic and other phactors. since the discovered high toxicity, cancerogenic and teratogenic effects of chloro-organic pesticides and the following prohibition of their production and use, the use of other types of pesticides especially phosphorus containing pesticides has grown considerably.

And while clinical manifestation of acute poisonings with pesticides are well studied and their diagnosis isn't causing problems, the cases with chronic intoxications, which etiology is mainly occupational are more rarely and more difficultly diagnosed.

MATERIALS AND METHODS

The examined patients are hospitalized in the occupational diseases unit for the period from 2011 to 2012 year. The following features were analyzed:

- Total count of patients hospitalized in the unit over the above mentioned period.
- Indications for hospitalization
- Final diagnoses registered according to the ICD - 10

Official medical documentation is our source of information:

- Register of patients admitted
- Medical history of every patient with diagnosis on admission and final diagnosis, anamnestic data, physical examination, paraclinical tests and specialist opinions.
- Annual report of the diagnostic and treatment of patients.

RESULTS

In 2011 383 patients were admitted in our unit. The count of the diagnosed and treated in the unit patients for 2012 is 438. After analyzing all the collected data it was established that none of the 821 hospitalized in the unit in 2011 and

2012 patients was diagnosed with chronic pesticide intoxication.

The following groups of occupationally exposed to pesticides people in high risk were established after analyzing the data published in specialized medical literature and studies:

1. Directly occupied with the production of pesticides workers.
2. Workers occupied with storage and transportation of pesticides.
3. Agricultural workers using the different types of pesticides.
4. The occupied with work in areas treated with pesticides.
5. High risk of intoxication is present in workers occupied with periodical destruction of big amounts of stored pesticides especially after expiring of their sell-by date.
6. Professionally exposed to pesticides are chemists, lab-workers and engineers working in military centres.
7. Medical and medical staff in contact with patients with acute pesticide poisonings during their transportation, treatment and diagnosis.
8. Lab-workers, chemists and other specialists working with pesticides in different agricultural institutions.

DISCUSSION

Some pesticides, especially the phosphorus containing and chloro-organic pesticides are with high toxicity for people (1,2,3,4).

The repeated and long-term exposure to pesticides in low concentrations that is seen in occupationally exposed workers causes various injuries of different organs and systems. Extremely vulnerable to those is the neuropsychology of people. This fact is not random at all. Almost all groups of pesticides, regardless of their physico-chemical content, have different but mostly high levels of lipophilicity and are lipotropic. This is mostly known about phosphorus containing and chloro-organic pesticides. Phosphorus containing pesticides influence the central and peripheral neural structures through inhibition of neural esterases (2,4). Unlike acute poisonings chronic ones are developed slowly in time and their early diagnosis is

highly impaired due to the non – specific character of clinical manifestations. The injuries of neural system due to long – term exposure to low concentrations of phosphorus containing pesticides objectified in medical literature are various:

- Disorders of memory and concentration
- Desorientation
- Deep depression
- Irritability
- Confusion
- Headaches
- Nightmares
- Prolonged time of reaction
- Sleepwalking
- Sleepiness or insomnia
- High risk of Alzheimer's disease (10)
- Attention deficit hyperactivity disorder (ADHD) in children (related to the influence of phosphorus containing compounds on mothers during pregnancy) (11,12).
- Schizophrenia and schizophrenic syndrome
- Extrapramide syndrome

A syndrome resembling flu was also described (7) and the US Environment protection agency includes the parathione in the group of probable cancerogenes for people (8). Dichlorphos is also considered as a potentially cancerogenic as well as some carbamide pesticides (2,9).

Other organs and systems are not rarely influenced by chronic pesticide poisonings. In the table below systemic and organ disorders caused by long-term exposure to phosphorus containing agents are listed.

Cardiocas- cular system	Hematologic disorders	Gastrointestinal tract and liver	Skin and mucosa	Respiratory tract
Low arterial blood pres- sure	Toxic anemia with methe- moglobinemia (methaphos)	Gastral secretion disorders	conjunc- tivitis	Chronic bronchitis
bradycardia		From functional liver disorders to chronic	contact derma- titis	Bronchial asthma
toxic cardi- opathy		toxic hepatitis (chlorphos, methaphos)		

Chloro-organic pesticides (COP) are unique by their high cumulative activity. They are classic polytropic poisons with high polymorphism of organic injury (1). Apart from their systemic toxicity and neurotoxicity the long – term exposure to COP is also related to myelotoxic effect. Such is most commonly seen in poisonings with dyens (aldrine, dyeldrine). Different systemic and organ disorders are also seen when long term exposure to COP is present (1,2):

- Early atherosclerosis and high risk of coronary ischemia
- Chronic toxic hepatitis that manifests itself without pain and has a benign development and good prognosis.
- Toxic polyneuropathy
- Toxic encephalopathy
- Encephalomyelopolyradiculoneuropathy (severe chronic intoxications)
- Hematologic disorders – anemia, agranulocytosis, pancytopeny
- Sensitisation to different agents

Due to the established for some COP embriotoxic, teratogenic and mutagenic effect their manufacturing and usage in the USA as well as the countries in the EU were prohibited (1,2,4,13).

Sensitisation to different agents is reported for almost all groups of pesticides, the prolonged contact with such might cause irritative skin and mucosa injuries – irritative conjunctivitis, rhinitis, bronchitis, dermatitis (1). COP are highly allergenic (2).

The long term exposure to carbamate pesticides is related to neurotoxicity, thyreoid insufficiency, hemopoetic disorders.

In cases of chronic intoxications with nitro- and chlorophenole pesticides hepatorenal syndrome, toxic cataract, thyreoid hyperfunction are reported.

The injuries cause by long term exposure to mercury containing pesticides is various as well. They are manifested with hemorrhagic gingivitis and stomatitis, toxic cerebrastenya and encephalopathy, anemia, toxic cardiopathy, psy-

choorganic syndrome, auditory and visual hallucinations.

The copper containing pesticides can cause chronic intoxications when there is a long term exposure to low concentrations. They are manifested with vegetovascular dystonia, toxic pneumosclerosis, hemolytic anemia, yellow – green coloring of the skin and teeth and also dark-red colouring of the gingiva.

Factors determining the lack of accurate diagnosis of chronic pesticide intoxications:

- Occupationally exposed to pesticide workers rarely seek medical help due to fear of losing their jobs.
- The employers are not fully interested in the adequate and ontime diagnosis of health problems amongst exposed to pesticides workers.
- The lack of professional qualification and clinical experience amongst general practitioners and doctors in occupational medicine units, they are the first ones the patients seek.
- The not perfect model of healthcare that does not allow intoxicated patients to be treated and diagnosed at the right places in the right time.
- Chronic problems cause by the organisation of inpatient and outpatient treatment of risk groups, all that gets the patient into a circle between the general practitioner, occupational medicine units and specialized toxicologic and pathologic medical help.

Factors hindering the early diagnosis of chronic pesticide intoxications:

- Non specific clinical manifestation especially in the early stages of chronic intoxication is rarely related to exposure to pesticides.
- Broad spectrum differential diagnosis in such cases. The difficulties often originate in the difficult differentiation of diseases with similar manifestation as well as the need of differentiation between different groups of pesticides.
- Lack of medical equipment for toxo-chemical analysis and detection of preparations as well as detection of a certain causing agent and it's metabolites in biologic material (blood, urine etc.)
- Lack of knowledge of the norms in occupational diseases medicine which hinders the timely diagnosis of poisonings.

CONCLUSION

1. The adverse effects of pesticides in the condition of a long term professional exposure is a problem that is not well studied.
2. The reasons for the low rate of accurate diagnosis of chronic pesticide intoxications are complex. Although a leading role must be given to the lack of effective control of risk factors of the working environment, the insufficient clinical experience and knowledge of medical workers that are directly engaged with the healthcare problems and problems of work safety.
3. Early diagnosis and effective prevention from toxic influences in different professions are possible only if the mentioned above factors are solved.
4. The key to solving of those problems is the thorough knowledge of etiology, pathogenesis, clinical manifestation of intoxications with different groups of pesticides and the methods of their diagnosis. This is followed by patients being referred to occupational diseases specialists working at regional clinical centres.

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SKIN AND MUCOUSE REACTIONS IN PATIENTS WORKING IN AGRICULTURE

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КОЖНО-ЛИГАВИЧНИ АЛЕРГИЧНИ ПРОЯВИ ПРИ СЕЛСКОСТОПАНСКИ РАБОТНИЦИ

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РЕЗЮМЕ

Уртикарията и ангионевротичният оток са системни реакции, които се развиват както по алергични, така и по неалергични механизми. Те могат да бъдат и професионално обусловени, съответно IgE и не IgE медиирани.

Заетите в различни сфери на земеделието лица, често се явяват професионално експонирани на различни фактори, които са в състояние да отключат изявата на разнообразни в патогенетично и клинично отношение алергични реакции.

В специализираната медицинска литература публикации за случаи на уртикария и ангионевротичен оток при ангажирани със земеделски дейности пациенти, обаче, почти липсват.

Ключови думи: уртикария, ангионевротичен оток, професионален контакт, земеделие.

ABSTRACT

Urticaria and angioneurotic edema are system reactions that are developed by allergic as well as non – allergic mechanisms. They could also be professionally determined and mediated by IgE or without it's mediation. People working in different agricultural fields are often exposed to various factors that are able to unlock the manifestation of different in their pathogenesis and clinical course allergic reactions. Publications of cases of urticaria and angioneurotic edema in patients occupied with agricultural work are almost lacking in specialized medical literature.

Key words: urticaria, angioneurotic edema, professional contact, agriculture

INTRODUCTION

It is known that agriculture is a field that includes various types of activities some of which include contact with materials leading to sensitization of the organism.

Professional etiology is not rare for some allergic diseases – bronchial asthma, hypersensitivity pneumonitis, rhinitis. Professional risk for some diseases such as byssinosis is too high (1,2,3). The reason for the bronchoobstructive syndrome in those patients is the non – immune release of bronchoconstrictive substances and mediators such as histamine and 5 – hydroxytryptamine (2).

Thermophilic actinomycetes cause immune inflammation of the lung parenchyma after inhalation of rotten hay dust in cow farms. That is how the best studied type of hypersensitivity pneumonitis also known as farmer's lung is developed (4,5).

Different in their clinical manifestation and pathogenesis allergic diseases and reactions could be developed separately or simultaneously in one and the same professionally exposed to agricultural allergens person. However such cases are rare in clinical practice. That is why their diagnosis is interesting from a scientific point of view.

Materials and methods:

Two clinical cases of patients are presented – one with urticaria and another with angioneurotic edema. They were both treated and diagnosed at the Department of occupational diseases and allergology at the University hospital in Plovdiv. Both of the patients are occupied with agricultural activities that determine their direct or indirect contact with allergens. The first patient had been professionally been exposed to wheat, corn, barley. The second of the patients had also been in contact with agricultural products – barley, groats, wheat, feed etc. The inhalation of dust that comed out of those crops was prevalent. The noted in the medical documentation of the first patient symptoms were obstructed nose, sore throat, sneezing, spastic caught with expectoration of

a small amount of mucous sputum. The symptoms exacerbated during work.

The physical examination revealed urticarial rash localized on the axillar area and lower limbs including the feet in the first patient.

The second patient was also complaining of symptoms of rhinitis – profuse watery nose discharge, sneezing, itchiness, nasal congestion episodes. The symptoms of rhinitis are often accompanied by respiratory complaints – shortness of breath, wheezes, tightness in the chest, irritating cough. The complaints manifest and exacerbate after massive exposition to grain dust.

The physical examination detects nasal obstruction, dysphonia, angioneurotic edema on the back. Coarse vesicular breathing with prolonged expiration and diffuse crackles was found on auscultation. After a thorough inquiry about their medical background of allergies allergic reactions due to use of medications or food, or to insects and other agents that most commonly provoke similar symptoms according to literature data are excluded for both patients.

Data of other diseases of general and systemic origin that might cause urticaria or angioedema are not present in medical documentation or reported by both patients (connective tissue diseases, autoimmune disorders, neoplasms, infections or parasites).

Urticarial rash in patient 1

Localized on the right axillar area



On the right foot



Angioneurotic edema in 2 patient



Complement system serum tests of patient 1

Complement component	method	result	Normal results (adults)
C 3	nephelometry	0.98 g/l	0,61 – 2,09 g/l
C 4	nephelometry	0.34 g/l	0,122 – 0,495 g/l

Complement system serum tests of patient 2

Complement component	method	result	Normal results (adults)
C 3	nephelometry	1.66 g/l	0,61 – 2,09 g/l
C 4	nephelometry	0.39 g/l	0,122 – 0,495 g/l

DISCUSSION

Urticaria and angioneurotic edema are systemic allergic reactions that could be developed through allergic and non – allergic mechanisms (1,3,4,5,6). Although they are not prevalent and frequent they are some of the professional allergic diseases established with studies on national and regional levels (1,3). A possible reason for the edema in patient 2 could be hereditary angioedema (4,5,6). The congenital angioedema was excluded on the basis of clinical and laboratory criteria such as the normal levels of C4. It is known that C4 is the most precise diagnostic test for hereditary angioneurotic edema and is in low levels even between exacerbations (4,5,6). It is absolutely possible that the professional contact with agricultural materials of the presented patients was the one unlocking the sensitization of the organism.

Urticaria and angioedema are developed along with other concomitant allergic problems present in the patients – allergic rhinitis, asthma, and hypersensitivity pneumonitis must be excluded after more precise diagnostic process. In some already published studies the simultaneous or consecutive onsets of several allergic diseases were found in children (10,16,17). There is also a considerable count of similar combinations of allergic syndromes in people in active age as well as professionally exposed patients (3,7,8,12,14,15). Combined manifestation with urticaria and angioneurotic edema is not rare as well (9,11,13).

However the role of the factors of the working environment that can unlock such problems are poorly studied. The activities the agricultural work involves also contribute to this, which could be seen by the presented by us cases. The criteria that support the professional etiology of the allergic diseases found in the patients are presented in the table.

They are in concordance with the used in professional diseases pathology criteria accepted as norms that determine whether a disease is professionally determined.

Criteria for diagnosis and evaluation of professional etiology of the objectified in the patients allergic pathology:

Work hygiene	Etiologic factors	Clinical manifestation (diagnosis, syndromes)
Objectifies the professional occupation and typical risk factors (in the presented cases this is a professional contact with agricultural products – seeds, grains, feed etc.)	Allergenes that the agricultural products contain (proteins), toxic pesticide substances used for cultivation, preservation, storage and transportation of the plants. Dust inhaled from grains and feed.	Urticaria Angioneurotic edema Bronchial asthma Allergic rhinitis Hypersensitivity pneumonitis

Gratis period	Conditions for acceptance of professional etiology
1) Not necessary for atopic diseases 2) For other types – from 1 to 10 years depending on the type and term of exposure	1) Proven professional contact 2) Praclinical tests: – Chest roentgenography – Functional lung tests – CT – scan – General and antigen specific IgE test – Immunologic tests – Otolaryngologic examination

CONCLUSIONS

1. Manifestation of systemic allergic reactions such as urticaria and angioneurotic edema in people working in agriculture is possible and should not be ignored.
2. Pathogenic mechanisms and clinical manifestation of allergic reactions could be too various.
3. Professional etiology of allergic diseases should be discussed on the basis of objective criteria officially accepted as norms.
4. The thorough knowledge of risk factors of work environment and mechanisms of allergic pathology results in more effective treatment of problems present in such patients.

The photographic material is published with the written consent of the patient. Her personal data is protected by Bulgarian law and normative legislation of the University hospital.

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The Bulgarian Medicine Journal, official edition of the Bulgarian Academy of Science and Arts, Science Division, Research Center for Medicine and Health Care is published in 4 issues per year. It accepts for publication reviews, original research articles, case reports, short communications, opinions on new medical books, letters to the editor and announcements for scientific events (congresses, symposia, etc) in all fields of fundamental and clinical medicine. The journal is published in English with exceptional reviews on significant topics in Bulgarian. The detailed abstracts and the titles of the articles, the names of the authors and institutions as well as the legends of the illustrations (figures and tables) are printed in Bulgarian and English. Bulgarian medicine is available online at the website of the Academy, publications section.

The manuscripts should be submitted in two printed copies, on standard A4 sheets (21/30 cm), double spaced, 60 characters per line, and 30 lines per standard page.

The size of each paper should not exceed 10 pages (up to 5 000 words) for original research articles, 12 pages for reviews (7 500 words), 3 pages for case reports, 2 pages for short communications, 4 pages for discussions or correspondence on scientific events on medical books or chronicles. The references or illustrations are included in this size (two 9x13 cm figures, photographs, tables or diagrams are considered as one standard page).

The abstracts are not included in the size of the paper and should be submitted on a separate page with 3 to 5 key words at the end of the abstract. They should reflect the most essential topics of the article, including the objectives and hypothesis of the research work, the procedures, the main findings and the principal conclusions. The abstracts should not exceed one standard typewritten page of 200 words.

Списание „Българска медицина“, издание на Българската Академия на Науките и Изкуствата, Отделение за наука, Научен център по медицина и здравеопазване, излиза в четири книжки годишно. „Българска медицина“ е достъпна онлайн на сайта на БАНИ, раздел издания.

В него се отпечатват оригинални научни статии, казуистични съобщения, обзори, рецензии и съобщения за проведени или предстоящи научни конгреси, симпозиуми и други материали в областта на клиничната и фундаменталната медицина. Списанието излиза на английски език с подробни резюмета на български и английски. Изключения се правят за обзорни статии по особено значими теми. Заглавията, авторските колективи, а също надписите и означенията на илюстрациите и в таблиците се отпечатват и на двата езика.

Материалите трябва да се предоставят в два еднакви екземпляра, напечатани на пишеща машина или на компютър, на хартия формат А4 (21 x 30 cm), 60 знака на 30 реда при двоен интервал между редовете (стандартна машинописна страница). Освен това могат да бъдат изпратени като прикачени файлове по електронната поща на адресите, посочени по-долу.

Обемът на представените работи не трябва да превишава 10 стандартни страници за оригиналните статии (или 5000 думи според стандарта на англосаксонските издания) 12 страници (7 500 думи) за обзорните статии, 3–4 страници за казуистичните съобщения, 4 страници за информации относно научни прояви в България и в чужбина, както и за научни дискусии, 2 страници за рецензии на книги (монографии и учебници). В посочения обем се включват книгописът и всичките илюстрации и таблици. В същия не се включват резюметата на български и английски, чийто обем трябва да бъде около 200 думи за всяко

The basic structure of the manuscripts should meet the following requirements:

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The title of the article, forename, middle initials (if any) and family name of each author; institutional affiliation; name of department(s) and institutions to which the work should be attributed, address and fax number of the corresponding author.

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Titles and subtitles should be standardized.

The original research reports should have the following structure: introduction (states the aim, summarizes the rationale for the study), subjects and materials, methods (procedure and apparatus in sufficient detail, statistical methods), results, discussion, conclusions (should be linked with the aims of the study, but unqualified statements not completely supported by research data should be avoided). These requirements are not valid for the other types of manuscripts. Only officially recognized abbreviations should be used, all others should be explained in the text. Units should be used according to the International System of Units (S. I. units). Numbers to bibliographical references should be used according to their enumeration in the reference list.

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REFERENCES

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EXAMPLES:

Reference to a journal article:

1. McLachan, S. , M. F. Prumel, B. Rapoport. Cell Mediated or Humoral Immunity in Graves' Ophthalmopathy? J. Clin. Endocrinol. Metab., 78, 1994, 5, 1070-1074.

Reference to a book chapter:

2. Delange, F. Endemic Cretenism. In: The Thyroid (Eds. L. Braveman and R. Utiger). Lippincott Co, Philadelphia, 1991, 942-955.

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ПРИМЕРИ:

Статия от списание:

1. McLachlan, S., M. F. Prumel, B. Rapoport. Cell Mediated or Humoral Immunity in Graves' Ophthalmopathy? J. Clin. Endocrinol. Metab., 78, 1994, 5, 1070-1074.

Глава (раздел) от книга:

2. Delange, F. Endemic Cretenism. In: The Thyroid (Eds. L. Braveman and R. Utiger). Lippincott Co, Philadelphia, 1991, 942-955.

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