Sympathetic Nerve Injury in Thyroid Cancer

Evangelos Diamantis[1], Paraskevi Farmaki[1], Spyridon Savvanis[2], Georgios Athanasiadis[3], Theodoros Troupis[1,*], Christos Damaskos[4]

ABSTRACT

The double innervation of the thyroid comes from the sympathetic and parasympathetic nervous system. Injury rates during surgery are at 30% but can be minimized by upwardly preparing the thyroid vessels at the level of thyroid capsule. Several factors have been accused of increasing the risk of injury including age and tumor size. Our aim was to investigate of there is indeed any possible correlations between these factors and a possible increase in injury rates following thyroidectomy.

Seven studies were included in the meta-analysis. Statistical correlation was observed for a positive relationship between injury of the sympathetic nerve and thyroid malignancy surgery ($p < 0.001$; $I^2 = 74\%$) No statistical correlations were observed for a negative or positive relationship between injury of the sympathetic nerve and tumor size. There was also no statistically significant value observed for the correlation of the patients' age with the risk of sympathetic nerve injury ($p = 0.388$). Lack of significant correlation reported could be due to the small number of studies and great heterogeneity between them.

KEYWORDS

thyroid neoplasm; sympathetic innervation; thyroidectomy

AUTHOR AFFILIATIONS

[1] Department of Anatomy, National and Kapodistrian University of Athens, Greece
[2] Department of Internal Medicine General Hospital of Athens "Elpis", Athens, Greece
[3] Thoracic Clinic, Red Cross Hospital, Athens, Greece
[4] Second Department of Propedeutic Surgery, Laiko General Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece
* Corresponding author: Mikras Asias 75 Street, Athens, Greece; e-mail: ttroupis@gmail.com

Received: 26 June 2017
Accepted: 26 October 2017
Published online: 23 April 2018

Acta Medica (Hradec Králové) 2017; 60(4): 135–139
https://doi.org/10.14712/18059694.2018.8

INTRODUCTION

Thyroid gland is considered as one of the most important glands of the human body (12). The complexity of the coordinated operations is reflected in its dual innervation which comes from the sympathetic and parasympathetic nervous system. Adrenergic postganglionic sympathetic fibers are vasomotor; they originate from the top and the middle cervical sympathetic ganglion and enter the gland along with its arteries. The preganglionic parasympathetic fibers originate from the vagus nerve and reach the gland with the branches of the laryngeal nerves. They also innervate the vasculature of the gland – regulating blood circulation – and indirectly affect the secretory function (20).

Knowing the topographical anatomy of the recurrent laryngeal nerve is crucial in the case of thyroid gland surgical removal, since a nerve injury may result in hoarseness or even mutism if there is a bilateral lesion. On the other hand, although not always identified due to anatomical variations, the external laryngeal nerves innervate the cricothyroid muscle (25). Taking into account its anatomical relationship to the superior thyroid artery, a possible injury during surgery could have severe complications in patients' quality life such as voice fatigue, decreased voice volume and range. Interestingly, the frequency of external branch of superior laryngeal nerve's palsy rages from 0.3% to 58% (21).

The thyroid carcinomas constitute about 0.6% of all malignant tumors in men and 1.6% in women, while in Europe, between 1978 and 1997, the appearance of thyroid carcinomas increased by about 3% (5). Prognosis and treatment depends on the type of thyroid cancer, tumor size and early diagnosis. Moreover, though patients' overall prognosis is considered excellent, it is widely accepted that thyroid carcinomas have a high risk of metastases occurrence, mainly through perineural filtration; cancerous cells could be installed on any tissue along the neural axis filter and then be transferred to the respective blood vessel (7, 3, 12). Moreover, once the nerve fibers from metastatic cells of the thyroid's primary tumors are affected, this metastasis can be applied to the same nerve fibers, resulting in their overstimulation or understimulation (6, 13). These distant metastases are used as prognosis markers. However, until now, the issue of whether the sympathetic innervation is associated with the prognosis of primary malignant tumors has not been clarified. Total thyroidectomy is considered the treatment of choice in thyroid gland malignancies (19, 2). Though only rarely mentioned, there is a possibility of injury to each specific nerve, especially in larger tumors. In our study, we want to examine the relationship between the malignancies of the thyroid gland and the injury risk to the sympathetic nerve aggregated.

MATERIALS AND METHODS

DATA COLLECTION

Information on clinical trials conducted between 2007 and 2015 was searched on the databases Scopus, Medline (PubMed), Cochrane Library and Clinicaltrials.org. The terms used had only the search link * AND, (e.g., Thyroid carcinoma * AND sympathetic innervation), so the results comprised all studies reporting in both search terms' sets. The data collection included all studies related to the types of thyroid malignancies, including thyroid cancer, the existence of sympathetic nerve trauma or injury absence. We also performed a search on the references of the retrieved studies and on the table of contents of periodicals related to the thyroid and its malignancies and surgical journals that publish articles on thyroidectomy. Finally, we studied the references of other systematic studies or meta- analyses of the same topic to find any additional sources within the timeline during the past three years.

STUDIES SELECTION

The parameters on which the articles were selected are listed below:

a. Inclusion of both genders in the research;
b. Reference of the average ages in the studied groups;
c. Type of carcinoma;
d. Existence of metastases;
e. Number of nodes (metastatic and non);
f. Existence and number of outbreaks;
g. Existence and number of nodules;
h. Existence of thyroidectomy;
i. Existence of temporary or permanent injury or paralysis of sympathetic innervation of the thyroid.

We did not include single case studies or single case analyses, since it would not be possible to extract sufficient information on group comparison from such studies. Finally, studies in the form of abstracts from conferences and scientific meetings were not included in the analysis. We did not include studies with less than 100 participants.

After thorough research and categorization of studies, the final number of studies used for the meta-analysis and further statistical correlation was seven. For all of the parameters tested, we calculated the frequencies and their distributions. In response to the patients' age and tumor size, we calculated the averages, which were then used in subsequent analysis.

STATISTICAL ANALYSIS

OpenMetaAnalyst for Windows 7 64-bit software – complete and free software designed and distributed from Brown University Public Health – and IBM SPSS statistical package version 22 were used for the analysis, while the description model used was the fixed effects model. To confirm the accuracy of the results, three different computer models, whose results converge, were used. The model chosen for the description of the analysis is the fixed effects model (9). The statistical correlations examined are listed below:

- The relationship of patient's age to the likelihood of sympathetic nerve of thyroid injury;
- The correlation of the type of thyroid carcinoma with the probability of sympathetic nerve injury;
- The correlation of tumor size with the possibility of sympathetic nerve injury during thyroidectomy;
- The correlation of metastatic lymph nodes with the possibility of sympathetic nerve injury;

– The relationship between the coexistence of thyroiditis in patients with thyroid cancer with sympathetic nerve injury.

The analyses were performed with χ^2 methods for proving the existence of possible correlation parameters, followed by ANOVA analysis, to detect any statistical difference between the types of parameters used. All parameters studied and analyzed were grouped into categories according to their means and distribution.

To assess the investigation sensitivity, the overall reasons mutandis (summary odds ratios (ORs)) were calculated, as well as their confidence intervals. The confidence interval was chosen in accordance with the limits set out in all normal Gauss distributions and, therefore, in our study, confidence intervals are at 95%. The heterogeneity between studies is estimated as a measure of heterogeneity and is denoted as I^2, which is a parameter calculated from a prior statistic called the Q statistic $\{I^2 = [(Q - df) / Q] \times 100\}$. This relationship describes the variability rate of the sample, which is due to the heterogeneity of the sample due to different sizes in the studies involved in the analysis rather than an error of the sampling. While the variable Q statistic depends on the number of the studies involved in the analysis, the I^2 values are between 0–100%. In a broader research context, values totaling more than 50% are considered to express great heterogeneity (6, 16).

RESULTS

The trials included in our meta-analysis study the permanent injury of the sympathetic nerve after surgery, the temporary injury of the sympathetic nerve, the presence of other side effects after surgery and the complete absence of injury and side effects. The seven studies fulfilling the requirements are shown in tables (Table 1).

The mean patients' age per study was 46.6131 years and the average tumor size was 1.69 cm. The average number of male patients is 46.69 men per survey, while the average number of female patients is 212.54 per survey – 4.55 times more than men. There were 123 breakouts on average in patients per study and 201.33 metastatic lymph nodes, contributing to the aggressiveness of the disease. The types of thyroid malignancies observed in this study were papillary carcinoma, follicular carcinoma and goiter, and there was one study that reported Graves's disease.

The sensitivity of our sample ranges from 0.382 to 0.472. Though the sensitivity of each survey sample does

Tab. 1 Studies included in the meta-analysis. M: men, W: women.

Study	Neoplasm type	Patients' age	Patients (N)	Tumor size	Breakouts (N)	Lymph nodes (N)	Thyroidotis	Metastases (N)	State of sympathetic innervation
Chang 2016 [6]	papillary thyroid carcinoma	46.2	613 (M: 55, W: 558)	0.8	152	239	293	1	Injury
Kwon 2015 [16]	papillary thyroid carcinoma	53.3	10 (M: 1, W: 9)	0.96	–	19	–	1	No injury
Gao 2015 [10]	papillary thyroid carcinoma	32.02	137 (M: 2, W: 135)	0.82	–	–	–	–	Injury
Kihara 2014 [15]	papillary thyroid carcinoma	59.9	18 (M: 3, W: 15)	–	–	–	391	–	Injury
Lang 2014 [17]	papillary thyroid carcinoma	46.1	1291 (M: 188, W: 1103)	0.8	425	845	Graves' disease	845	No injury
Giannopoulos 2013 [11]	follicular carcinoma	38.2	44 (M: 5, W: 139)	1.68	–	–	–	–	Injury
Wang 2014 [24]	papillary thyroid carcinoma	50	188 (M: 35, W: 153)	1.2	144	83	–	–	
Lee 2015 [18]	papillary thyroid carcinoma	49	34 (M: 10, W: 24)	2	17	22	–	43	Injury
Tamatea 2014 [23]	papillary thyroid carcinoma	42	8 (M: 2, W: 6)	0.57	–	–	218	–	No injury
Conzo 2014 [8]	follicular carcinoma	46.65	712 (M: 197, W: 524)	1.76	–	–	–	–	No injury
Boute 2013 [4]	follicular carcinoma	51	83 (M: 37, W: 46)	1	–	–	–	–	Injury
Perie 2013 [22]	Graves' disease, goiter	47.1	100 (M: 19, W: 81)	–	–	–	1, Graves' disease (27)	–	Injury

Tab. 2 Sensitivity results and confidence intervals for the studies of the meta-analysis.

Study	Sample sensitivity	Upper limit (95%)	Lower limit (95%)	Sample specialty	Upper limit (95%)	Lower limit (95%)
Boute 2013 [4]	0.098	0.037	0.233	0.786	0.637	0.885
Chang 2016 [6]	0.026	0.006	0.097	0.976	0.959	0.986
Gao 2015 [10]	0.143	0.020	0.581	0.974	0.923	0.992
Kihara 2014 [15]	0.875	0.266	0.993	0.156	0.046	0.417
Perie 2013 [22]	0.900	0.326	0.994	0.809	0.719	0.876
Tamatea 2014 [23]	0.075	0.015	0.300	0.995	0.924	1.000
Wang 2014 [24]	0.045	0.003	0.448	0.992	0.960	0.998

not seem to be great, it would not affect the analysis due to the small number of patients participating in the study. The sample specialty ranges from 0.860 to 0.869, with the majority of values above 0.65. The values of the sample specialty range at the same level, ensuring a common logic in the investigations, despite the different circumstances considered and the direction followed (Table 2).

In the independent variables model we used, if considered a negative correlation with the injury of the sympathetic nerve, we do not have a significant value to indicate the thyroidectomy in patients with thyroid malignancies is negatively associated with sympathetic nerve injury. This result is supported by calculations for the heterogeneity of individual research of meta-analysis. The value of Q = 22.950 indicates medium heterogeneity of individual investigations and that value is verified by the statistical variable I^2, where the significance value of the variable Q is less than 0.001, and the value I^2 = 74% indicates medium heterogeneity of individual studies. Such heterogeneity observed is due to the nature of the meta-analysis and the research limits we have set, allowing a small number of studies to be included in this study. Therefore, we found a positive correlation between malignancy of thyroid cancer and injury of the sympathetic nerve tends to be statistically significant.

Subsequently, the analysis for odds ratio factor was done. The value < 0.001 is similar to that of the positive correlation of thyroid malignancy presence and sympathetic nerve injury during thyroidectomy.

Then, we performed statistical analyses of the individual studies to find the existence of a particular association. Initially, the descriptive statistics values of the parameter used in the statistical analysis were calculated. The parameters that provided sufficient and adequate information to be readily available and give good quality results are: the type of malignancy, the age, the numbers of male and female patients, the size of the tumor in cm, the number of outbreaks and the number of nodes.

The other parameters – shown in Table 1 – not included in the statistical analysis did not provide enough information or had deficiencies per survey, or they did not show homogeneity and, therefore, we did not prefer them for our analysis.

We also wanted to examine the possible relationship between the tumor size and sympathetic nerve injury. Patients were divided into two groups: tumors having a volume of less than 1.7 cm and those greater than 1.7 cm. The significance value of this statistic was 0.618 indicating no correlation between the tumor size and the sympathetic nerve injury during thyroidectomy. In addition, we wanted to examine the existence of correlation between the age of patients and sympathetic nerve injury. Patients were grouped into two categories: those older than 46 years and patients aged less than 46 years. The statistical likelihood ratio was used, reaching a statistical significance value of 0.388, which is much greater than the significance threshold of 0.05.

Finally, we wanted to test whether there is a correlation between the type of thyroid malignancy and sympathetic nerve injury. The malignancy groups are those mentioned above (i.e., one group is that of patients suffering from papillary carcinoma, one of patients suffering from follicular carcinoma and one of patients with goiter). The significance value for this parameter was 0.877, which shows that the type of thyroid malignancy is not related to sympathetic nerve injury in patients (likelihood ratio).

DISCUSSION

The prognosis of primary malignant neoplasms of the thyroid gland is high, and it sometimes reaches 90% for a five-year survival (22). Though many factors have been proposed – such as patient's age, tumor differentiation grade, extrathyroidal extension in the surrounding tissue, the presence of distant metastatic foci, the size of the primary tumor and others – as indicators of survival, their prognostic value is debatable (1). Sympathetic innervation is directly involved with thyroid gland neoplasms, and often during a surgical intervention, its proper functioning could be affected either transiently or permanently, thus leading to complete loss of the gland functions. Taking all these into account, we conducted a meta-analysis of relevant studies during the past 3 years to investigate if there is a negative or positive correlation between the development of all types of thyroid neoplasms and sympathetic nerve injury risk.

Chang et al. examined the risk factors and incidence of central lymph node metastases (CLNMs) in 631 patients with papillary thyroid microcarcinoma (PTMC) who underwent thyroidectomy and central lymph node dissection (CLNM). Researchers conclude that male sex, tumor size ≥ 0.5 cm, extrathyroidal extension and multifocality are independent risk factors for CLNM in PTMC (6). Moreover, Kwon et al. proved the ultrasound-guided charcoal

tattooing localization is safe and feasible in patients with papillary thyroid carcinoma (16). The bilateral areolar approach endoscopic thyroidectomy has also been evaluated as an alternate therapeutic treatment in selected patients with low-risk papillary thyroid carcinoma (PTC). Though this approach was found feasible and safe, large comparative series and long-term follow-up studies are needed to verify its oncologic safety (10).

Another important consideration is the possible thickening of the recurrent laryngeal nerve after thyroidectomy. Kihara et al. showed no impairment in the vocal cords' function in the vast majority of patients with papillary thyroid cancer (15). Boute et al. mentioned that despite central compartment dissection being associated with total thyroidectomy, it does not increase the risk of recurrent laryngeal nerve paralysis or permanent hypoparathyroidism and was found to be responsible for increased rates of transient hypoparathyroidism in differentiated thyroid carcinoma of the follicular epithelium (4).

Any systematic review and meta-analysis has a potential weakness of missing unpublished trials and a potential individual trial heterogeneity difficult to account for in analysis. It is obvious from the published trials that before-and-after trials tend to overestimate effectiveness, and even variation in the length of a randomized trial may affect the ability to detect underlying benefits.

In conclusion, the corresponding correlation models examined showed no significant associations for the parameters studied probably due to the small number of available studies and great heterogeneity between the surveys. A research with broader limits can ensure a greater number of studies and a smoother distribution, leading to future clinical implications. In this way, more data would be available in the meta-analysis model, allowing accurate estimation of statistical variables and producing more reliable results, which can be used as a prediction database for risk of sympathetic nerve injury of patients with thyroid neoplasms.

ACKNOWLEDGEMENTS

All authors have contributed equally to the design and integration of this manuscript.

We have not received any funding for this study.

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Dose Dependent Prophylactic Efficacy of 6-Chlorotacrine in Soman-Poisoned Mice

Jiří Kassa*, Jan Korábečný

ABSTRACT

Aim: The influence of the dose on the ability of promising newly prepared reversible inhibitor of acetylcholinesterase (6-chlorotacrine) to increase the resistance of mice against soman and the efficacy of antidotal treatment of soman-poisoned mice was evaluated. Methods: The evaluation of the effect of pharmacological pretreatment is based on the identification of changes of soman-induced toxicity that was evaluated by the assessment of its LD_{50} value and its 95% confidence limit using probit-logarithmical analysis of death occurring within 24 hrs after administration of soman. Results: The dose of 6-chlorotacrine significantly influences the prophylactic efficacy of 6-chlorotacrine. Its highest dose was only able to significantly protect mice against acute toxicity of soman and increase the efficacy of antidotal treatment (atropine in combination with the oxime HI-6) of soman-poisoned mice. In addition, the highest dose of 6-chlorotacrine was significantly more effective to protect mice from soman poisoning than its lowest dose. Conclusion: These findings demonstrate the important influence of the dose of 6-chlorotacine on its prophylactic efficacy in the case of pharmacological pretreatment of soman poisoning in mice.

KEYWORDS

soman; 6-chlorotacrine; atropine; HI-6; pharmacological pretreatment; mice

AUTHOR AFFILIATIONS

Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, Hradec Králové, Czech Republic
* Třebešská 1575, Faculty of Military Health Sciences, 500 01 Hradec Králové, Czech Republic; e-mail: kassa@pmfhk.cz

Received: 10 November 2017
Accepted: 27 January 2018
Published online: 23 April 2018

Acta Medica (Hradec Králové) 2017; 60(4): 140–145
https://doi.org/10.14712/18059694.2018.9

INTRODUCTION

The highly toxic organophosphorus compounds, called nerve agents, are still considered to be the most dangerous chemical warfare agents. They exert their toxic effects mainly by inhibiting acetylcholinesterase (AChE, EC 3.1.1.7), the enzyme responsible for deactivating the neurotransmitter acetylcholine (ACh) at cholinergic synapses. Nerve agent-induced irreversible inhibition of AChE in the central as well as peripheral nervous system leads to accumulation of ACh in the central and peripheral cholinergic synapses and to subsequent stimulation of both central and peripheral muscarinic and nicotinic cholinergic receptors. Death occurs due to an acute cholinergic crisis, with signs and symptoms such as excessive salivation, lacrimation, urination, defecation, sweating, bronchoconstriction, neuromuscular block, generalized seizures, respiratory distress and respiratory failure (1–2).

The medical countermeasures of poisoning with organophosphorus compounds are usually based on a combined administration of a muscarinic cholinergic receptor antagonist to block the overstimulation of cholinergic receptors by accumulated ACh at muscarinic receptor sites and an oxime to reactivate nerve agent-inhibited AChE. Generally, anticholinergics (mainly atropine) are used for relieving muscarinic signs and symptoms whereas AChE reactivators (generally nucleophilic compounds with high affinity for phosphorus), called oximes, are used to repair the biochemical lesion by dephosphonylation of AChE and restoring its activity. Although the antidotes against nerve agents and organophosphorus insecticides have been developed based on the knowledge of above-mentioned basic mechanism of acute toxicity of organophosphorus compounds, their efficacy is limited (3–4).

One of the most resistant nerve agents is soman (pinacolyl methylfluorophosphonate). Its deleterious effects are extraordinarily difficult to counteract due to the very rapid dealkylation of the complex soman-AChE, called aging. The dealkylation of soman bound on the active site of AChE makes the nucleophilic attack of oximes almost impossible (1,5). In addition, the main action of soman is in the central nervous system where the reactivating efficacy of all oximes is low owing to their limited penetration through blood-brain barrier (6–7). The unsatisfactory efficacy of antidotal treatment available for acute nerve agent poisonings, especially in the case of soman and tabun exposure, has brought another approach how to protect the humans from nerve agent-induced acute lethal toxic effects – using pharmacological pretreatment in the case of the threat of exposure to nerve agents. The term "pharmacological pretreatment" generally represents the medical countermeasures applied relatively shortly before penetration of a toxic agent into the organism with the aim of protecting the organism against its acute toxicity and increasing the effects of post-exposure antidotal treatment (8–10).

Up to date, the most common principle of pharmacological pretreatment is the protection of AChE against nerve agent-induced irreversible inhibition that is focused on the use of reversible cholinesterase inhibitors. Among reversible inhibitors of AChE, the carbamate pyridostigmine bromide is generally accepted and commonly used for the pharmacological pretreatment of nerve agent poisonings. It is stockpiled by various armed forces for pretreatment purpose against nerve agent poisoning and has been used by several thousand servicemen during UN operation against Iraq in 1991 (11). However, pyridostigmine is only able to protect peripheral AChE from irreversible nerve agent-induced AChE phosphonylation, while nerve agents, especially fluorophosphonates, can cross the blood-brain barrier (BBB) and, thus, express their deleterious effects through their central toxic effects including centrally mediated seizure activity that can rapidly progress to *status epilepticus* and finally contribute to brain damage (12). Therefore, the shortage of effectiveness of pyridostigmine bromide alone to increase the resistance of nerve agent-exposed experimental animals was demonstrated (13).

Thus, the replacement of pyridostigmine bromide with sufficiently effective reversible inhibitors of AChE with low toxicity and ability to cross BBB has been an important goal for the pharmacological pretreatment of nerve agent poisonings because the small decrease of the brain AChE activity (up to 20%) was found to be beneficial for an increase in the efficacy of pharmacological pretreatment and it does not affect the behavioral and neurophysiological functions of experimental animals according to our neurobehavioral research (14). A few years ago, a novel reversible inhibitor of AChE – 6-chlorotacrine (6-chloro-1,2,3,4-tetrahydroacridine-9-amine hydrochloride) (Figure 1) was synthesized at our Department of Toxicology and Military Pharmacy to improve the efficacy of pharmacological pretreatment against nerve agents and potentially for the treatment of Alzheimer`s disease. Recently, a promising ability of 6-chlorotacrine to increase the resistance of soman-poisoned mice and the efficacy of post-exposure antidotal treatment (atropine in combination with the oxime HI-6) of soman-poisoned mice was found (15). However, the dose of 6-chlorotacrine used in this study was too small to reach optimal inhibition of the brain AChE.

In the present study, the influence of the doses of 6-chlorotacrine on its prophylactic effect in the case of soman poisoning was studied.

MATERIALS AND METHODS

ANIMALS

Male NMRI mice weighing 20–25g were purchased from VELAZ (Prague, Czech Republic). They were kept in an air-conditioned room (22 ± 2 °C and 50 ± 10% relative humidity, with lights from 7.00 hrs a.m. to 7.00 hrs p.m.) and allowed access to standard food and tap water *ad libitum*. The mice were divided into groups of eight animals (N = 8). Handling of experimental animals was done under the supervision of the Ethics Committee of the Faculty of Military Health Sciences in Hradec Králové (Czech Republic).

CHEMICALS

Soman was obtained from the Military Technical Institute in Brno (Czech Republic) and was 96.0% pure. Its purity

was assayed by acidimetric titration. The purity of the oxime HI-6 and 6-chlorotacrine (Figure 1) was higher than 98%. They were synthesized at the Department of Toxicology and Military Pharmacy of the Faculty of Military Health Sciences in Hradec Králové (Czech Republic). The purity of the oxime HI-6 and 6-chlorotacrine was analysed using HPLC. All other drugs and chemicals of analytical grade were obtained commercially and used without further purification. All substances were administered intramuscularly (i.m.) at a volume of 10 mL/kg body weight (b.w.).

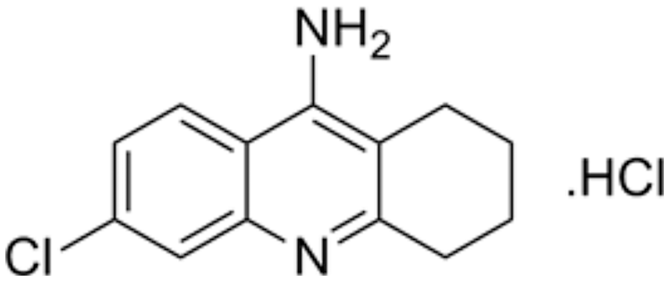

Fig. 1 Chemical structure of 6-chlorotacrine.

EVALUATION OF PROPHYLACTIC EFFICACY OF 6-CHLOROTACRINE

To evaluate prophylactic efficacy of tested doses of 6-chlorotacrine, it was administered i.m. at three doses corresponding to 5, 10 and 20% of its LD_{50} values 30 minutes before i.m. soman challenge. The LD_{50} value of 6-chlorotacrine (10.08 mg/kg) was assessed using probit-logarithmical analysis of death occurring within 24 hours after i.m. administration of 6-chlorotacrine at five doses with eight mice per dose (16) and published in our previous paper (15). The doses of tested reversible inhibitor of AChE were chosen to be sufficiently safe to avoid the potential adverse drug reactions in the peripheral as well as central compartment. Soman-induced toxicity was evaluated by the assessment of its LD_{50} value and its 95% confidence limit using probit-logarithmical analysis of death occurring within 24 hrs after administration of soman at five different doses with eight animals per dose (16). The efficacy of tested doses of 6-chlorotacrine was expressed as protective ratio (LD_{50} value of soman in pretreated mice / LD_{50} value of soman in non-pretreated mice).

EVALUATION OF THE INFLUENCE OF 6-CHLOROTACRINE ON THE THERAPEUTIC EFFICACY OF ANTIDOTAL TREATMENT

To evaluate the influence of 6-chlorotacrine on the therapeutic efficacy of antidotal treatment, the oxime HI-6 at a dose corresponding to 5% of its LD_{50} in combination with atropine at a dose corresponding to 10 mg/kg was administered i.m. 1 min after soman administration. In addition, 6-chlorotacrine was administered i.m. at three doses corresponding to 5, 10 and 20% of its LD_{50} value 30 minutes before i.m. soman challenge. Soman-induced toxicity was evaluated by the assessment of LD_{50} value and its 95% confidence limit using probit-logarithmical analysis of death occurring within 24 hrs after administration of soman at five different doses with eight animals per dose (16). The influence of tested doses of 6-chlorotacrine on the therapeutic efficacy of antidotal treatment of soman poisoning was expressed as protective ratio A (LD_{50} value of soman in pretreated mice with antidotal treatment/ LD_{50} value of soman in non-pretreated mice without antidotal treatment) and protective ratio B (LD_{50} value of soman in pretreated mice with antidotal treatment/ LD_{50} value of soman in non-pretreated mice with antidotal treatment). The differences between LD_{50} values were considered to be significant when $p < 0.05$ (16).

RESULTS

A comparison of the prophylactic efficacy of three doses of the reversible AChE inhibitor 6-chlorotacrine is presented in Table 1. All tested doses of 6-chlorotacrine were able to increase the resistance of experimental animals against acute toxicity of soman. However, there were marked differences in the prophylactic efficacy of 6-chlorotacrine depending on its dose used. Only the highest dose corresponding to 20% of its LD_{50} value was able to significantly increase the resistance of experimental animals against acute toxicity of soman ($p < 0.05$). Due to the prophylactic administration of 6-chlorotacrine at the highest dose, the LD_{50} value of soman was increased from 56.3 μg/kg to 110.5 μg/kg. When soman at the dose corresponding to its LD_{50} value for unprotected animals was administered to animals prophylactically protected by 6-chlorotacrine at a dose corresponding to 20% of its LD_{50} value, all animals survived within 24 hours.

A comparison of the benefit of all doses of reversible AChE inhibitor 6-chlorotacrine for the therapeutic efficacy of antidotal treatment of soman poisoning is presented in Table 2. All doses of 6-chlorotacrine markedly increased the efficacy of the antidotal treatment of soman-poisoned mice consisting of the oxime HI-6 and atropine. Nevertheless, only the medium and the highest dose of 6-chlorotacrine were able to significantly increase the efficacy of antidotal treatment of soman poisoning ($p < 0.05$). Due to the prophylactic administration of 6-chlorotacrine at the doses corresponding to 10% and 20% of its LD_{50} value, the protective ratio induced by antidotal treatment of soman poisoning was increased from 2.10 to 3.81 or 4.11, resp. On the other hand, the prophylactic administration of the lowest dose of 6-chlorotacrine did not significanly influence the therapeutic efficacy of chosen antidotal treatment of soman-poisoned mice.

Tab. 1 Prophylactic effect of 6-chlorotacrine administered at three different doses on the LD_{50} value of soman in mice. Statistical significance: * $p < 0.05$ (between non-pretreated mice and pretreated mice).

Pretreatment	LD_{50} (μg/kg) ± 95% CL	Protective ratio
–	56.3 (34.3–79.6)	–
6-chlorotacrine – 5% LD_{50}	68.1 (59.2–74.6)	1.21
6-chlorotacrine – 10% LD_{50}	80.6 (62.9–102.6)	1.43
6-chlorotacrine – 20% LD_{50}	**110.5 (85.4–142.5)***	**1.96**

Tab. 2 The influence of 6-chlorotacrine administered at three different doses on the ability of antidotal treatment to increase the LD_{50} value of soman in mice. Statistical significance: * $p < 0.05$ (between non-pretreated and non-treated mice and pretreated and/or treated mice), $^{x}p < 0.05$ (between non-pretreated and treated mice and pretreated and treated mice).

Pretreatment	Treatment	LD_{50} (μg/kg) ± 95% CL	Protective ratio A	Protective ratio B
–	–	87.0 (70.2–107.8)	–	–
–	HI-6 atropine	182.9 (150.8–221.7)*	2.10	–
6-chlorotacrine – 5% LD_{50}	HI-6 atropine	239.6 (163.0–313.7)*	2.75	1.31
6-chlorotacrine – 10% LD_{50}	**HI-6 atropine**	**331.3 (278.6–393.9)*,x**	**3.81**	**1.81**
6-chlorotacrine – 20% LD_{50}	**HI-6 atropine**	**357.5 (297.9–433.2)*,x**	**4.11**	**1.95**

DISCUSSION

The effective pharmacological pretreatment seems to be very important in the case of soman exposure because soman-induced deleterious effects are very difficult to counteract due to low reactivating efficacy of currently used oximes (17). The reason for the weak reactivating potency of the oximes is very rapid aging of phosphonylated AChE (18–19).

It is generally known that the therapeutic efficacy of antidotal treatment of nerve agent poisoning can be increased when it is combined with the pharmacological pretreatment by reversible AChE inhibitors (8, 20). The protection of AChE against irreversible inhibition focused on the use of reversible AChE inhibitors (mostly carbamates) is the most common principle of pharmacological pretreatment of nerve agent poisonings. They are able to inhibit AChE reversibly with spontaneous recovery of its activity. Recovered activity of AChE serves as a source of the active enzyme (8). Protection of AChE against inhibition – i.e. remaining intact AChE is a basic requirement for normal function of peripheral and central cholinergic nervous systems. Due to this pharmacological pretreatment, the enzyme AChE became resistant to nerve agent-induced irreversible inhibition (21).

The reversible cholinesterase inhibitor pyridostigmine bromide, that transiently carbamylates the active site of AChE to prevent any phosphonylation, has been used for more than 50 years in the palliative treatment of myasthenia gravis and other diseases (22). In addition, it was introduced in the 1980s for the pharmacological pretreatment of nerve agent poisonings (23). Pyridostigmine is rapidly absorbed following oral administration determined as inhibition of the blood cholinesterases. The maximum inhibition is achieved 2–3 hrs and lasts more than 8 hrs. The half-life of inhibition is about 20 hrs (21, 24, 25). The main reason for the widespread adoption of pyridostigmine as a prophylactic antidote against nerve agents is the fact that it does not influence the ability of the troops to perform the combat mission probably due to its inability to inhibit AChE in the central nervous system. Nevertheless, our results demonstrate the shortage of effectiveness of pyridostigmine bromide alone to increase the resistance of nerve agent-exposed experimental animals (13). Pyridostigmine is positively charged and, therefore, it does not readily cross BBB to afford the protection of brain AChE. In addition, a recent review emphasizes that this type of classic pharmacological pretreatment can produce behavioral impairment and region-specific alterations in ACh receptors at the doses required to afford protection against convulsant doses of nerve agents (26–27).

Therefore, the searching for less toxic, more effective and centrally active reversible inhibitors of AChE seems to be rationale to increase the effectiveness of pharmacological pretreatment of nerve agent poisonings. The administration of reversible inhibitors of AChE, that are able to cross BBB, should bring the protection of brain AChE from irreversible inhibition by nerve agents. This fact is important and useful for the increase of resistance of organism against nerve agents and the increase of the efficacy of post-exposure antidotal treatment. Of course, it is necessary to be careful with the dosage of centrally acting prophylactic drug. The doses of reversible inhibitors of AChE must be sufficiently safe to avoid peripheral as well as central adverse drug reactions and to maintain battle readiness of troops. Physostigmine is one of the most important representative of central inhibition of AChE (21). However, it produces marked behavioral impairment at doses sufficient to contribute to protection against a convulsant dose of soman (27). Recently, some alternative substances with known anti-cholinesterase activity have been studied to evaluate their prophylactic efficacy in comparison with pyridostigmine bromide (28–31). Some of them are already in clinical use or have been developed as potential therapeutics for other indications such as myasthenia gravis (32) or Alzheimer's disease (AD) (33–34). Among them, some substituted analogues of tacrine, a reversible inhibitor of AChE that was launched in 1993 as the first drug for the symptomatic treatment of AD (35), seem to be promising, sufficiently effective reversible inhibitors of AChE, suitable for the pharmacological pretreatment of nerve agent poisonings. Especially, tacrine derivatives substituted in the position 6 of the tetrahydroacridine moiety (such as 6-chlorotacrine) were found to be very promising reversible inhibitors od AChE because they exerted relative steric freedom and favorable electron-attracting effect that represents a possibility of a hydrophobic interaction between some amino acid residues and substituents in position 6 of tacrine in the active site of AChE (36). The IC_{50} value of 6-chlorotacrine was calculated for human AChE and corresponds to 0.2 ± 0.001 μM. It means that 6-chlorotacrine is very strong inhibitor of AChE (37). It is able to increase the resistance of experimental animals against

lethal toxicity of soman and to increase the therapeutic efficacy of standard antidotal treatment of acute soman poisoning. It was found to be more effective and less toxic than commonly used pyridostigmine bromide (15).

The effect of reversible inhibitors of AChE administered prior nerve agent exposure strongly depends on their ability to protect enough peripheral and central AChE from irreversible inhibition by nerve agents. Therefore, it is important to find the optimal dose of each reversible inhibitor of AChE (including 6-chlorotacrine) to reach the maximal prophylactic efficacy. The optimal dose should as effective as possible but, at the same time, sufficiently safe. Our results clearly demonstrated the influence of the dose of 6-chlorotacrine on its ability to increase the resistance of experimental animals against acute toxicity of soman and to increase the efficacy of post-exposure antidotal treatment. When 6-chlorotacrine was administered at the maximal therapeutic dose corresponding to 20% of its LD_{50}, its prophylactic efficacy was markedly higher than the efficacy of its lower doses corresponding to 5 or 10% of its LD_{50}. Generally, the tacrine analogues exert the prophylactic efficacy due to their potency to reversibly inhibit AChE in the peripheral and central nervous systems but they must be administered at sufficiently effective and sufficiently safe dose. As the acute toxicity of effective tacrine analogues studied is usually lower compared to commonly used pyridostigmine, their safe optimal dose is higher and more effective.

CONCLUSION

Our results show that centrally acting reversible inhibitors of AChE are still promising drugs for pharmacological pretreatment of nerve agent exposure, significantly more effective than commonly used pyridostigmine bromide when they are administered at optimal doses. Nevertheless, the basic principle of pharmacological preatreatment of nerve agent poisonigs – the protection of AChE from nerve agent-induced irreversible inhibition by administration of reversible AChE inhibitors is somewhat limited, especially by relatively high toxicity of sufficiently effective reversible inhibitors of AChE and by the risk of potential behavioral impairment at doses required to afford sufficient protection against convulsive doses of nerve agents.

ACKNOWLEDGEMENTS

The authors express their appreciation to Mrs J. Uhlirova for her skill technical assistance. The study was funded by the grant of Ministry of Defense of the Czech Republic – "Long-term organization development plan Medical Aspects of Weapons of Mass Destruction of the Faculty of Military Health Sciences, University of Defence".

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Prevalence of Fibromyalgia Syndrome and Its Correlations with Arrhythmia in Patients with Palpitations

Ayhan Aşkın[1,*], Ece Güvendi[2], Ayten Özkan[3], Ersin Çağrı Şimşek[4], Uğur Kocabaş[5], Aliye Tosun[1]

ABSTRACT

Objective: It is aimed to determine the prevalence of fibromyalgia syndrome (FMS) and its correlations with arrhythmia in patients with palpitations.

Material and Methods: Sixty-two patients who underwent electrophysiological study (EPS) due to palpitation complaints in Cardiology department and 40 healthy controls were included in the study. The precise diagnosis of arrhythmia was established using EPS. All participants were screened for FMS using American College of Rheumatology 2010 Fibromyalgia diagnostic criteria. Clinical assessments included measurement of severity of pain, fatigue and morning fatigue with visual analog scale (VAS), functional status with Fibromyalgia Impact Questionnaire (FIQ), and anxiety/depression with Hospital Anxiety and Depression Scale (HAD).

Results: FMS was diagnosed in 22 of the 62 patients (36%), and 4 of the 40 healthy controls (10%) ($p < 0.05$). Mean HAD scores of the patients were significantly higher than the controls ($p < 0.05$). The frequency of FMS was statistically higher in EPS+ and EPS– patients with palpitations than in controls ($p < 0.05$) (38%, 33%, 10%, respectively), but there was no difference between EPS+ and EPS– groups. There were no statistical differences between the 3 groups, in terms of pain intensity, fatigue level, FIQ and HAD scores ($p > 0.05$). EPS+ patients with FMS had higher fatigue levels, HAD and FIQ scores than EPS– patients, although statistically insignificant. HV durations were statistically longer in the EPS– subgroup ($p < 0.05$) but other EPS data were similar.

Conclusion: FMS frequency and HAD anxiety scores were found to be higher in patients with palpitation complaints. However, we found no association between arrhythmia, EPS parameters and FMS. In our clinical practice we should keep in mind to carry out assessments in terms of FMS in patients with palpitation.

KEYWORDS

fibromyalgia; arrhythmia; electrophysiological study; anxiety

AUTHOR AFFILIATIONS

[1] Department of Physical Medicine and Rehabilitation, Katip Çelebi University, Faculty of Medicine, Izmir, Turkey
[2] Department of Physical Medicine and Rehabilitation, Katip Çelebi University Atatürk Training and Research Hospital, Izmir, Turkey
[3] Department of Physical Medicine and Rehabilitation, Selçuk State Hospital, Izmir, Turkey
[4] Department of Cardiology, University of Health Science, Tepecik Training and Research Hospital, Izmir, Turkey
[5] Department of Cardiology, Katip Çelebi University Atatürk Training and Research Hospital, Izmir, Turkey
* Corresponding author: Katip Çelebi Üniversitesi Atatürk Eğitim ve Araştırma Hastanesi AMATEM binası FTR Ek Servisi, Karabağlar/İzmir, Turkey; ayhanaskin@hotmail.com

Received: 19 July 2017
Accepted: 18 October 2017
Published online: 23 April 2018

Acta Medica (Hradec Králové) 2017; 60(4): 146–151
https://doi.org/10.14712/18059694.2018.10

INTRODUCTION

Fibromyalgia syndrome (FMS) is a form of extraarticular or soft tissue rheumatism which is characterized with widespread pain, fatigue, sleep disturbances and cognitive disorders (1). It is more frequently seen in women within age range of 20 and 55 years. Mean prevalence of FMS was reported as 1.78% (2). Although its pathogenesis is still unclear, chronic stress and neuroendocrine abnormalities were suggested as causative factors, and it has been shown in many studies that the autonomic nervous system dysfunction plays an important role in the development of the disease (3–6).

The autonomic nervous system is a regulatory system that protects the homeostasis within the body and plays an important role in response to stress. Sympathetic/parasympathetic balance enables the continuity of the normal autonomic nervous system. As a result of sympathetic hyperactivation and/or parasympathetic dysfunction, the body may not be able to tolerate excessive stress, and it may result in sleep disorders, stiffness, tender points, and exercise intolerance (6, 7). Cohen et al. (8) found that autonomic dysregulation may have implications regarding the symptomatology, physical and psychological aspects of health status in FMS, while Furlan et al. (6) reported that sympathetic activity was increased by disruption of autonomic balance during rest in female patients with FMS.

Pathological alterations in the sympathetic and parasympathetic systems are known to result in various types of arrhythmias (9). Arrhythmia risk has been investigated in patients with FMS, and an increase in the autonomic activity and fluctuations in the heart rates were reported (7). However; there are conflicting results in the literature regarding relationship between arrhythmia and FMS. Doğru et al. (10) found that sympathetic activity significantly increased whereas parasympathetic activity decreased in patients with FMS which led to the changes in autonomic circadian activity. These changes caused an increase in the prevelance of arrhythmia and the ratio of supraventricular tachycardia was found as increased in patients when compared to healthy controls. Sarıfakıoğlu et al. (11) have also showed that the risk of atrial fibrillation was increased in patients with FMS. In contrast to these data, Kulshreshtha et al. (12) reported that the autonomic reflex arc and cardiac autonomic function were normal in patients with FMS. Yolbas et al. (13) also reported that long QT and P wave dispersions, which indirectly indicate atrial and/or ventricular arrhythmias, are seen in the patients with FMS with the same frequency as the controls.

Anxiety and depression are common in patients with FMS. Numerous studies suggested that accompanying anxiety results in cardiac autonomic disorders and increases the risk of coronary artery disease in healthy individuals (14). Sarıfakıoğlu et al. (11) did not find any relation between arrhythmia and anxiety-depression, but reported that the risk of atrial fibrillation was increased in patients with FMS. However, Zamuner et al. (15) reported that the disruption of neurocardiac integrity in FMS have an impact on pain and quality of life.

In this study, we aimed to determine the prevelance of FMS and its correlations with arrhythmia in patients with palpitations. Our secondary aim was to investigate the relations between arrhythmia, FMS and anxiety-depression.

MATERIALS AND METHODS

The study has been designed as cross-sectional study. All participants were informed about the study and their consents were obtained. The study has been performed according to the Declaration of Helsinki and approved by the ethical committee of our hospital (Approval number: 2015/100).

PARTICIPANTS

Sixty-two patients who underwent electrophysiological study due to palpitations in Cardiology department and 40 healthy controls were included in the study. Patients with previously diagnosed cardiac pathologies (hypertension, valve diseases, myocardial infarction, rheumatic heart diseases, cardiac insufficiency, cardiomyopathy, cardiac pacemakers), thyroid dysfunction, malignancy, diabetes mellitus, peripheral vascular disease, chronic lung disease, psychiatric disease, renal disease, pregnancies or using medications including beta blockers, calcium channel blockers or anti-arrhythmic drugs were not included in the study. The demographic data (age, gender, educational status, and marital status), body mass index, symptom durations, and medical histories (allergy history, medications, past operations and accompanying diseases) of all participants were recorded.

CLINICAL ASSESSMENTS

All participants were screened for FMS using American College of Rheumatology 2010 Fibromyalgia diagnostic criteria. These criteria includes the "widespread pain index" displaying the number of painful body parts and the "symptom severity scale" indicating cognitive symptoms, sleep, fatigue and somatic symptoms (1). Clinical assessments included measurement of severity of pain, fatigue and morning fatigue with visual analog scale (VAS), functional status with Fibromyalgia Impact Questionnaire (FIQ), and anxiety/depression with Hospital Anxiety and Depression Scale (HAD).

Fibromyalgia Impact Questionnaire is a specific measure that assesses the physical function and health status in patients with FMS. FIQ is composed of 10 items. In the inquiry, the first item contains a scale on which 11 daily activities are evaluated over 0–3. The second item determines the number of days in which the patient felt well during the last week, while the third item questions the number of days in which the patient was unable to go to work or do housework due to the illness during the past week. The other seven items evaluate the severity of pain, the ability to do work, fatigue, and sleep quality, stiffness, anxiety, and depression within the last week, using VAS. The total FIQ score is maximum 100 points. High scores indicate low functionality level (16).

Hospital Anxiety and Depression Scale is an assessment scale developed to identify the risk regarding anxiety and depression, and measures their levels and severities. Its subscales are anxiety (HAD-A) and depression (HAD-D). It contains 14 questions in total. Seven of them (odd numbers) measure anxiety and the other seven (even numbers) measure depression. The lowest and highest scores that a person can obtain from either subscale are 0 and 21, respectively (17).

ELECTROPHYSIOLOGICAL STUDY (EPS)

Electrophysiological study is an invasive procedure based on the assessment of intracardiac electrogram records. Cardiac electrical potentials are obtained from the sinus node, right atrium, atrioventricular (AV) node and the right ventricle using catheters placed as a standard (18–20). In addition, while the intracardiac electrograms are being obtained from the inside of the heart using these catheters, stimulation can be performed as well. "PA = Intra-atrial communication time, A = Atrial Activity, AH = Atrial-his communication time, H = His branch activity, HV = His-ventricular communication time, V = Ventricular activity" are the values measured as a standard within the EPS procedure. The PR interval in the superficial ECG is the sum of the intervals of PA, AH and HV. Data obtained as the basal cycle length is the RR interval in the ECG. Even though these measured times may vary depending on the age and sex, normal values are considered as AH 60–125 ms and HV 35–55 ms (20). The AH interval can be affected by autonomic changes especially. Sympathetic stimulation shortens the interval, whereas parasympathetic stimulation extends it. The HV interval is not affected by autonomic changes and measurement techniques. In the assessment of atrioventricular node functions, atrial-his (AH) and his-ventricular (HV) periods are used among the basal communication times. The determination of the moment, when a tissue with the ability to transmit stimulation within the heart can no longer transmit such stimulation, is called the "refractory period measurement". The measurement of the refractory period in the atrioventricular node is called "AV Wenckebach". Even though it varies depending on the gender and age, it is considered to be below 450 ms as a normal value (20).

STATISTICAL ANALYSIS

SPSS 16.0 (IBM Corporation, Armonk, New York, United States) program was used to analyze the variables. The Shapiro-Wilk test was used to check whether the data is normally distributed. One-way analysis of variance (ANOVA) test was used to compare three independent groups with each other based on the quantitative data, and the Pearson Chi-Square test was used to compare categorical variables with each other. In the subgroup analysis, two independent groups were compared with each other according to the quantitative results using Mann Whitney U test. Correlation analysis of clinical parameters was carried out using Pearson correlation test. Quantitative variables were displayed in tables as mean±standard deviation (std) and median range (maximum-minimum), and categorical variables were displayed as n (%). The variables were examined at 95% confidence level and p value < 0.05 was considered significant.

RESULTS

Sixty-two patients (mean ages; 47.22 ± 11.24 years) with palpitations and 40 healthy controls (mean ages 45.87 ± 12.40 years) were included in the study. FMS has been diagnosed in 22 patients (36%), and in 4 controls (10%) ($p < 0.05$). EPS data yielded arrhythmias in 52% of the patients, whereas 48% had no abnormality. All the patients with positive EPS had supraventricular tachycardias. Mean HAD-anxiety scores (6.51 ± 4.24) of the patients

Tab. 1 Demographic and clinical characteristics of the patients with palpitation complaints.

	Patients with Palpitation complaints (n = 62)		
	n	%	Mean ± Standard deviation
Age (years)			47.22 ± 11.24
Gender			
Female	36	58	
Male	26	42	
BMI (kg/m^2)			25.56 ± 3.50
Education status (years)			8.01 ± 2.93
Marital status			
Married	45	73	
Single	17	27	
FMS			
Yes	22	36	
No	40	64	
Symptom Period (months)			58.37 ± 74.47
Pain severity			2.24 ± 3.02
Fatigue severity			3.24 ± 3.07
Morning fatigue severity			2.85 ± 3.15
FIQ			20.59 ± 14.70
HAD			
Depression			4.70 ± 4.08
Anxiety			6.51 ± 4.24
EPS			
Positive	32	52	
Negative	30	48	
EPS Results			
BCL			819.12 ± 154.05
AH			102.32 ± 25.71
HV			42.96 ± 4.36
Wenckebach			364.35 ± 73.74
Ventricular Refractor			220.96 ± 39.11

N: number, % percentage
BMI: Body Mass Index, FMS: Fibromyalgia syndrome, FIQ: Fibromyalgia Impact Questionnaire, HAD: Hospital Anxiety Depression Scale, EPS: Electrophysiological Study, BCL: Basal Cycle Length, AH: duration between Atrial-his bundle, HV: duration between his-ventricle

with palpitations were significantly higher than the control (4.60 ± 4.31) ($p < 0.05$). Demographical data, clinical characteristics, and EPS results of the patients are shown in Table 1.

When the patient group was divided into two subgroups as the ones with positive EPS (EPS+) and negative EPS (EPS–); no statistical difference was found between the 3 subgroups (EPS+, EPS– and the control group) in terms of demographical characteristics ($p > 0.05$). The prevelance of FMS was statistically higher in EPS+ and EPS– groups than in controls (38%, 33%, 10%, respectively) ($p < 0.05$), but there was no significant difference between EPS+ and EPS– groups. There was no statistical difference between the 3 groups, in terms of pain intensity, fatigue level, FIQ and HAD scores ($p > 0.05$). Comparison of demographical and clinical parameters of the patients and the controls based on their EPS results has been summarized in Table 2.

When the patients with FMS were divided into two subgroups as EPS+ and EPS–, no statistically significant difference was found in-between ($p > 0.05$), even though EPS+ FMS patients had higher pain severities, fatigue levels, HAD and FIQ scores. According to the EPS results of both subgroups, HV durations were statistically longer in the EPS– subgroup ($p < 0.05$). Other EPS data were similar. The comparison of the demographical and clinical characteristics of the patients with FMS based on the EPS results is displayed in Table 3.

No significant correlation was detected between EPS scores and pain severity, fatigue level, FIQ, HAD scores ($p > 0.05$).

DISCUSSION

In our study, FMS frequency and HAD anxiety scores were found to be higher in patients with palpitations than in controls. In the EPS– FMS patients, it was found that HV times were prolonged. However, these patients showed similar characteristics with the EPS+ patients. No correlation was found between EPS results and clinical parameters.

It is known that the basic pathophysiological mechanisms in FMS are closely associated to neurohormonal disorders and autonomic dysfunction. Hypothalamic-pituitary-adrenal axis changes and the effects of sympathetic nervous system on homeostasis have been shown in FMS physiopathology (21). It is thought that autonomic dysfunction may be effective in the pathogenesis of major symptoms such as sleep disorders, stiffness, tender points, exercise intolerance (6, 7), and Vincent et al. (22) reported that autonomic symptoms such as dizziness, palpitation, sensitivity to sound and light, and gastrointestinal symptoms, were also frequently seen in these patients other than characteristic symptoms. Martinez-Lavin et al. (23) found that sleep disorders and fatigue were associated with increased sympathetic activation during the night period in patients with FMS, whereas Doğru et al. (10) reported increased sympathetic activation in these patients compared to the control group especially at night, and decreased parasympathetic activity. In our study we could not find statistical difference or any clinical relations between the groups, in terms of pain intensity, fatigue level, fibromyalgia symptom severity and EPS parameters.

Tab. 2 Comparison of demographic and clinical characteristics of the patients and of the controls.

	EPS (+) (n = 32)			**EPS (-) (n = 30)**			**Controls (n = 40)**			
	n	%	Mean ± Std	n	%	Mean ± Std	n	%	Mean ± Std	p
Age (years)			44.56 ± 11.25			50.06 ± 10.69			45.87 ± 12.40	0.152
Gender										0.104
Female	22	72		14	47		21	53		
Male	10	28		16	53		19	47		
BMI (kg/m^2)			25.20 ± 3.94			25.95 ± 2.98			24.44 ± 2.89	0.167
Education status (years)			7.84 ± 3.05			8.20 ± 2.83			8.02 ± 2.97	0.894
Marital status										0.873
Married	24	75		21	70		30	75		
Single	8	25		9	30		10	25		
FMS										0.015*
Yes	12	38		10	33		4	10		
No	20	62		20	67		36	90		
Pain severity			1.87 ± 2.82			2.63 ± 3.23			1.95 ± 2.34	0.494
Fatigue severity			3.71 ± 3.41			2.73 ± 2.62			2.05 ± 2.93	0.070
Morning fatigue severity			3.25 ± 3.41			2.43 ± 2.84			1.70 ± 2.71	0.097
FIQ			20.84 ± 15.28			20.32 ± 14.31			17.66 ± 14.57	0.615
HAD										
Depression			5.50 ± 4.66			3.86 ± 3.23			3.90 ± 3.10	0.129
Anxiety			6.53 ± 4.77			6.50 ± 3.67			4.60 ± 4.31	0.094

N: number, % percentage, * statistically significant ($p < 0.05$)
EPS: Electrophysiological Study, BMI: Body Mass Index, FMS: Fibromyalgia syndrome, FIQ: Fibromyalgia Impact Questionnaire, HAD: Hospital Anxiety Depression Scale
Chi-Square test, Single variance analysis

Tab. 3 Comparison of demographic and clinical characteristics of the patients with fibromyalgia based on the electrophysiological study results.

	EPS (+) (n = 12)			EPS (-) (n = 10)			
	n	%	Median (min-max)	n	%	Median (min-max)	p
Age (years)			45.5 (32–63)			55 (53–64)	0.014*
Gender							0.015*
Female	12	100		6	60		
Male	0	0		4	40		
BMI (kg/m^2)			27.5 (19–32)			25.3 (23–34)	0.146
Education status (years)			8 (2–11)			8 (3–11)	0.728
Marital status							0.364
Married	9	75		9	90		
Single	3	25		1	10		
Symptom Period (months)			36 (12–200)			27 (2–48)	0.111
Pain severity			4 (1–10)			7 (2–9)	0.059
Fatigue severity			8 (3–10)			5 (3–9)	0.094
Morning fatigue severity			7 (0–10)			6 (3–10)	0.547
FIQ			37.2 (18–51)			34.2 (23–57)	0.999
HAD							
Depression			9 (3–17)			6 (1–10)	0.073
Anxiety			9 (4–17)			9 (0–15)	0.817
EPS Results							
BCL			792 (520–1030)			875 (633–1094)	0.552
AH			97 (84–140)			90 (64–160)	0.642
HV			41 (35–48)			48 (38–50)	0.003*
Wenckebach			320 (280–420)			320 (290–550)	0.842
Ventricular Refractor			210 (200–250)			210 (210–230)	0.370

N: number, % percentage, * statistically significant ($p < 0.05$)
EPS: Electrophysiological Study, BMI: Body Mass Index, FMS: Fibromyalgia syndrome, FIQ: Fibromyalgia Impact Questionnaire, HAD: Hospital Anxiety Depression Scale, BCL: Basal Cycle Length, AH: duration between atrial-his bundle, HV: duration between his-ventricle
Chi-Square test, Mann Whitney U test

It was also thought that autonomic dysfunction, which is considerably important in pathogenesis, has a relation with the risk of cardiovascular disease in patients with FMS, and increased cardiac dysfunction was investigated by various studies in such patients. Meeus et al. (7) found that there is an increase in the autonomic activity and there are fluctuations regarding the stable and unstable heart rates of patients with FMS. Contrary to this, Dursun et al. (24) found that the heart rate turbulence parameters reflecting cardiac autonomic activity did not change in female patients with FMS compared to healthy controls. In our study, EPS parameters (especially AH interval) were used in order the detect autonomic changes but no significant clinical data could be identified.

Palpitation (sensation of fast or irregular heartbeat) is one of the most frequent causes of admission to the hospital, and history, physical examination, 12-lead electrocardiogram and holter recordings are most commonly used for diagnostic purposes (25). Apart from these, electrophysiological study (EPS) carried out with invasive procedures is a widely used method for determining etiology of palpitation (26). In a study conducted by Tsiachris et al. (27), EPS has been shown to be a highly reliable and advanced diagnostic method for the diagnosis of palpitation and has been shown to improve the quality of life by providing a successful guidance in arrhythmia mechanism and in patient management. In their study, they did not find any EPS data to explain the palpitation in only 16.6% of patients with palpitation symptoms. EPS was carried out in our study as well for etiology and diagnosis purposes, on the patients admitted with palpitation complaints and EPS data which could explain the palpitation was detected in 52% of the patients, whereas 48% of the patients had no abnormality regarding EPS. Similar to our data, in a study, in which 172 patients with palpitation symptoms but who have normal electrocardiogram data were assessed through EPS, positive EPS data has been found in 50% of the patients (28).

There is very limited data in the literature regarding relationship between arrhythmia and FMS. In our study, FMS was detected in 36% of the patients who admitted with palpitation complaints and supraventricular tachycardia was found in all EPS+ patients. In a similar study, Doğru et al. (10) which evaluated 50 individuals with FMS and 30 healthy controls, the prevalence of supraventricular extra systole was found as 48% in the FMS group and this ratio was found as 23.3% in the control group. Sarıfakıoğlu et al. (11) have also showed that the risk of atrial fibrillation was increased in patients with FMS. In contrast to these data, Yolbas et al. (13) reported similar frequencies of arrhythmias in patients with FMS when com-

pared with controls. Likewise, we also found no significant correlation between EPS scores and clinical parameters.

Cardiovascular diseases are often accompanied by anxiety and depression, and these are generally known to increase the symptoms of the disease. In the studies conducted, it has been detected that 30% of the patients admitted to the hospital due to myocardial infarction have depressive findings, and 15–20% of them have been diagnosed with major depression (29). It is also known that there is an increase in anxiety symptoms after acute cardiovascular events (30). Likewise, in our study HAD-anxiety score average was significantly higher in all patients admitted with palpitation complaints than in the control group. However, there was no statistically significant difference, even though EPS (+) patients with FMS had higher pain severities, fatigue levels, HAD and FIQ scores. The relation between anxiety-depression and cardiac events in patients with FMS has been addressed in a very few amount of the studies. Zamuner et al. (15) indicated that disruption of neurocardiac integrity in patients with FMS have an impact on pain and quality of life. In a study where the relation between anxiety, depression and arrhythmia have been investigated in 59 patients diagnosed with FMS, higher anxiety-depression scores were obtained when compared to the control group, but its relation with arrhythmia has not been suggested (11).

Our study has several limitations. First is the small number of patients and controls. Secondly, only EPS data was evaluated in our study, without using the ECG and the 24-hour Holter ECG data, which are the starting criteria of arrhythmia assessment. EPS was also not performed in the control group.

CONCLUSION

FMS frequency and HAD anxiety scores were found to be higher in patients with palpitation complaints. However, we found no association between arrhythmia, EPS parameters and FMS. In our clinical practice we should keep in mind to carry out assessments in terms of FMS in patients with palpitation. Further studies with larger sample sizes are needed to clarify the relationship between FMS and arrhythmia.

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Treatment of Multifocal Multisystem BRAF Positive Langerhans Cell Histiocytosis with Cladribine, Surgery and Allogenic Stem Cell Transplantation

Filip Gabalec[1,*], Martin Šimkovič[1], Alžběta Zavřelová[1], Petra Kašparová[2], Paula Morávková[3], Marcela Kopáčová[3], Václav Masopust[4], Pavel Žák[1], Jan Čáp[1], Jakub Radocha[1]

ABSTRACT

Langerhans cell histiocytosis (LCH) is a very rare disease in adults and as well a very rare cause of sellar expansion. The clinical presentation can be heterogeneous, from a single bone lesion to potentially fatal, widespread disease. We describe the difficulties with the diagnosis and treatment of LCH as well as successful treatment with cladribine chemotherapy and allogeneic stem cell transplantation.

KEYWORDS

histiocytosis; transplantation, stem cell; cladribine; hypopituitarism

AUTHOR AFFILIATIONS

[1] 4th Department of Internal Medicine – Haematology, University Hospital Hradec Králové, Czech Republic and Charles University, Faculty of Medicine in Hradec Králové, Czech Republic
[2] Fingerland's Department of Pathology, University Hospital Hradec Králové, Czech Republic and Charles University, Faculty of Medicine in Hradec Králové, Czech Republic
[3] 2nd Department of Internal Medicine – Gastroenterology, University Hospital Hradec Králové, Czech Republic and Charles University, Faculty of Medicine in Hradec Králové, Czech Republic
[4] Neurosurgery Clinic, Central Military Hospital Prague, Prague, Czech Republic
* 4th Department of Internal Medicine; University Hospital Hradec Králové
Sokolská 581, 500 05 Hradec Králové, Czech Republic; e-mail: filip.gabalec@fnhk.cz

Received: 24 October 2017
Accepted: 20 December 2017
Published online: 23 April 2018

Acta Medica (Hradec Králové) 2017; 60(4): 152–156
https://doi.org/10.14712/18059694.2018.11

BACKGROUND

Langerhans cell histiocytosis (LCH) is a clonal proliferative disease. LCH is a very rare disease in adults with an incidence 1–2 adults per million per year. The clinical presentation can be heterogeneous, from a single bone lesion to potentially fatal, widespread disease. The most frequently affected systems are bones, lung, and skin. LCH that presents as endocrine disorders or as a cause of a sellar expansion is rather rare and can be often misdiagnosed. Central nervous system is affected in 6% of patients with LHC, mostly the hypothalamo-pituitary region and pineal gland, cerebellum and basal ganglia (1). Usually, patients suffer from central diabetes insipidus and pituitary stalk is thicker on imagining. We describe the difficulties with the evaluation and the treatment of LCH as well as successful treatment with cladribine chemotherapy and allogeneic stem cell transplantation.

CASE PRESENTATION

42 years old Caucasian woman has had amenorrhea for 2 years and higher intake of fluids (5–7 liters/day) for 6 years prior to referral. Non-functioning pituitary expansion with pituicytoma appearance was found on MRI. She was followed up for almost 3 years. Panhypopituitarism developed during the follow-up and replacement therapy with desmopressin (180 μg/day), levothyroxine (100 μg/day), ethinylestradiol/levonorgestrel (30 μg / 150 μg/day) and hydrocortisone (30–50 mg/day) was initiated. She did not take other medication. The patient was referred to biopsy of sellar expansion, as slow enlargement of the pituitary expansion occurred. No relevant family history was reported. Her past medical history included a notion of asymptomatic mild mitral valve insufficiency. She is a non-smoker, not drinking alcohol and she has no allergies or autoimmune disorders. Physical examination was physiological except obesity. No sensoric or motoric neurological deficit was found.

INVESTIGATION

An open (craniotomy) biopsy of the suprasellar lesion (Fig. 1) was inconclusive for the first time and complicated with transient manifest left hemiparesis due to small acute ischemia in basal ganglia (revealed by MRI). The biopsy was repeated in one month. This time histology showed small foci with large cells positive for CD1a and S100 in imunohistochemical analysis (Fig. 2). Also, positivity for CD68 and mild positivity for BRAF was present. GFAP was negative. The conclusion of Langerhans cell histiocytosis was made by two independent pathologists. She was admitted to our centre for further evaluation. Staging of the disease revealed suprasellar expansion, skeletal and retrobulbar infiltration, lymphadenopathy along the right internal carotid artery and retroperitoneal and mediastinal lymphadenopathy with FDG avidity on fluorodeoxyglucose-positron emission computer tomography scanning (FDG-PET/CT) (Fig. 3A). The bone marrow biopsy did not reveal an infiltration of bone marrow. Retrospectively, BRAF mutation expression in codon 600 and 601 was evaluated by PCR with consequent reverse hybridization (kit BRAF 600/601 StripAssay – ViennaLab). Mutation in gene BRAF, NM_004333.4: c.1799T>A, p.V600E was present.

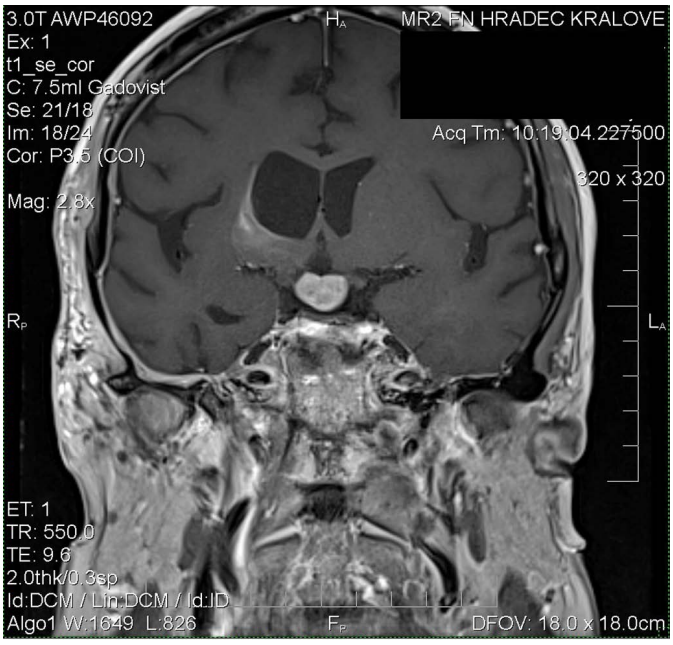

Fig. 1 Magnetic resonance imagining showing suprasellar expansion due to Langerhans cell histiocytosis.

TREATMENT AND FURTHER INVESTIGATION

The patient received five cycles (six planned) of cladribine monotherapy (0.14 mg/kg for 5 days in each cycle). However, prolonged neutropenia and gastrointestinal and repeated urinary infections led to premature termination of the treatment. A complete regression of previously described lymphadenopathy and tumour infiltration was achieved on subsequent PET/CT restaging. Unfortunately, new FDG positive lesion approximately 40 cm in proximal jejunum was found with bowel dilatation up to 7 cm (Fig. 3B). Small lymphadenopathy with an accumulation of FDG was found locally. Double balloon enteroscopy showed tight stenosis (4 mm) of the oral jejunum due to tumour infiltration (Fig. 4). Prestenotic dilatation with impacted food was present. Biopsy confirmed infiltration by Langerhans cell histiocytosis. As no other infiltration was found on PET/CT and bone marrow biopsy was negative, resection of infiltrated part of jejunum with end-to-end anastomosis was performed and the patient was indicated for myeloablative allogeneic stem cell transplantation from matched unrelated donor. A conditioning regimen BuFluTG included (Fludarabine 40 mg/m^2 from day −7 to day −3, i.v. Busulfan 3.2 mg/kg from day −7 to day −5 and rATG (Thymoglobulin) 3 mg/kg from day −2 to day −1. She received transplantation from the male 10/10 donor (dose 6.0 × 10/6/kg CD34+ cells). Transplantation was complicated with sepsis, originated from the gastrointestinal tract. Frequent monitoring of sodium and therapy amendment was required due to hypopituitarism and higher require-

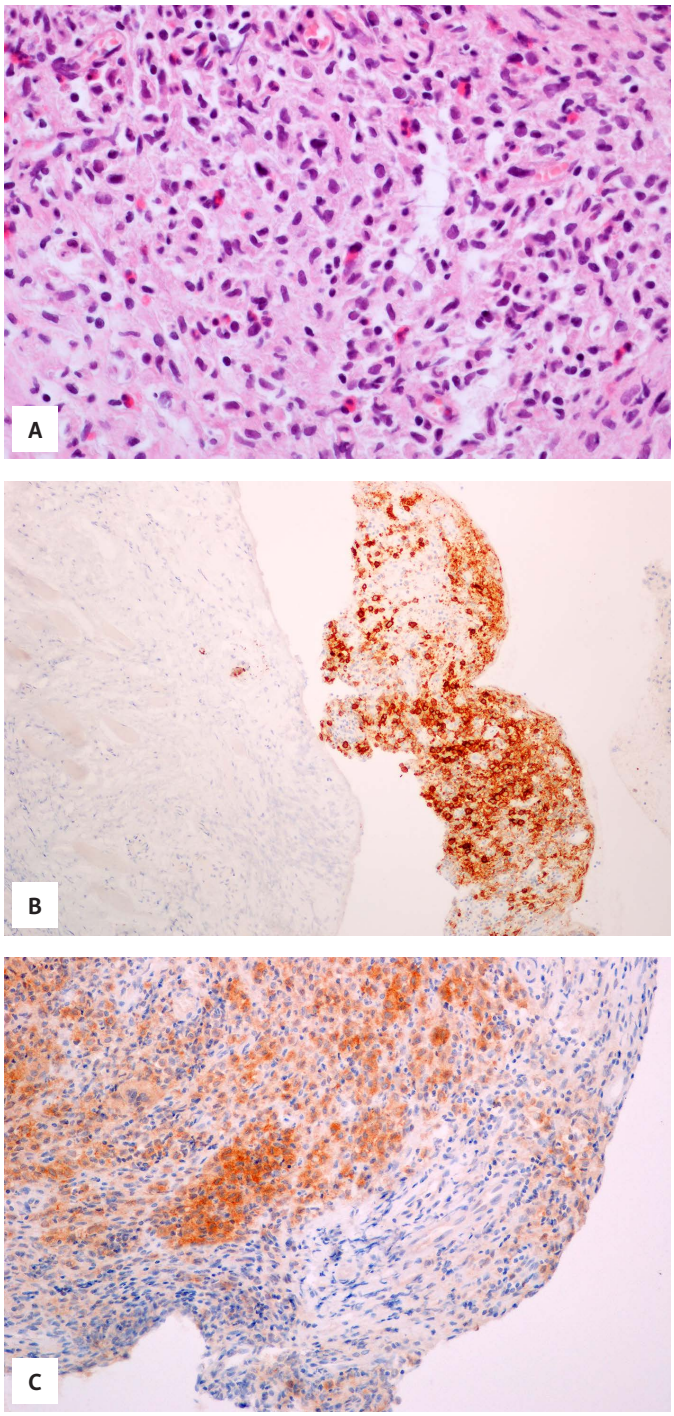

Fig. 2 Biopsy specimens taken from the suprasellar lesion.
A. Langerhans cells demonstrate characteristic nuclei with prominent nuclear folds. H&E ×40 B. Expression of CD1a – anti-CD1a antibody stain ×10. C. Weak BRAF positivity. Immunohistochemical staining ×10.

ments of hydrocortisone during sepsis. Engraftment in thrombocytes was on day 9 and leucocytes on day 12.

OUTCOME AND FOLLOW-UP

The patient is now followed-up more than 130 days after transplantation without serious complications and signs of the disease. PET/CT was negative after surgery (Fig. 3C) and in 3 months after transplantation. Next PET/CT is planned at 12 months after transplantation.

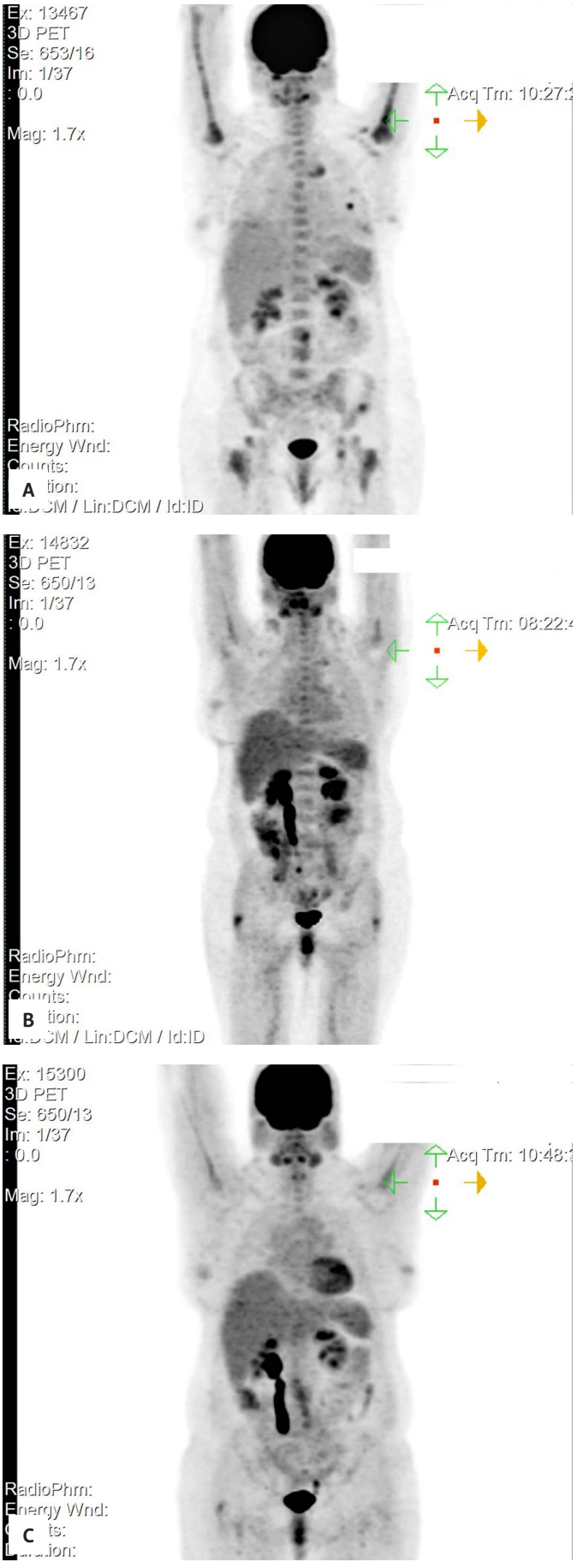

Fig. 3 A. Fluorodeoxyglucose-positron emission tomography scanning (FDG-PET) before treatment. B. FDG-PET after Cladribine chemotherapy – regression of the previous tumor, new infiltration of jejunum is present. C. FDG-PET after jejunum resection.

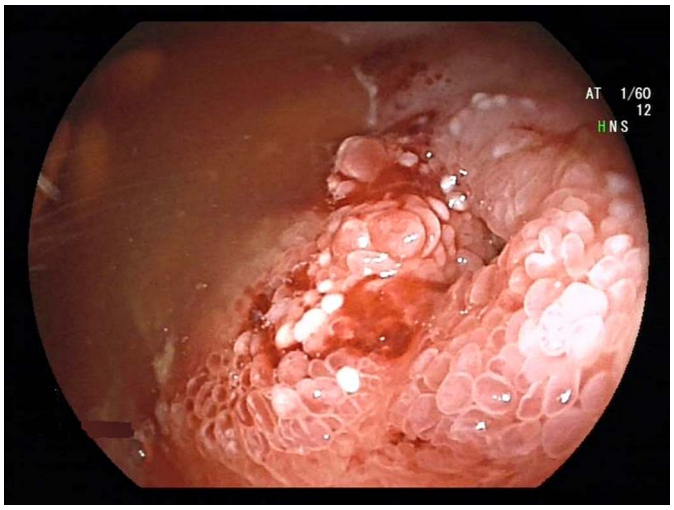

Fig.4 Double balloon enteroscopy showed tight stenosis (4 mm) of the oral jejunum due to tumour infiltration.

DISCUSSION

Langerhans cell histiocytosis (LCH) is a rare histiocytic disorder of unknown etiology. LCH is characterized by monoclonal proliferation of Langerhans cells derived from myeloid progenitor cells that express the CD34 surface antigen. As in our case, somatic oncogen $BRAF^{V600E}$ mutation is present in 25–60% of cases. Patients with the $BRAF^{V600E}$ mutation have a higher occurrence of diabetes insipidus and have more severe disease than those with wild-type BRAF. Also, resistance to combined therapy (vinblastine and corticosteroids) is more common. Second-line chemotherapy and rescue therapy is necessary in 19% of cases and reactivation is more common (2).

Hypothalamus-pituitary-adrenal axis infiltration is present in up to 50% of LCH. The most common disorder is diabetes insipidus (DI). The frequency of DI is 30–40% and 94% if other pituitary hormone deficiencies exist. The diagnosis can be difficult. It is important to exclude LCH before making the diagnosis of idiopathic DI. Whole body imaging is indicated in the presence of thickened pituitary stalk to find potential extracerebral place for biopsy (3). FDG-PET seems to be a good tool in these cases. Also, FDG-PET scanning is used for assessing the extent of LHC, its progression and the response to treatment (4, 5).

Our patient had hypopituitarism. Other hormone deficiencies are present less frequently – in up to 20% of LCH cases and in up to 60% when DI is present. These deficiencies are often irreversible even after treatment of LCH. Prompt hormone replacement therapy is mandatory, and continuous follow-up is needed to detect deficiencies that might evolve later (6).

There is no specific therapy or universally accepted guidelines for treatment of this orphan disease in adults. Validated therapeutic options are outlined in recent review by Haroche et al. (7). The primary treatment modalities for LCH include local excision of the lesion, corticoid therapy, chemotherapy, radiotherapy, and immunotherapy with anti-CD1a monoclonal antibodies or BRAF inhibition if present. In general, the localized disease requires minimal treatment and is considered as having a good prognosis. Multi-systemic and multi-focal disease with involvement of liver, spleen, lung and bone-marrow are considered as a higher risk. The data regarding the treatment of the central nervous system involvement with LCH are very limited. No drug or regimen has been proved superior to the others. Cladribine (2-CdA) is a promising agent in this setting as previously reported also with a review of previous studies (8). Cladribine has good bioavailability in the CNS. A total of six cycles of cladribine monotherapy can be administered with respect to the good profile of toxicity in this indication. However, in our case, only five cycles were administrated and chemotherapy stopped earlier due to prolonged neutropenia and infectious complications. As the patient had persistent disease after front-line treatment, she was considered as a high risk further. Surgery seemed as an optimal option to solve out the bowel stenosis due to the tumour. Gastrointestinal involvement in LCH commonly includes duodenum or colon in a multifocal pattern with superficial erosions or bleeding ulcers. It appears usually in multi-systemic diseases. Isolated LCH in a small bowel is very rare. Case reports were reviewed by Shankar et al. (9), reporting only one case in adults (10).

Myeloablative allogeneic stem cell transplantation (allo-HSCT) is an option in high-risk LCH with persistent disease and was reported in the pediatric population (11–13). Data for adults are rather scarce. The worlds' first adult case of multisystem LCH with pulmonary involvement and thrombocytopenia with absent radii successfully treated with allo-HSCT was reported in 2006 (14). The other was reported in Spain (15). No trials exist for treatment of LCH in adult population. With limited accuracy the treatment strategies are translated from pediatric experience to adult population. Even in the pediatric population the data are limited. Steiner et al. described successful treatment of 7 out of 9 children with allo-HSCT (12). Cooper et al. reported other 3 cases of LCH successfully treated with allo-HSCT (13). All previously mentioned patients were under the age of 24 months. Reduced intensity conditioning is preferred in these cases to reduce toxicity. In our case, front-line therapy with cladribine was unsuccessful, so we continued with surgery and allo-HSCT. In the future, the position of allo-HSCT may have limited indication for LCH following the discovery and wider use of BRAF inhibitors and the increased use of targeted therapies like vemurafenib for example.

LEARNING POINTS

- Histiocytosis is an orphan disease and should be considered in differential diagnosis as any organ or system can be affected.
- Histiocytotis should be excluded in cases of diabetes insipidus of unknown aetiology and whole body imaging is indicated in the presence of thickened pituitary stalk to find potential extracerebral place for biopsy.
- Rare small bowel infiltration by Langerhans cell histiocytosis is presented.
- Allogeneic haematopoetic stem cell transplantation is an option for the second-line treatment.

FUNDING STATEMENT

Supported by program PROGRES Q40/08 and by MH CZ – DRO (UHHK, 00179906).

DECLARATION OF INTEREST

There is no conflict of interest.

PATIENT CONSENT

Written informed consent has been obtained from the patient. Signed copy of the consent is available to the publisher.

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A Large Laryngeal Mucocele Causing Progressive upper Airway Obstruction and Cervical Swelling

Ioannis Aidonis[1], Nikolaos Lazaridis[2], Maria Piagkou[3,*], Nikolaos Anastasopoulos[2], Konstantinos Natsis[2]

ABSTRACT

Laryngocele (LC) is an uncommon clinical entity, occasionally associated with fatal complications. If its neck becomes obstructed, mucous accumulates and then a laryngeal mucocele (LMC) is formed. Reports of LMCs are rare in the literature. A fluid-filled combined LMC in a 48 year-old Greek construction worker with presenting symptoms of cervical swelling and dysphonia is described. The male patient was surgically treated via an external approach. A LC rarely becomes symptomatic and infection unusually occurs. Magnetic resonance imaging depicts in detail the size, extension and structure of the neck mass and remains the diagnostic gold standard, providing superior soft-tissue discrimination, in cases of a concurrent laryngeal tumor. Histopathological examination confirms diagnosis, since there is always a high index of suspicion for malignancy. Established guidelines regarding surgical treatment of a LC do not exist. Although during the last two decades micro laryngoscopy with CO_2 laser has gained popularity for the treatment of an internal LC, the external approach still remains the method of choice in cases of a combined LMC.

KEYWORDS

laryngeal mucocele; upper airway obstruction; cervical swelling; surgical treatment; external approach

AUTHOR AFFILIATIONS

[1] Bioclinic Thessaloniki, Greece
[2] Department of Anatomy and Surgical Anatomy, Faculty of Health Sciences, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3] Department of Anatomy, Faculty of Health Sciences, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
* Corresponding author: 9 Kontoyiannaki Street, N. Psychico, 11526, Athens, Greece; e-mail: mapian@med.uoa.gr

Received: 18 August 2017
Accepted: 30 November 2017
Published online: 23 April 2018

Acta Medica (Hradec Králové) 2017; 60(4): 157–159
https://doi.org/10.14712/18059694.2018.12

INTRODUCTION

Benign dilatation of the laryngeal saccule may end up in a laryngocele (LC) formation, an uncommon air and/or fluid-filled laryngeal entity, extending upward into the false vocal fold, although still retaining its communication with the laryngeal lumen (1). If the LC's neck becomes obstructed, mucosal glands, lubricating the vocal cords, will continue to produce mucous, thus their secretion will be accumulated ultimately forming a laryngeal mucocele (LMC) (1, 2). In the current report a case of a LMC, which became clinically evident over a six month period, as a progressive neck swelling associated with dysphagia and symptoms of upper airway obstruction, is described. The male patient underwent an uneventful surgical excision of the neck mass, via an external approach, which still remains the treatment of choice.

CASE REPORT

A 48 year-old Greek construction worker with a 2-month history of progressive dysphagia, hoarseness, dysphonia and moderate breathing difficulty visited the Department of Otolaryngology of Bioclinic Hospital. The main reason for seeking medical attention was the formation of a left firm compressible round soft neck mass, developed over a 6-month period (Fig. 1A). On palpation of the neck, cervical swelling was identified. Videolaryngoscopy with a flexible rhinolaryngoscope revealed a mass protruding from the left ventricular fold, obscuring the laryngeal inlet. Movement of the vocal cords was normal. A computed tomography (CT) scan exactly depicted the extension and content of internal and external components of the LC (Fig. 1B). A Magnetic Resonance Imaging (MRI) evaluated a paralaryngeal cystic fluid-filled dilatation, outpouching from the left laryngeal ventricle. It measured approximately 57 × 36 × 50 mm, expanded laterally under the platysma muscle, while part of it protruded medially into the laryngeal ventricle (Fig. 1C). No regional lymph nodes were detected. Diagnosis of a LMC was based on the above clinical and imaging findings, confirmed by histopathological examination, which showed no evidence of malignancy (Fig. 2). The male patient was a smoker for

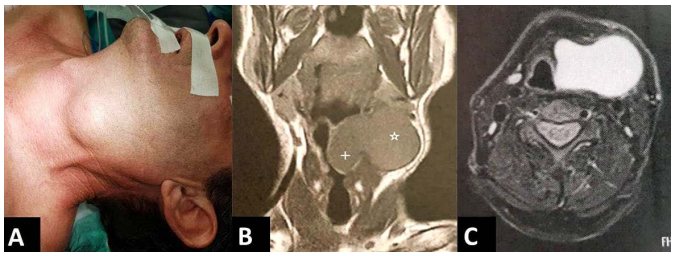

Fig. 1 A. The left firm compressible round soft neck mass. B. Preoperative magnetic resonance imaging (MRI) coronal view of the neck, demonstrating a large left combined laryngeal mucocele. Asterisk indicates the external portion, herniating through the thyrohyoid membrane, and the cross sign indicates the internal portion. C. MRI, axial view of the neck, exactly depicts the extension and content of both internal and external components of the large fluid-filled saccular cyst.

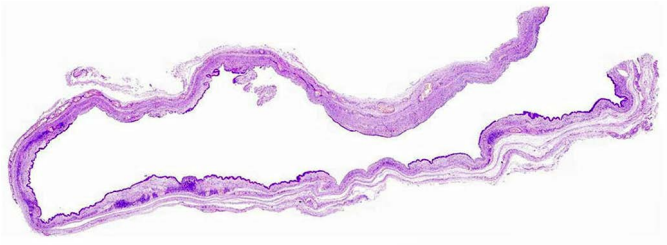

Fig. 2 Cystic lesion (mean diameter 4cm) with a thin wall and smooth inner surface. Variable types of cells (respiratory, squamous and oncocytic) and a rich vascularity are present. The cyst has focally fibrotic and hemorrhagic wall with mucinous debris. Submucosal accumulations were evident subepithelially. No signs of malignancy were detected (Hematoxilin & Eosin, panoramic)

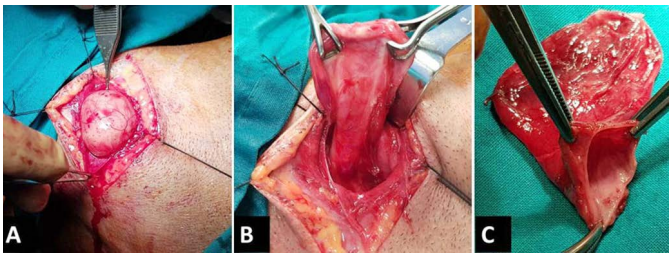

Fig. 3 A, B. Intraoperative views during lesion removal via an external approach. C. The surgical specimen.

30 years (approximately 30 cigarettes per day). Because of the nature and the extent of the lesion, surgical resection was performed via an external approach (Figs. 3A, B). The lesion was carefully dissected from the adjacent thyrohyoid membrane and the entire mucous-filled saccular mass was removed (Fig. 3C). No tracheostomy was performed. Patient's symptoms had subsided in one-month follow-up visit and his voice was normal. Vocal cord function was unaffected. This work is conformed to the standards set by the Declaration of Helsinki (2000) and the procedure has been approved by the Ethics Committee of our University.

DISCUSSION

The incidence of laryngocele is reported to be up to 1 in 2.5 million people annually (2). Male to female ratio is 5:1 with peak incidence during the 5th or 6th decade of life (3). A laryngocele could be classified as internal, which is entirely confined to the endolarynx, or combined, when it herniates laterally, ultimately forming a mass in the neck. Combined and unilateral laryngocele is the commonest type (4).

Etiology of the LC development still remains uncertain, although different theories have been proposed about its development. A LC can be congenital, acquired or due to a mechanical obstruction. It may occur in individuals with congenitally large saccule or those with congenital laryngeal tissue weakness (3). Singers, chronic coughers, wind instrument players, glass blowers and public speakers are more prone to develop a LC, due to the extended and increased intralaryngeal pressure. A strong correlation between tobacco use, alcohol and laryngeal cancer has been proven, since histologic changes of the vocal fold epithelium are detected among smokers and drinkers (5). Amyloi-

dosis, chordoma and laryngeal cancer (squamous cell carcinoma) are also indirectly associated with LC formation via increased intraventicular pressure and obstruction (6, 7, 8). Although, CT scan is helpful in differentiating a LC from a saccular cyst of the larynx and usually uncovers evidence of an occult laryngeal tumor, MRI is superior, because it provides excellent soft-tissue discrimination and distinguishes malignancy from mucus or inflammation (3).

A LC is usually asymptomatic with hoarseness being the most frequent presenting symptom. Neck swelling and dysphonia are typical symptoms; other clinical manifestations include cough, foreign body sensation, progressive dyspnea, even acute inspiratory occlusion requiring emergent tracheotomy (9). Infection, airway obstruction, dysphagia and dysphonia indicate surgical intervention. Cases of LC enlargement and worsening of symptomatology are associated with carcinomas (2). Because of the rare incidence of a LC, the best treatment method still remains controversial (1). Traditionally, the size and type of the lesion indicate the route of surgical approach. During the last 20 years, endoscopic resection with CO_2 laser has become popular for the internal LC cases (10). Nevertheless, probability of incomplete resection of large lesions limits the usage of the endoscopic approach (3). Surgical treatment of combined cases still remains a surgical dilemma, although it is generally performed via an external approach, which has the benefit of easy access to the lesion with low recurrence rate. Disadvantages include the high morbidity due to the superior laryngeal nerve injury and the increased need for tracheotomy, resulting in prolonged hospitalization (4). Ciabatti et al. (11) removed a large combined LC using transoral robotic surgery. Although this minimally invasive approach seems to be a very promising alternative solution for benign and malignant diseases, more experience should be gained to consolidate a safe and successful outcome. In LM cases, the external approach still remains the preferred method of treatment.

CONCLUSIONS

Concluding, diagnosis of a combined LMC was made in our case on the basis of clinical findings, imaging, laryngoscopy and histopathological examination. MRI depicted in detail the size, extension and structure of the neck mass and remains the diagnostic gold standard, providing superior soft-tissue discrimination. Histopathology of the specimen confirms diagnosis in LMC cases, since a high index of suspicion for malignancy should be always present. Surgical resection via an external approach remains the therapeutic option of choice.

CONSENT

Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Severe Self-Inflicted Acute Ocular Siderosis Caused by an Iron Tablet in the Conjunctival Fornix

Ioannis Asproudis*, Paraskevas Zafeiropoulos, Andreas Katsanos, Christos Kolettis

ABSTRACT
We present the case of a female patient who grinded a ferrous sulfate tablet and placed it at the conjunctival fornix of her left eye. She rapidly developed severe ocular siderosis, with profoundly decreased visual acuity, corneal opacities, cataract, retinal degeneration and ultimately phthisis bulbi. To our knowledge, this is the first report on the consequences of application of an iron tablet on the conjunctiva.

KEYWORDS
siderosis; siderosis bulbi; self-inflicted injury; phthisis; oedipism; deferoxamine

AUTHOR AFFILIATIONS
Ophthalmology Clinic of the Medical School of the University of Ioannina, Ioannina, Greece
* Corresponding author: Stavros Niarchos Avenue, Ophthalmology Clinic, University Hospital, 45500 Ioannina, Greece; e-mail: asproudisi@yahoo.gr

Received: 13 August 2017
Accepted: 25 January 2018
Published online: 23 April 2018

Acta Medica (Hradec Králové) 2017; 60(4): 160–162
https://doi.org/10.14712/18059694.2018.13

CASE REPORT

A 45-years old white female patient presented to the emergency service claiming she had injured her left eye when she accidentally fell the previous day. Her general medical history was only significant for schizophrenia under medication. The patient appeared well oriented in time, place and person and behaved appropriately in all occasions.

Snellen best corrected visual acuity (VA) was 10/10 in her right eye (OD) and light perception in her left eye (OS). Intraocular pressure was 13 mmHg in OD, but could not be measured in OS due to hypotony. The examination of OD was unremarkable. The OS had excessive palpebral edema, chemosis and a dark brown material mostly along the temporal part of the limbus. This material was initially thought to be part of the ciliary body or iris. The cornea was edematous with an unusual brown hue, whitish stromal infiltrates, epithelial erosions and brownish endothelial precipitates. Fibrinous material was noted in the anterior chamber. The crystalline lens was diffusely yellow with cortical and anterior subcapsular opacities. Fundoscopy was not possible. Computed tomography of the orbits revealed a poorly defined radiopaque area within the structures of the anterior segment in OS (Fig. 1). Surgical exploration revealed no site of injury or foreign body. Large areas of necrotic, brown, frail conjunctiva and sclera were identified, especially underneath the superior eyelid. Careful dissection of the necrotic tissue was performed (Fig. 2).

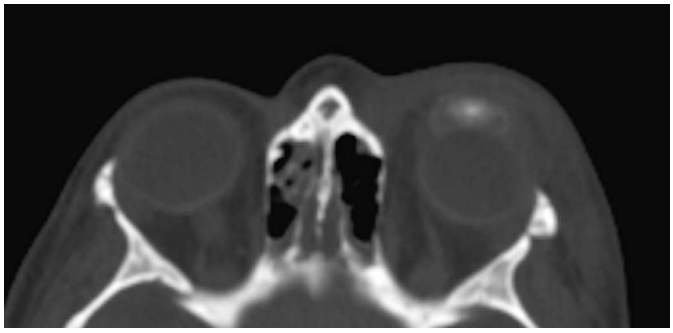

Fig. 1 Poorly-circumscribed radiopaque area on computed tomography scan in left eye. Edema of the peribulbar soft tissues is noted.

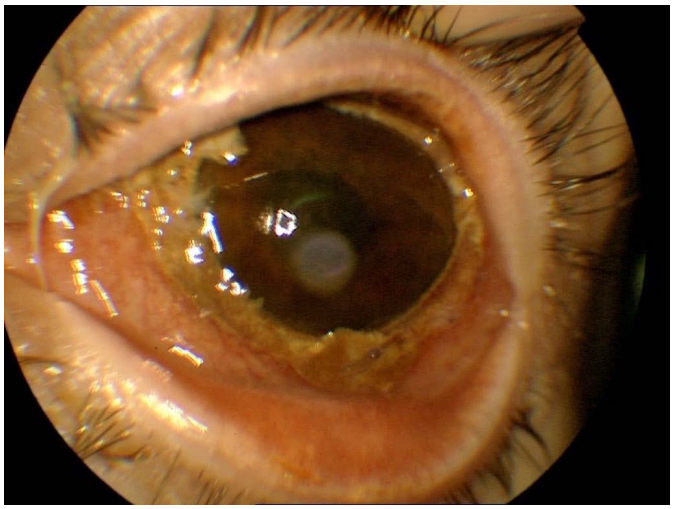

Fig. 2 Slit lamp photograph of the patient's left eye after surgical exploration and debridement. Chemosis, hyperaemia and corneal infiltration are shown. Residual necrotic conjunctival tissue appears brown.

On further questioning, the patient eventually admitted she had grinded a single ferrous sulfate tablet and placed the powder at the superior conjunctival fornix of her left eye. Self-inflicted ocular siderosis was diagnosed and urgent psychiatric evaluation was arranged. The patient was started on cefuroxime intravenously, methylprednisolone *per os*, atropine eyedrops b.i.d., chloramphenicol-dexamethasone eyedrops t.i.d. and deferoxamine mesylate 10% eyedrops q.i.d. After several days of in-patient treatment the VA in OS was still light perception, the anterior chamber was shallow with fibrinous material and the eye remained hypotonous. Phthisis ensued within the next couple of weeks.

DISCUSSION

To the best of our knowledge, there has been no previous report of self-inflicted ocular siderosis using iron tablets. Ocular self-mutilation has been described in schizophrenia, drug-induced psychosis, manic phase of bipolar disorder, depression, and in certain organic diseases (Lesch-Nyhan syndrome, delirium tremens, encephalitis) (1). The different forms of self-inflicted injuries include blunt trauma, penetrating wounds, and unilateral or bilateral enucleation (oedipism) (1).

Ocular siderosis is an uncommon, potentially blinding condition usually caused by an iron-containing retained intraocular foreign body (IOFB) (2, 3). Cell death results from the toxic effect of iron on cellular enzyme systems (2, 4). The condition may appear from few days up to many years following the injury and any ocular tissue from the cornea to the optic nerve may be affected (2, 3). The clinical severity depends on the size and shape of the IOFB, its iron content and the time it remains in the eye. The most common manifestations are cataract formation (especially anterior subcapsular), diffuse pigmentary changes of the retinal pigment epithelium, iris heterochromia often in conjunction with a tonically dilated pupil, secondary glaucoma, iritis and cystoid macular edema (4). Retinal arteriolar attenuation, proliferative vitreoretinopathy and retinal detachment have also been described (2, 3, 4).

There are two reasons why our patient suffered a rapid and severe deterioration. Firstly, the quantity of iron in the tablet (256.3 mg of iron sulfate corresponding to 80 mg of elemental iron) is significantly larger than that of a typical IOFB. Secondly, the speed of iron release from a tablet is much faster than that of an IOFB: at least in the gastrointestinal tract, these tablets dissolve within approximately 7 hours.

The management of IOFB-induced ocular siderosis involves the removal of the foreign body (2, 4). In our case, when surgical exploration was undertaken, practically all ocular tissues were already saturated with iron as sug-

gested by their brown colour and brittle texture (Fig. 2). Deferoxamine is a chelator with high iron affinity that has been used subconjunctivally or topically as 10% solution for the management of ocular siderosis (5). In our case, its use proved ineffective because irreversible tissue damage had already occurred at presentation.

Our case illustrates the challenges of dealing with self-inflicted ocular injuries in psychiatric patients. It further illustrates that upon contact with the eye, ferrous sulfate tablets can cause severe tissue necrosis that can rapidly culminate to irreversible blindness despite all efforts. Ophthalmologists should have a high index of suspicion when treating ocular injuries in psychiatric patients. Prompt intervention and close collaboration with psychiatrists may reduce the risk of significant handicap.

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Giant Metastatic Liver Tumor of Unknown Primary Origin: Thoracic Autopsy Solves the Mystery

Antigony Mitselou[1], Stamatis S. Papadatos[2], Savvina Tsoultsidou[1], Kleio Fragkouli[1], Michail Mitsis[3], Theodoros Vougiouklakis[1], Vasiliki Galani[4,*]

ABSTRACT

A 59-year-old male patient was hospitalized in the Internal Medicine Department for investigation of hepatic metastases from an unknown primary neoplasm. During the hospitalization the patient died from acute myocardial infarction. The autopsy revealed a 8.2 kilograms (kg) liver that was diffusely infiltrated by whitish metastatic masses. No other tumor was detected, apart from a 2.5 centimeters (cm) pulmonary nodule next to the right intermediate bronchus that was histologically compatible with small cell lung cancer (SCLC). Despite the fact that hepatic metastases from SCLCs are common, diffuse metastatic hepatomegaly from a malignant pulmonary nodule are rarely seen. Given that the most common cause of malignancy-related death is lung cancer, early diagnosis and appropriate management of pulmonary nodules is of paramount importance.

KEYWORDS

pulmonary nodule; lung cancer; liver metastasis

AUTHOR AFFILIATIONS

[1] Department of Forensic Medicine and Toxicology, University of Ioannina, Greece
[2] 3rd Department of Internal Medicine, National and Kapodistrian University of Athens School of Medicine, Sotiria General Hospital, Athens, Greece
[3] Department of Surgery, University of Ioannina, Greece
[4] Department of Anatomy-Histology-Embryology, University of Ioannina, Greece
* Corresponding author: Medical School, University of Ioannina, Panepistimioupoli, 45110, Ioannina-Greece; e-mail: vgalani@cc.uoi.gr

Received: 26 June 2017
Accepted: 22 January 2018
Published online: 23 April 2018

Acta Medica (Hradec Králové) 2017; 60(4): 163–166
https://doi.org/10.14712/18059694.2018.14

INTRODUCTION

A pulmonary nodule is a well-circumscribed radiological opacity which diameter does not exceed 3 cm (1). Due to the recent advances in medical imaging, the incidental detection of pulmonary nodules has tremendously increased (2). Malignant nodules account for 1.1% to 12% of these nodules (3) and their early detection is a great challenge for the clinicians. The liver is one of the most frequent site of metastasis from primary malignant solid tumors. Small-cell lung cancer (SCLC) comprises 15% of all lung cancers (4) and is known for its aggressive biological behavior. Liver metastasis is seen in about 50% of patients with extensive-stage SCLC and is a poor prognostic indicator (5). SCLC metastases in the liver grow according to different metastatic patterns, yet the excessive hepatomegaly due to metastatic spread is quite uncommon.

We present an unusual case of a coin-like primary pulmonary lesion and a huge metastatic liver that was necrotomically proven to be a SCLC.

CASE REPORT

A 59-year-old obese male was admitted to the Internal Medicine department for further investigation of metastatic liver disease of unknown primary origin. The patient had reported a history of coronary artery disease with percutaneous transluminal coronary angioplasty (PTCA) and was an active smoker. Since the patient died a few hours after admission, it was impossible to conduct further investigation and an autopsy was ordered. At autopsy, acute myocardial infarction was determined as the cause of death. Additionally, the macroscopic examination revealed a giant liver (8.2 kg) which parenchyma was almost completely replaced with metastatic tumor (Figure 1, Figure 2). A 25-mm white nodule was identified next to the right intermediate bronchus without peripheral nodules. An enlarged lymph node adjacent to the nodule was identified as well (Figure 3). No other lymph nodes were identified as enlarged or abnormal in the necrotomic examination. The other organs showed no modifications. Microscopic examination of the nodule revealed small round or oval cells. The nuclei were finely granular and hyperchromatic with unrecognizable cytoplasm, as well as absent or inconspicuous nucleoli. In some areas the cells had an elongated shape. There were areas of necrosis and vessel and lymphatic invasion (Figure 4). The same cells were also seen in the metastatic liver masses (Figure 5) and in the examined lymph node. Immunohistochemically, the cells were strongly positive for TTF-1 and CD56, moderately positive for chromogranin, synaptophysin and negative for CD45 and CD99. The proliferation index was 70–80% of the malignant cells (Figure 6). The macroscopic and microscopic features were consistant with SCLC presenting as a solitary pulmonary nodule with metastases in the liver and lymph nodes.

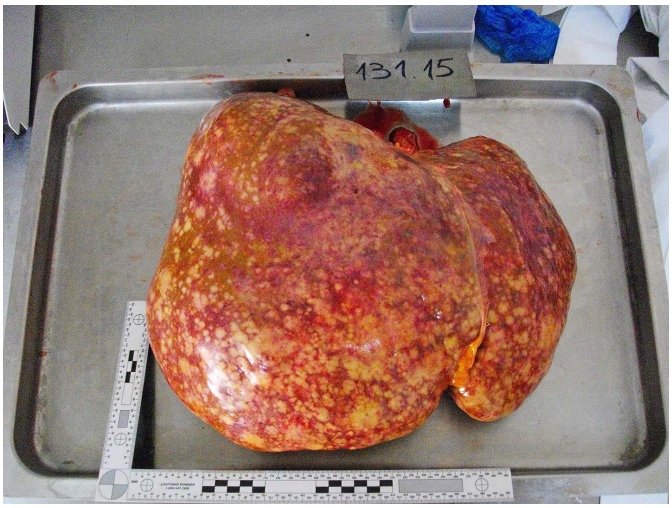

Fig. 1 Diffuse hepatomegaly with varying sized metastatic nodules.

Fig. 2 The liver contained multiple and variable nodules ranging from 0.5 cm to 3 cm in size.

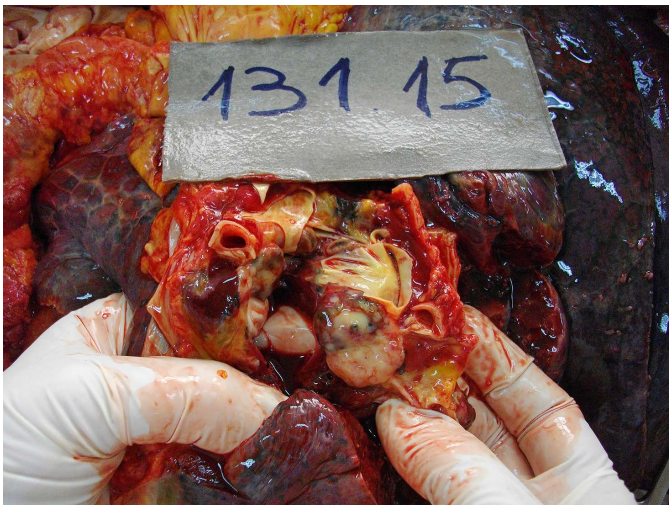

Fig. 3 Macroscopic appearance of the 25-mm white-tan, soft, friable nodule with little areas of necrosis, was identified next to the right intermediate bronchus.

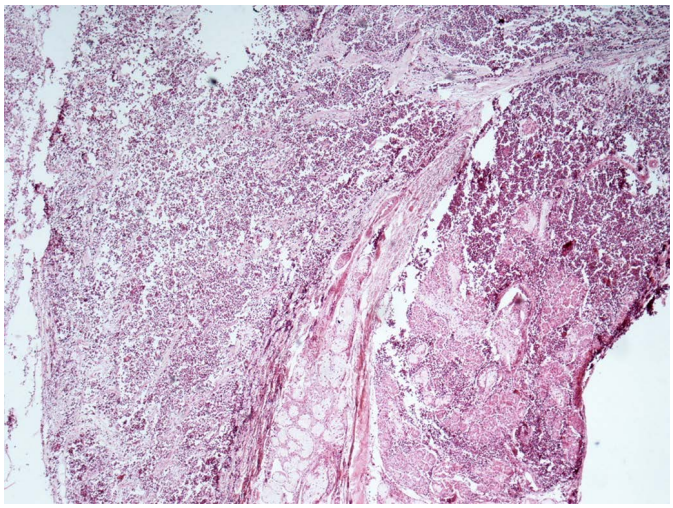

Fig. 4 Small cell carcinoma showing cells with darkly staining nuclei and scanty cytoplasm (hematoxylin-eosin stain at 4×).

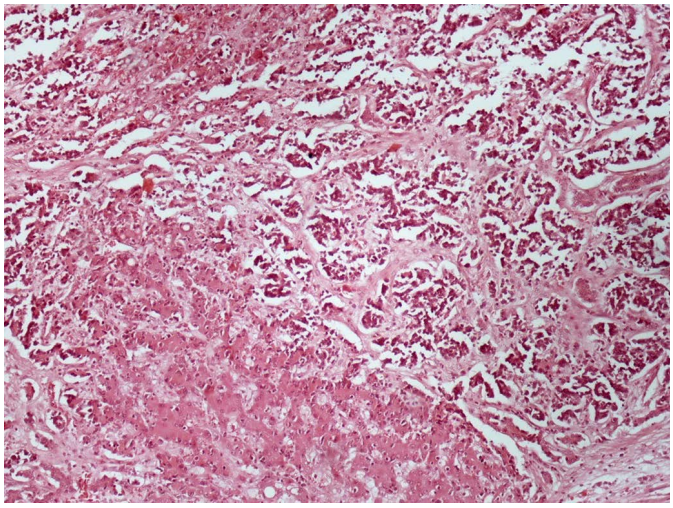

Fig. 5 Microscopic findings from the same case demonstrate a diffuse pattern of small cells infiltrating through the hepatic parenchyma (hematoxylin-eosin stain at 4×).

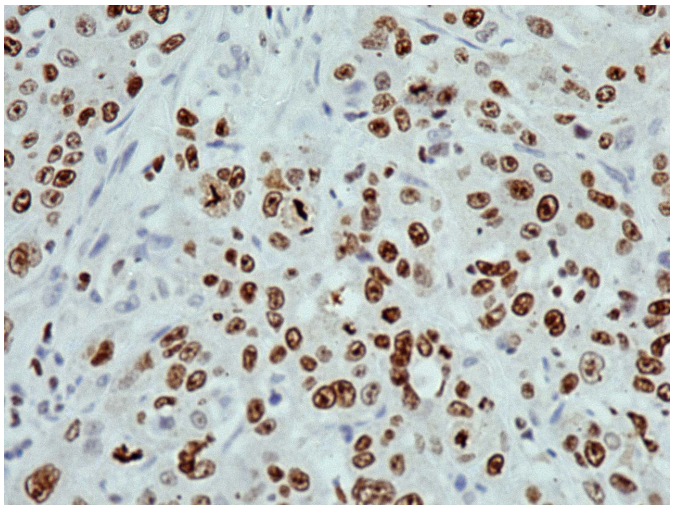

Fig. 6 TTF-1 positive staining in small cell lung cancer (Immunohistochemical stain for thyroid transcription factor-1, 40×).

DISCUSSION

Lung cancer is one of the most commonly diagnosed malignant tumor and one of the most common causes of cancer-related mortality, including the malignant mesothelioma (6, 7). SCLC accounts for approximately 15% of bronchogenic carcinomas and is strongly related to tobacco use (8). The likelihood of malignancy in solitary pulmonary nodules is generally related to the patient's characteristics – namely sex, age, family history of lung cancer and presence of emphysema – as well as the radiological characteristics of the nodule (9). Metastatic spread of cancer to distant organs is the main reason for the majority of malignancy-related deaths. Having studied 17,431 deceased lung cancer patients, Riihimaki et al. found out that the most usual metastatic sites of SCLC tumors are liver and central nervous system, yet liver and bone metastases signal poor survival, compared with nervous system metastases (10). Furthermore, in a retrospecrive study by Nakazawa et al. it was shown that liver metastasis was strongly correlated with poorer performance status of the patients (11). Additionally, Bremnes et al. have reported that liver metastasis was an independent prognostic factor in ED-SCLC (12). Hepatic metastases are present in about 50% of patients with extensive-stage SCLC (E-D SCLC) and they can be met either as nodular lesions or as diffuse malignant infiltration of the hepatic sinusoids (5). Excessive hepatomegaly with almost complete parenchymal replacement with metastatic tumor is rarely reported. Biliary tract obstruction due to metastases to lymph nodes in the *porta hepatis* or hepatic parenchyma is well described and regularly met in clinical practice (13) yet fulminant and subfulminant hepatic failure with encephalopathy resulting in hepatic coma is developed in only 7% of the patients (14).

CONCLUSION

The aggressiveness of SCLC is well documented and, in many cases, metastatic lesions are diagnosed prior to the discovery of the primary tumor. It is therefore of paramount importance for the clinicians to take it into consideration in the investigation of distant metastases of unknown primary origin.

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SGLT-2 Inhibitors: Are They a Promising Treatment Option in T2DM Patients with NAFLD?

Dimitrios Patoulias*

ABSTRACT
Sodium glucose co-transporter type 2 inhibitors (SGLT-2 inhibitors) are a class of antidiabetics, recently approved for the treatment of patients with T2DM. They feature cardioprotective and renoprotective action, while they exert beneficial effects on metabolic parameters. Non-alcoholic fatty liver disease (NAFLD) is a frequent co-morbidity in diabetic patients. Its prevalence reaches up to 70%. Since there is no specific treatment approved for NAFLD, both experimental and clinical studies have been recently conducted highlighting the efficacy and safety of SGLT-2 inhibitors mainly in animal models and secondarily in patients with T2DM and NAFLD. This class of antidiabetics seems very attractive, improving both glycemic control and liver function tests, while inhibiting NAFLD progression. However, further investigation is required to establish them as a first-line treatment option in T2DM patients with NAFLD, after thorough assessment of their efficacy and safety in clinical practice.

KEYWORDS
SGLT-2 inhibitor; NAFLD; T2DM

AUTHOR AFFILIATIONS
Department of Internal Medicine, General Hospital of Veria, Veria, Greece
* Corresponding author: M. Alexandrou 3B, Pefka, Thessaloniki, Postal code 57010; e-mail: dipatoulias@gmail.com

Received: 8 September 2017
Accepted: 6 February 2018
Published online: 23 April 2018

Acta Medica (Hradec Králové) 2017; 60(4): 167–170
https://doi.org/10.14712/18059694.2018.15

ABBREVIATIONS

SGLT-2, sodium-glucose co-transporter type 2; DM, diabetes mellitus; T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; TC, total cholesterol; FPG, fasting plasma glucose; FFA, free fatty acids

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is very common among patients with type 2 diabetes mellitus (T2DM). Prevalence of NAFLD in patients with T2DM rises up to 70%, while prevalence of non-alcoholic steatohepatitis (NASH) in T2DM asymptomatic individuals is as high as 20% (1). As NAFLD can lead to NASH, and the latter to cirrhosis and hepatocellular carcinoma (HCC), physicians should pay extra attention in those cases of concomitance.

SGLT-2 inhibitors are a class of antidiabetic drugs, recently approved for the treatment of patients with T2DM. A few experimental and clinical studies have been conducted aiming at investigating whether SGLT-2 inhibitors can offer as therapeutic option in patients with both T2DM and NAFLD.

Although there is no approved treatment for NAFLD, the presence of a drug class that improves both glycemic control and liver function tests, and inhibits the progression of NAFLD, seems very attractive in clinical practice. Through this brief communication, I aim at presenting both the relevant experimental and clinical data, discussing the potential beneficial effects of SGLT-2 inhibitors on those patients.

PATHOPHYSIOLOGY

SGLT-2 inhibitors mediated glucosuria leads to decrease in blood glucose levels, and finally to suppression of insulin secretion by pancreatic β-cells. Glucagon levels are elevated due to the paracrine effect of insulin and the direct effect of SGLT-2 inhibitors on the pancreatic α-cells. This hormonal imbalance (increased glucagon/insulin ratio) induces increase in hepatic gluconeogenesis, glucogen breakdown and lipolysis (2).

Daniele et al. documented that dapagliflozin shifts energy metabolism in patients with T2DM from glucose to lipid oxidation. The researchers noticed the presence of increased plasma ketone concentration in patients treated with dapagliflozin. Increased b-oxidation and increased fasting plasma glucagon levels constitute a potential mechanism of increased total ketone levels in those subjects treated with SGLT-2 inhibitors. Ferrannini et al. came to a similar conclusion, with the use of empagliflozin in patients with T2DM (3, 4).

In another interesting study by Ferrannini and colleagues in patients with T2DM, it was observed in the acute phase that empagliflozin led to improved glycemia, decrease in tissue glucose disposal and improved β-cell function and cellular insulin sensitivity, along with enhanced postprandial GLP-1 response. Chronic study confirmed the shift in substrate utilization from carbohydrate to lipid – as indicated by the increase in FFA concentrations –, with maintenance of the results obtained in the acute phase of the study (5).

Kamei and colleagues showed in their recently published study that tofogliflozin administration in patients with T2DM led significant decrease in body weight, body mass index (BMI), body fat mass and visceral fat area, besides the significant improvement in glycemic control and serum uric acid levels. After univariate analyses, the authors concluded that tofogliflozin seems a more reasonable therapeutic approach in obese individuals with recently diagnosed T2DM, suggesting in fact the phenotype of patients that may benefit most from SGLT-2 inhibitor initiation (6).

Collective and careful interpretation of the above leads to the conclusion that SGLT-2 inhibitors mediate metabolism shift from glucose to lipid oxidation, enhance β-cell function, improve cellular glucose sensitivity and lead to significant decrease in body weight, mainly due to reduction in body fat mass and visceral fat area. Thus, it seems reasonable that SGLT-2 inhibitor administration in patients with concomitant T2DM and NAFLD will be much beneficial, with significant improvement in all metabolic parameters.

EXPERIMENTAL DATA

Honda et al. (7) investigated the effect of ipragliflozin on diet induced obese (DIO) mice, using an amylin liver NASH model (AMLN) for 20 weeks. The researchers found that ipragliflozin reduced levels of total cholesterol (TC), fasting plasma glucose (FPG), insulin, free fatty acids (FFA) and aminotransferase, while it also improved insulin resistance. At pathogenesis level, ipragliflozin improved steatosis grade, decreasing lobular inflammation and lipid droplets deposition, while it improved fibrosis stage in treated mice, as well.

Komiya et al. (8) conducted an experimental study in obese mice with insulin resistance, concluding that ipragliflozin decreased liver weight, hepatic lipid accumulation and triglyceride (TG) deposition, serum ALT levels, along with glucose intolerance, findings supportive of beneficial effects on hepatic steatosis. Downregulation of lipogenic genes and genes involved in macrophages activation explain ipragliflozin effects on hepatic steatosis and inflammation. In a clinical study following the animal study, the authors documented improvement of liver dysfunction in patients with T2DM, without significant weight reduction. They suggest that SGLT-2 inhibitors may serve as optional treatment for hepatic steatosis in T2DM individuals, due to their multifactorial effects on metabolic parameters.

In another DIO mouse model, Nakano et al. (9) documented that after a 4-week course of treatment, remogliflozin decreased serum ALT and AST levels, total liver weight, while it also reduced hepatic inflammation and oxidative stress, as indicated by reduced mRNA expression of TNF-a, MCP-1 and TBARS, respectively. Remogliflozin also improved hepatic steatosis, by reducing TG content in hepatic tissue.

Tahara et al. (10) investigated the therapeutic impact of ipragliflozin on high-fat diet and streptozotocin–nicotinamide-induced type 2 diabetic mice. The administered SGLT-2 inhibitor improved significantly glycemic control, lipid levels, hepatic lipid content, total body and liver adipose tissue weight and serum levels of AST and ALT. Oxidative stress and inflammatory process were significantly improved, dose-dependently, by ipragliflozin. Thus, ipragliflozin ameliorates hepatic steatosis and progression to NASH, finding indicative of potential use in diabetic patients with NAFLD.

In another experimental study in rats fed on choline-deficient L-amino acid-defined diet, with induced hepatic steatosis and fibrosis, Hayashizaki-Someya et al. (11) documented that ipragliflozin: a) suppressed body weight gain, b) reduced hepatic TG content, c) decreased hepatic fibrosis score in a dose-dependent manner and d) did not affect serum AST and ALT levels. The authors suggest fat utilization as the main factor that led to decrease in steatosis. In accordance with the previous studies was that conducted by Qiang et al. (12). The researchers developed a rodent model with T2DM and NASH and evaluated the efficacy of luseogliflozin on metabolic parameters. In mice treated with the SGLT-2 inhibitor, the authors noticed normalization of serum ALT levels, decrease in hepatic steatosis and improvement in inflammatory and fibrotic changes.

Ji et al. (13) evaluated the beneficial effect of canagliflozin on metabolic parameters performing an experimental study in DIO mice, which did not feature or develop T2DM. It decreased body weight in DIO mice, reduced serum levels of TC and TG, but more significantly, it improved hepatic steatosis, reversing the effects of high fat diet on liver weight and liver to body weight ratio. Up-regulation of PPARα1 and down-regulation of DGAT2, PPARγ1 and PPARγ2 may explain this effect on hepatic steatosis.

Jojima et al. (14) confirmed the synergistic effect of empagliflozin and linagliptin on inhibition of NASH progression in a mouse model of NASH and DM, however lacking obesity and insulin resistance. The authors found that empagliflozin reduced serum ALT, liver to body weight ratio and hepatic TG content, while it also decreased significantly hepatic inflammation. However, anti-fibrotic effect of the combination was more efficient than empagliflozin or linagliptin alone. Another interesting finding was that the combination significantly reduced FAS and ACC1 mRNA expression, genes both involved in lipogenesis, while neither empagliflozin nor linagliptin affected expression of ACOX1 and PPAR-α, genes involved in β-oxidation, in contrast with Ji et al.

Obara et al. (15) identified an effect of tofogliflozin on the progression of NAFLD towards cirrhosis and hepatocellular carcinoma (HCC). The researchers administered tofogliflozin in diabetic and obese mice for 2 weeks, while they were also administered diethylnitrosamine-containing water. They concluded that tofogliflozin improved hepatic steatosis and inflammation, reduced serum glucose and FFA levels, while it did not affect insulin resistance. A much more interesting finding was that the administered SGLT-2 inhibitor suppressed the development of hepatic paraneoplastic lesions, due to attenuation of NAFLD progression and not due to direct effect on HCC cells.

The main results of the above discussed experimental studies are summarized on table 1.

CLINICAL DATA

There are only a few clinical data regarding the usefulness of SGLT-2 inhibitors in NAFLD until now. In their clinical study in 11 patients with T2DM and NASH, Tobita et al. (16) affirmed that dapagliflozin after 24 weeks of treatment reduced body fat mass and percent body fat, improved liver tests and glycemic parameters. The authors associated: a) lower ferritin levels with improvement in hepatic fibrosis and b) improvement in glycemic control and lower insulin levels with decrease in insulin resistance. Despite the small sample of patients, the short-term study and the absence of liver biopsies, the above results are promising as for the use of SGLT-2 inhibitors in patients with metabolic syndrome. In a recently published randomized controlled trial, patients with T2DM and NAFLD were randomized to receive either ipragliflozin or pioglitazone for 24 weeks. Improvement in NAFLD values and glycemic control were equal between the two groups. However, only ipragliflozin reduced significantly body weight and abdominal fat area, enhancing its usefulness as treatment option in patients with T2DM and NAFLD (17).

In a pooled analysis, Leiter et al. (18) documented that canagliflozin was superior to placebo or sitagliptin regarding the improvement of liver function tests in individuals with T2DM, finding consistent with substantial improvement in glycemic parameters and reduction in total body weight. Finally, Okhi et al. (19) administered

Tab. 1 Effect of SGLT-2 inhibitors on hepatic steatosis, hepatic fibrosis and tumorigenesis (experimental models).

	Administered SGLT-2 inhibitor	Hepatic steatosis	Hepatic fibrosis	Tumorigenesis
Honda et al (7)	Ipragliflozin	✓	✓	
Komiya et al (8)	Ipragliflozin	✓	✓	
Nakano et al (9)	Remogliflozin	✓		
Tahara et al (10)	Ipragliflozin	✓	✓	
Hayashizaki-Someya et al. (11)	Ipragliflozin			
Qiang et al. (12)	Luseogliflozin	✓	✓	
Ji et al. (13)	Canagliflozin	✓		
Jojima et al. (14)	Empagliflozin	✓	✓	
Obara et al. (15)	Tofogliflozin	✓		✓

an SGLT-2 inhibitor in 24 patients with T2DM and NAFLD previously receiving either a DPP-4 inhibitor or a GLP-1 analog. After 320 days of administration, the researchers observed not only a further improvement in glycemic control, but also decrease in serum ALT levels and body weight and improvement in FIB-4 index. SGLT-2 inhibitors seem an interesting alternative in patients with NAFLD and T2DM, who did not respond to incretin-based therapies.

CONCLUSION

Based upon the above mentioned, primarily, experimental and, secondly, clinical data, although it is early to reach a safe conclusion, SGLT-2 inhibitors share some certain characteristics: they act against inflammation and oxidative stress, decrease hepatic steatosis, prevent the progression towards hepatic fibrosis or even reverse fibrotic changes in liver parenchyma and they possibly act against tumorigenesis. According to available data, they seem superior to other antidiabetics, and they can act synergistically, as well. Further investigation regarding their efficacy and safety in well-designed randomized controlled trials in humans will clarify whether they can be established as first-line therapeutic option or not.

I consider that this class of antidiabetics constitutes a very attractive therapeutic option in patients with T2DM and NAFLD.

CONFLICT OF INTEREST

The author declares that he does not have any conflict of interest, including specific financial interests or relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

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