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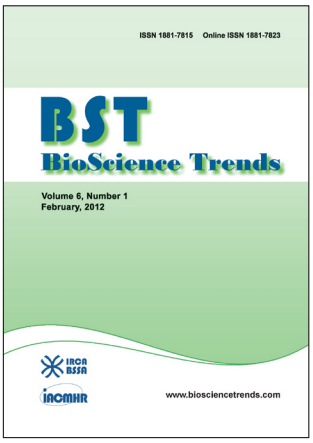

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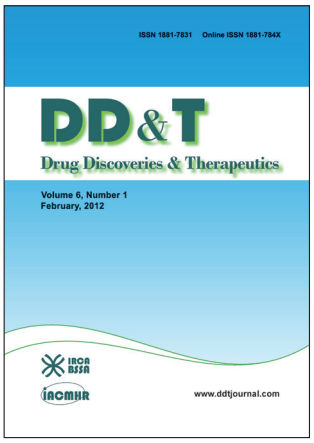

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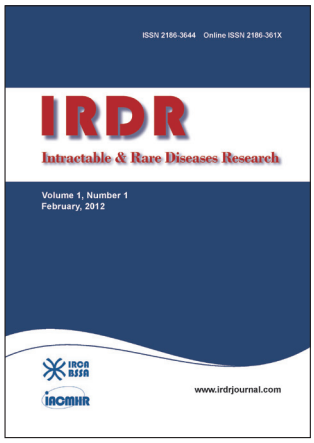

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A brief introduction to China's new Drug Administration Law and its impact on medications for rare diseases

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Summary **The Drug Administration Law of the People's Republic of China (amended in August 2019) is a major piece of legislation governing drug administration in China. This law seeks to improve health legislation; to achieve this, it defines the concepts of fake drugs and inferior drugs and their differences, it emphasizes the regulation of reliability, it standardizes online drug sales, it encourages technological innovation, and it specifies legal liability and punishment through legislative amendments. The Drug Administration Law is characteristic of responsive legislation, and it guarantees a right to health for citizens. The law provides for priority review and approval of new drugs for diseases like rare diseases, it encourages the domestic manufacturing and development of new drugs for rare diseases, and it provides a firm legal basis for medications to treat rare diseases under the Healthy China strategy.**

Keywords: Drug Administration Law, characteristics of legislation, implementation of the law, rare disease, orphan drug

1. Introduction

The Drug Administration Law of the People's Republic of China is the basic law governing drug manufacturing, usage, and administration. The newly amended Drug Administration Law of the People's Republic of China (herein after referred to as the "new Drug Administration Law") was passed in August 2019, and it took effect on December 1, 2019. The amendment signals that China has upgraded its "drug legislation" with numerous innovations. More importantly, the new Drug Administration Law has now provided a legal framework for a priority review system for pediatric drugs, drugs in short supply, drugs for serious infectious diseases, and drugs for rare diseases. The Law actively promotes and provides for resources to research and develop those drugs, and especially those for rare diseases, thus providing legal support to ensure the supply of medications for rare diseases.

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2. Main aspects of the legislation

2.1. *Legal protection of the "right to health" for Chinese citizens*

The right to health is a basic right specified in the Chinese Constitution (*1*). The Law clearly stipulates that the new Drug Administration Law is intended "to ensure drug safety and protect the public's legitimate rights and interests and to protect and promote public health" in Chapter 1, Article 1; similarly, Article 3 states that "drug administration shall focus on public health". Chapter 2, Article 16 states that the State will implement priority review and approval for drugs in short supply that are urgently needed clinically, for new drugs to prevent and treat serious infectious diseases, for drugs to treat rare diseases, and for pediatric drugs. Ethics review, informed consent, clinical risks, and other aspects of "conducting clinical trials on drugs" are stipulated in detail. The new Drug Administration Law is based on a right to health, protecting the legitimate rights of Chinese citizens. This concept is intended to facilitate the adoption of national strategies like "Healthy China" and also to accord with international norms of human rights protection in China.

2.2. *Reinforcing regulation during and after drug administration*

The Chinese Government has promoted in-depth reforms "to streamline administration, delegate powers, and improve regulation and services" to spur innovation in regulatory concepts, systems, and methods, and to establish and improve a new regulatory system connecting the entire regulatory process before and after drugs are marketed (*2*). The new Drug Administration Law stipulates that "departments of drug administration will create records of the reliability of drug safety, increase the frequency of supervision and inspections for entities with a record of unreliability, and potentially implement joint punishment pursuant to State regulations". The Law specifies a system for sharing information and a drug traceability system. Regulation of reliability and regulation of information are used to achieve drug traceability, to promote regulation of drug safety, and to improve regulatory efficiency.

2.3. *Implementation of internet-related provisions to safeguard the drug supply*

Internet-related provisions are crucial to reforming the drug supply. The supply of quality medical resources has been effectively promoted through close coordination with the healthcare industry and innovation (*3*). Chapter 6, Articles 61 and 62 of the new Drug Administration Law describe "online drug sales" in detail. The Law encourages the online sales of drugs, but it prohibits the online sales of drugs specially administered by the State. Online drug distributors are required to have the same qualifications as offline entities. Entities operating third-party platforms where drugs are available must file for approval, have their qualifications reviewed, and follow other legal procedures so that regulations of drug sales online and offline are standardized. Other laws and regulations like the Measures for the Supervision and Administration of Drug Sales Online have been issued to further standardize and facilitate the healthy development of drug sales online.

2.4. *Reinforcing drug administration and intensifying punishments for unlawful acts*

The new Drug Administration Law specifies liabilities for pharmaceutical companies and legal sanctions to crack down on corruption in the pharmaceutical industry, and it stresses the primary responsibility of pharmaceutical companies, and especially persons responsible. Drawing upon legislative concepts in the Law for the Protection of Consumer Rights and Interests, the new Drug Administration Law includes liability for compensation. A system of civil liability for drug harms has been established, and the administrative liability of drug regulators is specified.

3. Major results of the legislation

3.1. *Developing and improving extensive laws and regulations on drug administration*

The objectives, chapters, structure, rationale, and principles of the new Drug Administration Law have been substantially revised from the former version. The former Drug Administration Law had 10 chapters with 104 articles (2015 Revision) while the new Drug Administration Law has 12 chapters with 155 articles. Entities authorized to market drugs, drug stockpiles and supply, drug post-marketing management, and other chapters have been added to the new Law (see Table 1).

3.2. *Promoting the optimization of scientific and rigorous health legislation*

The Basic Medical Care and Health Promotion Law is intended to legally specify for the first time that health is a basic right of citizens. The Healthy China strategy has been implemented (*4*), and it clearly specifies important issues like the National Essential Drugs System and the "drug supply". The new Drug Administration Law takes the national legislative framework of the Vaccine Administration Law into account and it excludes regulations related to vaccines, a special type of drug (*5*), in order to allow the strictest regulation and administration of vaccines. The new Drug Administration Law focuses on the link between drug legislation and the scope of specially targeted laws, helping to improve health legislation in China.

3.3. *Embodying the rule of law and flexibly controlling and regulating drug administration*

The pharmaceutical industry is at the leading edge of scientific research and innovation. Problems spanning law and science and technology are continually appearing with the rapid development of information technology and artificial intelligence (*6*). The new Drug Administration Law has reiterated the legal concept of drugs and for the first time has adopted a "general" legislative approach to the definition of drugs instead of a "specific" approach. The legal concept of a "drug" was defined in the Supplementary Provisions of the old version of the Law, but it is now defined in Article 2 under Chapter 1, General Principles. Article 5 in the same chapter encourages the development of and innovation in drugs. The amendment of the law is an extensive revision. Accordingly, the entire text of the amended law needs to be read (*7*).

Amendment of the legislation was approached scientifically, i.e. "from tweaks to extensive revision", and various aspects of the Drug Administration Law,

Table 1. Table comparing the revised chapters in the new and former versions of the Drug Administration Law

Name of the law	new Drug Administration Law		former Drug Administration Law	
Chapters and number of articles	Chapter 1 General Principles	15	Chapter 1 General Principles	6
	Chapter 2 Drug Research, Development, and Registration	14	–	-
	Chapter 3 Entities Authorized to Market Drugs	11	–	-
	Chapter 4 Drug Manufacturing	10	Chapter 2 Control over Drug Manufacturers	7
	Chapter 5 Drug Distribution	18	Chapter 3 Control over Drug Distributors	8
	Chapter 6 Pharmacy Administration in Medical Institutions	8	Chapter 4 Control over Pharmaceuticals in Medical Institution	7
	Chapter 7 Post-marketing Management	7	Chapter 5 Post-marketing Management	23
	–		Chapter 6 Control over Drug Packaging	3
	Chapter 8 Drug Pricing and Advertising	8	Chapter 7 Control over Drug Pricing and Advertising	8
	Chapter 9 Drug Stockpile and Supply	6	–	-
	Chapter 10 Supervision and Administration	6	Chapter 8 Supervision and Administration	9
	Chapter 11 Legal Liability	48	Chapter 9 Legal Liability	28
	Chapter 12 Supplementary Provisions	4	Chapter 10 Supplementary Provisions	5
Total	12 chapters, 115 articles		10 chapters, 104 articles	

from concepts to content, have been altered. This helped to improve the logical consistency of the regulatory system in the Law, the completeness of revisions, and the feasibility of content stipulated in the Law. As a result, pharmaceutical affairs legislation in China has been revised and improved and better legal safeguards have been put in place for domestic drug regulation (*8*).

3.4. *Actively responding to Chinese society's concerns about medications*

Transitioning to a responsive legislative approach is inevitable as the rule of law develops in China (*9*). The core of a responsive legislative approach is: to respond to society, to reduce repression but to increase awareness, efforts, and openness in accordance with legislative objectives, to combine openness and legitimacy, to provide a legal foundation, to provide substantive justice, to serve the public interest, and to uphold society's ideals (*10*). The new Drug Administration Law focuses on societal needs like being able "to purchase anticancer drugs on the overseas market on behalf of a party wishing to do so". For the first time, the Law defines the scope of fake drugs and inferior drugs and it differentiates their legal attributes to help identify fake drugs and inferior drugs more precisely and to punish their sellers. Drugs that are marketed overseas but that have not been approved domestically are no longer treated as fake drugs. Punishment imposed on offenders who import small quantities of drugs marketed overseas that have not been approved domestically may be reduced in some cases; if no harm is caused or treatment is not delayed, offenders can be exempted from punishment. Legally addressing societal issues in pharmaceuticals, meeting vast legislative needs, and reinforcing the public's respect for the law will help to shape the practical aspects of legislation and increase its effectiveness.

4. Impact on medications for rare diseases

4.1. *Current medications for rare diseases in China*

Rare diseases are not clearly defined in China, and patients depend almost entirely on imports. This represents a major obstacle to protection of the right to health of patients with rare diseases. One major problem is the limited manufacture of drugs to treat rare diseases. Companies lack the initiative to develop, license, and manufacture drugs for rare diseases because of the small population of patients with rare diseases and unclear benefit, the long cycle of clinical trials and unpredictable results, unclear import or regulatory policies concerning drugs in certain categories, and the uncertainty of medical insurance reimbursement. A second major problem is the lack of medications for rare diseases. There is no medication to treat 95% of the rare diseases in China. Of the 121 diseases included in the First Rare Diseases List, 44 can be treated with 88 medications but only 35 of those medications are available in China. Many innovative drugs to treat rare diseases are not imported, and medications are unavailable to many patients (*11*). A third major problem is the cost of drugs for rare diseases. Of 55 medications for rare diseases that are available in China, only 29 are included in the National Health Insurance Reimbursed Drug List, and only 9 are reimbursed at A level. High drug prices directly limit reimbursement for drugs to treat rare diseases and hamper obtaining approval for marketing, further influencing the manufacturing and development of those drugs. The societal dilemma is the limited applicability of "orphan drugs" to treat rare diseases compared to more widely applicable drugs.

4.2. *Basis for policies related to medications for rare diseases in China*

Social trends are encouraging the development of innovative drugs and accelerating review and approval, so the Chinese Government has initiated innovations in its policies on drugs to treat rare diseases. In 2015, the National Medical Products Administration (NMPA) issued the "Notice on Polices for Drug Registration, Review, and Approval". The Notice clearly stated that applications could be submitted for innovative orphan drugs individually and that their review and approval would be accelerated. In October 2017, the General Office of the CPC Central Committee and General Office of the State Council of the People's Republic of China issued their "Opinions on further reform of the review and approval system and encouraging innovations in drugs and medical devices". The document stipulated that drugs and medical devices to treat rare diseases that had been approved abroad could be conditionally approved for use in China, and it clearly supported the development of drugs and medical devices to treat rare diseases. In December 2017, the NMPA issued "Opinions on encouraging drug innovation and implementing priority review and approval"; rare diseases were included in the scope of priority review and approval. The document also stated that "applications to reduce the number of clinical trial subjects or for exemption from clinical trials could be submitted for rare diseases and other specific diseases". The Center for Drug Evaluation will decided whether to approve an application or not based on technical review and the actual status of Chinese patients. In May 2019, the National Health Commission, Ministry of Science and Technology, Ministry of Industry and Information Technology, NMPA, and National Administration of Traditional Chinese Medicine jointly published the First Rare Diseases List, which included 121 diseases. This is an important step in supporting medications for rare diseases. Areas and regions like Shanghai, Guangdong Province, and Shandong Province have also actively explored measures to encourage the development of drugs to treat rare diseases and the prevention and treatment of rare diseases via local legislation. A point worth mentioning is that these efforts involve inherent problems like fragmented policies, defective regulations, and limited legal reach. The problems of development and approval of "orphan drugs" cannot be solved by local regulation alone.

4.3. *Reinforcing the law basis for approval of drugs to treat rare diseases*

Chapter 9, Article 96 of the new Drug Administration Law stipulates that the State will encourage the research, development, and manufacture of drugs in short supply and implement priority review and approval for drugs in short supply that are urgently needed clinically, for new drugs to prevent and treat serious infectious diseases, and for drugs to treat rare diseases. The Law provides special legal protections for medications that are useful in treating rare diseases. From a legal perspective, reforms have resulted in a drug review and approval system that aims to encourage the pharmaceutical industry to independently conduct research, development, and innovation. The reforms "to streamline administration, delegate powers, and improve regulation and services" in drug regulation are based in law and filled the gap caused by the fact that drugs to treat rare diseases in China are almost entirely imports and the lack of specialized legislation governing research and development. From society's perspective, the Law has helped to unshackle medications for rare diseases and to enhance the ability of Chinese pharmaceutical companies to share information internationally and engage in technical cooperation. This has increased the accessibility to medications for rare diseases and thus better protects the right to health of patients with rare diseases both at home and abroad.

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Review

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Quality of life in refractory generalized myasthenia gravis: A rapid review of the literature

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Summary **Generalized myasthenia gravis (GMG) is a neuromuscular transmission disorder that creates a fluctuating weakness of the voluntary muscles. This study is aimed at understanding the effect that refractory GMG has on the quality of life of patients who suffer from it, and the effect of eculizumab on it. A systematic literature search was conducted in MEDLINE (Ovid), EMBASE and the Cochrane Database of Systematic Reviews (Ovid). Eligibility criteria were verified via the title and summary and afterward through the full text. The risk of bias of the included randomized clinical trials was evaluated and the data were synthesized in a descriptive manner. Nine studies were identified that evaluated the quality of life of patients with GMG. Regarding the effect of eculizumab, two studies were identified. The quality of life in patients with GMG is lower compared to ocular myasthenia gravis (MG) and MG in remission, especially in the domains of physical function, physical role, bodily pain, vitality, and social function. Patients treated with eculizumab had a better perception of their quality of life compared to those who received placebo. GMG affects the quality of life more than other types of MG. This outcome is of great importance for the choice of therapeutic options in patients with refractory GMG. Eculizumab generates improvements in the perception of patients' quality of life compared to placebo, making it a relevant therapeutic option in the management of refractory GMG.**

Keywords: Myasthenia gravis, refractory generalized myasthenia gravis, quality of life, generalized myasthenia gravis

1. Introduction

Myasthenia gravis (MG) is a disorder of neuromuscular transmission in which autoantibodies bind to acetylcholine receptors that are found in the postsynaptic membrane in the neuromuscular junction, making it difficult to transmit impulses to skeletal muscles and causing weakness (*1,2*). Its prevalence varies between 15 to 179 cases per million and the mortality rate varies between 0.06 to 0.89 per million person-years (*3*).

This disease is characterized by fluctuating weakness of the voluntary muscles, which worsens with activity and as the day progresses (*2,4*). It is classified as generalized or as ocular. In patients with ocular MG, the weakness affects only extraocular muscles and manifests itself with symptoms such as diplopia and palpebral ptosis (*5*). MG is considered generalized (GMG) when weakness involves the bulbar muscles, extremities or axial muscles. Weakness in the bulbar muscles causes difficulty in speaking, swallowing and chewing. In the muscles of the upper extremities, the weakness causes difficulty in raising the arms and performing daily tasks, such as combing, and when there is weakness in the muscles of the lower extremities, gait becomes unstable or irregular. Axial muscle involvement causes weakness in the back and neck, which can lead to painful spasms in these regions of the body. Weakness in these muscles is usually also accompanied by weakness in extraocular muscles (*5,6*).

All these symptoms affect the quality of life of patients with GMG, and therefore the management

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of MG aims to restore muscle strength and decrease disease activity. There are mainly four types of therapy to achieve these goals: *i*) symptomatic therapy with acetylcholinesterase inhibitors; *ii*) chronic immunosuppressive therapies; *iii*) rapid immunomodulatory therapies; and *iv*) Thymectomy (*7,8*). However, between 10 and 30% of patients with MG do not respond adequately to conventional immunosuppressive therapy, so they require management with different types of biological therapy such as rituximab and eculizumab (*7*).

Therefore, this study was aimed at understanding the effect of refractory GMG on the quality of life of patients suffering from this disease (objective 1) and evaluating the effect of eculizumab on the quality of life of patients with refractory GMG (objective 2).

2. Materials and Methods

A systematic literature search was carried out through the design of two generic search strategies, delimited by key terms for each of the proposed objectives (see Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=48*). The following databases were searched: MEDLINE (OVID), EMBASE and the Cochrane Database of Systematic Reviews (OVID). Two independent researchers (LvdW and NG) performed an initial screening of the results by reviewing the title and summarizing the references obtained. Disagreements between the researchers were resolved by consensus. Subsequently, the eligibility criteria were verified (see Table S2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=48*) using the full text of the preselected references.

The management and extraction of the data obtained from each included study were carried out using a form designed in Excel®. This form included information on the main outcome of each study and the results of the quality of life outcome with its respective measurement scale. The risk of bias of the randomized clinical trials (RCTs) that were included was assessed, using the risk of bias tool designed by the Cochrane Collaboration. The data were synthesized descriptively by means of graphs or tables and grouped by the different scales of measurement of quality of life.

3. Results

After removing duplicates, a total of 193 references were identified. Following the review of the full text, 12 studies were included in the synthesis of evidence (see Figure 1). Table S3 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=48*) shows the list of included and excluded studies for each objective, and Table S4 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=48*) shows the characteristics of the included studies.

3.1. *Quality of life of patients with GMG*

The outcome of quality of life was identified in nine cross-sectional studies that focused on patients with GMG. No specific evidence was found for the subtype of refractory GMG. Five of the studies used the 36-Item Short Form Health Survey (SF-36) (*9-13*), three used the 15-item MG Quality of Life scale (MG-QOL15) (*14-16*) and one used a specific Italian disease questionnaire (IMGQ) (see Table S4, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=48*) (*17*).

3.1.1. *Quality of life of patients with GMG measured with the SF-36 scale*

The SF-36 scale has a score of 0 to 100 that reflects the overall state of health and classifies it from worst to best state of health, in which lower scores represent

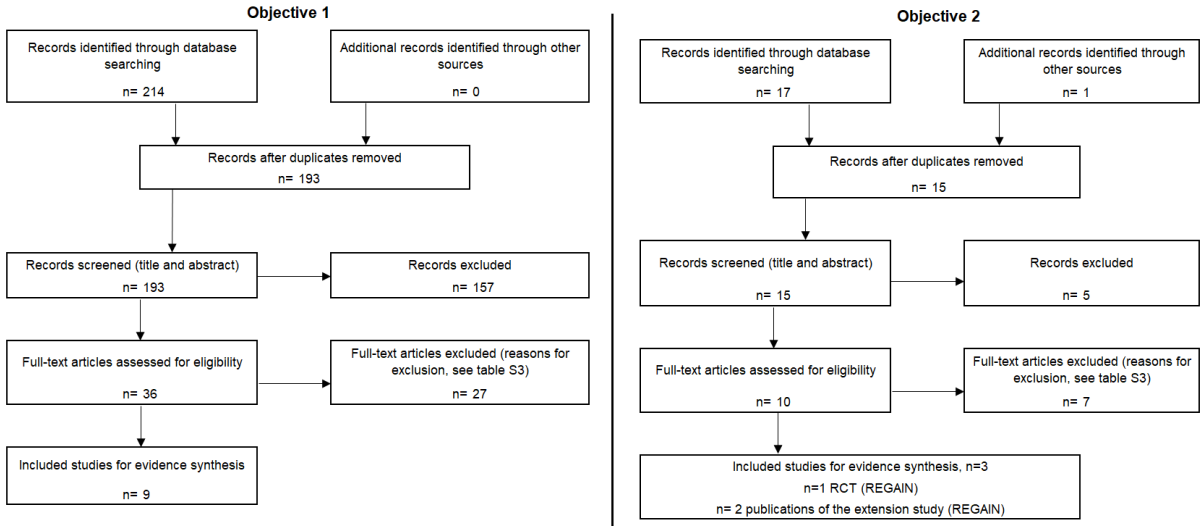

Figure 1. Flowchart search screening and selection of evidence.

a worse state of health. It contains 36 questions organized in eight domains: physical function, physical role, bodily pain, general perception of health, social function, emotional role, mental health, and vitality; in some cases the first four domains are grouped into a general physical component and the remaining ones make up the mental component (*10*). In the study by Boldingh *et al*., patients with GMG had a lower score in the physical and mental component (mean values of 48 and 61 points, respectively) than patients with ocular MG, in remission and residual ocular. When comparing these scores with the results of patients with ocular MG (patients who began with and remained with only ocular symptoms throughout the course of the disease), residual ocular (patients with residual ocular symptoms after having generalized disease) and in remission, the differences were statistically significant ($p < 0.001$) (see Figure 2) (*10*).

Likewise, in the study by Yang *et al*. (*12*), a comparison was made between two types of MG (GMG and ocular MG) by domain and quality of life component. Statistically significant differences were observed in the domain of physical function ($p < 0.001$), physical role ($p < 0.001$), bodily pain ($p = 0.028$), vitality ($p = 0.018$), social function ($p = 0.003$), and in the physical and mental health component in general ($p < 0.001$ and $p = 0.022$, respectively) (*12*).

Two studies were also identified that compared the results (*11,12*) by domain and overall quality of life score in patients with GMG (*11,12*), with a reference population (VR) of the study of Cohen *et al*. (*18*). In the first study, despite the small sample size ($n = 20$ patients with GMG), statistically significant differences were identified when comparing the two populations in the domains of physical function (GMG: 57 ± 22 compared with VR: 84.5 ± 22.9), physical role (GMG: 47± 40 compared with VR:81.2 ± 33.8) and general perception of health (GMG: 49 ± 11 compared with VR: 72.2 ± 20.2) (*11*). In Figure 3, the results of the second study are observed, also with a small sample size ($n = 29$ patients GMG), in which a lower score is also reported in the population with GMG in most domains, except for the mental health domain (*12*).

Finally, Basta *et al*. (*9*) performed a disease severity analysis according to the Myasthenia Gravis Foundation of America (MGFA) classification, where patients from Category II onward are considered to have GMG. It was identified that patients with severe MG have worse scores in all domains in the physical and mental health component in general, as well as in the overall quality of life score ($p = 0.001$).

3.1.2. *Quality of life of patients with GMG measured with the MG-QOL15 scale*

The MG-QOL scale consists of 15 items, each rated from 0 to 4 according to its frequency, with a maximum score of 60. The higher the score, the worse the quality of life perceived by the patient (*19*). In the study by Hernández *et al*. (*14*) the quality of life of three types of MG was compared, finding that patients with GMG have a higher score on the MG-QOL scale (19.3 ± 10.5), that is, a worse perceived quality of life, compared to the reported scores in patients with MG in remission and ocular MG (3.6 ± 5.2 and 8.8 ± 7.4, respectively).

The study by Hoffmann *et al*. in 2016 sought to assess the impact of fatigue, measured with the Chalder Fatigue Scale (CFQ). In general, it was shown that the quality of life scores were significantly higher in patients who displayed fatigue (CFQ ≥ 4) in the three types of MG, patients with GMG having a worse perception of quality of life (mean of 22.7) compared with patients with MG in remission (mean of 8.6) and

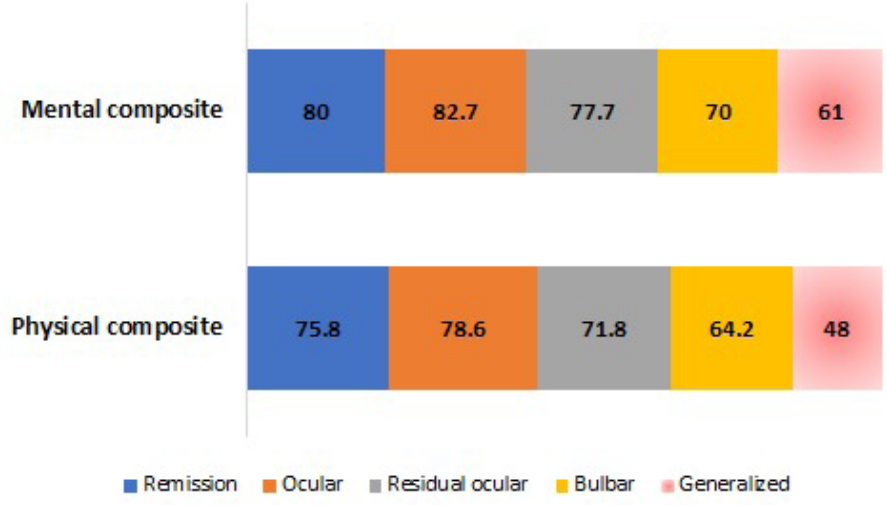

Figure 2. Score of the physical and mental component of the SF-36 scale by type of myasthenia gravis, in Boldingh *et al.* (*10*). Mean values. MG: Myasthenia gravis. Source: Adapted from Boldingh, *et al*. An up-date on health-related quality of life in myasthenia gravis – results from population-based cohorts. Health Qual Life Outcomes. 2015; 13:115.

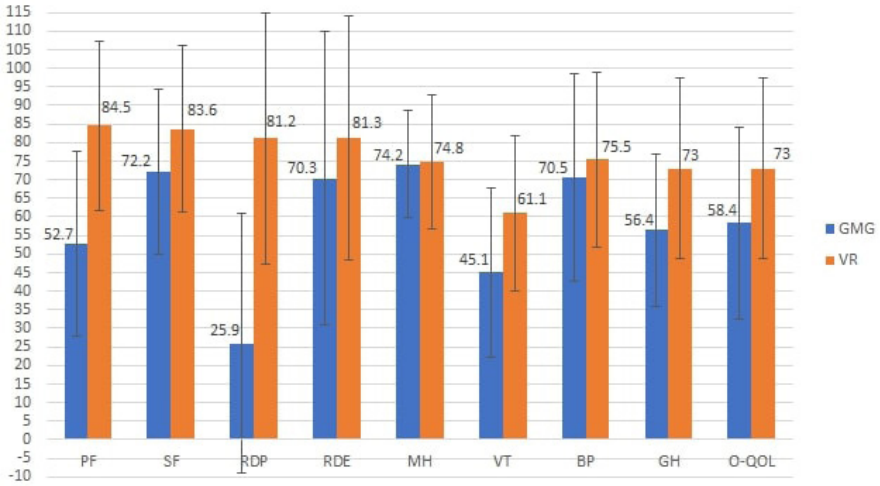

Figure 3. Score using the SF-36 scale by domain in the GMG population and reference population, in Paul *et al.* (*12*). Mean ± standard deviation. GMG: Generalized myasthenia gravis, VR: Reference values, O-QOL: Overall quality of life related to health, PF: physical function, SF: social function, RDP: Role disruption–physical, RDE: Role disruption–emotional, MH: mental health, VT: vitality, BP: body pain, GH: general perception of health. Source: Adapted from Paul RH, *et al*. Quality of life and well-being of patients with myasthenia gravis. Muscle Nerve. 2001; 24:512-516.

ocular MG (mean of 19.3) (*15*).

Finally, in India Kumar *et al*. (*16*) performed an analysis of quality of life by the severity of the disease according to the MGFA classification, finding average values on the MG-QoL15 scale of 3.5, 9.4 and 15.9 in MG grades I, II and III/IV, respectively. A correlation was identified between the severity of the disease and the quality of life scores. Patients with grade I/II had a better perception of quality of life compared to the more severe degree of the disease (MGFA III/IV).

3.1.3. *Quality of life of GMG patients measured with the IMGQ questionnaire*

The IMGQ is a specific self-reported questionnaire for the disease, which assesses the perception of the quality of life. Its items are classed as good or bad (*17*). This questionnaire was used by Cioncoloni *et al*., whose study aimed to assess the changes in motor function that determine to a large extent the loss of a good quality of life in patients with MG. As a result of the evaluation of 41 patients (12 with ocular MG and 29 with GMG), 18 patients of the 29 with GMG reported their perception of quality of life as excellent or good, and 11 as poor, while none of those in the group with ocular MG reported a poor quality of life. Patients with a poor perception of quality of life showed a significantly lower overall IMGQ questionnaire score and a higher score on the scale of evaluation of symptoms and activities of daily living (the myasthenia gravis-specific activities of daily living scale (MG-ADL)) in comparison with patients who displayed an excellent or good perception of quality of life. In addition, in the analysis of variables associated with a poor quality of life, it was identified that the variables such as difficulty chewing solid foods and breathing while at rest (items of the MG-ADL scale) show a significant association with a poor perception of quality of life ($p < 0.005$) (OR: 6.57, 95% CI: 1.19 to 36; and OR:14, 95% CI: 1.11 to 176, respectively) (*17*).

3.2. *Effect of eculizumab compared with placebo on the quality of life of patients with refractory GMG*

In the second objective, the outcome of quality of life was evaluated in a randomized, double-blind and multicenter phase III clinical trial (REGAIN) (*20*), with a low risk of bias (Table S5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=48*), and its extension study (two publications) (*21,22*).

This RCT evaluated the efficacy and safety of eculizumab compared with placebo in patients with refractory GMG from 17 centers in 17 countries in North America, Latin America, Europe, and Asia. Patients were randomly assigned to the eculizumab group or the placebo group until week 26. Administration for both eculizumab and placebo was performed as follows: 900 mg on the first day in week 2 and week 3; in week 4 they received a dosage of 1,200 mg, and after this the patients received 1,200 mg of maintenance every two weeks (*20*). The following efficacy outcomes were evaluated: activities of daily living measured with the MG-ADL scale; severity or disability of the disease with the Quantitative Myasthenia Gravis (QMG) scale; clinical signs and symptoms with the Myasthenia Gravis Composite (MGC) scale; and quality of life (*20*).

The quality of life outcome was analyzed using a statistical model of worst-rank ANCOVA, in order to explain the potential effect of rescue medication in efficacy evaluations, as well as other negative outcomes (death, MG crisis, rescue use or treatment interruption). In this analysis (worst-rank) the patients were ranked

from 1 (best outcome) to 125 (worst outcome), taking into account the time function until the event for the worst outcome category, and the change of the baseline until week 26 for the best outcome category (*20*). In addition to this first analysis, two additional analyses were performed:

i) Sensitivity analysis: patients with poor or negative outcomes were ranked according to the change from baseline until week 26 and not as a function of time until the event. For this approach, the worst-case ANCOVA analysis and a repeated measures analysis were used with the changes observed at each visit, adjusting them according to the use or non-use of immunosuppressive therapies as the covariate.

ii) Post-hoc sensitivity analysis: patients who left without experiencing death, myasthenic crisis, rescue therapy or worsening of the disease were included in the group of patients who did not experience negative outcomes. Patients who died (none in REGAIN), experienced myasthenic crises, or presented exacerbation of MG with or without the need for rescue therapy, were assigned to the worst ranks (death was classified as the worst outcome, followed by myasthenic crises, the requirement of rescue therapy, and interruption due to disease progression).

The outcome of quality of life was evaluated with the MG-QoL15 scale; the average baseline score for the intervention group was 33.6 ± 12.2 and that for the placebo group was 30.7 ± 12.7 (*20*). In Table 1, the results of the ANCOVA analysis and repeated measures (worst-rank) of the change of the baseline until week 26 are observed. In the analysis of repeated measures without immunosuppressive therapies as a covariate, patients who received eculizumab showed a better score in week 4 compared to that of the baseline, and a greater effect at week 12 and 26. The average change between baseline until week 26 was greater for the eculizumab group in the analysis of repeated measures with and no immunosuppressive therapies as covariate (*20*) (Table 1).

Between the REGAIN study and its extension, a blind induction phase of 4 weeks was performed in order to preserve the masking nature of the REGAIN study. During this phase, the researchers, patients, and personnel of the study remained blind to all treatment assignments. Patients who had been assigned to eculizumab in REGAIN received eculizumab 1200 mg (4 vials) on the first day and in week 2, and placebo (4 vials) in weeks 1 and 3. Patients who had been assigned to placebo in REGAIN received eculizumab (900 mg, 3 vials) + placebo (one vial) every week. After this phase (week 4), all patients received eculizumab with an open-label (1,200 mg) every 2 weeks (*22*).

Two baselines were taken into account in the extension study for data analysis: the first day of the REGAIN study, and the last evaluation available before the first infusion of eculizumab in the extension study. These analyses were based on repeated measurement models. With regards to the change measured from the first baseline (first day of the REGAIN study), the result of quality of life on the MG-QoL15 scale reported by patients who received eculizumab during the 26 weeks of follow-up was identified as being maintained during the extension (cut-off point for analysis at three years) (*22*).

In the repeated measures analysis, performed to evaluate the change from the baseline of the extension study until week 130, no significant differences were found in the eculizumab/eculizumab group (average change of −1.2 from the start to the week 130 of the extension study, $p = 0.4756$), while patients who received placebo during the REGAIN study experienced a rapid improvement in quality of life when they started treatment with eculizumab, displaying an average change of -5.4 from the baseline to the 130th week of the extension study ($p < 0.0001$) (*22*).

Table 1. Quality of life results (MG-QoL15) of the REGAIN study

Items	Eculizumab ($n = 62$)	Placebo ($n = 63$)	Difference (95% CI)	p value
Worst-rank ANCOVA analysis score *	55.5 (4.6)	69.7 (4.5)	-14.3 (-27 to -1.6)	0.0281
Sensitivity analysis with the worst-rank ANCOVA analysis score *	55.6 (4.6)	69.5 (4.5)	-13.9 (-26.6 to -1.2)	0.0328
Sensitivity analysis with the change in scale score - ANCOVA **	-11.3 (1.5)	-6 (1.5)	-5.2 (-9.4 to -1.0)	0.0152
Sensitivity analysis with a model of repeated measures with immunosuppressive therapy as a covariate **	-13.8 (1.6)	-6.7 (1.6)	-7.1 (-11.3 to 3)	0.0009
Sensitivity analysis with a model of repeated measures without the use of immunosuppressive therapy as a covariate **	-12.6 (NR)	-5.4 (NR)	-7.2 (NR)	0.0010
Post-hoc sensitivity analysis of worst-rank ANCOVA *	54.6 (4.5)	70.6 (4.5)	-16 (-28.6 to -3.4)	0.0134

NR: Data not reported in the study. *, Change in the score from the baseline until week 26 – worst-rank ANCOVA presented as the mean of the range of least squares. **, Change in score from baseline until week 26 or last observation of the MG-QoL15 scale score given as the mean of least squares. Source: Howard JF, *et al*. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. Lancet Neurol. 2017; 16:976-986.

Table 2. Results of the outcome of fatigue (Neuro-QOL) of the patients of the REGAIN study and its extension

Items	Intervention (95% CI)	Control (95% CI)
Mean difference between baseline and week 26 of the REGAIN study	-16.3 (-20.8 to -11.8)	-7.7 (-12.1 to -3.3)*
Mean difference between the baseline of the REGAIN study and week 4 of the extension study	-17.8 (-22.5 to -13)	-17.4 (- 22 to -12.9)
Mean difference between the baseline of the REGAIN study and week 54 of the extension study	-17.5 (-22.5 to -12.5)	-15.7 (-20.5 to -10.9)

*p = 0.0081. Source: Andersen H, Mantegazza R, Wang JJ, O'Brien F, Patra K, Howard JF Jr; REGAIN Study Group. Eculizumab improves fatigue in refractory generalized myasthenia gravis. Qual Life Res. 2019; 28:2247-2254.

Andersen *et al*. (*21*) performed an analysis with the data from the REGAIN study and its extension by correlating the fatigue data, measured by the Quality of Life in Neurological Disorders (Neuro-QOL) subscale (higher scores indicate greater fatigue) and quality of life scores (MG-QOL15). In Table 2, the fatigue results of the patients of the REGAIN study and their extension are observed. They also identified a strong, statistically significant correlation between fatigue scores and quality of life scores when the change from baseline to week 26 of the REGAIN study was measured. This correlation was evident in both the eculizumab group and the placebo group (*21*).

4. Discussion

This study sought to identify the effect or impact of refractory GMG on the quality of life of patients. As a result of the search, 9 cross-sectional studies were identified that assessed the quality of life of patients with GMG; however, no specific evidence was identified for the subtype of refractory GMG. In most of the included studies, comparisons are made between different types of MG, identifying a greater impact on the quality of life in GMG versus ocular MG and MG in remission (*10,13,14*). The differences are especially found in the domains of physical function, physical role, bodily pain, vitality and social function (*13*). The connection of the domains of quality of life may be related to that found by Oosterhuis H, in his study of the natural course of MG in 1989, in which he noted that when GMG is associated with predominant bulbar muscle weakness, it results in severe restrictions in the activities of daily life and, when severe, it can cause myasthenic crises or require respiratory assistance (*23*). Likewise, in the study of Basta *et al*. (*9*) a relationship between the severity of the disease and worse scores on the quality of life scales was described, both in the physical component and in the mental health component. Similar results were identified in the study of Kumar *et al*. (*16*) where it was observed that patients with mild grades of the disease have a better quality of life compared to those with more severe grades.

Although no studies were found aimed at identifying predictive factors in the perception of quality of life of patients with GMG, it has been found in populations with MG that the change in life, that is, the change in occupation or work due to physical difficulty, and depression are significant factors in predicting the low quality of life of these patients. Since this disease is a chronic condition and there is no curable alternative, patients are more vulnerable to suffer, in addition to motor disorders, depression and low self-esteem that affect the mental health component, compromising the overall quality of life (*24*). As a result, it is essential to keep these factors in mind with any subtype of the disease, in order to propose and implement strategies that help positively influence the quality of life of these patients and their caregivers.

On the other hand, some authors have observed that the measurement of the quality of life in patients with MG at the time of evaluating and choosing a therapeutic option is of great help, because this measurement evaluates the impact of therapy at intermediate levels of clinical improvement and morbidity, in addition to supplementing the information provided by the analysis of clinical outcomes (*25,26*). Likewise, patients with MG are often treated successfully, using therapies such as pyridostigmine, therapeutic plasma exchange (plasmapheresis or PLEX/PEX), corticosteroids, azathioprine, cyclosporine, and thymectomy, thus improving their life expectancy (*27*). However, a group of patients continues to perceive a reduction in their quality of life, especially patients with generalized symptoms or active disease (*10*), particularly patients with the disease that is resistant to treatment, who require more aggressive therapeutic options to prevent life-threatening myasthenic crises (*28*).

There are few therapeutic options for this last subtype of the disease (refractory GMG) becoming an unfulfilled requirement (*29*). Therapies such as rituximab have shown some benefits in small studies and individual cases of refractory MG, and other therapies are under investigation, such as rozanolixizumab and abatacept (*30*). Currently, eculizumab is the only alternative with phase III studies approved for these symptoms. Because of this, the second research question in the present study was in

order to understand the effect of eculizumab on the quality of life in patients with refractory GMG. As a result of the search, three publications were identified: an RCT, the REGAIN study, and its extension.

Overall, it was shown that patients treated with eculizumab had a better perception of their quality of life when compared to the control group (placebo) and this effect was maintained during the three-year extension phase (*20,22*). In addition, a statistically-significant rapid improvement in the quality of life score was demonstrated in the group of patients who received placebo during the REGAIN study and started treatment with eculizumab at the beginning of the extension phase (*22*). Andersen *et al*. (*21*) found a correlation between fatigue scores and quality of life, that is, greater fatigue resulted in lower quality of life, and the REGAIN study also identified that fatigue reported by patients improved more in the group treated with eculizumab than in the control group (*22*).

As a result eculizumab has become a novel therapy for this group of patients and in recent years has received approval by regulatory bodies in some countries such as the United States for the management of GMG (*29,31*), in the European Union for refractory GMG (*29,32*) and in Japan for the treatment of GMG with symptoms difficult to control with high doses of immune therapy (IgG or PLEX) (*29,33*), in addition to its inclusion in clinical guidelines as a therapeutic option for the management of patients with severe and refractory GMG (*29,34*).

5. Conclusion

The effects of health-related quality of life are higher in patients with GMG compared to other types of MG, especially in the domains of physical function, pain, vitality, and social function. The measurement of this outcome in patients with MG, in the evaluation and choice of therapeutic options, is of great importance, especially in patients with refractory GMG where these options are scarce. In recent years, eculizumab has become a novel therapy for patients with refractory GMG because it generates improvements in the perception of patients' quality of life compared to placebo.

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Original Article

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Clinical investigation of pituitary incidentalomas: A two-center study

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Summary **Recent advances in imaging technology resulted in an increase in pituitary incidentalomas (PIs) detection. PIs were reported to be present in 1.6% persons with magnetic resonance imaging of the brain. Whereas, there were few studies about PIs with detailed investigation. We aimed to investigate the clinical and endocrinological characteristics of PIs. We evaluated 65 patients diagnosed with PIs who underwent detailed clinical and endocrinological evaluations. Of the 65 patients, 33 (50.8%) had non-functional pituitary adenomas (NFPAs), 11 (16.9%) had Rathke's cleft cysts (RCCs), 7 (10.8%) had functional pituitary adenomas (FPAs), 6 (9.2%) had benign extra-pituitary tumors (BEPTs), and 8 (12.3%) had malignant tumors (MTs). Compared with patients with NFPAs, those with MTs were significantly younger and had a significantly lower body mass index, lower prevalence of hypertension, and lower prevalence of dyslipidemia. Patients with MTs had significantly higher prevalence of central diabetes insipidus than those with NFPAs. In addition, patients with NFPAs had significantly higher prevalence of pituitary apoplexy than those with FPAs, BEPTs, and MTs. In conclusion, our study demonstrated clinical and endocrinological characteristics of PIs. Highly detailed clinical and endocrinological investigations should be performed for PIs. In addition, MTs should be considered in the differential diagnosis for young and lean patients with central diabetes insipidus.**

Keywords: Pituitary incidentaloma, hormonal deficiency, pituitary apoplexy

1. Introduction

Pituitary incidentalomas (PIs) are tumors of the pituitary gland discovered unexpectedly upon imaging that are not due to symptoms related specifically to the lesion (*e.g.*, visual loss) or a clinical manifestation of hormonal disorders (*1*). Vernooij and colleagues reported analysis of patient with magnetic resonance imagings of the brain showed PIs were present in 1.6% persons (*2*). The previous autopsy investigations also showed the prevalence of PIs were 2.7-24.0 % (*3-5*). Whereas, adrenal incidentalomas, the same endocrine incidental tumors as PIs, have been investigated in several studies (*6-9*), even though their prevalence investigated by autopsy were reported to be lower than that of PIs (1.1-5.0 %) (*10-12*). However, few studies have focused on PIs (*2,13,14*).

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The Endocrine Society (Washington, DC, USA) produces guidelines for the clinical management for several endocrine diseases including PIs authored by Freda and colleagues (*1*). They recommended that patients with PIs undergo thorough history-taking and a complete physical examination, including evidence of asymptomatic hormonal disorders.

Here, we demonstrate the findings of an analysis of the clinical and endocrinological characteristics of PIs with highly detailed evaluations in two university hospitals.

2. Materials and Methods

2.1. *Ethical approval of the study protocol*

The study protocol was approved by the Ethics Review Committees of Fukuoka University (Fukuoka, Japan) and Yamagata University (Yamagata, Japan). Written informed consent was obtained from the patients for participation in the study. The study was carried out according to the principles of the Helsinki Declaration.

2.2. *Subjects*

The study cohort comprised 65 individuals found to have PIs at Yamagata University Hospital or Fukuoka University Chikushi Hospital (or individuals with PIs detected at other hospitals and who were then transferred to these two institutions) from April 2015 to March 2018.

PIs were diagnosed with the criteria: detected incidentally upon imaging examinations undertaken for monitoring of non-endocrine diseases; general health status; various symptoms not considered to have a relationship with the lesion, defined by The Endocrine Society produces guidelines (*1*). All study participants underwent endocrinology evaluations and laboratory tests.

2.3. *Methods and disease definitions*

We collected data on age, sex, tumor diameter, medical history, physical examination, laboratory tests, and endocrinological evaluations for all patients.

Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or use of antihypertensive drugs. Diabetes mellitus was defined as any combination of: fasting plasma glucose ≥ 126 mg/dL; random plasma glucose ≥ 200 mg/dL; glycated hemoglobin ≥ 6.5%; or use of antidiabetic agents. Dyslipidemia was defined as any combination of total cholesterol ≥ 220 mg/dL; low-density lipoprotein-cholesterol ≥ 140 mg/dL; high-density lipoprotein-cholesterol < 40 mg/dL; triglyceride ≥ 150 mg/dL; or use of lipid-lowering drugs.

2.4. *Functional pituitary adenomas*

The diagnosis of functional pituitary adenomas (FPAs) was performed in accordance with previous reports (*15-19*). In detail, a growth hormone (GH)-producing adenoma was diagnosed based on a combination of increased GH levels, unsuppressed GH levels after a 75-g oral glucose tolerance test, increased insulin-like growth factor (IGF)-1 levels, and pathology studies in patients who underwent surgical treatment. A prolactin (PRL)-producing adenoma was diagnosed by a combination of increased prolactin levels, unchanging prolactin levels after a thyrotropin-releasing hormone test and GH-releasing peptide-2 test, or pathology studies in patients who underwent surgical treatment. An adrenocorticotropic hormone (ACTH)-producing adenoma was diagnosed based on a combination of increased levels of ACTH and cortisol in the morning, loss of diurnal ACTH and cortisol rhythm, high urinary levels of free cortisol, autonomic secretion of ACTH and cortisol confirmed by a 0.5 mg/8 mg dexamethasone suppression test, increased ACTH levels after a 1-desamino-8-D-arginine vasopressin test, and pathology studies in patients who underwent surgical treatment. A luteinizing hormone (LH)- or follicle stimulating hormone (FSH)-producing adenoma was diagnosed by a combination of increased LH or FSH levels, existing secondary hypergonadism, and pathology studies in patients who underwent surgical treatment. A thyroid-stimulating hormone (TSH)-producing adenoma was diagnosed by a combination of increased or normal TSH levels with mild hyperthyroidism, and pathology studies in patients who underwent surgical treatment.

2.5. *Hormonal deficiency*

The diagnosis of hormonal deficiency was performed in accordance with previous reports (*20-23*). In detail, GH deficiency was diagnosed by no or inadequate changes in GH levels after a GH-releasing peptide-2 test/insulin tolerance test/arginine test. ACTH deficiency was diagnosed by a combination of reduced ACTH levels and cortisol levels in the morning, and no or inadequate changes in ACTH levels or cortisol levels after a corticotropin-releasing hormone test. Deficiency in LH or FSH was diagnosed by a combination of reduced LH levels or FSH levels, no or inadequate changes in LH levels or FSH levels after a LH-releasing hormone test, and existing secondary hypogonadism. TSH deficiency was diagnosed by a combination of reduced TSH levels, no or inadequate changes in TSH levels after a thyrotropin-releasing hormone test, and existing secondary hypothyroidism. PRL deficiency was diagnosed by a combination of reduced PRL levels, no or inadequate changes in PRL levels after a thyrotropin-releasing hormone test. Central diabetes insipidus was diagnosed by a combination of increased urinary volume, low urinary osmolality and low ADH levels compared with serum osmolality, no or inadequate changes in ADH

levels after a 5% NaCl loading test/water restriction test, and increased ADH levels and decreased urinary volume after a 1-desamino-8-D-arginine vasopressin test.

2.6. *Pituitary apoplexy*

Pituitary apoplexy was diagnosed by the symptoms of sudden and severe headache and existing abrupt hemorrhage and/or infarction of the pituitary gland (*24*).

2.7. *Statistical analyses*

Data are the mean ± standard deviation. Statistical analyses were performed using STATA® SE version 13.1 (Stata Corporation, College Station, TX, USA). Significance of differences between mean values was estimated by the Student's *t*-test. $P < 0.05$ was considered significant.

3. Results

Table 1 shows the clinical characteristics of the 65 patients who formed the study cohort. Their mean age was 55.6 ± 16.5 years; 34 patients (52.3%) were men and 31 (47.7%) were women. All patients underwent a detailed physical examination. 20 PIs (30.8%) were detected upon monitoring for headache, 10 (15.4%) at general check-up, 9 (13.8%) at monitoring for other diseases, and 8 (12.3%) at check-up for vertigo. In addition, 7 PIs (10.8%) were detected upon monitoring after trauma, 5 (7.7%) at check-up for hand paraesthesia, 3 (4.6%) upon monitoring for drowsiness, 1 (1.5%) at check-up for nausea, and 1 (1.5%) upon cancer staging (Table 2). The mean diameter of PIs was 22.2 ± 12.0 mm. Among the study cohort, 12 (18.5%) had diabetes mellitus, 32 (49.2%) had hypertension, and 45 (69.2%) had dyslipidemia.

The hormonal deficiency of these patients are shown in Table 3. 10 patients (15.4%) had a deficiency of ACTH, 16 (24.6%) had a deficiency of TSH, 21 (32.3%) had a deficiency of LH, 22 (33.8%) had a deficiency of FSH, 24 (36.9%) had a deficiency of GH, 2 (3.1%) had a deficiency of PRL, and 3 (4.6%) had central diabetes insipidus.

In regard to diagnosis, 33 patients (50.8%) had non-functional pituitary adenomas (NFPAs), 11 (16.9%) had Rathke's cleft cysts (RCCs), 7 (10.8%) had FPAs, 6 (9.2%) had benign extra-pituitary tumors (BEPTs), and 8 (12.3%) had malignant tumors (MTs). This information (along with the sub-classifications of FPAs, BEPTs, and MTs) is shown in Table 4.

We also investigated the clinical and endocrinological characteristics of respective tumor groups (Table 5). Patients with MTs were significantly younger (37.1 ± 16.1 *vs*. 60.1 ± 13.7 years, $p < 0.001$), had a significantly lower body mass index (19.5 ± 2.7 *vs*. 22.8 ± 2.9, $p = 0.005$), as well as lower prevalence of hypertension (12.5% *vs*. 51.5%, $p = 0.047$) and dyslipidemia (37.5% *vs*. 72.7%, $p = 0.044$) than patients with NFPAs. Significantly more patients with MTs had central diabetes insipidus than patients with NFPAs (25.0% *vs*. 0.00%, $p = 0.011$). Patients with NFPAs had significantly greater prevalence of pituitary apoplexy (12.1%) than that of patients with FPAs (0%), BEPTs (0%), or MTs (0%) ($p = 0.044$ for all).

Table 1. Clinical characteristics of patients with pituitary incidentalomas

Items	*n* = 65
Age (years)	55.6 ± 16.5
Sex (M/F)	34/31
Tumor diameter (mm)	22.2 ± 12.0
Body mass index (kg/m^2)	23.0 ± 3.5
Estimated glomerular filtration rate (mL/min/1.73 m^2)	80.1 ± 18.3
Glycated hemoglobin (%)	6.01 ± 1.23
Fasting plasma glucose (mg/dL)	101.0 ± 25.2
Aspartate transaminase (U/L)	26.6 ± 12.5
Alanine aminotransferase (U/L)	28.3 ± 22.4
γ-glutamyl transferase (U/L)	32.3 ± 25.9
Low-density lipoprotein-cholesterol (mg/dL)	123.0 ± 34.4
High-density lipoprotein-cholesterol (mg/dL)	52.7 ± 15.6
Triglyceride (mg/dL)	166.2 ± 136.7
Systolic blood pressure (mmHg)	121.4 ± 20.0
Diastolic blood pressure (mmHg)	74.8 ± 14.0
Morbidity due to hypertension (%)	49.2
Morbidity due to diabetes mellitus (%)	18.5
Morbidity due to dyslipidemia (%)	69.2

Data are the mean ± standard deviation.

Table 2. Reasons for imaging examinations leading to detection of pituitary incidentalomas and the prevalence of pituitary apoplexy

Items	Number (%)
Headache	20 (30.8%)
General check-up	10 (15.4%)
Other diseases	9 (13.8%)
Vertigo	8 (12.3%)
After trauma	7 (10.8%)
Hand paraesthesia	5 (7.7%)
Drowsiness	3 (4.6%)
Nausea	1 (1.5%)
Cancer staging	1 (1.5%)
Pituitary apoplexy	5 (7.7%)

Table 3. Morbidity due to hormonal deficiency of patients with pituitary incidentalomas

Items	Number (%)
Deficiency of ACTH	10 (15.4%)
Deficiency of TSH	16 (24.6%)
Deficiency of LH	21 (32.3%)
Deficiency of FSH	22 (33.8%)
Deficiency of PRL	2 (3.1%)
Deficiency of GH	24 (36.9%)
Central diabetes insipidus	3 (4.6%)

ACTH, adrenocorticotropic hormone; FSH, follicle stimulating hormone; GH, growth hormone; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid stimulating hormone.

4. Discussion

Recent advances in imaging technology (especially those in magnetic resonance imaging) have resulted in an increase in PI detection, but few studies have focused on PIs (*12-14*). Freda and colleagues, in their guideline on the definition and recommendations for clinical management of PIs, emphasized the importance of thorough evaluation of PIs (*1*). In addition, tumors of the pituitary gland could lead to pituitary apoplexy. Thus, by undertaking highly detailed evaluations, we investigated the clinical and endocrinological characteristics of PIs, including the prevalence of pituitary apoplexy as well as the prevalence of hormonal disorders of respective tumor groups.

As the result of our investigations, the number of NFPA was about half of all incidentalomas. Previously, it was reported that 77-81% patients with PIs were diagnosed as NFPAs (*13,14*). The reason of disparity might be that all patients underwent highly detailed investigations in our study. Another reason might be that almost all cases of our study (except for 8 patients with RCCs and 1 patient with Prolactinoma) underwent a surgical procedure (including biopsy of the pituitary gland) and pathological investigations.

With respect to characteristics of respective tumor group, compared with patients with NFPAs, patients with MTs were significantly younger and had a significantly lower body mass index, lower prevalence of hypertension, and lower prevalence of dyslipidemia. In addition, compared with patients with NFPAs, significantly more individuals with MTs had central diabetes insipidus. On the other hand, with respect to tumor diameter, there was no significant difference between patients with NFPAs or those with MTs, RCCs, FPAs, or BEPTs. With regard to the function of the anterior pituitary gland, there were also no significant difference between patients with NFPAs and patients with RCCs, FPAs, BEPTs, or MTs. Considering MTs are commonly thought to increase in size rapidly, the reasons why there were no significant differences in both tumor diameter and the prevalence of anterior pituitary dysfunction between patients with NFPAs and those with MTs are not entirely clear, but may reflect the difficulty of making the diagnosis without undertaking a detailed physical examination or pathology studies. Conversely, the prevalence of pituitary apoplexy in patients with NFPAs was significantly higher than that of patients with FPAs, BEPTs, or MTs. McCabe and colleagues reported an increase of mRNA expression

Table 4. Diagnosis of patients with pituitary incidentalomas

Items	Number (%)
Non-functional pituitary adenoma	33 (50.8%)
Rathke's cleft cyst	11 (16.9%)
Functional pituitary adenoma	7 (10.8%)
GH-producing adenoma	2 (3.1%)
PRL-producing adenoma	4 (6.2%)
FSH-producing adenoma	1 (1.5%)
Benign extra-pituitary tumor	6 (9.2%)
Meningioma	3 (4.6%)
Craniopharyngioma	3 (4.6%)
Malignant tumor	8 (12.3%)
Astrocytoma	1 (1.5%)
Glioma	1 (1.5%)
Glioblastoma	1 (1.5%)
Germinoma (unclassifiable)	2 (3.1%)
Malignant lymphoma	2 (3.1%)
Metastasis of squamous cell carcinoma of the lung	1 (1.5%)

Table 5. Clinical characteristics of patients with pituitary incidentalomas

Items	Non-functional pituitary adenoma	Rathke's cleft cyst	Functional pituitary adenoma	Benign extra-pituitary tumor	Malignant tumor
Number	33	11	7	6	8
Age (years)	60.1 ± 13.7*	52.4 ± 15.7	58.7 ± 16.7	54.3 ± 19.4	37.1 ± 16.1*
Male (%)	48.5	54.5	71.4	33.3	62.5
Tumor diameter (mm)	21.4 ± 4.9	16.4 ± 8.9	32.0 ± 28.9	26.5 ± 10.0	23.5 ± 11.1
Body mass index (kg/m^2)	22.8 ± 2.9$^{\$}$	24.4 ± 4.2	24.4 ± 2.0	24.4 ± 3.6	19.5 ± 2.7$^{\$}$
Hypertension (%)	51.5$^{\#}$	45.4	71.4	66.7	12.5$^{\#}$
Diabetes mellitus (%)	18.2	18.2	28.6	16.7	12.5
Dyslipidemia (%)	72.7$^{\&}$	81.8	85.7	50.0	37.5$^{\&}$
Deficiency of ACTH (%)	18.2	18.2	14.3	16.7	50.0
Deficiency of TSH (%)	24.2	18.2	14.3	16.7	50.0
Deficiency of LH (%)	33.3	27.3	28.6	16.7	50.0
Deficiency of FSH (%)	33.3	27.3	28.6	33.3	50.0
Deficiency of PRL (%)	3.0	9.0	0	0	0
Deficiency of GH (%)	36.4	36.4	28.6	16.7	62.5
Central diabetes insipidus (%)	0¶	0	0	16.7	25.0¶
Pituitary apoplexy (%)	12.1$^{\Sigma\ddagger\int}$	9.0	0$^{\Sigma}$	0‡	0$^{\int}$

Patients with malignant tumors (MTs) were significantly younger and had significantly lower body mass index, less hypertension, and less dyslipidemia than those with non-functional pituitary adenomas (NFPAs) ($^{*\$\#\&}$). Patients with MTs had significantly more central diabetes insipidus than those with NFPAs (¶). Patients with NFPAs had significantly more pituitary apoplexy than those with functional pituitary adenomas (FPAs), benign extra-pituitary tumors (BEPTs), and MTs ($^{\Sigma\ddagger\int}$). The significance of differences between means was estimated by the Student's *t*-test.

of vascular endothelial growth factor in patients with NFPAs compared with that in patients with other types of pituitary-gland tumors and healthy controls (*25*), which could support our results regarding the prevalence of pituitary apoplexy. In addition, we found no significant difference in the prevalence of pituitary apoplexy between patients with NFPAs or RCCs. Some scholars have reported that pituitary apoplexy can occur in patients with RCCs (*26-28*). Indeed, Hama and co-workers reported inflammation in the epithelium of RCCs (*29*), which could support the prevalence of pituitary apoplexy observed in patients with RCCs in the present study. This finding has not been reported adequately.

This study had one limitation. Our study cohort was small because PIs are relatively rare which caused difficulty in our analysis. Future studies with much larger study cohorts are needed to confirm our results.

In conclusion, our study provided insights into the clinical and endocrinological characteristics of PIs. PIs should undergo highly detailed clinical and endocrinological investigations. In addition, our study also indicated that young and lean patients with central diabetes insipidus might warrant particularly careful investigation considering MTs.

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Coronary artery disease and mesenteric artery stenosis - Two sides of the same coin? - Long term prospective analysis

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Summary **Coronary artery disease (CAD) patients might have concomitant mesenteric artery stenosis (MAS). Identification of risk factors predicting mesenteric artery involvement might guide screening high risk individuals. A dilemma of intervention in radiologically severe MAS exists. This prospective study included CAD patients undergoing a coronary angiogram. A concomitant mesenteric angiogram was performed to diagnose MAS. Clinically relevant MAS (CR-MAS) was defined as *i*) presence of classical mesenteric angina with any degree of MAS or *ii*) severe stenosis (> 70%) involving two or more vessels. Risk factors for CR-MAS were studied and followed up prospectively. One hundred and three patients were included in the study. Left anterior descending artery was the most common involved coronary artery and was affected in 73% (n = 76). Mesenteric angiogram revealed 42.7% (n = 44) to have MAS. CR-MAS was present in 21 patients (20.4%). Involvement of celiac axis, superior mesenteric artery and inferior mesenteric artery was 22, 39 and 15 respectively. Multivariate analysis showed mesenteric angina ($p < 0.01$), diabetes mellitus ($p < 0.01$) and peripheral artery disease ($p < 0.01$) to be independent predictors of CR-MAS. At a median follow-up of 36 months (range 29-48 months), there was no acute mesenteric ischemia. In patients with CR-MAS, 16 (76.2%) had symptomatic improvement and 5 (23.8%) had stable symptoms. Three patients underwent angioplasty of superior mesenteric artery for persistent symptoms. Chronic CAD patients had a high prevalence of MAS. Mesenteric angina, diabetes mellitus and peripheral artery disease are independent predictors of CR-MAS. Intervention for MAS should be dictated by symptoms and not radiological severity. Lifestyle modification and medication for atherosclerotic ischemic heart disease probably prevents acute mesenteric ischemia in CAD patients.**

Keywords: Mesenteric ischemia, coronary artery disease, angiography, chronic mesenteric ischemia, mesenteric artery stenosis

1. Introduction

Stenosis or occlusion of the mesenteric arterial circulation is the predominant causative factor of chronic mesenteric ischemia (CMI) (*1*). While acute mesenteric ischemia has a dramatic presentation, CMI has an insidious course (*2*). The natural course of CMI is gradual with non-specific abdominal pain and unremarkable physical findings. Hence diagnosis of CMI is often delayed by months or even years (*3*).

Atherosclerosis is the leading cause of CMI (*3,4*). The process of atherosclerosis is a generalized process affecting all vascular beds (such as renal artery, coronary artery, peripheral artery *etc*.) (*5*). However, variability of sequence and severity of affectation at various sites exists. Numerous studies have demonstrated asymptomatic arterial narrowing in vascular territory during evaluation of symptomatic lesions involving another region (*6,7*).

Limited prospective data exists regarding mesenteric arterial stenosis (MAS) (*5,8,9*). Asymptomatic MAS

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has been studied during evaluation of arterial occlusion at distant sites. While Thomas *et al*. (*8*) showed that 6% of patients with asymptomatic mesenteric artery stenosis (MAS) developed mesenteric ischemia during a mean follow up period of 2.6 years, other studies have reported no incidence of acute mesenteric ischemia despite radiological MAS (*5,9*). Thus, management of asymptomatic yet significant MAS is controversial.

On the other hand, MAS with mesenteric anginal symptoms or narrowing of two or more of the three mesenteric arteries are considered for revascularization (*4,10*). The rationale is based on the assumption that these patients are more likely to have acute mesenteric ischemia.

The incidence of MAS in ischemic heart disease has not been studied before. Incidence is likely to be high given that 33% of patients undergoing surgery for CMI have coronary artery disease (CAD) (*11*). We believe that CAD is likely to be associated with MAS given the universal nature of the atherosclerotic process. The non-specific abdominal symptoms of CMI make it difficult for the physician to differentiate from other more common causes like drug induced gastritis. Lack of reliable non-invasive investigation to accurately identify CMI compounds the difficulty. Possible identification of risk factors for MAS in CAD patients might help in risk stratification and guide appropriate management. We also wanted to study the course of asymptomatic MAS and clinically relevant MAS (CR-MAS) on medical management.

Thus, we decided to prospectively study the incidence and predictive risk factors for CR-MAS in CAD patients. The natural course of patients with asymptomatic mesenteric involvement and CR-MAS was also studied.

2. Patients and Methods

A prospective study of patients with suspected chronic stable angina admitted for coronary angiogram in the Department of Cardiology from January 2013 to June 2014 was done. Institutional ethics committee approval was obtained. After obtaining informed consent, patients were enrolled for the study.

2.1. *Criteria for suspecting chronic stable angina*

Canadian Cardiovascular Society (CCS) class III angina on maximal medical treatment for minimum period of 3 months (*12*).

2.2. *Exclusion criteria*

The exclusion criteria were *i*) age < 18 years; *ii*) patients with normal coronaries on angiogram; *iii*) Mehran risk score (*13*) > 5 for contrast induced nephropathy.

A detailed history was taken, with emphasis on the risk factors for atherosclerosis and abdominal symptoms was done followed by complete clinical examination. Allen test was performed to confirm the patency of the palmar arches.

Mesenteric angina was defined as abdominal pain occurring after 15-60 minutes following ingestion of a meal and may last from 1-4 hours (*3*).

Patients were subjected to basic investigation including renal parameters. Echocardiogram was performed as a part of cardiac evaluation. Risk stratification for contrast induced nephropathy was done based on the Mehran Risk Score (*13*).

2.3. *Procedure for angiogram*

Pre-procedure hydration was maintained by administration of intravenous fluids. Right radial artery was the preferred site of catheterization. Catheterization was done by Seldinger technique and coronary study was performed in the usual way. If coronary arteries were involved, then mesenteric angiogram was done by selective cannulation of the three mesenteric arteries performed in two views - antero-posterior and lateral views. If selective cannulation could not be obtained, the non-selective study was performed by pressure injection using a pigtail catheter. After the procedure, a compression dressing was applied. During the procedure, patient ECG, pressure and oxygen saturation was continuously monitored. Patient was monitored for anaphylactic reaction. Patients were discharged after 24 hours. If therapeutic intervention was planned, it was performed after 3 days.

2.4. *Grading of severity of stenosis*

i) Normal – 0-30% narrowing of mesenteric vessel; *ii*) Mild stenosis – any degree of narrowing without fulfilling the criteria for severe stenosis; *iii*) Severe stenosis – any one of the below: ≥ 70% narrowing; any degree of narrowing with post stenotic dilatation or presence of collaterals.

2.5. *Criteria for clinically relevant MAS*

Clinically relevant MAS was defined as any degree of narrowing of mesenteric vessel(s) with at least one of the following conditions (*10*): *i*) presence of classical mesenteric angina with any degree of MAS; *ii*) severe stenosis involving two or more vessels.

2.6. *Management of specific conditions*

i) Coronary artery disease: As per our institute protocol, only diagnostic angiogram is performed in chronic stable angina at first sitting. Therapeutic angioplasty and bypass surgery were advised as indicated.

ii) Mesenteric artery disease: Patients with CR-MAS

were assessed and offered mesenteric revascularization after management of CAD. All patients, including patients without MAS initially, were questioned about history of mesenteric angina and clinically examined. Re-evaluation of mesenteric arteries (ultrasound doppler or computed tomography) during follow up was done in cases of new-onset symptoms.

Medical management (lifestyle medication, control of co-morbid disease, antiplatelet, statins) was instituted in patients with any degree of MAS including those with CR-MAS who refused intervention (*10*). Patients with persistent abdominal anginal symptoms despite medical management for 6 months were advised to have endovascular or surgical intervention.

iii) Peripheral vascular disease: Symptomatic individuals with critical limb ischemia were advised to have revascularization (endovascular or surgical bypass). Amputation at appropriate level was advised for non-salvageable limb or non-reconstructible lesions with critical limb ischemia.

2.7. Follow up

Patients were followed up every 6 months. Thorough clinical examination including history of abdominal symptoms was done. ECG and echocardiogram were done every 6 and 12 months respectively. Intervention was performed as per the indication mentioned above. Medical co-morbidities were assessed and treated accordingly. All patients were advised about lifestyle modification and diet as per the AHA Diet and Lifestyle modification Recommendation: Revision 2006 (*14*). These included: Maintaining healthy body weight; Diet rich in vegetables, fruit, whole grain, high-fiber foods; Restricting salt intake (< 6 grams/day); Avoiding usage and exposure to tobacco in any form; Minimizing beverages and food with added sugars; Limiting intake of saturated fat.

2.8. *Statistical analysis*

Data was entered and analysis was performed using the Statistical Package for the Social Sciences (SPSS for Windows, Version 22.0; SPSS Inc, Chicago, Ill). χ^2 or Fisher's exact test was used for univariate comparison; continuous variables were analyzed by using the unpaired Student's t test. Multivariate logistic regression analysis of factors significant on univariate analysis was performed to identify predictive risk factors for CR-MAS. All p values less than 0.05 were considered statistically significant.

3. Results

From January 2013 to June 2014, a total of 110 patients underwent coronary angiogram for suspected ischemic heart disease in the Department of Cardiology. Seven patients had a normal coronary study. Thus, one hundred three patients were included for further analysis.

3.1. *Demographic features*

The mean age of the population under study was 56.31 years. Sixty-six patients were male (64.1%). Comorbidities were present in 82 patients (79.6%). The incidence of diabetes, hypertension, previously diagnosed ischemic heart disease, peripheral arterial disease and dyslipidemia was 39.8%, 22.3%, 22.3%, 20.4% and 31.1% respectively. Twenty-six (25.2%) patients had mesenteric angina. The classical triad of mesenteric angina, sitophobia and weight loss was present in only 2 patients. Table 1 describes the demographic features of the study population.

3.2. *Coronary artery disease*

With respect to the coronary arteries the prevalence of single vessel, double vessel and triple disease was 36% (n = 37), 43% (n = 44) and 21% (n = 22), respectively. Left anterior descending artery was the most common involved coronary artery and was affected in 73% (n = 76) of the study population. Isolated left anterior descending artery affectation was present in 19 patients with isolated left circumflex and right coronary artery involvement in 14 and 5 patients respectively. Table 2 depicts the distribution and severity of coronary artery involvement.

3.3. *Mesenteric vascular disease*

Mesenteric angiogram revealed 42.7% (n = 44) to have MAS. Twenty-one (20.4%) patients had CR-MAS (19 – mesenteric angina with any degree of MAS; 2 – severe stenosis of two or more mesenteric arteries). The prevalence of single vessel mesenteric disease and multivessel disease among the study population was 19.4% (n = 20) and 23.3% (n = 24), respectively. All lesions were ostio-proximal in location (Figure 1). Table 2 depicts the distribution and severity of mesenteric artery involvement. One patient had a concomitant abdominal aortic aneurysm (Figure 2).

Stenosis of superior mesenteric artery was present in 89% (n = 39) of those with mesenteric involvement and 38% of the study population. Of these 39, isolated superior mesenteric artery stenosis was present in 16 (41%) patients. The next common artery to be involved was celiac axis followed by inferior mesenteric artery in 22 (21.4%) and 15 (14.6%) patients respectively. Severe triple vessel stenosis was present in 8 (7.7%) patients.

3.4. *Risk factors for mesenteric artery involvement*

The mean age of patients with and without mesenteric vessel involvement was 60.63 years and 53.08 years

Table 1. Demographic Characteristics of study population

Items	Study population (n = 103)	Mesenteric vascular disease (n = 44)	No Mesenteric vascular disease (n = 59)	p Value
Age	56.31 ± 10.4	60.6 ± 10.9	53.0 ± 8.7	< 0.01
Gender (M/F)	66/37	24/20	42/17	0.82
Mesenteric angina (Y/N)	26/77	19/25	7/52	0.01
Diabetes Mellitus (Y/N)	41/62	29/15	12/47	< 0.01
Hypertension (Y/N)	23/80	10/34	13/46	0.93
Known Ischemic heart Disease (Y/N)	23/80	13/31	10/49	0.13
Peripheral Arterial Disease (Y/N)	21/82	19/25	2/57	< 0.01
Dyslipidemia (Y/N)	32/71	15/29	17/42	0.56
Family History (Y/N)	16/87	6/38	10/49	0.65
Smoking (Y/N)	40/63	20/24	20/39	0.23
Alcohol (Y/N)	33/70	14/30	19/40	0.96
BMI (Underweight/Normal/Overweight)	8/46/49	6/29/9	2/17/40	< 0.01
Waist/Hip ratio	8/56/39	7/30/7	1/26/32	< 0.01

BMI, body mass index.

Table 2. Severity of involvement of Coronary and Mesenteric artery stenosis

Items	RCA involvement	LAD involvement	LCX involvement	Celiac Axis involvement	SMA involvement	IMA involvement
No involvement	51	27	40	81	64	88
Mild disease	22	32	28	16	18	13
Severe disease	30	44	35	6	21	2

IMA, inferior mesenteric artery; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; SMA, superior mesenteric artery.

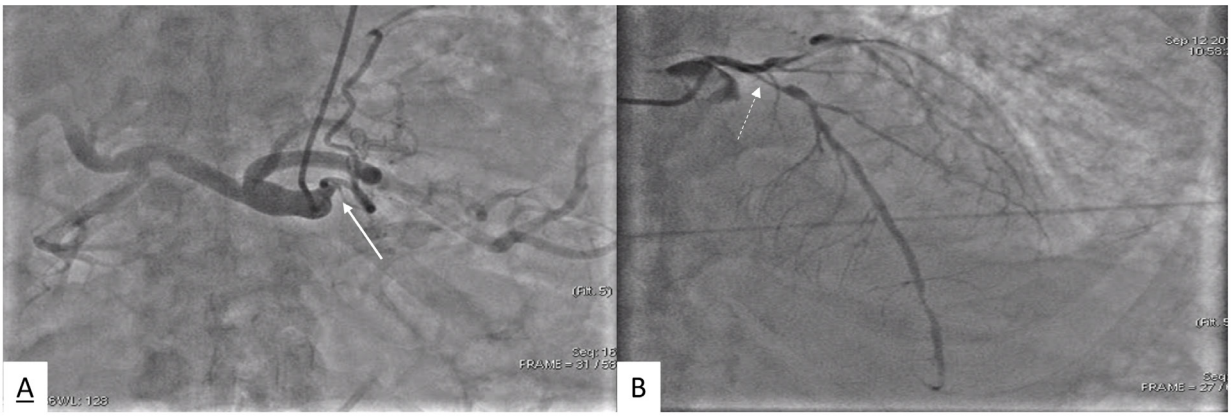

Figure 1. Conventional coronary and mesenteric angiogram. (**A**) Critical Celiac axis stenosis with post-stenotic dilation; (**B**) Left anterior descending artery ostio-proximal critical lesion with distal LAD involvement in the same patient.

respectively. Seven out of 26 patients with mesenteric angina had no evidence of mesenteric vascular disease. Nineteen and twenty-five patients had symptomatic and asymptomatic MAS respectively. Among those with MAS, 70% were diabetic and 43% had prior history of peripheral arterial disease. Comparison of clinical and biochemical parameters between group 1 and group 2 is depicted in Table 1.

Presence of MAS was not associated with the number of coronary arteries involved. However, involvement of left anterior descending artery was significantly associated with presence of MAS (p = 0.04).

Univariate analysis revealed age greater than 65 years, abdominal symptoms, BMI, serum albumin, ESR, diabetes mellitus, peripheral artery disease, prior ischemic heart disease, and left anterior descending artery involvement to be significant predictor of CR-MAS. Multivariate analysis of significant factors showed presence of abdominal symptoms (p < 0.01), diabetes mellitus (p < 0.01) and peripheral artery

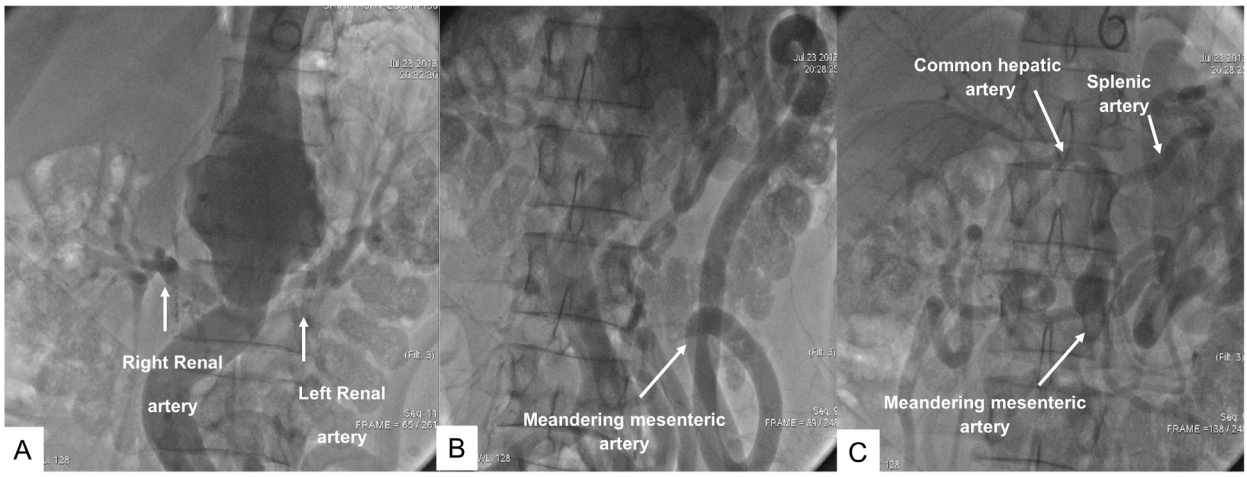

Figure 2. Photo panel of conventional angiogram of a patient with concomitant abdominal aortic aneurysm (AAA). (**A**) AAA with Complete occlusion of Celiac axis and superior mesenteric artery origin. Severe bilateral renal artery stenosis also noted - Lateral View; (**B**) Dilated Collateral (Meandering mesenteric artery) arising from Inferior Mesenteric Artery; (**C**) Retrograde filling of Celiac Axis and Superior Mesenteric Artery.

Table 3. Multivariate Regression Analysis for risk factors predicting CR-MAS in CAD patients

Items	Regression Coefficient	Relative Risk	95% CI	*p* value
Age > 65 years	1.106	3.024	0.51 - 17.87	0.222
Mesenteric Angina	2.365	10.645	1.79 - 63.27	0.009
Diabetes Mellitus	2.174	8.796	1.98 - 38.89	0.004
Peripheral Vascular Disease	3.105	22.307	2.93 - 169.53	0.003
BMI < 18.5	1.678	5.357	0.35 - 80.72	0.225
W/H ratio	2.922	18.583	0.69 - 497.74	0.082
Erythrocyte Sedimentation Rate	0.090	0.914	0.19 - 4.20	0.908
Serum Albumin	0.868	0.420	0.06 - 2.58	0.349
Left anterior descending artery involvement	1.214	3.368	0.67 - 16.84	0.140

BMI, body mass index; W/H, waist/hip.

disease ($p < 0.01$) to be independent predictors of CR-MAS (Table 3).

3.5. *Follow up*

Among the 103 patients, 75 individuals required percutaneous intervention and 12 others required coronary artery bypass surgery for coronary revascularization. All twenty-one patients who were advised mesenteric revascularization at index presentation refused intervention for mesenteric disease.

At the end of 36 months of median follow up (range 29-48 months), there were 12 deaths. All were related to acute coronary syndrome. Among the remaining 91 patients, 38 had mesenteric vessel involvement during the index angiogram.

Of the 21 patients with CR-MAS, 16 had improvement in abdominal symptoms, and 5 had stable symptoms. Patients with improved abdominal pain also had weight gain. Three patients out of the five with persistent symptoms underwent angioplasty for an isolated SMA lesion. There was no procedure related complication. All three had good symptomatic relief in the form of pain relief and weight gain at median follow up of 13 months (6-15 months).

One patient with initially asymptomatic MAS developed symptoms of mesenteric angina at 2 years of follow up. He refused evaluation of the mesenteric anginal symptoms and was managed conservatively.

Major vascular events during follow-up in patients with MAS are summarized in Figure 3.

4. Discussion

Significant proportion of CAD patients had MAS (42.7%). The high prevalence of mesenteric disease, in comparison with previous studies, might be due to the variability in defining and diagnosing mesenteric artery disease (*15*). It is possible that angiogram, the gold standard in vascular imaging, could have detected mild stenosis missed by other imaging modalities. In order to further ascertain the individuals who might require active follow-up or intervention, we identified these clinically relevant lesions based on criteria laid out for the need

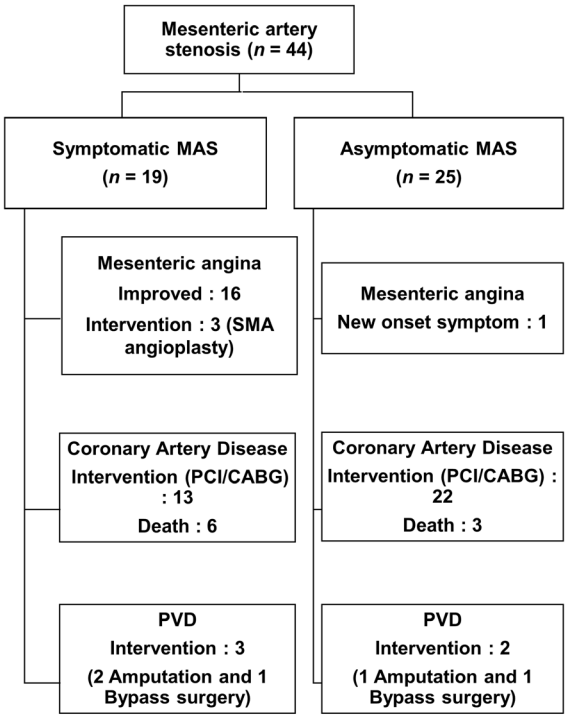

Figure 3. Flowchart depicting the major vascular events in the follow-up period in MAS patients.

for revascularization (*10*). The incidence of clinically relevant MAS was 20.4%. Known risk factors of the atherosclerotic process like advanced age and diabetes mellitus predicted CR-MAS (*16*). Increased risk of MAS in CAD patients with peripheral arterial disease highlights global involvement of the atherosclerotic process in this subgroup of individuals.

Among CR-MAS, 16 patients had symptomatic improvement and only 5 patients had persistent symptoms. Multiple factors might be responsible. Attention to coronary artery involvement is usually given priority. By the time stabilization is achieved, patients might get adapted and also alter their eating habits (*17*). The progression of the atherosclerotic process is also likely to be reduced given the considerable overlap of medical management of coronary artery disease and mesenteric artery disease.

The course of CR-MAS on medical management also exemplifies the controversies involved in their management. Some authors recommend intervention if patient is symptomatic or having significant involvement of two or more arteries (*10*). We did not have acute mesenteric ischemia in 21 patients and only 3 patients (14.2%) underwent intervention. Consideration has to be given to the degree of nutritional compromise, and number and severity of comorbidities these patients already suffer from. Given the morbidity of surgery and good proportion of CR-MAS that had symptomatic improvement, we believe that a trial of medical management has to be given before intervention is contemplated. Thus, symptoms of MAS should dictate intervention and not radiological findings.

Advances in endovascular techniques, expertise and stents have seen the gap between a morbid open revascularization and relatively safe endovascular revascularization being narrowed (*18*). Though the long term outcome of severe multivessel disease is better with surgical revascularization, endovascular intervention has an important role in single vessel disease (*19*). The superior mesenteric artery is considered as the primary culprit for the symptoms (*20*). It was also the most involved artery in our study. The possibility of addressing the ostio-proximal superior mesenteric artery lesions alone may be adequate in treating symptoms, like in three of our patients, thus making an endovascular approach more attractive in this subgroup (*21*).

Though new onset or worsening mesenteric angina was seen, acute mesenteric ischemia was not seen at 3 years of follow up. Contradictory results in the incidence of acute mesenteric ischemia during follow up of MAS have been reported (*5,8,9*). We believe that strict adherence to a healthy lifestyle with antiplatelet agents and statins reduce the progression of atherosclerosis allowing time for rich collateral formation. Goals of lifestyle modification were consuming a healthy diet and achieving healthy body weight. Apart from ensuring normal blood pressure, glycemic control and correcting dyslipidemia, being physically active was stressed (*14*). Educating the CAD patients about the possibility of concomitant MAS is also important to ensure timely recognition and intervention.

There were limitations to our study. Patients who had symptoms of mesenteric angina but no MAS might have other causes of abdominal pain like biliary colic or gastritis. Investigations to rule out such conditions were not performed. Biochemical tests for mucosal ischemia and endoscopy to rule out drug induced gastritis could have made the diagnosis of mesenteric ischemia more specific. Similarly, repeat contrast study of patients who worsened could have identified the status of mesenteric arteries.

5. Conclusion

Significant proportion of CAD patients have MAS. Nearly one-fifth of CAD patients have CR-MAS. Mesenteric angina, diabetes mellitus and peripheral artery disease are independent predictors of CR-MAS. Intervention for MAS should be dictated by symptoms and not radiological severity. Lifestyle modification and medication for atherosclerotic ischemic heart disease probably prevents acute mesenteric ischemia in CAD patients.

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Medical students' knowledge and opinions about rare diseases: A case study from Poland

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Summary **While genetics constitutes an important part of medical education, one can observe a lack of knowledge about rare diseases (RD) among medical students and healthcare professionals. Meanwhile, many RD are life threatening and chronically debilitating conditions that significantly reduce patients' quality of life. Most RD patients experience various psychiatric symptoms, behavioral changes and mental retardation. Consequently, physicians should be educated on RD. Thus, the aim of this paper is to assess the knowledge about RD among future physicians. The study was conducted among 346 medical students of Poznan University of Medical Sciences. It showed that while 99.4% of respondents had heard the term 'rare disease' and 90.5% knew its main cause, only 11.5% correctly estimated the prevalence of RD. Moreover, only 35.3% knew what percentage of RD is of genetic character and 24.9% that RD are most common among children. Additionally, very few students knew the number of RD patients in Poland (5.2%). Most respondents believed that it is primarily geneticists (76.6%) and pediatricians (74.3%) who should be uniquely educated and trained in RD. Interestingly, although 95.4% of respondents perceived their knowledge about RD as insufficient or very poor and 92.2% did not feel prepared for caring for RD patients, 45.7% believed that it is not necessary to add an extra course on RD into medical curricula. Thus, as most future physicians do not possess knowledge about RD, there is an urgent need to raise the awareness on RD among medical students and educate them about such diseases.**

Keywords: Rare diseases, medical education, medical students' knowledge

1. Introduction

While there is no accepted definition of rare diseases (RD) it is widely recognized that they affect a small percentage of the population. For example, in the European Union a disease is considered to be rare when the number of people affected is less than 5 per 10,000, *i.e.* no more than 1 in 2,000, while in the United States RD are defined as conditions that affect fewer than 200,000 people and in Japan it is 1 in 2,500 people (*1*). While there have been listed about 6,000-8,000 RD so far (*2*), their prevalence differs significantly between countries or regions and many of them affect only a few people in the world. At the same time it is estimated that approximately 300-350 million people, *i.e.* 6-8% of the global population, suffer from RD worldwide, including 25-30 million in the US and 27-36 million in the EU (*3*). Moreover, it is said that one in every seventeen EU citizens will be affected by RD during their lifetime. These numbers alone suggest that RD patients are numerous and that most healthcare professionals will encounter such patients during their professional career. For all these reasons, in 2009, the Council of the European Union recognized that RD pose a serious 'threat to the health of EU citizens' (*4*). Consequently, they are now considered an important medical and social problem and an urgent public health issue (*5*).

Nevertheless, while some countries, *i.e.* the USA, the United Kingdom, Australia, Japan or Taiwan, have adopted legislation on RD and orphan drugs and promote investments in RD research, many other countries, including Poland, still lack a national strategy

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for RD (*6*). Meanwhile, approximately 2.3-3 million people suffer from RD in our country (*7,8*). Moreover, in Poland there is neither a center for registration of RD nor a central register of drugs for such diseases. Yet another problem patients and their caregivers have to struggle with is lack of education of healthcare professionals on RD. Consequently, they are often left without a diagnosis and proper treatment. It is important because while RD are life threatening and chronically debilitating diseases that significantly reduce patients' quality of life, their early detection, diagnosis and therapy enable the patient's daily functioning and increase their quality of life.

Thus, although during the past few years there have been an increasing number of campaigns and initiatives aiming at addressing RD, including celebration of Rare Disease Day on February 29th (which was first organized in 2008 in numerous European countries and in Canada), and creation of many websites dedicated to RD and their particular types, still there can be observed a lack of knowledge about RD among the general population (*9,10*), medical students (*11-16*) and healthcare professionals, including physicians and pharmacists (*17-22*). Consequently, RD patients and their families report that due to physicians' lack of knowledge and disease-related experience they have to become self-experts on their own disease and educate physicians about their condition (*18,23,24*). At the same time, healthcare professionals themselves declare that lack of knowledge is the biggest problem they face while caring for RD patients (*17,19,21,22*). Meanwhile, as RD are very complex and require multi-professional care, it is essential that not only primary-care physicians but also various specialists, including geneticists, neurologists, psychiatrists, immunologists, pediatricians, pharmacists, nurses, dieticians and physiotherapists must be engaged in education on RD (*11,13,25*).

Concurrently, it is important to remember that one of the main factors leading to increased stress in RD patients and their caregivers is delays in receiving a correct diagnosis (*19, 26-28*). For example, a European survey conducted in 2008 showed that after the occurrence of the first symptoms 25% of patients with RD were waiting for an appropriate diagnosis between five and thirty years and 40% of them received an incorrect diagnosis (*26*). The *Rare Disease Impact Report* published in 2013 shows that in the US it takes an average of 7.6 years to receive a proper diagnosis and in the United Kingdom 5.6 years. Moreover, before patients get one, they usually visit up to eight physicians and receive two or three misdiagnoses (*23*). In Australia, some parents of children with RD, while waiting ten or even eighteen years for diagnosis, reported visiting more than ten physicians, and 27.3% had received misdiagnosis (*20*). Walshe and Yealland (*29*) reported that only 30% of patients with Wilson disease were diagnosed at presentation. It is important, because a delay or misdiagnosis leads to worsening of symptoms and disease progression (physical, cognitive, psychological deterioration of the patient or even death or birth of other affected children), results in delays in appropriate treatment or in receiving unnecessary medical interventions, including tests, readmissions and surgeries, and cause additional medical costs (*26*). Therefore, the aim of this paper is to assess the knowledge and awareness about RD among future physicians. Our findings may serve as a point of departure for revision of medical university curricula.

2. Materials and Methods

The study was conducted between January and March 2019 among medical students of Poznan University of Medical Sciences, Poland. The participants were recruited during regular classes. The survey was conducted with a standard questionnaire that was constructed from themes based on a review of the literature and the study aim. It included 28 questions: 22 items referring to respondents' knowledge of and attitudes towards RD and 6 questions that addressed their demographic data. The questionnaire consisted of four groups of questions. The first group were ten questions on the definition, frequency, number and estimated prevalence of RD. The respondents were also asked whether they have ever heard the term 'rare disease'. Other questions referred to epidemiological issues: in which age group RD are the most common, how many people suffer from RD worldwide, in the EU and in Poland. Two questions referred to the etiology of RD. Additionally, students were asked to indicate RD from a list comprised of twenty eight diseases: eighteen rare diseases and ten more common disorders. The rare diseases included were chosen because they are either commonly known rare diseases (*i.e.* haemophilia, progeria, Huntington disease or sickle cell anemia) or students had an opportunity to learn about them during their studies (*i.e.* Niemann-Pick disease, neurofibromatosis, Pompe disease, Gaucher disease).

The second group of six questions concentrated on organizational issues. The respondents were asked about the name of the European website providing information about RD and orphan drugs and whether Poland has a national register of RD patients. They were also asked about the percentage of RD that can be treated with the drugs and whether orphan drugs are reimbursed in Poland. Finally, the respondents were asked whether RD should be considered as an important aspect of Polish healthcare policy and which specialists should possess special knowledge about RD.

The third group of questions contained six questions related to the participants' self-assessment of knowledge and competence in the field of RD. The respondents were asked if they had any classes on RD, how they perceive their knowledge about RD and whether they

would like to broaden it. The authors also wanted to know whether future physicians believed that there is a need to include an obligatory course on RD in medical curricula. Finally, the respondents were asked where they got the information on RD from and whether they felt prepared to care for RD patients.

The last group of questions consisted of six items related to gender, year of studies, marital status, dwelling place, whether the respondents had ever met a patient with RD and had a relative suffering from RD.
Ethics approval and research governance approval were obtained from the Poznan University of Medical Sciences Bioethics Committee (1018/18).

3. Results

Out of all 523 students approached, the questionnaire was completed by 346 students (66.1%). 177 students who did not complete the questionnaire refused to participate in the study and their refusals were motivated by an unwillingness to discuss their knowledge on RD and/or lack of interest in the study. Feedback on surveys from fifth year students was 201/272 (73.9%) and from sixth year – 145/251 (57.7%). The sample consisted of 212 females (61.3%) and 134 males (38.7%), all of Polish origin (Table 1). 58.1% of respondents were fifth year students and 41.9% sixth. The majority of the respondents were single (60.7%), while 27.4% were cohabiting and 10.7% were married. Most respondents lived either in large agglomerations with a population of over 500 thousand (43.1%) or in big towns with 101-500,000 inhabitants (20.2%). 72.5% of the respondents declared having met a patient suffering from RD and 78.3% did not have a RD relative in his or her family.

Table 1. Socio-demographic characteristics of students

Characteristics	N (%)
Year of study	
5	201 (58.1)
6	145 (41.9)
Gender	
Female	212 (61.3)
Male	134 (38.7)
Marital status	
Single	210 (60.7)
Cohabiting	95 (27.4)
Married	37 (10.7)
Widowed	0 (0)
Divorced	4 (1.2)
Domicile	
Under 10,000 inhabitants	41 (11.8)
10-50,000 inhabitants	54 (15.6)
51-100,000 inhabitants	32 (9.3)
101-500,000 inhabitants	70 (20.2)
Over 500,000 inhabitants	149 (43.1)
Have you ever met a person suffering from RD	
Yes	251 (72.5)
No	62 (18)
I do not know	33 (9.5)
Is anyone in your family suffering from RD?	
Yes	25 (7.2)
No	271 (78.3)
I do not know	50 (14.5)

While almost all of the respondents declared having heard the term 'rare disease' (99.4%) and knew its main cause (90.5%), only 11.5% correctly estimated the prevalence of RD (Table 2). Even fewer knew the number of RD (10.4%). Moreover, only ¼ knew that RD are most common among children (24.9%). Very few students were also aware of the number of patients suffering from RD in the world (9.5%), in the EU (9%) and in Poland (5.2%). One third of future physicians knew what percentage of RD is of genetic character (35.3%).

The respondents were also presented a list of twenty eight diseases and asked to select those they considered to be rare (Table 3). The most recognized RD were: Pompe disease (72.8%), Gaucher disease (69.1%) and Niemann-Pick disease (65.6%). Least often were indicated acromegaly (17.3%) and sickle cell anemia (13.9%). On the other hand, the most common diseases that were mistaken with RD were Munchhausen syndrome (50.9%), fibromyalgia (33.2%), halitosis (27.8%) and acquired immunodeficiency syndrome (24.9%).

Future physicians also lacked practical information on RD (Table 4). For example, barely 19.4% knew the name of the European website providing information about RD and orphan drugs. Moreover, 61.6% falsely believed that Poland has a central register of RD patients and less than one third knew the percentage of RD that have an approved drug treatment (31.5%); 59.2% knew that only some orphan drugs are reimbursed in our country.

Finally, the authors also evaluated students' perception of their knowledge about RD (Table 5). Surprisingly, although 95.4% of future physicians perceived their knowledge about RD as insufficient (56.6%) or very poor (38.7%) and 92.2% did not feel well prepared for caring for RD patients, almost half of the respondents (45.7%) believed that it is not necessary to add an extra course on RD into medical curricula. At the same time, 75.1% declared eagerness to increase their knowledge about RD and 78% believed that RD constitute a serious public health issue and should be prioritized. What is important from an educational perspective is that while 51.7% of the respondents declared getting their knowledge about RD from mandatory courses and 22% from elective courses, the Internet was a major source of information for 59.8%.

At the same time, the respondents believed that it is primarily geneticists (76.6%) and pediatricians (74.3%) who should be uniquely educated and trained in RD (Table 6). Thus, although RD patients frequently experience psychological distress, behavioral changes and mental deterioration and are often confused with psychiatric patients, only 22.5% of future physicians

Table 2. Students' knowledge about rare diseases

Items	N (%)
Have you ever heard the term 'rare diseases'?	
Yes	344 (99.4)
No	2 (0.6)
Rare disease is the one that affects less than:	
1 person in 1,000	24 (6.9)
1 person in 2,000	**40 (11.5)**
1 person in 3,000	11 (3.2)
1 person in 5,000	12 (3.5)
1 person in 10,000	196 (56.7)
I do not know	63 (18.2)
What is the estimated number of rare diseases?	
100-500	25 (7.2)
1,000-2,000	50 (14.5)
3,000-5,000	46 (13.3)
6,000-8,000	**36 (10.4)**
9,000-1,000	18 (5.2)
Over 10,000	119 (34.4)
I do not know	52 (15)
At what age group do rare diseases most frequently appear?	
Newborns	166 (48)
Children	**86 (24.9)**
Adolescents	10 (2.9)
Adults	8 (2.3)
They are present in all age groups equally	37 (10.7)
I do not know	39 (11.2)
How many people suffer from rare diseases worldwide?	
10-15,000,000	63 (18.2)
50-75,000,000	65 (18.8)
100-150,000,000	69 (19.9)
200-250,000,000	28 (8.1)
300-350,000,000	**33 (9.5)**
Over 500,000,000	27 (7.8)
I do not know	61 (17.65)
How many people in the EU suffer from rare diseases?	
5,000,000	106 (30.6)
15,000,000	53 (15.3)
20,000,000	34 (9.85)
30,000,000	**31 (9)**
40,000,000	29 (8.4)
Over 50,000,000	20 (5.8)
I do not know	73 (21.1)
How many people suffer from rare diseases in Poland?	
500-1,000	23 (6.6)
10-15,000	67 (19.4)
50-75,000	58 (16.8)
100-150,000	58 (16.8)
300-500,000	55 (15.9)
1,000,000	6 (1.7)
2-3,000,000	**18 (5.2)**
Over 5,000,000	6 (1.7)
I do not know	55 (15.9)
What is the most common cause of rare diseases?	
Infectious and bacterial	2 (0.6)
Genetic	**313 (90.5)**
Autoimmune	12 (3.5)
Mitochondrial	1 (0.3)
Environmental	2 (0.6)
I do not know	16 (4.6)
What percentage of rare diseases are of genetic origin?	
5-10%	11 (3.2)
20%	52 (15)
50%	126 (36.4)
80%	**122 (35.3)**
100%	18 (5.2)
I do not know	17 (4.9)

Correct answers are written in bold characters.

Table 3. Which of the following diseases are considered to be rare in Poland?

Items	N (%)
Sickle cell anemia	48 (13.9)
Cystic fibrosis	84 (24.3)
Acromegaly	60 (17.3)
Haemophilia	82 (23.7)
Down syndrome	18 (5.2)
Niemann-Pick disease	227 (65.6)
Halitosis	96 (27.8)
Glaucoma	16 (4.6)
Progeria	195 (56.4)
Neurofibromatosis	103 (29.8)
Craniodiaphyseal dysplasia	147 (42.5)
Cerebral palsy	30 (8.7)
Fibromyalgia	115 (33.2)
Huntington disease	160 (46.2)
Duchenne muscular dystrophy	154 (44.5)
Acquired immunodeficiency syndrome	86 (24.9)
Munchausen syndrome	176 (50.9)
Mucopolysaccharidoses	161 (46.5)
Achondroplasia	90 (26)
Crohn's disease	16 (4.6)
Pompe disease	252 (72.8)
Gaucher disease	239 (69.1)
Fragile X syndrome	133 (38.4)
Marfan syndrome	99 (28.6)
Schizophrenia	8 (2.3)
Alzheimer's disease	15 (4.3)
Osteogenesis imperfecta	188 (54.3)
Phenylketonuria	140 (40.5)

Table 4. Students' knowledge about healthcare system for RD patients

Items	N (%)
What is the name of the European website providing information about RD and orphan drugs?	
Rare Disease Foundation	8 (2.3)
NORD	4 (1.1)
EURORDIS	21 (6.1)
R.A.R.E	27 (7.8)
Orphanet	**67 (19.4)**
Global Genes	6 (1.7)
I do not know	213 (61.6)
Is there a central register of RD patients in Poland?	
Yes	213 (61.6)
No	**14 (4)**
I do not know	119 (34.4)
What percentage of rare disease can be treated with drugs?	
0%	1 (0.3)
5%	**109 (31.5)**
10%	61 (17.6)
15%	65 (18.8)
20%	31 (9)
50%	5 (1.4)
I do not know	74 (21.4)
Are orphan drugs reimbursed in Poland?	
Yes	7 (2)
Yes, some	**205 (59.3)**
No	28 (11)
I do not know	96 (27.7)
Do RD constitute a serious public health issues?	
Absolutely yes	59 (17)
Yes	211 (61)
No	54 (15.6)
Definitely no	0
I do not know	22 (6.4)

Table 5. Students' self-assessment of their knowledge about RD

Items	N (%)
How would you rate your knowledge about rare diseases?	
Very good	0
Fair enough	16 (4.6)
Insufficient	196 (56.7)
Very poor	134 (38.7)
Do you feel prepared for caring for a patient with a rare disease?	
Definitely yes	1 (0.3)
Rather yes	15 (4.3)
Rather not	144 (41.6)
Definitely not	175 (50.6)
I do not know	11 (3.2)
Would you like to broaden your knowledge about rare diseases?	
Yes	260 (75.1)
No	38 (11)
I do not know	48 (13.9)
Do you think that there should be a mandatory course on rare diseases in medical curricula?	
Definitely yes	23 (6.6)
Rather yes	138 (39.9)
Rather not	136 (39.3)
Definitely not	22 (6.4)
I do not know	27 (7.8)
Have you had any classes about rare disease during your studies?	
Yes	264 (76.3)
No	60 (17.3)
I do not know	22 (6.4)
Where do you get your knowledge about RD from?	
Mandatory courses at the university	179 (51.7)
Faculty courses at the university	76 (22)
Scientific literature and research	67 (10.7)
Scientific conferences, symposia	35 (10.1)
Internet	207 (59.8)
Other	9 (2.6)
I do not search for such information	41 (11.8)

Table 6. Which physicians should be uniquely trained in RD?

Speciality	N (%)
Family physician	158 (45.7)
Pediatrician	257 (74.3)
Neurologist	126 (36.4)
Geneticist	265 (76.6)
Psychiatrist	78 (22.5)
Immunologist	171 (49.4)
Other:	
Neonatologist	24 (6.9)
Oncologist	4 (1.2)
Gynecologist	3 (0.9)
Every physician regardless of specialization	84 (24.3)

said that also psychiatrists should be trained in RD. Surprisingly, however, 24.3% believed that all physicians, regardless of their specialization, should possess such knowledge.

4. Discussion

While our understanding of genetically based human diseases has increased significantly and genetics constitutes an important part of medical education, still many educational programs lack specific courses dedicated to RD. Consequently, medical students often do not receive necessary training in RD (*11-16*).

This research confirms that medical students receive little education on RD and that the problem of inadequate training in RD during medical studies does exist. Although nearly all students taking part in the study were aware of such diseases and knew that the vast majority of RD are caused by genetic factors, they lacked basic knowledge about their epidemiology. Moreover, many had even problems with distinguishing rare from common diseases. Most respondents also lacked knowledge about the organization of the health care system for RD patients in our country, as neither did they know the name of the most important website that gathers and provides information about RD and orphan drugs nor about the Polish central register of RD patients. Nevertheless, what is most alarming is that while the vast majority of future physicians were aware of their insufficient knowledge about RD and did not feel prepared for caring for such a patient, still many did not see the need to add extra classes on RD into medical curricula. What is even more intriguing is that while most respondents perceived RD as a serious public health problem, they stressed that it is mainly geneticists and pediatricians who should be uniquely educated and trained in RD.

These results confirm the findings from previous studies, such as, for example, other research conducted among Polish medical students in Szczecin revealed that although the majority (87.8%) had heard about RD during their studies, only 20.7% knew their correct definition. 58.1% did not know their prevalence and 67.4% the number of RD (*16*). Only 25% of healthcare students from la Rioja, Spain knew the definitions of RD and orphan drugs and the majority did not believe that research or funding for RD should be prioritized (*12*). While medical students in Serbia recognized many problems related to drug provisions for RD patients they demonstrated a moderate level of knowledge about RD and did not consider them an important social problem (*15*). Also, many Pakistani pharmacy students from Karachi lacked knowledge about RD (*14*). All these results confirm that RD receive little attention in medical curricula and that the problem of inadequate training in RD during medical studies is a fact.

This is even more important because while knowledge about RD is not retained throughout medical studies, it also does not improve during clinical courses. Thus, as many young physicians acknowledge that they are not adequately trained in RD, they are also afraid that they cannot serve the needs of patients suffering from these diseases. This is why the Council of the European Union stresses the need for 'adequate

education and training for all health professionals to make them aware of the existence of these diseases and of resources available for their care' and 'development of medical training in fields relevant to the diagnosis and management of rare diseases, such as genetics, immunology, neurology, oncology or pediatrics' (*4*). Thus, it acknowledges the need for future healthcare professionals to be well prepared for a possible encounter with RD patients. The problem is, however, that many universities neglect training their students in RD.

Consequently, during their medical encounter RD patients and their families have to struggle with an insufficiency or lack of knowledge about RD among healthcare professionals. This, in turn, results in lack of or delayed diagnosis, as sometimes patients have to wait up to ten or even twenty years for the correct diagnosis (*19,20,22,23,26-28*). Delayed diagnoses or misdiagnoses usually result in many unnecessary visits to different health specialists, delays in appropriate treatment or in receiving unnecessary medical interventions, including tests, medications, readmissions and surgeries, which cause additional medical costs, worsening of symptoms and disease progression (physical, cognitive, psychological deterioration of a patient or even death) (*26*).

Moreover, although most RD are of genetic character, due to many psychiatric, emotional and behavioral symptoms they cause they present a challenge for psychiatrists who, are a core part of the treatment team for both the RD patient and his or her family. For that reason there is a great need to alter the awareness about RD among physicians in general and psychiatrists in particular (*30*). As many RD have multiple neuropsychiatric manifestations and serious psychosocial implications, both for the patient and the family, psychiatrists are among those medical specialists who should be uniquely educated and trained in the field of RD, especially because that approximately 75% of all RD appear in early childhood leading to permanent disability or premature death. That is why a timely and correct diagnosis is critical for the effective clinical management of RD characterized by psychiatric symptoms and, often, the psychiatrist is the best place to offer this. After that the treatment will require an integrated and collaborative approach between multiple specialists.

Thus, it should be emphasized that since many RD patients experience various psychiatric symptoms, behavioral changes and mental retardation, psychiatrists seem to be those medical practitioners who should be uniquely educated and trained in RD. The reason is that although approximately 80% of RD are of genetic character, living with RD is a serious source of stress and has a detrimental effect on mental well-being for both RD patients and their carers (*24,26-28*), leading to attentional problems, anxiety, low mood, emotional exhaustion, neurocognitive dysfunctions, depression or suicidal ideations. Moreover, mental health problems are often significantly higher in patients suffering from RD than in the general population (*23,30-35*). Research on phenylketonuria indicates that more than one third of patients meet the criteria for psychiatric diagnosis (*36*). Akil *et al*. (*37*) reported psychiatric symptoms at the onset in two thirds of patients with Wilson disease. In the case of cystic fibrosis it has been suggested that all patients and their families should have a chance to be under a psychiatrist's care (*38*). Yet another reason why psychiatrists should be educated and trained in RD is that such diseases are frequently characterized by various neuropsychiatric symptoms, including apathy, social withdrawal, psychosis, delusions or hallucinations, anxiety or aggressive behaviors. Consequently, RD patients are often misdiagnosed as psychiatric patients and often assigned such labels as having schizophrenia, bipolar disorder or other neurodegenerative diseases, including Alzheimer's or Parkinson's disease. Reports show that patients suffering from Niemann-Pick disease type C or classical hyperhomocysteinaemia were misdiagnosed with late-onset schizophrenia (*39*). Some metabolic disorders (but also genetic ones) in their initial phase are manifested by psychiatric symptoms, which in some cases are the only manifestation of the disease (*39-41*).

Although this study brings new insight into the state of knowledge of Polish medical students about rare diseases, it also has a few limitations. Since only 66.1% of medical students from Poznan Medical University of Medical Sciences completed the questionnaire, the results may hinder generalization of the entire population of future doctors. Consequently, a more in-depth study would be required to help clarify the issues of education for RD. However, some advantages of this study should also be acknowledged. Most importantly, as RD seem to be neglected by medical education and there is a scarcity of previous work on the topic, it gives some highlight on the knowledge of medical students about RD. Hopefully, this study may not only stimulate further research on the topic but also provoke discussion on the need of better education of future physicians, including psychiatrists on RD.

5. Conclusions

While the majority of future physicians lack basic knowledge both about the epidemiology and the prevalence of rare diseases and the organization of the Polish health care system for RD patients, there is an urgent need to raise awareness on RD among medical students and educate them about such diseases. It is of special importance because while most of the respondents did not feel prepared for caring for such patients, they did not believe that RD constitute an important public health issue and that they should be well educated in this area.

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Brief Report

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Microglia express TMEM119 in the brains of Nasu-Hakola disease

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Summary **We previously identified an evolutionarily conserved protein named transmembrane protein 119 (TMEM119) as the most reliable maker for human microglia. Recent studies showed that under homeostatic conditions, microglia intensely express TMEM119, whereas the expression levels are greatly reduced in disease-associated microglia (DAM) activated at the site of neurodegeneration. Nasu-Hakola disease (NHD) is a rare autosomal recessive disorder, pathologically characterized by leukoencephalopathy, astrogliosis, axonal spheroids, and accumulation of microglia. However, it remains unknown whether microglia are homeostatic or activated in NHD brains. In the present study, we identified TMEM119 on microglia in NHD brains by immunohistochemistry. TMEM119 was expressed on microglia in NHD brains as well as in the brains of non-neurological controls (NC) and Alzheimer's disease (AD) patients, although TMEM119-immunolabeled areas exhibited great variability from case to case without significant differences among the study population. These results suggest that TMEM119 expression on microglia might play a key role in steady-state brain maintenance in NHD, AD and controls.**

Keywords: Alzheimer's disease, disease-associated microglia (DAM), microglia, Nasu-Hakola disease, TMEM119

1. Introduction

Microglia are resident myeloid cells of the central nervous system (CNS) that play a principal role in the maintenance of normal tissue homeostasis and plasticity (*1*). Microglia are ontogenetically and functionally distinct from monocyte-derived macrophages that infiltrate the CNS under pathological conditions (*2-4*). Microglia are originated from primitive hematopoietic progenitor cells present in the yolk sac during embryonic development (*5*). The establishment of specific markers that distinguish resident microglia from circulating blood-derived macrophages in human brains is essential for exact evaluation of microglial contributions to the human brain pathology.

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To identify microglia-specific markers, we performed a comparative analysis of five comprehensive datasets of mouse microglia transcriptome characterized by microarray and RNA-Seq technologies (*6*). Then, we identified transmembrane protein 119 (TMEM119) as the most reliable marker for human microglia. The resting microglia presenting with a ramified morphology have a capacity to constantly migrate and scavenge invading pathogens, apoptotic debris, unwanted synapses, and pathologically accumulated protein aggregates by sensing them with a panel of pattern recognition receptors (PRRs) (*7-9*). The homeostatic mouse microglia express high levels of Cx3cr1, P2ry12, and Tmem119 (*3,9*). At sites of neurodegeneration, they are transformed into disease-associated microglia (DAM), also called as the microglial neurodegenerative phenotype (MGnD), which upregulate the expression of Tyrobp, Apoe, and Trem2 and reduce the expression of Cx3cr1, P2ry12, and Tmem119 (*9-11*). These cells are named as the stage 1 (Trem2-independent) DAM (*9,10*). Trem2 signaling further induces the expression of Lpl,

Cst7, Axl, and Itgax. They are termed as the stage 2 (Trem2-dependent) DAM (*9,10*).

Nasu-Hakola disease (NHD), also designated polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), is a rare autosomal recessive disorder, characterized by progressive presenile dementia and formation of multifocal bone cysts, caused by loss-of-function mutations of either *TYROBP* (*DAP12*) or *TREM2* (*12*). TREM2 and DAP12 constitute a receptor/adaptor signaling complex expressed exclusively on osteoclasts, dendritic cells, macrophages, and microglia. Although NHD patients are clustered in Japan and Finland, approximately 200 NHD cases are presently reported worldwide. Clinically, NHD patients show recurrent bone fractures during the third decade of life, a frontal lobe syndrome during the fourth decade of life, and progressive dementia and death until the fifth decade of life (*13*). Pathologically, the brains of NHD patients exhibit extensive demyelination designated leukoencephalopathy, astrogliosis, axonal spheroids, and accumulation of microglia predominantly in the frontal and temporal lobes and the basal ganglia (*14*). At present, molecular mechanisms responsible for development of leukoencephaolpathy in NHD brains remain unknown. Because NHD is a pathological entity of microgliopathy where microglia act as a key regulator of leukoencephalopathy, we propose the hypothesis that TMEM119 expression on microglia might play a central role in maintenance of homeostasis in NHD brains. In the present study, we have attempted to clarify the expression pattern of TMEM119 on microglia in NHD brains, compared with AD brains.

2. Materials and Methods

2.1. *Human brain tissues*

The brain autopsies were performed at the National Center Hospital, National Center of Neurology and Psychiatry (NCNP), Japan, Kohnodai Hospital, National Center for Global Health and Medicine (NCGM), Japan, and affiliated hospitals of Research Resource Network (RRN), Japan. The comprehensive examination by established neuropathologists (YS and TI) validated the pathological diagnosis. The Ethics Committee of NCNP for the Human Brain Research, the Ethics Committee of NCGM on the Research Use of Human Samples, and the Human Research Ethics Committee of Meiji Pharmaceutical University approved the present study. Written informed consent was obtained in all cases at autopsy, following the regulation of the institutional ethics committees.

For immunohistochemical studies, serial sections of the frontal lobe were prepared from four subjects who died of non-neurological causes (NC), ten Alzheimer's disease (AD) patients, and five NHD patients, as listed in Table 1. The homozygous mutation of a single base deletion of 141G (c.141delG) in exon 3 of DAP12 was identified in NHD1, NHD2, and NHD5, while the genetic analysis was not performed in NHD3 or NHD4. All AD cases were satisfied with the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria for diagnosis of definite AD, categorized into the stage C of amyloid deposition (*15*). They were classified into the stage VI of neurofibrillary degeneration, following the Braak's staging (*16*).

Table 1. Study Population

Case No.	Age (year-old)	Sex (male, female)	Pathological Diagnosis
NC1	63	M	prostate cancer and acute myocardial infarction
NC2	67	M	dissecting aortic aneurysm
NC3	57	M	alcoholic liver cirrhosis
NC4	61	M	rheumatoid arthritis and interstitial pneumonia
NHD1	42	M	Nasu-Hakola disease (DAP12 c.141delG)
NHD2	48	F	Nasu-Hakola disease (DAP12 c.141delG)
NHD3	44	M	Nasu-Hakola disease (mutation not analyzed)
NHD4	32	F	Nasu-Hakola disease (mutation not analyzed)
NHD5	38	M	Nasu-Hakola disease (DAP12 c.141delG)
AD1	68	F	Alzheimer's disease (C/VI)
AD2	70	F	Alzheimer's disease (C/VI)
AD3	68	F	Alzheimer's disease (C/VI)
AD4	56	M	Alzheimer's disease (C/VI)
AD5	59	M	Alzheimer's disease (C/VI)
AD6	81	M	Alzheimer's disease (C/VI)
AD7	68	F	Alzheimer's disease (C/VI)
AD8	80	M	Alzheimer's disease (C/VI)
AD9	72	M	Alzheimer's disease (C/VI)
AD11	77	F	Alzheimer's disease (C/VI)

The study population is composed of four subjects who died of non-neurological causes (NC), five Nasu-Hakola disease (NHD) patients, and ten Alzheimer's disease (AD) patients. The homozygous mutation of c.141delG in exon 3 of DAP12 was identified in NHD1, NHD2, and NHD5. All AD cases were satisfied with the stage C of amyloid deposition in the CERAD criteria and the stage VI of neurofibrillary degeneration in the Braak's staging.

2.2. *Immunohistochemistry*

After deparaffination, tissue sections were heated in 10 mM sodium citrate buffer, pH 6.0 by autoclave at 110℃ for 15 min in a temperature-controlled pressure chamber (Biocare Medical, Pacheco, CA, USA). They were treated at room temperature (RT) for 15 min with 3% hydrogen peroxide-containing methanol to block the endogenous peroxidase activity. They were then incubated with phosphate-buffered saline (PBS) containing 10% normal goat serum at RT for 15 min to block non-specific staining, followed by incubation in a moist chamber at 4℃ overnight with a rabbit polyclonal anti-human TMEM119 antibody numbered HPA051870 (Sigma, St. Louis, MO, USA) (*6*) or a rabbit polyclonal anti-Iba1 antibody (FUJIFILM Wako Pure Chemical, Osaka, Japan).

After washing with PBS, tissue sections were incubated at RT for 30 min with a horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (Nichirei, Tokyo, Japan), followed by incubation with diaminobenzidine tetrahydrochloride (DAB) substrate (Vector, Burlingame, CA, USA). They were processed for a counterstain with hematoxylin. Negative controls underwent all the steps except for exposure to primary antibody. In limited experiments, double immunolabeling was performed using HPA051870 followed by incubation with a HRP-conjugated anti-rabbit secondary antibody and exposure to DAB, and in combination with a mouse monoclonal antibody against amyloid-β peptide (12B2; Immunobiological Laboratories, Gunma, Japan), followed by incubation with an alkaline phosphatase-conjugated anti-mouse secondary antibody (Nichirei) and exposure to Warp Red chromogen (Biocare Medical).

2.3. *Quantification of TMEM119 immunoreactivity*

To quantify immunolabeled areas, the images derived from three fields each of the frontal cortex were captured at a 200 X magnification on the Olympus BX51 universal microscope. They were then processed for quantification by using ImageJ software (National Institute of Health, Bethesda, MD, USA). The differences in the TMEM119-positive areas among NC, NHD, and AD subjects were evaluated statistically by one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test.

3. Results and Discussion

By immunohistochemistry, we found that TMEM119 was intensely expressed predominantly on ramified microglia located in the frontal cortex and white matter of NHD (Figure 1, panels A, C, E), AD and NC brains (Figure 2, panels A, C, F). Iba1 immunolabeling was always more intense when compared with that of TMEM119 in these brains (Figure 1, panels B, D, F; Figure 2, panels B, D). Multipolar fine process-bearing ramified microglia were well stained by TMEM119 antibody (Figure 2, panel F), while amoeboid microglia exhibited a less intense immunoreactivity for TMEM119. Clusters of TMEM119-expressing microglia were often incorporated into the amyloid-beta (Aβ) deposition in AD brains (Figure 2, panel E). Overall, TMEM119-immunolabeled areas or Iba1-positive areas were not significantly different among NHD, AD and NC brains (Figure 3, panels A, B). Approximately 32.5% of $Iba1^+$ microglia expressed TMEM119 in NHD brains, 35.0% in AD brains, and 32.8% in NC brains.

By the data-mining approach, we previously identified an evolutionarily conserved protein TMEM119 as the most promising marker specific for human microglia (*6*). TMEM119 discriminates resident microglia from blood-derived macrophages in the human brain (*6,17*). Several recent studies revealed that TMEM119 is highly expressed on microglia under homeostatic conditions, while its immunoreactivity is greatly reduced in disease-associated microglia named DAM, accumulating at sites of neurodegeneration (*9-11*). DAM cells are activated sequentially by TREM2-independent and TREM2-dependent pathways (*9,10*). DAM cells sense neurodegeneration-associated molecular patterns (NAMPs), which serve as danger signals expressed on apoptotic bodies of dying neural cells, myelin debris, lipid degradation products, and extracellular protein aggregates (*9*). On the other hand, NAMPs trigger resting microglia into a DAM phenotype with decreased expression of TMEM119 (*9*). We found that TMEM119 is expressed on a subset of microglia profoundly in NHD brains as well as in NC and AD brains, and TMEM119-immunolabeled areas exhibited great variability from case to case without significant differences among the study population. Furthermore, Iba1-positive areas were always more intense when compared with that of TMEM119 in these brains, suggesting the possibility that there exist a number of Iba1-positive TMEM119-negative DAM-like microglia not only in NHD and AD brains but also in NC brains. For instance, we estimated that approximately one-third of $Iba1^+$ microglia express TMEM119 in NHD brains. However, it is possible that an apparent inconsistency between microglial TMEM119 and Iba1 immunolabeling areas is attributable to a difference in the density of antigenic epitopes, the avidity of antibodies, or both. Furthermore, our results indicated that it is difficult to identify DAM solely by the loss of TMEM119 immunoreactivity on microglia in human brains.

TMEM119 is originally identified as an osteoblast induction factor (OBIF), composed of a type I transmembrane protein predominantly expressed in mouse osteoblasts with the location chiefly in the plasma membrane (*18*). OBIF-deficient mice exhibit severe bone hypoplasia accompanied by decreased

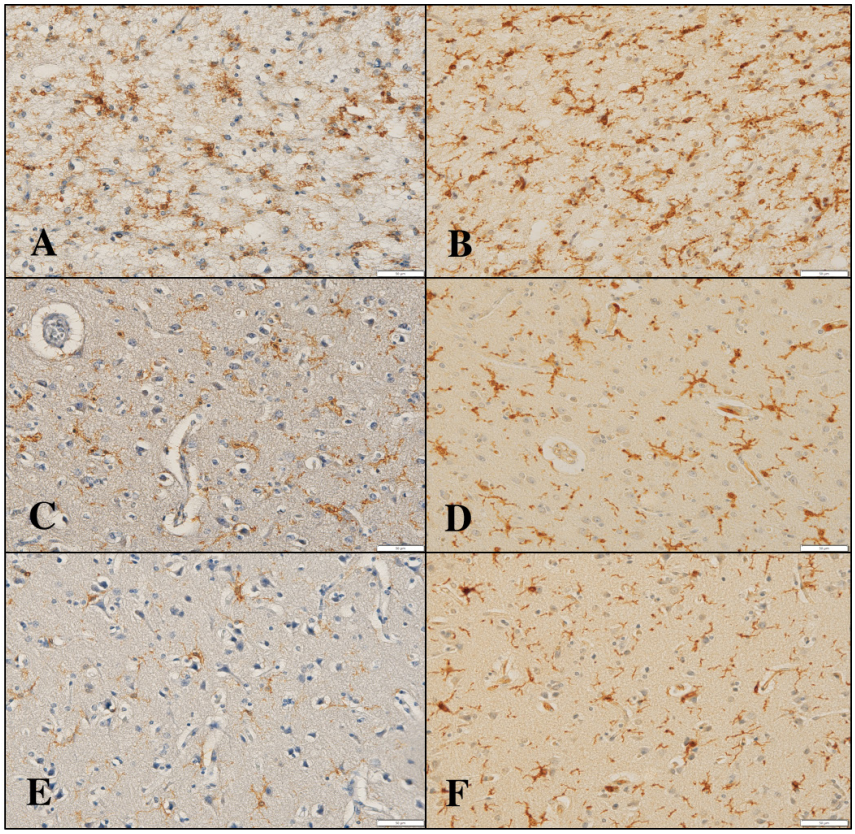

Figure 1. TMEM119 expression in the frontal cortex of NHD brains. (A) TMEM119, the frontal cortex, NHD5, **(B)** Iba1, the same field of **(A)**, **(C)** TMEM119, the frontal cortex, NHD2, **(D)** Iba1, the same field of **(C)**, **(E)** TMEM119, the frontal cortex, NHD4, and **(F)** Iba1, the same field of **(E)**. Scale bars indicate **(A-F)** 50 μm.

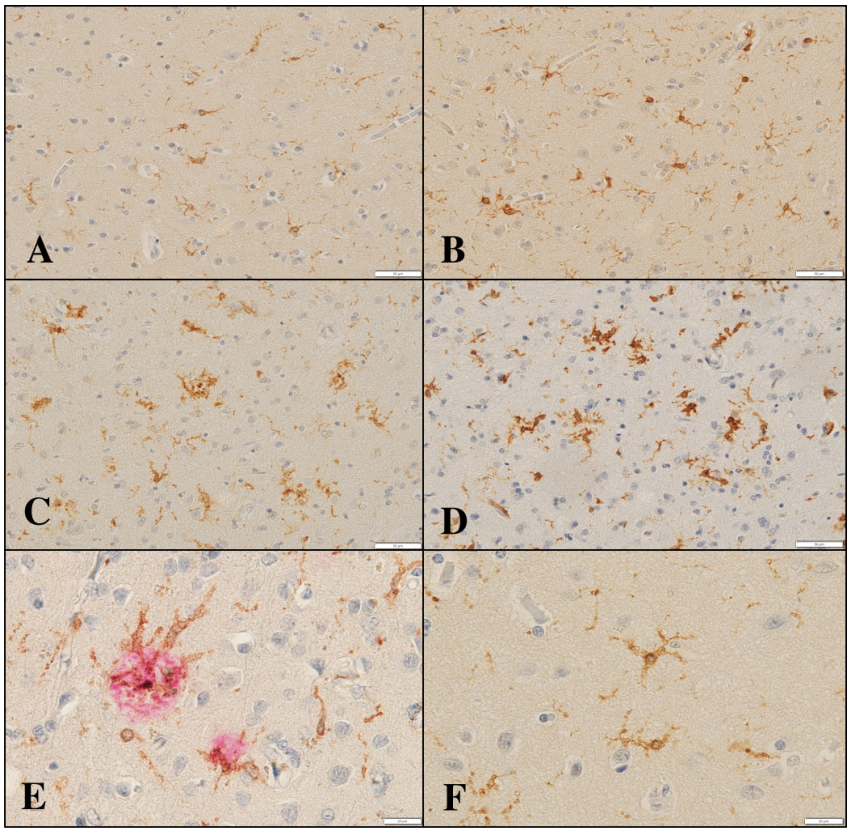

Figure 2. TMEM119 expression in the frontal cortex of NC and AD brains. (A) TMEM119, the frontal cortex, NC1, **(B)** Iba1, the same field of **(A)**, **(C)** TMEM119, the frontal cortex, AD8, **(D)** Iba1, the same field of **(C)**, **(E)** TMEM119 (brown) and Aβ (red), the frontal cortex, AD8, and **(F)** TMEM119, the frontal cortex, NC1. Scale bars indicate **(A-D)** 50 μm and **(E, F)** 20 μm.

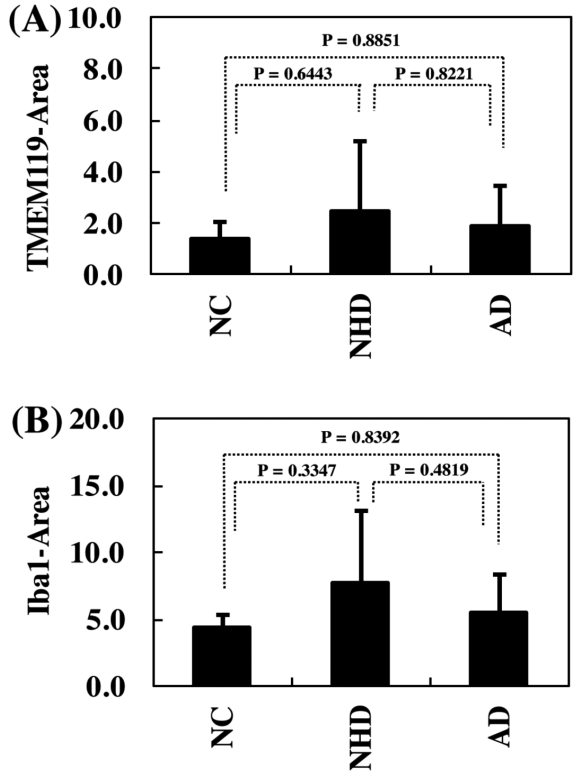

Figure 3. TMEM119-immunolabeled areas of the frontal cortex. The differences in the TMEM119-immunolabeled or Iba1-immunolabeled areas (pixel^2) of the frontal cortex among NC, NHD and AD subjects were evaluated statistically by one-way ANOVA followed by post-hoc Tukey's test. **(A)** TMEM119 and **(B)** Iba1.

expression of osteoblast marker genes (*19*). OBIF, induced by parathyroid hormone, interacts with several key regulators in the BMP2 signaling pathway, such as Smad1/5 and Runx2, to induce osteoblast differentiation (*20*). OBIF elevates the expression of ATF4, a transcription factor that plays a central role in osteoblast differentiation (*21*). However, at present, precise biological functions and endogenous ligands of TMEM119 and OBIF in human microglia and osteoblasts remain largely unknown.

In conclusion, TMEM119 is expressed on homeostatic microglia in NHD, AD, and NC brains. TMEM119-immunolabeled areas are not significantly different among the study population. TMEM119 expression on microglia might play a key role in steady-state brain maintenance. TMEM119 immunohistochemistry serves as a useful tool for investigating the biology and pathology of human microglia. However, it is difficult to identify DAM solely by the loss of TMEM119 immunoreactivity in these brains.

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Case Report

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Malignant transformation of hepatic adenoma complicated by rupture and hemorrhage: An extremely rare clinical entity

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Summary **Hepatic adenomas (HAs) are rare benign tumors of the liver and comprise 2% of all liver tumors with an annual incidence of 3-4/100,000 per year in Europe and North America. These tumors may be clinically silent or present with abdominal pain. Although rare, the most important complications associated with this tumor is haemorrhage and malignant transformation to hepatocellular carcinoma. The reported risk of malignant transformation is believed to be 4.2%. We present an extremely rare case report of a young woman on the oral contraceptive pill (OCP) with malignant transformation of a hepatic adenoma complicated additionally by tumor rupture and intraperitoneal bleed. This article therefore highlights the need to carefully evaluate any liver lesion in a young female on the OCP to be a possible adenoma and if confirmed to be so, to consider the potential risks associated with it as well as the need for follow-up imaging in order to avoid life threatening complications.**

Keywords: Hepatic adenoma, inflammatory adenoma, hepatocellular carcinoma, oral contraceptive pill, hemorrhage, malignant transformation

1. Introduction

Hepatic adenomas (HAs) are rare benign tumors of the liver resulting from monoclonal proliferation of liver cells and comprise 2% of all liver tumors with an annual incidence of 3-4/100,000 per year in Europe and North America. Risk factors for the development and progression of HAs include- use of estrogen containing contraceptive pills, androgen or anabolic steroid intake (used primarily for management of Fanconi syndrome, impotence, body building and in trans-sexuals), conditions leading to impaired glycogenesis and excessive hepatic intracellular glycogen deposits such as glycogen storage diseases (GSD), familial adenomatous polyposis and metabolic syndromes like diabetes mellitus, insulin resistance, dyslipidemia and high blood pressure (*1*). Two recognized complications for HAs are hemorrhage and malignant transformation to hepatocellular carcinoma (HCC). It has been observed that HAs less than 5 cm tend not to bleed, while the reported risk of a malignant transformation of an HA to HCC is very low (4-5%), and the reported mean size of solitary HA with features of malignant transformation is 10.5 cm (range: 4.5-18 cm) (*2*).

We present a unique case of a solitary HA in a young woman on the oral contraceptive pill (OCP) that underwent malignant transformation, which subsequently ruptured and presented with intra-abdominal hemorrhage. An extensive online search failed to reveal a similar documented case with both combinations of complications, and we felt our case report would be valuable for the worldwide medical fraternity.

2. Case Report

A young female in her mid-30s on the oral contraceptive pill presented with acute onset of abdominal pain, early satiety and indigestion. She had no relevant past or family history. On physical examination the patient appeared anemic. Her blood work revealed very low hemoglobin (70 g/L), elevated Alkaline Phosphatase (160 U/L) and elevated Alkaline Transferase (629 U/

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L) and normal Total bilirubin (17 umol/L). She was referred for ultrasound examination of the abdomen for further evaluation.

Ultrasound of the abdomen revealed a 10 cm poorly defined hypoechoic lesion in the right lobe of the liver close to the capsule (Figure 1A). No significant vascularity was noted within the lesion on colour doppler. Additionally, turbid free fluid was noted in the pelvis in the right lower quadrant and in the posterior cul-de-sac (Figure 1B) raising the possibility of intrabdominal hemorrhage. The patient was immediately referred to our University Hospital for further evaluation and management.

The patient was further evaluated by contrast enhanced abdominal and pelvic computed tomography (CT). On non-contrast CT (Figure 2A), the liver appeared non-cirrhotic and demonstrated a 10.4 × 9.5 × 10.7 cm predominantly hyperdense lesion occupying majority of the right lobe of the liver. In addition, subcapsular hematoma involving the right lobe of the liver was noted, as well as adjacent intraperitoneal hemorrhage into the right subphrenic space with extension into the peritoneal cavity, with significant hemoperitoneum in the right lower quadrant and pelvis. On the post contrast image (Figure 2B), the lesion was noted to involve the entire right lobe (segments 5 to 8) and portions of segment 4a of the liver. In addition, peripheral areas of soft tissue enhancement were demonstrated in a patchy distribution. No additional lesions were noted in the liver. No intra or extra-hepatic vessel occlusion were seen. With the combination of these findings on CT along with the background history of OCP usage, the possibility of a ruptured adenoma was raised.

The patient was taken up for immediate surgery and an extended right hepatectomy including portions

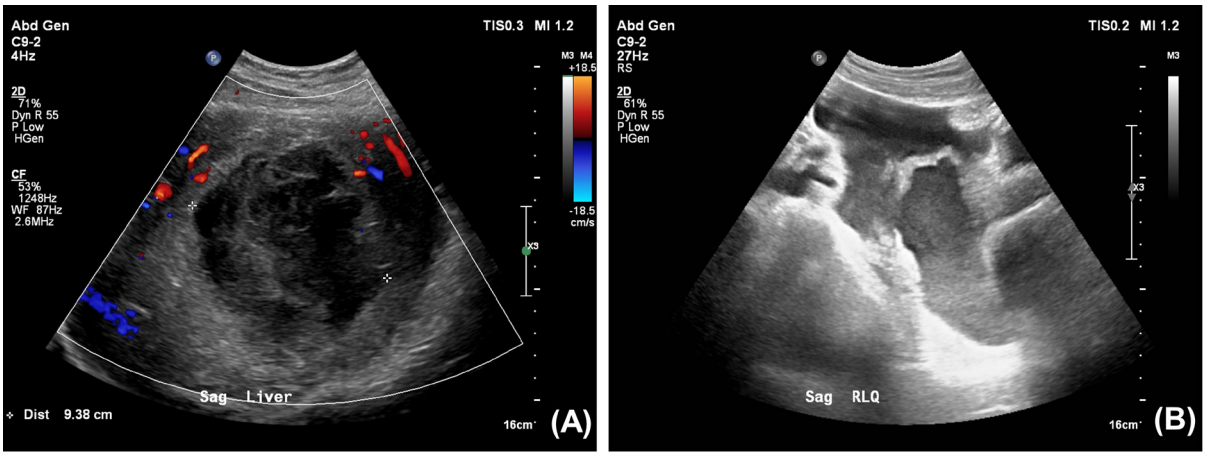

Figure 1. Ultrasound of the abdomen revealed a poorly defined non-vascular hypoechoic lesion (**A**) in the right lobe of the liver close to the liver capsule. Additionally, turbid free fluid was noted in the pelvis in the right lower quadrant (**B**) in the posterior cul-de-sac raising the possibility of intrabdominal hemorrhage.

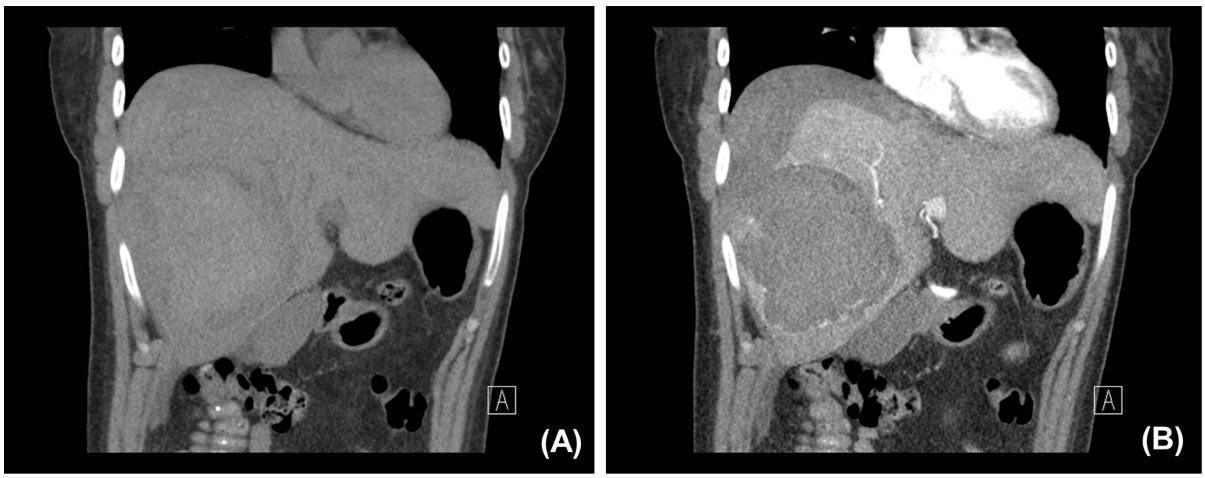

Figure 2. Non-contrast coronal reformatted CT image (**A**), showing non-cirrhotic liver with a 10 cm hyperdense lesion occupying the majority of the right lobe of the liver, in addition, subcapsular hematoma involving the right lobe of the liver as well as adjacent intraperitoneal hemorrhage into the right subphrenic space as well as into the peritoneal cavity was also identified. Post contrast coronal reformatted CT image (**B**) showing peripheral areas of soft tissue enhancement were demonstrated in a patchy distribution. In addition, a liver capsule defect was clearly visible, confirming lesion rupture and subsequent intraperitoneal hemorrhage.

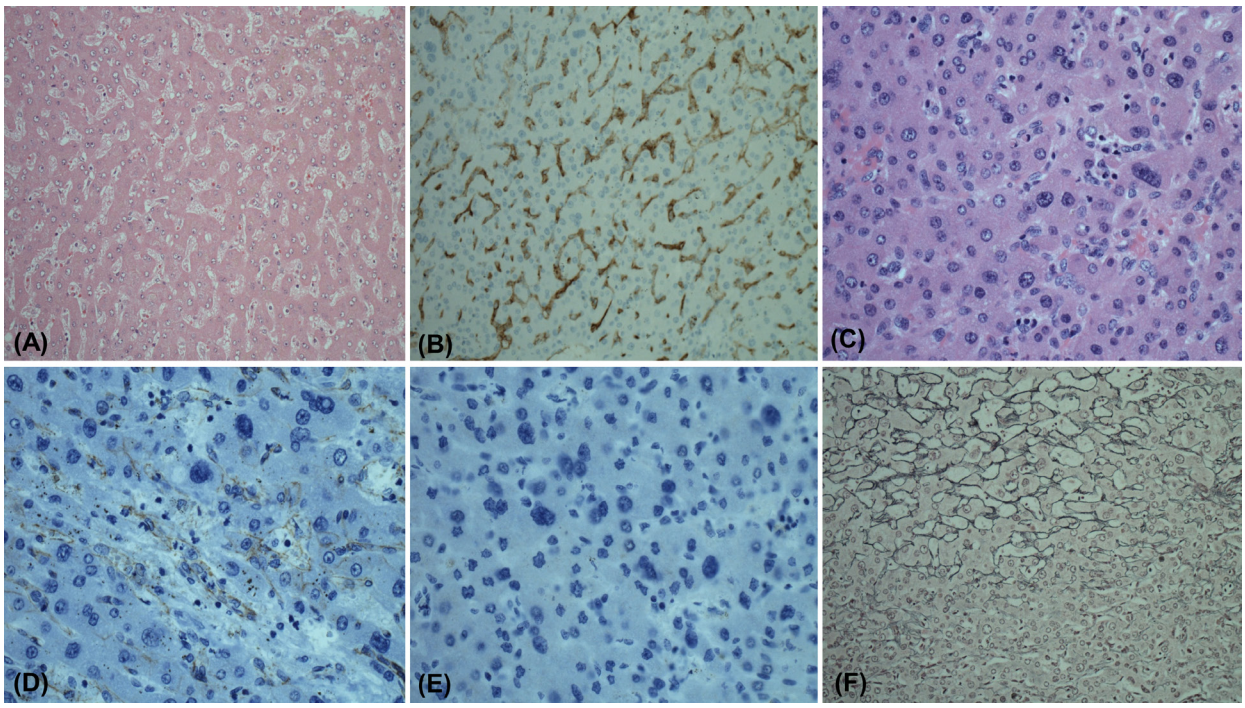

Figure 3. The adenoma at 100× (**A**), section shows liver parenchyma with no intervening portal tracts and normal trabecular pattern, one to two cell width. The HCC 200× (**B**), section shows liver parenchyma with visible marked atypia and patchy expanded trabeculae. Retic interface (**C**), section shows preservation of normal reticulin pattern in the adenomatous area while the malignant area shows reticulin loss. CD34 (**D**), sections positive expression of CD34 indicative of arterialization of the sinusoids. Negative Glypican3 (**E**), section shows malignant area with no expression of Glypican3. Negative Beta Catenin (**F**), sections shows malignant area with no expression of Beta Catenin.

of segment IVa and cholecystectomy was carried out. A final diagnosis of a ruptured poorly differentiated hepatocellular carcinoma arising within a hepatic adenoma was reached on pathological analysis of the resected specimen of the liver (Figure 3A-F).

The patient's postoperative course was unremarkable. She was advised to discontinue OCP usage.

Post-operative follow-up CT done 1.5 months later revealed no residual or recurrent tumor in the liver. A 9 × 5.3 cm fluid attenuating lesion was noted at the site of the hepatectomy margin, consistent with a post-operative seroma. In addition, compensatory hypertrophy of the left lobe of the liver was also noted. Post-operative alpha fetoprotein (AFP) was normal.

3. Discussion

HAs usually tend to be solitary (70-80%) and less commonly multiple (20-30%) (*3*).The term hepatic adenomatosis may be used by some authors when there are ten or more adenomas, and some in the past even considered hepatic adenomatosis to be a separate clinical and histological distinct entity from solitary HAs and multiple HAs (< 10) (*4*). Nevertheless, in recent years the term has lost its individuality and recent molecular classification has shown that the term HA comprises both solitary and multiple adenomas (< 10 and ≥ 10) (*5*). Complications with HAs tend to occur when the tumors outgrow their blood supply and include rupture, hemorrhage, thrombosis, infarction, malignant transformation and rarely even cystic degeneration (*6*).

HAs can be classified into six major molecular subgroups namely: HNF1α inactivated HA, inflammatory HA, b-catenin exon 3 mutated HA, b-catenin exon 7/8 mutated HA, Sonic Hedgehog activated HA and unclassified HA. The b-catenin exon 3 mutated HAs have a greater propensity for malignant transformation, while the Sonic Hedgehog activated HAs are at risk for bleeding (*7*). The Bordeaux group (*8*), identified useful immunohistochemical markers for identifying HA subtypes, namely, β-catenin and glutamine synthetase (GS) for β-catenin activated HA, liver-fatty acid binding protein (LFABP) for HNF-1α inactivated adenomas, serum amyloid A (SAA) and C- reactive protein (CRP) for the inflammatory subtype of HA. However, no markers were identified for the unclassified subtype. Risk factors for malignant transformation of HAs include- large size (> 5 cm), multiplicity, β-catenin mutated subtype of HA and the male gender (*9*).

The commonly used imaging modalities for evaluation of HAs are ultrasound, contrast enhanced CT and magnetic resonance imaging (MRI). Typically, HAs (< 3-5 cm in size) on ultrasound appear isoechoic to background liver parenchyma, but these tumors can appear hypoechoic in patients with steatosis. However, HAs containing glycogen (*e.g.* in patients with GSD) or fat such as in HNF-1α subtype, generally appear hyperechoic. Large HAs (> 5 cm) tend to be

Table 1. Magnetic resonance imaging (MRI) features of various hepatic adenoma (HA) subtypes

HA Subtype	MRI Features
Inflammatory HA	• T1 hypointense and T2 hyperintense • On out-of-phase T1 sequence: may occasionally demonstrate heterogenous loss of signal intensity because of intralesional microscopic fat. • Demonstrate a rim of higher T2 signal at the periphery than the centre of the lesion, termed the 'atoll sign'. • On dynamic imaging appears as a hypervascular mass with hyperenhancement during the arterial phase and persisting hyperenhancement or isoenhancement on the portal and delayed phases. • On hepatobiliary phase: peripheral hyperintensity with central low signal intensity may be seen reflecting the abnormal ductal dilatation and altered biliary excretion.
HNF-1α-Mutated HAs	• T1 hyperintense and T2 iso-hypointense • On out-of-phase sequence: may demonstrate diffuse areas of loss of signal intensity because of intralesional microscopic fat. • On dynamic imaging: appears as a hypervascular mass but to a lesser extent than inflammatory HCAs, and may become hypointense (washout) to liver parenchyma on the portal venous and delayed phases. • On hepatobiliary phase: appears homogenously hypointense in almost all cases.
β-Catenin-Mutated HAs	• T1 heterogeneously hypointense and T2 heterogeneously hyperintense • On out-of-phase sequence: may demonstrate heterogenous areas of loss of signal intensity because of intralesional microscopic fat. • On dynamic imaging appears as a homogenous or heterogenous hypervascular mass with persistent or non-persistent enhancement during the delayed phase. • On hepatobiliary phase: can appear hypointense or hyperintense.
Unclassified HAs.	• As imaging experience is limited with this subtype no specific MRI pattern has yet been proposed.

heterogenous in appearance because hemorrhage or necrosis. Colour doppler has poor sensitivity and may demonstrate predominantly peripheral vascularity (*10*). On non-contrast CT, HAs appear as well circumscribed hypodense lesions (because of intra-tumoral fat, chronic hemorrhage or necrosis). In cases of acute hemorrhage, the tumor appears hyperdense. Calcifications may be seen in 5-10% of the cases. On post intravenous contrast administration these tumors show enhancement in the arterial phase becoming isodense to background liver parenchyma on the portal venous and delayed phases, although some lesions can show washout on the portal or delayed phases (*11*). MRI features of HAs can vary depending on the subtype and has been elaborated in detail in Table 1 (*3,12*).

Management strategies for HAs depend on the underlying risk factors and size. In patients on hormone replacement therapies (including estrogen and androgen) complete cessation is recommended as studies have shown a regression rate of almost 80% and even complete resolution of the tumor in some patients on stopping OCPs (*13*). In obese patients, weight loss and even bariatric surgery has proved to be beneficial. For patients with GSD, alterations in dietary habits can cause tumor regression. HAs < 5 cm in size are managed conservatively as they tend to have a benign and uncomplicated clinical course and hence are followed up every 6 months by CT/MRI for the first two years and then annually. In hemodynamically stable patients with bleeding HAs, transarterial embolization (TAE) is considered as the first treatment option, with studies showing a 75% tumor regression rate (*13*). Limited available data has shown that ablative techniques (*e.g.* microwave ablation, percutaneous irreversible electroporation, and thermal ablation) are efficacious for small adenomas (< 5 cm) especially in patients with comorbidities. As per current guidelines, surgical resection is recommended for HAs > 5 cm in size and those adenomas that don't regress or increases in size following OCP cessation during the 6-month follow-up interval (*13*). Surgery is also recommended for HAs demonstrating increase in size, belonging to the β-catenin subtype, rising alpha fetoprotein (AFP) levels, those with features of malignant transformation and HAs in males, as the latter are prone to have the β-catenin subtype. The main indications for liver transplantation include-multiple HAs not amenable to surgical resection with suspicious or confirmed malignant transformation, and the presence of portosystemic venous shunt (*13*).

4. Conclusion

Malignant transformation of HAs is proven and occurs in 4-5% of all cases. Our case is unique, as the HA not only underwent malignant transformation but was further complicated by rupture and intra-abdominal bleed. By identifying the risk factors for HAs and recognizing the imaging characteristics, an early and accurate diagnosis can be reached thereby enabling urgent intervention which could potentially be lifesaving.

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Case Report

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Management of cyclical pelvic pain by multiple ultrasound-guided superior hypogastric plexus blocks in a rare case of Mayer-Rokitansky-Küster-Hauser syndrome - A case series of three blocks in a patient

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Summary **Mayer-Rokitansky-Küster-Hauser syndrome is an uncommon disorder of mullerian agenesis where patients face multiple challenges like difficulty or inability to conceive and have sexual intercourse and chronic abdominal pain. This is a case report of a patient with Mayer-Rokitansky-Küster-Hauser syndrome who presented to the pain clinic with severe cyclical pelvic pain unresponsive to conservative treatment. This case was successfully managed with three ultrasound-guided superior hypogastric plexus blocks. This case illustrates that acute pelvic pain in MKRS patients can be effectively treated with bedside ultrasound-guided superior hypogastric plexus blocks. However, a GnRh analogue or hysterectomy is recommended for definitive treatment.**

Keywords: Superior hypogastric plexus block, ultrasound, Mayer-Rokitansky-Kuster-Hauser syndrome, pelvic pain

1. Introduction

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is an uncommon disorder of mullerian agenesis with a reported incidence of 1 in 4,500 live births and is diagnosed when a female presents with primary amenorrhoea with normal secondary sexual characteristics and a normal female genotype around puberty (*1*). It is caused by embryologic mullerian agenesis or atresia of the vagina, uterus, or both. MRKH is classified as type I or type II (*2*). MRKH type I is restricted to abnormalities of the reproductive system whereas MRKH type II has associated system anomalies (*2*). Patients face multiple challenges like difficulty or inability to conceive and have sexual intercourse; if rudimentary mullerian structures with active endometrium are present, they experience severe cyclical or chronic abdominal pain due to ovulation or endometriosis.

According to searches of PubMed and Google Scholar, management of abdominal pain in these patients has not been previously described. Reported here is the case of a patient with MRKH who presented to the pain clinic with severe cyclical pelvic pain unresponsive to conservative treatment. This case was successfully managed with repeated ultrasound-guided superior hypogastric plexus blocks.

2. Case Report

A 27-year-old married nulliparous female with a weight of 50 kg and height of 155 cm who had previously been diagnosed with MRKH type II was referred to the pain clinic for management of pelvic pain. She had a history of cyclical pain of 3-4 days duration every month. The pain was severe (8-9) on a numerical rating scale (NRS), aching in nature, infraumblical, and radiated to the back and perineal region. Pain was not present in any other region of the body. There was no relief after oral antispasmodic and non-steroidal anti-inflammatory drugs and partial relief with intravenous diclofenac.

The patient's past history indicated that pubarche occurred at age 12 with primary amenorrhoea and

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complete development of secondary sexual characteristics. She underwent vaginal dilatation at another hospital a year earlier, after which she developed a rectovaginal fistula and subsequently underwent diversion colostomy. Contrast-enhanced computerized tomography (CECT) of the abdomen revealed an ectopic right kidney with a left renal cortical cyst and uterine agenesis with an absent right ovary. Routine blood tests including serum estradiol and levels of follicular stimulating hormone, luteinizing hormone, and testosterone were within normal limits.

The woman was seen as an outpatient by Gynecology at this Hospital for severe pelvic pain (8 on the numerical rating scale). Pain had started in the morning and was not relieved with oral pain medications or injectable diclofenac. She was referred to the pain clinic for further management. A bedside ultrasound-guided superior hypogastric plexus block was planned for immediate pain relief. An intravenous line was inserted, and routine monitors were attached. Her heart rate was 120 beats/minutes and blood pressure was 134/84 mmHg. The procedure was explained to the patient and consent was obtained. She was then placed in a supine position in an sterile environment. A 2-5 MHz curvilinear probe (FUJIFILM SonoSite Edge) was used to identify the division of the abdominal aorta into common iliac arteries, which occurs at the fifth vertebral body. After subcutaneous local anesthesia with 2% lignocaine, a 9-cm 22-gauge spinal needle was inserted 3 cm below the umbilicus *via* the out-of-plane technique until bony contact at the anterior part of the fifth lumbar vertebral body. After hitting the vertebral body, the needle was withdrawn 1-2 mm, and 20 ml of 0.2% ropivacaine and 30 ug of clonidine was given after negative aspiration. The uniform spread of each drug was confirmed sonographically. Post-procedure, pain on the NRS decreased to 1-2. Her pulse rate decreased to 88 beats/min and blood pressure was 110/70 mmHg. She was observed in the recovery room for two hours. Her vital signs remained stable. She was satisfied after the procedure and did not complain of excessive sedation or drowsiness. There was no bruising or soreness at the injection site.

She was discharged after two hours and remained pain free for the full month but was seen again after a month with pain of 8-9 on the NRS. The block was repeated using a similar procedure and dose (Figure 1 and Figure 2). She had immediate pain relief post-procedure. MRI revealed slight deviation of uterine tissue to the left with an endometrial thickness of 5.8 mm. The right ovary and atretic cervix and upper vagina were not evident. MRI findings differed from those of a previous MRI at another hospital that found no uterine tissue. She was advised to receive injectable leuprolide (a GnRH analogue) to decrease the plasma level of estradiol in order to provide longer relief of abdominal pain. After a month, she was seen again

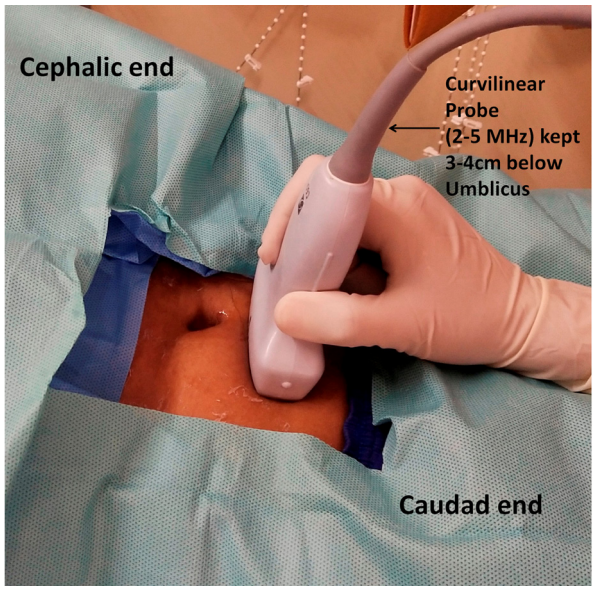

Figure 1. Curvilinear ultrasound probe (2-5MHz) kept in the transverse plane 3-4 cm below the umbilicus.

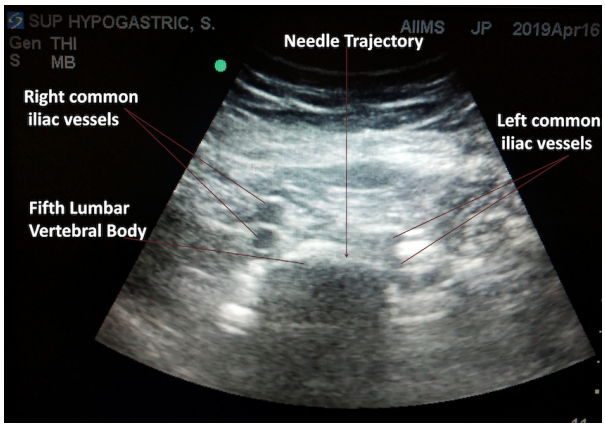

Figure 2. Transverse ultrasound image showing the body of the fifth lumbar vertebra along with the common iliac vessels and needle trajectory out-of-plane.

for a third time with pelvic pain of 7 on the NRS. A third block was similarly administered with ultrasound guidance and yielded excellent results. The patient has been followed up for the last three months and has been pain-free.

3. Discussion

MRKH syndrome is a rare condition with a reported incidence of 1:4,500. It represents the second most common cause of primary amenorrhea (*3,4*). MRKH syndrome is a complex malformation of complete agenesis of any of the structures derived from the paramesonephric müllerian duct (uterus, cervix, and upper 2/3 of the vagina) in females with a normal genotype, phenotype, and endocrine status (*5*). The typical syndrome (type I) is characterized by abnormalities restricted to the reproductive system. MRKH syndrome type II is associated with kidney

abnormalities in 40% of cases, with hearing problems in 10%, and with skeletal abnormalities in 10-12%. The most important step in the effective management of müllerian agenesis is correct diagnosis of the underlying condition, evaluation for associated congenital anomalies, and psychosocial counseling in addition to treatment or intervention to address the functional effects of genital anomalies. Abnormalities in the reproductive tract include a shortened vaginal canal, single midline uterine remnant, or uterine horns (with or without an endometrial cavity). The challenge is to recognize a remnant uterus and rudimentary müllerian structures, which are difficult to interpret on ultrasonography and may be particularly misleading before puberty (*6*). Thus, MRI should always be advised for assessment and should be interpreted by an expert radiologist since identification of uterine remnants is sometimes difficult, as it was in the current case (*7*). Severe abdominal pain is present in 70-80 % of patients. Abdominal pain in these patients is either because of endometriosis from retrograde menstruation due to obstructed uterine horns or monthly ovulation (*2*). Severe abdominal pain in patients with MRKH is described in the literature, but to the extent known no source has described pain management for those patients.

The superior hypogastric plexus (SHP) is located at the aortic bifurcation anterior to the peritoneum (*1,8*). The superior hypogastric plexus is an inferior continuation of the prevertebral sympathetic trunk and extends from the level of L-4 to S1. Primary visceral afferent nociceptive fibers from the upper vagina, cervix, uterus, fallopian tubes, bladder, and right colon travel through the SHP to the dorsal horn of the spinal cord. An SHP block (SHPB) is generally performed for relief of pain arising from lower abdominal structures (*9*). It is usually performed under fluoroscopy and is reported to be effective in relieving about 70-80% of pain (*9*). However, a special area is required for this. Other disadvantages include radiation hazards, discitis in a transdiscal approach, and inability to identify vascular and other peritoneal structures. Recently an ultrasound-guided technique for SHPB has been described for patients with cancer (*10*). The procedure was verified in a cadaveric study and confirmed using fluoroscopy (*11*). The advantages are that it is simple, fast, and non-invasive, it causes less pain since it is performed in the supine position, and it spares somatic nerve roots. To minimize the risk of injury to the bowel, patients are advised to take a tablet of dulcolax a day prior to the procedure. This same protocol was followed in the current case for every block except the first.

A previous report described successful management of interstitial cystitis in these patients *via* SHPB using pulsed radiofrequency (*12*). For chronic pelvic pain, ablation of the uterosacral nerve *via* a laparoscopic or open technique has also been reported (*13,14*). PubMed and Google Scholar were searched using the keywords "superior hypogastric plexus block," "ultrasound," and "chronic abdominal pain," but yielded no hits regarding SHPB for noncancer abdominal pain in females with MRKH syndrome. In the current case, cyclical abdominal pain did not respond to conventional oral or intravenous pain medications, but an SHPB provided complete pain relief. An immediate bedside ultrasound-guided superior hypogastric plexus block was performed since cyclical pain was present for only 2-3 days, and delay of another day would have been inappropriate in this patient.

Injectable leuprolide, a GnRh analogue, prolongs activation of GnRH receptors, which leads to desensitization and consequent suppression of gonadotrophin secretion and results in good pain relief when pain is secondary to endometriosis. However, inability to identify uterus remnants is common, as in the current case, so this treatment is frequently delayed. Definite treatment of cyclical abdominal pain is removal of the uterus, for which the current patient is scheduled.

In conclusion, acute pelvic pain in patients with MKRH syndrome can be effectively treated with a bedside ultrasound-guided superior hypogastric plexus block. However, a GnRh analogue or hysterectomy is recommended for definitive treatment.

Informed consent: Informed consent was obtained from the patient prior to publication of this article

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Case Report

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A case of recurrent progressive multifocal leukoencephalopathy after human stem cell transplant, with detection of John Cunningham virus and human herpesvirus 6 on cerebrospinal fluid, treated with Mirtazapine, Olanzapine and Foscarnet

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Summary **We reported the case of a John Cunningham virus (JCV) and human herpesvirus 6 (HHV-6) mediated progressive multifocal leukoencephalopathy (PML) after human stem cell transplant, reactivated 6 months later in absence of immunosuppressive therapy, successfully treated with anti-5HT2A receptors agents and antiviral therapy. Few cases of JCV and HHV-6 coinfection associated PML are described in literature and the role of HHV-6 in the pathogenesis and prognosis of PML is not completely clear. Our case suggests that, in a possible PML, the research of HHV-6 and JCV should be always performed on cerebrospinal fluid (CSF) and on blood samples and in case of detection of HHV-6 DNA a "chromosomally integrated human herpesvirus 6" (ciHHV-6) should be excluded. Furthermore we recommend to start an appropriate therapy with antiviral and anti-5HT2A receptors agents in case of possible PML due to JCV and HHV-6 coinfection.**

Keywords: Neurovirology, human herpesvirus 6, CNS infection

1. Introduction

John Cunningham virus (JCV) and human herpesvirus 6 (HHV-6) are two ubiquitous viruses which can be reactivated in conditions immunosuppression, such Natalizumab therapy, HIV or human stem cell transplant (HSCT) (*1-3*). Although HHV-6 reactivation may be common after allogeneic HSCT, it only rarely causes central nervous system (CNS) complications (*4*).

Progressive multifocal leukoencephalopathy (PML) is a rare but fatal disease of the CNS secondary to JCV infection and reactivation (*5*), with an incidence of 35.4 per 100.000 person-years in transplanted patients (*6*), one-year mortality rate of the cases present in literature is 65.4% and mean time of death in the first year estimated at 4.1 months (*3*).

We present a case recurrent PML due to a possible JCV with HHV-6 coinfection after HSCT, successfully treated with Mirtazapine, Olanzapine and Foscarnet.

2. Case Report

A 44 year old Caucasian woman was admitted to our departmet on July 2017 for gradual onset of posterior headache, hearing loss and left homonymous hemianopia.

Remote pathological history was significant for a diagnosis of subcutaneous panniculitis-like T-cell lymphoma in 2013, treated with allogeneic HSCT from an HLA-identical sister in 2015 (conditioning regimen: Tiotepa; Busulfan and Fludarabine), complicated with Chronic Graft versus Host Disease (GVHD), treated with Corticosteroids, Mycophenolate Mofetil, Cyclosporine-A, FEC (Fluorouracil, Epirubicin, and Cyclophosphamide) and Imatinib with no clinical benefit. Tacrolimus, started in february 2017, with a

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partial response.

A brain magnetic resonance imaging (MRI) showed non-enhancing T2 hyperintensities in the right occipital lobe, ipsilateral temporal lobe, thalamus and internal capsule with restricted diffusion signal (Figure 1a, 1b, 1c, and 1d). Cerebrospinal fluid (CSF) examination demonstrated total protein of 0.75 g/L, normal cell count (2 leucocyties), Polymerase Chain Reaction (PCR) on CSF detected HHV-6 (< 350 copies/mL) and JCV (< 350 copies/mL), PCR on blood sample detected HHV-6 (< 240 copies/mL). PML was diagnosed, a possible JCV and HHV-6 coinfection was suspected, Tacrolimus therapy was discontinued and Mirtazapine and Foscarnet were administered. Immunophenotype on blood serum showed an inversion in CD4/CD8 ratio (790/uL÷1710/uL). On discharge from the hospital only left homonymous hemianopia persisted.

Her clinical picture remained stable for over six months and an MRI performed in October 2017 didn't show any PML pathological activity. On December 2017 she was hospitalized for Influenzavirus B pneumonia and dischrged after 7 days, without sign of infection. On February 2018 she presented to our department symptomatic for headache, chest pain, left arm numbness, nausea and vertigo, nystagmus on left lateral gaze, weakness of both upper limbs, apraxia, dysmetria and left homonymous hemianopia followed by two episodes of myoclonic movements of the left side of the face and left arm occurred. On EEG a focal motor status epilepticus was recorded and successfully treated with Levetiracetam.

A brain MRI showed increased areas of pathological signal, compared to the MRI of October 2017. Besides, new lesions showing restricted diffusion were found, suggesting a re-activation of the pathological process (Figure 1e, 1f, 1g, and 1d).

Immunophenotype on blood serum showed a reduction in CD4+ count, an inversion in CD4/CD8 ratio (330/uL÷1000/uL), severe depression of B lymphocytes (100/uL) and normal NK count.

CSF analysis showed hyperproteinorrachia at 1.57 g/L and normal white blood cell count and oligoclonal bands (OCB) in a 'mirror-pattern'. CSF PCR showed presence of JCV (< 350 copies/mL) and absence of HHV-6. PCR for JCV was also positive in both serum and urine samples. A diagnosis of PML reactivation was established and Olanzapine was administered.

Later she developed respiratory failure due to Pneumocystis Carinii pneumonia, successfully treated with intravenous Trimethoprim/Sulfamethoxazole. There was partial resolution of neurological symptoms as well, on discharge from the hospital only left homonymous hemianopia, left arm weakness and numbness persisted.

A brain MRI performed in April 2018, showed a reduction of the pathological areas on FLAIR sequences and absence of pathological activity on DWI sequences, the MRI performed in November 2018 and April 2019 were stable (Figure 2).

3. Discussion

Although data are limited, some evidence suggest that HHV-6, in conjunction with JCV, infection or reactivation is associated with the demyelinating lesions of PML (*2*).

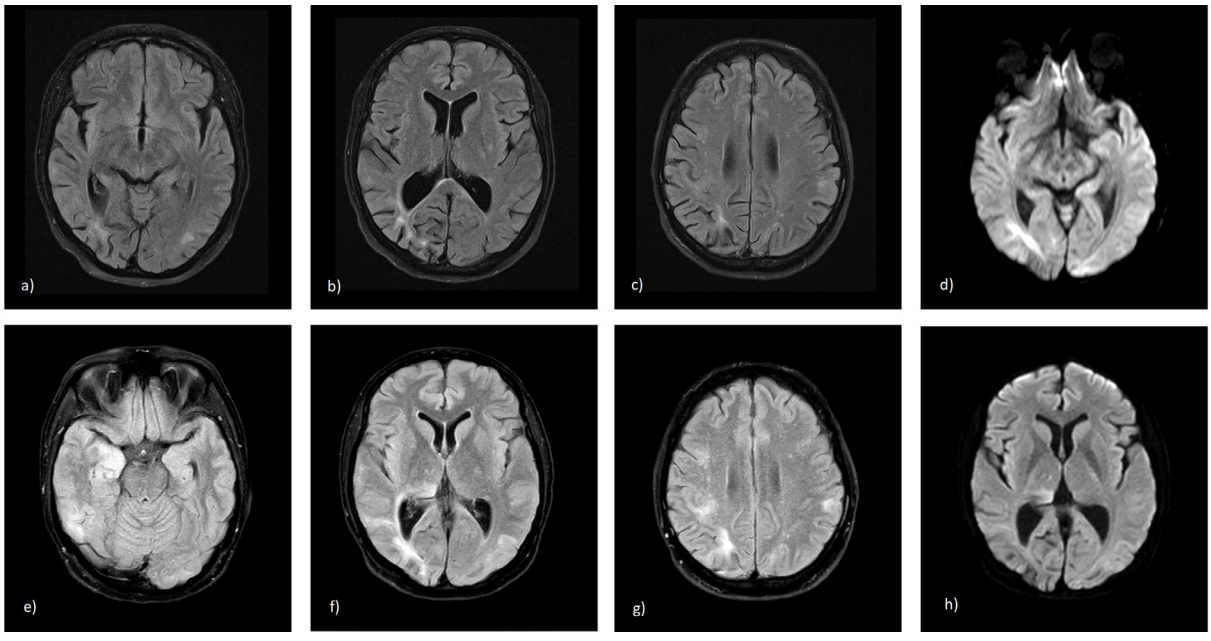

Figure 1. MR imaging of brain of the second hospitalization (**e, f, g, h**), when compared with first hospitalization (**a, b, c, d**) showed an increase in number and volume of the multifocal areas of high signal on FLAIR sequence (**a, b, c, e, f, g**) with restricted patchy diffusion on the axial DWI sequence (**d, h**).

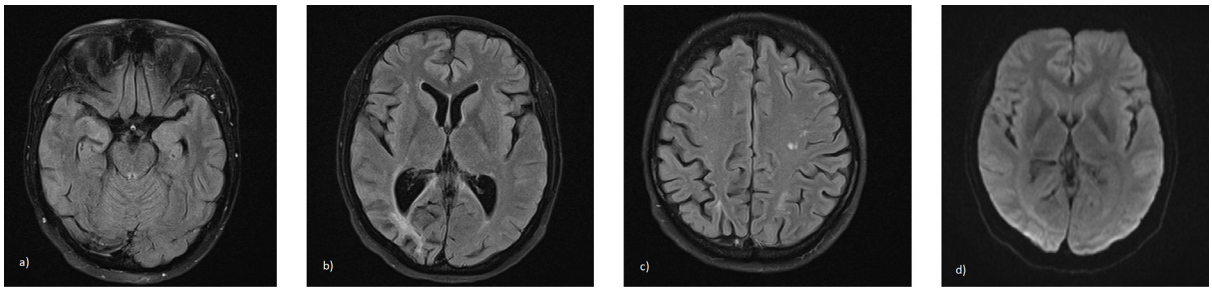

Figure 2. Brain MRI performed after the second hospitalization, showed a reduction of the pathological areas on FLAIR sequences (a, b, c) and absence of pathological activity on DWI sequences (d).

In some case reports JCV and HHV-6 coinfection was demonstrated by two-step in-situ PCR procedure performed on brain tissue samples, and single cases of coinfection of JCV and HHV-6 have been described in PML affected patients (*7,8*). Futhermore, a great frequency of HHV-6 and JCV genomes was present in numerous positively stained cells of oligodendroglial morphology in PML lesional white matter, and no HHV-6 antigens were detectable in adjacent uninvolved tissue in brain tissues of PML patients and in control tissues (*9,10*). Finally Yao demonstrated that superinfection with HHV-6 resulted in increased JCV copy number over time compared to JCV-infected SV40- transformed human glial cell line (SVG cells) alone (*2*).

CSF detection of HHV-6 is an unusual evenience in PML, Nakamichi *et al*. did not find HHV-6 DNA in any of the 299 CSF samples of 255 patients suspected of having PML, 31 of which were JCV positive on CSF (*11,12*). When HHV-6 DNA is detected on blood or other biological fluid, a "chromosomally integrated human herpesvirus 6" (ciHHV-6) must be excluded, this is a condition in which the complete HHV-6 genome is integrated into the telomere of a host cell chromosome, and it can be inherited in a Mendelian manner. Individuals with ciHHV-6 have one or more HHV-6 genomic copies per white blood cell, which corresponds to > 5.5 log10 copies/mL of whole blood, in absence of leukopenia, and the high viral DNA loads persist over time. DNA PCR testing of hair follicles or nails can confirm ciHHV-6 status, because only ciHHV-6 individuals have detectable HHV-6 DNA in these tissues (*13*).

Current prophylaxis and treatment of PML are focused on immune reconstitution, restoration of immune responses to JC virus infection, and eventual suppression of immune reconstitution inflammatory syndrome (*14*). There have been some case reports that have reported successful treatment with antipsychotic agents, such as Ziprasidone, Risperidone, and Olanzapine, as these agents can block the serotonin 5HT2A receptor, which has been shown to be a cellular receptor for JCV on glial cells, according to Elphick and colleagues (*15*).

In our case a brain biopsy and DNA PCR testing of hair follicles or nails was not performed but, the relatively low number of HHV-6 copies in serum and CSF let us suppose an absence of ciHHV-6. For the presence of HHV-6 and JCV on CSF, a PML due to JCV and HHV-6 coinfection was considered and the patient was treated with foscernet associated with anti 5HT2A receptor drugs, namely olanzapine and mirtazapine, with a good clinical outcome, despite the important hematologic comorbilities.

The relatively low number of HHV-6 copies in CSF and serum during the first hospitalization, the good response to therapy with foscarnet and anti 5HT2A receptors drugs and the absence of HHV-6 in CSF in the second hospitalization, associated with literature's findings, can indicate a pathogenetic role of HHV-6 in this patient.

In a possible PML due to HHV-6 and JCV coinfection, brain biopsy and PCR testing of hair follicles or nails are suggested to confirm the diagnosis, but not always possible. In these cases we recommend to consider an ex juvantibus antiviral therapy.

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Case Report

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Anaplastic glioneuronal tumor with *KIAA1549/BRAF* fusion

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Summary **Glioneuronal tumors are usually low-grade and have favorable prognosis. The anaplastic glioneuronal tumor with *KIAA1549/BRAF* fusion has not yet been documented. This article reports a case of glioneuronal tumor with anaplasia and *KIAA1549/BRAF* fusion to illuminate the importance of *KIAA1549/BRAF* fusion in high-grade glioneuronal tumors. A ten-year-old boy presented with one year of headache and three months of blurry vision and proptosis. Ophthalmologic evaluation revealed bilateral papilledema. Magnetic resonance imaging showed a large mixed cystic and solid mass in the left frontal lobe of cerebrum. Histologic analysis demonstrated a neoplasm with pseudopapillary growth pattern, focal necrosis, microcalcification, and brisk mitotic activity with a high Ki67 labeling index of focally up to 20%. Immunohistochemical assessment identified a mixed glial and neuronal neoplastic cell population. Molecular studies revealed a *KIAA1549/BRAF* fusion. The histological and molecular changes are consistent with an anaplastic glioneuronal tumor with *KIAA1549/BRAF* fusion. In view of the fact that the effective, targeted therapies for the tumors with *KIAA1549/BRAF* fusion are available, detection of *KIAA1549/BRAF* fusion for high-grade glioneuronal tumors is clinically helpful.**

Keywords: Glioneuronal tumor, *KIAA1549/BRAF* fusion, anaplasia, central nervous system

1. Introduction

Glioneuronal tumors are a group of neoplasms composed of mixed glial and neuronal cells. According to the WHO classification, this group of tumors includes papillary glioneuronal tumor, rosette-forming glioneuronal tumor, diffuse leptomeningeal glioneuronal tumor, ganglioglioma, dysembryoplastic neuroepithelial tumor (DNT), and desmoplastic infantile ganglioglioma (*1*). These tumors are low-grade, except for anaplastic ganglioglioma (WHO grade III) and a minor subset of diffuse leptomeningeal glioneuronal tumors, and have favorable prognosis.

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Several underlying genomic alterations have been identified in glioneuronal tumors (*1*). Gangliogliomas possess *BRAF* V600E mutation and *BRAF* fusion to *FXR1*, *KIAA1549* or *MACF1*. Reduced expression of *LDB2* gene is also found in the neuronal component of this type of tumors. Anaplastic gangliogliomas harbor *CDKN2A* deletion or gain/amplification of *CDK4*. *PIK3CA* and *FGFR1* mutations as well as *KIAA1549/BRAF* fusion are identified in some of rosette-forming glioneuronal tumors (*2*). Approximate three-fourths of diffuse leptomeningeal glioneuronal tumors show *KIAA1549/BRAF* fusion, and either 1p or 1p19q chromosomal deletion is identified in some of these tumors as well. *BRAF* V600E mutation is documented in approximately 30% of DNTs. Minority of desmoplastic infantile gangliogliomas have *BRAF* V600E mutation. Papillary glioneuronal tumors exhibit *SLC44A1-PRKCA* fusion in a high portion of the cases, and *FGFR1* N546K mutation is also reported in one case of this tumor (*3*).

KIAA1549/BRAF fusion has not been reported in

high-grade glioneuronal tumors so far in the literature. Here, we discuss a case of glioneuronal tumor with anaplasia and *KIAA1549/BRAF* fusion.

2. Case Report

2.1. *Clinical history*

A ten-year-old boy presented to hospital with one year of headache and three months of blurry vision and proptosis. Ophthalmologic examination demonstrated bilateral papilledema. Magnetic resonance imaging (MRI) with and without contrast showed a mixed cystic and solid mass measuring 7.7 × 7.2 × 8.9 cm in the left frontal lobe with significant mass effect upon the left lateral ventricle, 1.7 cm of left to right midline shift, and right lateral hydrocephalus (Figure 1). A near total resection was performed. The resulted tissue was submitted for pathology evaluation. The patient is currently receiving continuous care in an outside hospital and in a stable condition.

2.2. *Histopathology*

Intraoperative cytological study showed mixed cell population including cells with regularly shaped nuclei, moderate eosinophilic cytoplasm, and cell processes (astrocytes); cells with small hyperchromatic nuclei and small amount of eosinophilic cytoplasm, and no obvious cell process (oligodendrocyte-like cells); and cells with round or oval nuclei and scant or inconspicuous cytoplasm (neurocytes). There were no bipolar cells in the cytological smear preparation. Histological examination of the tumor using hematoxylin and eosin staining revealed a hypercellular neoplasm with diffuse pseudopapillary growth pattern. The neoplastic cells around hyalinized vessels were flattened or cuboidal with moderate nuclear pleomorphism and cell processes and in single or pseudostratified layer overlying hyalinized vascular cores. In the interpapillary area, there were small to medium size neoplastic cells, and some of them had eccentrically located nuclei and eosinophilic cytoplasm. There were no definitive nucleolus-bearing dysplastic ganglioid cells in the specimen examined. Multifocal microcalcifications were present, and scattered hemosiderin-laden macrophages were noted. Focal necrosis was evident. There was focal brisk mitotic activity in the tumor. Neither Rosenthal fibers nor eosinophilic granular bodies were present. Immunohistochemical studies revealed that Ki67 labeling index was diffusely increased with several foci of up to 20%. Neoplastic cells mostly surrounding the vascular cores co-expressed glial fibrillary acid protein (GFAP) and S100 (data not shown), while neoplastic cells mostly residing in the interpapillary area were reactive with OLIG2 antibody. Multiple foci of neoplastic cells majorly located in the interpapillary area expressed synaptophysin, neuron specific enolase (NSE) (data not shown), and NeuN (data not shown). There were no cells expressing neurofilament protein (NFP) by immunohistochemical study. Chromochranin-A expression was absent. There was a tendency that the GFAP-positive cells were more concentrated perivascularly, while the cells expressing synaptophysin, NSE, NeuN, and OLIG2 mostly resided in the interpapillary area (Figure 2).

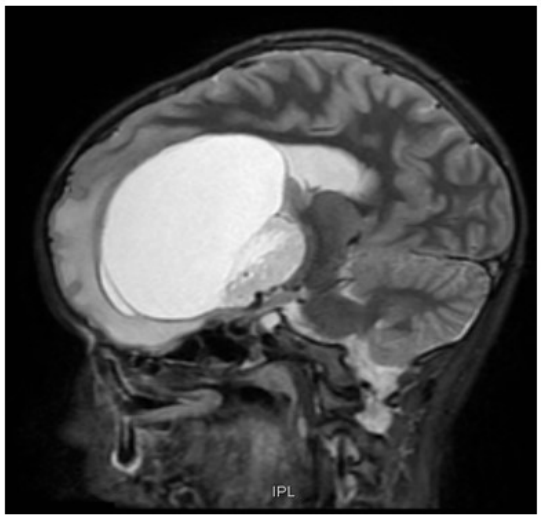

Figure 1. Magnetic Resonance Imaging, T2-weighted, showed a cystic and solid mass in the left frontal lobe.

2.3. *Molecular and cytogenetic study*

Molecular and cytogenetic studies were performed on this case. The next-generation DNA sequencing that evaluates 30 genes for point mutations, small insertions, and deletions as well as 24 genes for copy number changes carried out the molecular studies by the Molecular & Genomic Pathology Laboratory, University of Pittsburgh (*4*). These genes include *IDH1/2*, *ATRX*, *BRAF*, *KRAS*, *PTEN*, *TERT*, *p53*, *AKT1*, *CDK6*, *CIC*, *CDKN2A*, *CTNNB1*, *DDX3X*, *EGFR*, *FUBP1*, *H3F3A*, *HRAS*, *KLF4*, *KRAS*, *MET*, *MYC*, *MYCN*, *NF1/2*, *NRAS*, *PIK3CA*, *PTCH1*, *RB1*, *SETD2*, and *SMO*. This sequencing also screened for 16 subtypes of *BRAF* and *FGFR3* gene fusions as well as *EGFRvIII* structural alterations. Methylation status of the *MGMT* gene promoter was assessed with methylation specific polymerase chain reaction (MS-PCR) by the Molecular & Genomic Pathology Laboratory, University of Pittsburgh. Cytogenetic studies for chromosomal abnormalities, particularly 1p and/or 19q deletion, were performed using the nuclear in situ hybridization technique by the Cytogenetics Laboratory, Robert Wood Johnson Medical School. The above studies demonstrated a *KIAA1549/BRAF* fusion. There were no other molecular and cytogenetic alterations (Table 1).

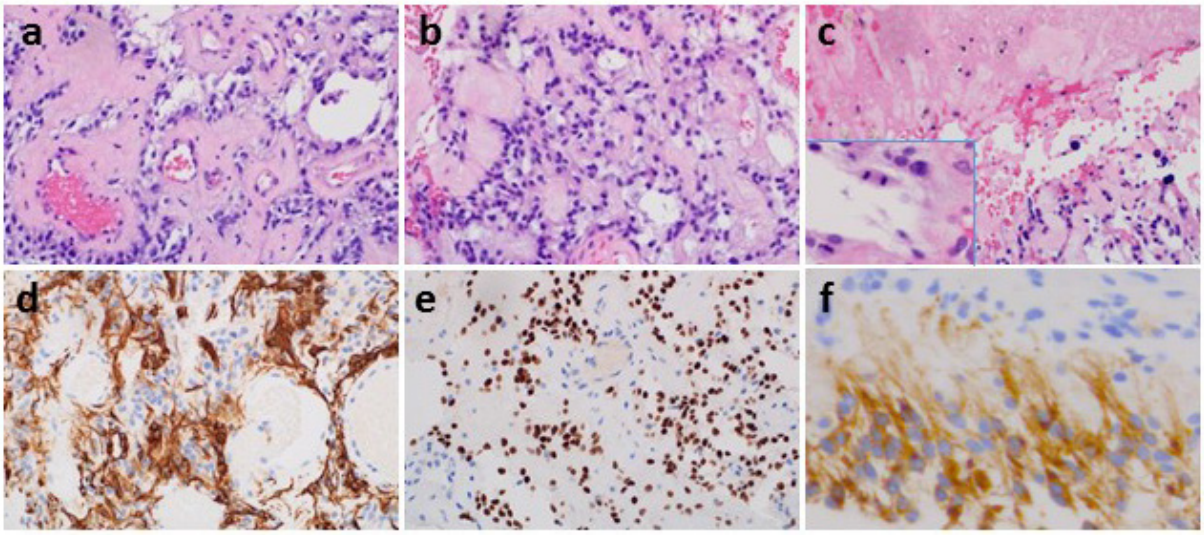

Figure 2. The lesion showed a cellular tumor with mixed cell population, pseudopapillary growth pattern (**a** and **b**), focal necrosis (**c**), and brisk mitotic activity (insert [3 mitotic figures] in **c**). Immunohistochemical studies demonstrated a mixed glial and neuronal cell population (**d-f**). **a-c**) H+E, 400×; **d**) GFAP immunostaining, 400×; **e**) OLIG2 immunostaining, 400×; **f**) Synaptophysin immunostaining, 600×.

Table 1. Molecular and cytogenetic study

Test	Result
Next-generation DNA Sequencing (GlioSeq)	*KIAA1549-BRAF* fusion
Methylation-specific PCR	Negative for MGMT promoter hypermethylation
Nuclear in situ hybridization	Negative for 1p and/or 19q chromosomal deletion

3. Discussion

The histopathological and molecular findings of this tumor are consistent with an anaplastic glioneuronal tumor with *KIAA1549/BRAF* fusion. Previous studies have revealed that the primary central nervous system (CNS) tumors can harbor *KIAA1549/BRAF* fusion (*5*). The *KIAA1549/BRAF* fusion has been considered as a characteristic genomic alteration of pilocytic astrocytoma in recent years. However, our case does not have the essential histological features such as bipolar glial cells, Rosenthal fibers, eosinophilic granular bodies, and unique biphasic histologic pattern to satisfy the classification of pilocytic astrocytoma. Instead, this tumor contains significant glial and neuronal components and exhibits a diffuse pseudopapillary growth pattern, which is more like those seen in a papillary glioneuronal tumor. Interestingly, the *KIAA1549/BRAF* fusion is also commonly seen in glioneuronal tumors. However, up to date, this genomic change has only been identified in several low-grade glioneuronal tumors such as ganglioglioma, rosette-forming glioneuronal tumor, and diffuse leptomeningeal glioneuronal tumor (*1,2,6*). Our case demonstrates that the *KIAA1549/BRAF* fusion exists in a high-grade glioneuronal tumor.

The v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) gene is located at chromosome 7 (7q34) and encodes BRAF protein. The latter is a serine/threonine protein kinase in the downstream of RAS-RAF-MEK-ERK signaling pathway, also known as MAPK/ERK pathway, and a signal transducer between extracellular growth stimulus and cellular response. Three RAF proteins (ARAF, BRAF, and CRAF/c-RAF-1) are the first activators of RAS downstream. RAS-GTP association and binding of RAS-binding domain to the N-terminal regulatory region of RAF kinase result in recruitment of the RAF proteins to cell membrane and structure alteration of the RAF protein, leading to RAF activation. RAF subsequently activates MEK and ERK via phosphorylation, which in turn activates transcription factors Elk-1, c-Fos, and c-Myc; promoting cellular differentiation and proliferation (*7*). BRAF is the most potent activator of MEK/ERK. Regulation of the MAPK/ERK pathway is important for the balance between extracellular signaling and gene transcription. Dysregulation of this pathway leads to tumorigenesis (*8*). The *KIAA1549/BRAF* fusion results in a tandem duplication involving chromosome 7q34. This fusion retains the C-terminal BRAF protein kinase and the substituted N-terminal which does not have the BRAF auto-regulatory domain, leading to constitutive activation of the BRAF kinase domain and hyperactivity of the MAPK/ERK pathway (*9*).

The clinical prognostic significance of *KIAA1549/BRAF* fusion for primary brain tumors seems to be dependent on histological type, location, and age of diagnosis. There is a debate over the clinical relevance of *KIAA1549/BRAF* fusion status. Some studies have reported better clinical outcome for a pediatric low-grade astrocytoma with *KIAA1549/BRAF* fusion (*10-12*), while others failed to find similar results (*9,13,14*). Despite the inconclusive prognostic significance of *KIAA1549/BRAF* fusion, detection of its presence is still useful in view of that the effective, targeted therapies for *KIAA1549/BRAF* fusion are clinically available (*15*).

Acknowledgements

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Letter

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A curious case of disseminated cysticercosis in an immunocompetent adult

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Summary **Cysticercosis is an infection with the larval stage of *Taenia Solium* which is estimated to affect over 50 million people worldwide. We report a case of disseminated cysticercosis in an immunocompetent 68-year-old male who presented with back pain, presumed to be musculoskeletal in nature initially. Magnetic-resonance-imaging of the lumbar spine revealed intramuscular (paraspinous and psoas muscles) cysts, innumerable small cystic lesions bilaterally throughout the cerebellar and cerebral hemispheres, midbrain, and right ventricle suggestive of cysticercosis. Treatment with albendazole with dexamethasone for 3 months led to resolution of the cysts with complete resolution of symptoms. Despite its importance, current data on prevalence of this infection, disease burden and the incidence of hospitalization remains incomplete. Mandatory reporting of diagnosis would enable complete understanding of epidemiology of the disease. In this case we have emphasized the importance of early diagnosis of a systemic condition that could have caused serious implications if left untreated.**

Keywords: Disseminated, cysticercosis, immunocompetent

Cysticercosis is an infection with the larval stage of pork tapeworm, *Taenia solium*. It is estimated to affect over 50 million people worldwide (*1,2*). It is common among immigrant population in the United States (US), with > 2,000 cases per year leading to hospital charges of nearly 100$ million per year. Endemic regions include Sub-Saharan Africa, Latin America and some parts of Asia (*3-6*). The usual sites of involvement are the central nervous system (97.46%), the eye (1.4%), skeletal, heart muscles, skin and subcutaneous tissue (1.14%) (*7*).

Here, we report an uncommon case of disseminated cysticercosis (DCC) in immunocompetent adult. A 68-year-old immigrant man presented to primary care clinic with chronic back pain with an acute worsening in the past four weeks. His pain started in his left hip with radiation to left lower leg along with associated numbness and tingling, and weakness. He denied any fever, chills, saddle anesthesia, bladder incontinence, or bowel incontinence. He has no other medical co-morbidities. Review of symptoms was non-significant. Vital signs were normal. On physical exam he had an antalgic gait with tenderness along his left paraspinal muscles with limited extension and flexion of the left leg. The straight leg test was performed and was positive at 20 degrees in addition to decreased strength (4/5) in his hip flexors and quadriceps in the left leg. Complete blood count with differential and basic metabolic panel were within normal limits. Further workup with magnetic resonance imaging (MRI) of the lumbar spine revealed L5 disc protrusion compressing the nerve root in addition to intramuscular (paraspinous and psoas muscles) cysts suggestive of cysticercosis (Figure 1A). MRI of the brain revealed innumerable small cystic lesions bilaterally throughout the cerebellar and cerebral hemispheres, midbrain, and right ventricle consistent with neurocysticercosis in the vesicular stage (Figure 1B). Cysts were additionally seen in the posterior neck musculature and the right temporalis muscle. He was started on albendazole 400 mg twice daily with dexamethasone 6mg twice daily for 3 months which he tolerated well. Repeat MRI of brain and lumbar spine two months after his first MRI showed resolution of the cysts (Figure 1C) with complete resolution of symptoms. The Center for Disease Control and prevention has designated cysticercosis as "one of the five neglected parasite infections". Likewise, the World Health Organization has

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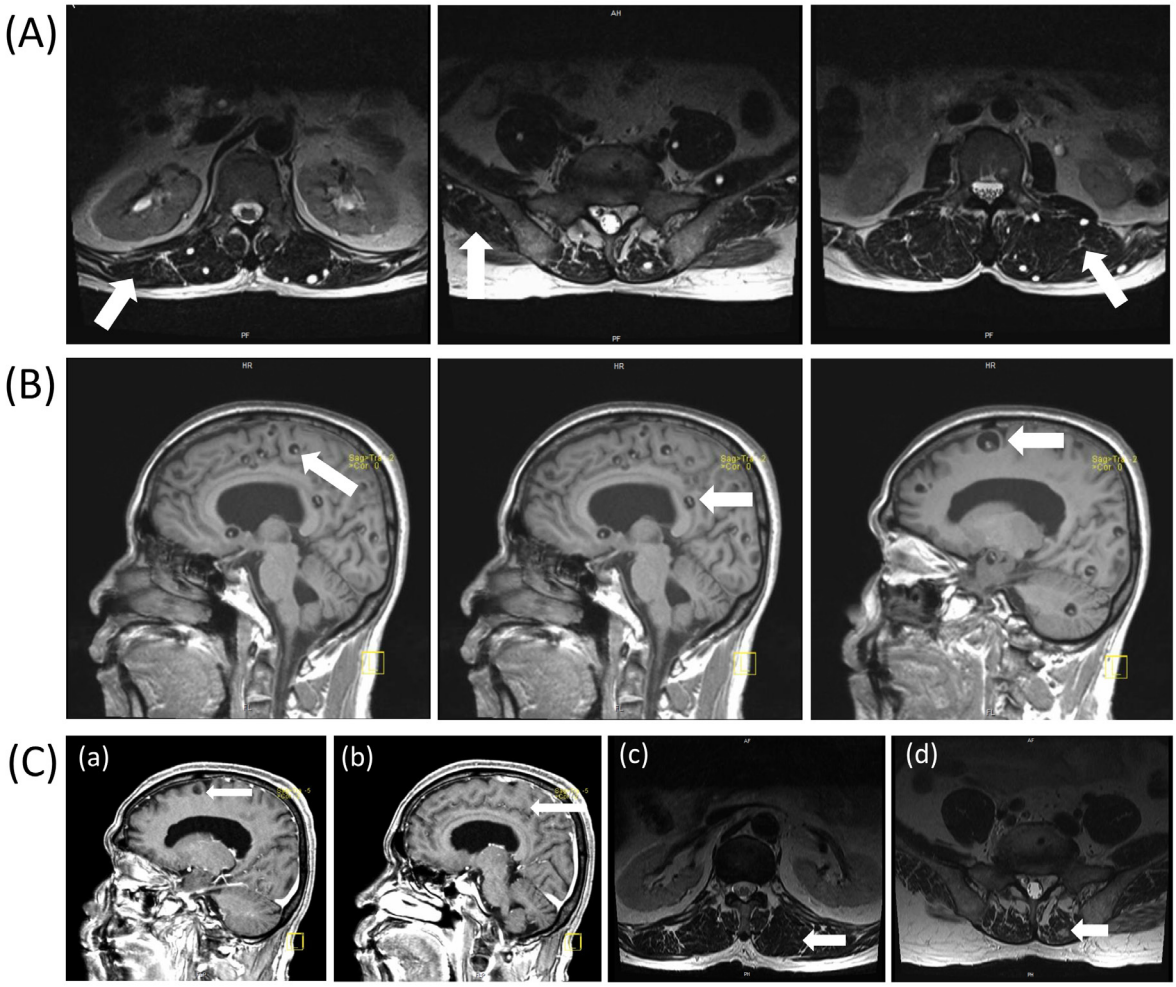

Figure 1. (A) Lumbar MRI shows innumerable intramuscular cysts most suggestive of cysticercosis (red arrows); (B) Brain MRI numerous to count small cystic lesions throughout the bilateral cerebral and cerebellar hemispheres, midbrain, and right ventricle; (C) Brain MRI shows interval decrease in size of the innumerable small cystic lesions throughout the bilateral cerebral and cerebellar hemispheres (a, b); No edema surrounding enhancing lesions. Lumbar MRI shows significant interval decrease in size and number of the innumerable intramuscular cysts (c, d).

designated it as "one of the seventeen neglected tropical diseases" worldwide. A survey was conducted in ED of eleven institutions throughout the US. It was noticed 2.1% of 1,801 patients presenting with seizures were diagnosed with neurocysticercosis. Despite its importance, current data on prevalence of this infection, disease burden and the incidence of hospitalization remains incomplete. Mandatory reporting of diagnosis would enable complete understanding of epidemiology of the disease. In this case we have tried to emphasize the importance of early diagnosis of a systemic condition that could have caused serious implications if left untreated.

DCC is a condition with multiple organ involvement. Although cysticercosis is common, DCC is an uncommon manifestation. It is usually seen in immunocompromised individuals, DCC in immunocompetent state is extremely rare with less than 50 cases being reported in literature, the majority being seen in India. Management of DCC includes albendazole (15 mg/kg/day) for 28 days or praziquantel (10-15 mg/kg/day) for 7-21 days. Further, symptomatic treatment of seizures with antiepileptics and glucocorticoids to decrease the host response and inflammatory changes is recommended.

Our patient was immunocompetent who initially presented with back pain, which was presumed to be musculoskeletal in nature. He was being treated with pain medications along with physical therapy for 4 weeks when he presented again with antalgic gait. At that time spine MRI was done for further evaluation which showed multiple paraspinous and psoas muscle cysts. This emphasize the fact that a high suspicion of disseminated cysticercosis should be considered in immigrant population. When there is suspicion of cysticercosis, a whole body MRI should be done for detection of disease burden and to plan the appropriate therapy accordingly.

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Letter

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Successful anaesthesia management of a child with hunter syndrome for adenotonsillectomy

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Summary **Airway management in a child with hunter syndrome is a challenge to the anesthetists. Various methods to achieve this are reported in literature. Here we describe another method in a three year old male child posted for adenotonsillectomy and myringotomy. After check videolaryngoscopy with C Mac blade size 2, vocal cords were not visible even with various monoevres. Thus a larger blade size 3 was used to place it under the epiglottis after which posterior part of vocal cords became visible and bougie guided endotracheal intubation was successful. Thus we recommend that in a child with hunter syndrome if vocal cords are not visible, a larger blade can be utilized to place under the epiglottis to visualize the vocal cords for successful endotracheal intubation.**

Keywords: Endotracheal intubation, hunter syndrome, airway, C Mac blade

Mucopolysaccharidoses (MPS) is a rare, inherited, lysosomal storage diseases characterized by accumulation of glycosaminoglycans in various tissues including airways which makes airway management a challenging (*1,2*). Amongst the seven types of reported MPS, highest incidence of difficult intubation is encountered in MPS II also called hunter syndrome (*3,4*). Here we describe successful anaesthesia management in a child with hunter syndrome and sleep apnea.

A three year-old, 14 kg male child, diagnosed case of hunter syndrome was scheduled for adenotonsillectomy with bilateral myringotomy and grommet insertion. He presented with breathlessness with lower respiratory chest infection. On admission, child had yellow thick nasal discharge and was breathing through mouth in sitting position. He had respiratory rate of 24/min with bilateral coarse crepitations on auscultation with 96% room air saturation and was receiving oral antibiotics and salbutamol nebulisation. He had history of recurrent chest infections, mouth breathing, snoring, episodes of sleep apnea, frequent night awakenings and delayed milestones. On examination child had large head, coarse facies, macroglossia, receeding mandible and short neck with limited neck extension. He could not sleep in supine position and needed pillow under the shoulders. Enzyme replacement therapy was not being taken by the patient due to financial constraints. Cardiovascular system examination revealed no abnormality and echocardiocardiography was normal. His preoperative routine investigations including hemogram and urine analysis were within normal limits. Computed tomography of head and neck revealed nasopharyngeal airway lumen of 2 mm, grade IV adenoid hypertrophy, grade II tonsillar hypertrophy and no hydrocephalus. Surgery was rescheduled after resolution of chest infection. Risk of difficult airway was explained to parents with informed written consent for tracheostomy.

On the day of surgery, before shifting to the operating room, a 24 gauge cannula was secured on left dorsum of wrist, xylometazoline drops were instilled in both nostrils and nebulization with 3 mL of 2% xylocaine was done. Difficult airway cart was prepared and airway adjuncts were kept ready according to the age and weight of the patient.

Plan A was induction of anaesthesia with sevoflurane in 100% oxygen and a check laryngoscopy

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with a CMAC videolaryngscope under spontaneous breathing. Plan B was intubation with oral pediatric fiberoptic bronchoscope (FOB) under spontaneous ventilation. Plan C was insertion of air Q ILA and FOB guided intubation through it under spontaneous ventilation. If at the time of induction of anesthesia, difficult mask ventilation would be encountered, it was planned to insert air Q ILA as plan A. In case of failure, tracheostomy was planned as the last resort.

In the operating room, video games on phone were shown to the child and standard monitors (pulse oximeter, ECG, BP cuff) were attached. Intravenous glycopyrolate 0.1 mg and ketamine 15 mg were administered and child was placed supine with a roll under his shoulders without a pillow. Inhalational anaesthesia was induced with 100% oxygen at 4 L/min fresh gas flow with gradual increase in sevoflurane concentration by face mask. Size 2 oral airway was inserted for effective mask ventilation. Check laryngoscopy with C-MAC blade 2 revealed cormack lehane (CL) grade III b view with no improvement after optimal external laryngeal manipulation (OELM) (Figure 1A). 10% lignocaine was sprayed over epiglottis, arytenoids and below the epiglottis to anesthetize vocal cords (Figure 1B) and bag-mask assisted spontaneous ventilation was resumed. Repeat laryngoscopy was performed with CMAC size 3 blade which was now kept below posterior surface of epiglottis to reveal posterior part of larynx. Bougie guided endotracheal intubation with size 5mm south pole preformed cuffed endotracheal tube was successfully inserted into trachea at first attempt (Figure 1C). Bilateral air entry was confirmed and atracurium 7 mg was administered. ETT was fixed in midline and stomach was decompressed with a suction catheter. Lungs were ventilated with volume control mode 150 mL tidal volume with oxygen:air (FiO2 0.5) and 2-5% sevoflurane dial concentration to achieve 1.0-1.3 MAC. Intraoperatively peak airway pressure remained at 20 mmHg. Intravenous dexamethasone 4mg and paracetamol 200 mg were administered for analgesia. At the end of surgery, no active bleeding from tonsillar fossa was ensured, gauze soaked with 0.25 % ropivacaine was kept in peritonsillar bed bilaterally for a minute. Surgery lasted for one hour and was uneventful. Sevoflurane was switched off, and at start of spontaneous ventilation; muscle relaxation was reversed with injection neostigmine and glycopyrrolate. After achieving adequate tidal volume, trachea was extubated when child was fully awake and placed in lateral position. He was shifted to high dependency pediatric unit for overnight monitoring which was uneventful. Postopertive analgesia was managed with paracetamol and ketoralac. The child was discharged after three days and was asked to follow up in the genetics clinic of our hospital for further management. Consent from the parents was obtained for publishing this case report.

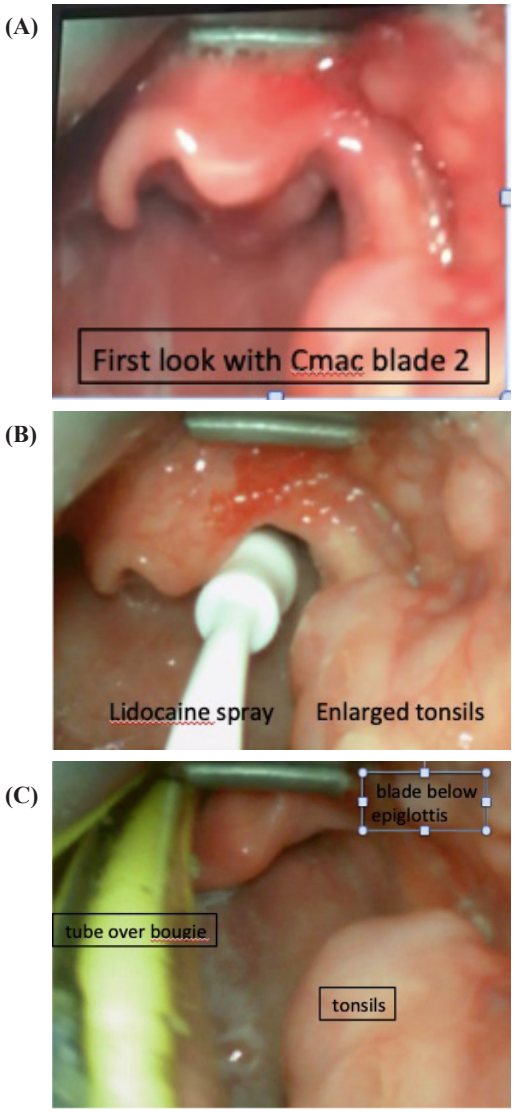

Figure 1. (A), CL IIIb view with Cmac blade 2 with OELM; (B), Lignocaine spray around vocal cords; (C), Visible posterior larynx with Cmac blade 3 kept below epiglottis.

Difficulty in airway management reported in these patients include endotracheal intubation with a smaller size ETT after multiple attempts at intubation, stylet guided endotracheal intubation, view of CL grade 3 or 4 on laryngoscopy, ill fitting LMA, ineffective ventilation with LMA, difficult or unsuccessful FOB guided endotracheal intubation and obstructed laryngeal inlet due to incidental epiglottis polyp covering the larynx (*5-10*).

In the present patient as laryngoscop view of CL grade IIIb did not improve with OELM due to large and fixed epiglottis, we planned to lift posterior surface of epiglottis with blunting of airway reflexes with topical lignocaine. During second laryngoscopy with size 3 C Mac blade posterior surface of epiglottis was lifted along with OELM which helped to visualize posterior part of glottis and bougie guided endotracheal intubation was successful. Ketamine administration helped in

maintaining spontaneous ventilation and deepening of anaesthesia without respiratory depression.

To conlcude, airway in a patient of hunter syndrome can be secured by placing the a larger videolaryngoscope blade at posterior surgace of epiglottis to view posterior part of larynx to facilitate bougie guided intubation. An opioid free anesthesia is helpful to prevent postoperative respiratory obstruction due to presence of airway abnormality and sleep apnea.

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