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Access to orphan drugs in the Middle East: Challenge and perspective

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Summary An orphan drug is a drug developed specifically to treat a rare medical condition. With a combined population of less than 400 million, about 2.8 million patients are estimated to be suffering from a rare disease in the Middle East. Some disorders such as hemoglobinopathy, glucose-6-phosphate dehydrogenase deficiency, autosomal recessive syndromes, and several metabolic disorders have a presence throughout the Middle East. In order to promote the treatment of these diseases, Middle Eastern governments need to facilitate education and training of healthcare personnel; develop and execute a method for obtaining and paying for orphan drugs; and, finally, provide tax, marketing, and other incentives to domestic and international firms to develop drugs specifically for the diseases of most importance to Middle Eastern patients.

Keywords: Orphan drug, rare disease, genetic disorder, Middle East

1. Introduction

An orphan drug is a drug developed specifically to treat a rare medical condition (1). Because the high cost of drug development tends to discourage pharmaceutical companies from developing products for very small populations of patients, public-sector involvement becomes critical to the success of orphan-drug markets. Legislation has been implemented by the United States, the European Union, Japan, Singapore, Taiwan, South Korea, and Australia that offers subsidies and other incentives to encourage the development of orphan drugs (2). Some companies, such as Genzyme, acquired by Sanofi-Aventis in 2011, have even thrived under such legislation, focusing their efforts on developing treatments for rare diseases as a profitable business strategy (3). Myozyme (alglucosidase alfa, recombinant human GAA) and Lumizyme (alglucosidase alfa), both Genzyme products and the first two therapies available for Pompe disease, were approved as orphan drugs by the U.S. Food and Drug Administration (FDA) in 2006 and in 2010, respectively (4,5). They significantly improve survival for those patients suffering from this rare condition, affecting 5,000-10,000 people worldwide (6). Other recently approved orphan drugs include Glaxo’s Lexiva (fosamprenavir) for HIV infection, Genzyme’s Fabrazyme (agalasidase beta) for Fabry disease, and Novartis’s Visudyne (verteporfin) for age-related macular degeneration (2).

As high-quality healthcare becomes a growing priority in developing countries, it is not surprising to see a rising interest in rare diseases and potential treatments in those countries as well. Public awareness of rare diseases is growing in China, where at least 10 million people (out of over 1.3 billion), i.e., approximately 0.7% of the Chinese population, are estimated to be living with osteogenesis imperfecta, Fabry disease, hemophilia A and B, albinism, acromegaly, and other rare conditions (7). At this point in time, however, Chinese patients do not have good access to orphan drugs, nor are Chinese pharmaceutical companies participating in new orphan drug development (7). Meanwhile, rare-disease patients and their advocates in a number of Middle Eastern countries are finding themselves in a similar situation to those in China. As living standards improve in the Middle East, healthcare providers face higher expectations for better quality healthcare products and services (8). With a combined population of less than 400 million, about
2.8 million patients are estimated to be suffering from a rare disease in the Middle East (7,9).

2. Rare diseases in the Middle East

The Middle East, in its "narrow" definition, consists of 16 countries (in declining order by population: Egypt, Iran, Turkey, Iraq, Saudi Arabia, Yemen, Syria, United Arab Emirates or UAE, Israel, Jordan, Lebanon, Oman, Kuwait, Qatar, Bahrain, and Cyprus) plus the Palestinian territories of the West Bank and the Gaza Strip. Using the 0.7% prevalence rate for China and the United Nations' population estimates for countries in the Middle East, estimated numbers of patients with rare diseases are shown for the Middle Eastern countries in Figure 1.

The population of the region is characterized by large family size, older maternal and paternal age, and a high rate (25-60%) of consanguineous marriages (10). Hence, the risk for genetic disorders may be higher than in other regions of the world. Indeed, such disorders account for the majority of rare diseases in the Middle East and are responsible for the lion's share of infant mortality, morbidity, and handicaps in Arab countries (10). Genetic disorders such as hemoglobinopathy, glucose-6-phosphate dehydrogenase deficiency, autosomal recessive syndromes, and several metabolic disorders have a presence throughout the Middle East (10). Patients and their advocates have pushed for awareness of hypoparathyroidism (lack of parathyroid hormone) (11) and beta thalassemia (a blood disorder that reduces the production of hemoglobin) (12). Other genetic disorders, e.g., glutaric aciduria type I (an organic acid disorder where individuals cannot metabolize the amino acids lysine, hydroxylysine, and tryptophan), may be more specific to certain countries and subpopulations (in this case, Israel) (13).

Yet other rare diseases may not have a genetic cause but rather result from viral or bacterial infections or allergies. Behçet's disease, characterized by genital ulcers, skin lesions, and uveitis, though very rare in the United States, is more common in the Middle East and...
Asia, suggesting a tropical-area cause (14). The lichen planus pigmentedus presents with hyperpigmented, dark-brown macules in sun-exposed areas of the body and is more common in the Middle East than in Europe. It may be caused by a viral infection or topical agent (15). Leishmaniasis, a sand-fly-transmitted disease, leads to symptoms ranging from cutaneous lesions to fatal visceral disease. It has been designated as one of the most neglected tropical diseases by the World Health Organization (16). Pemphigus is the general designation for a group of autoimmune skin diseases that cause ulceration and crusting of the skin (17). It is present in people of Middle Eastern or Jewish descent (18).

3. Diagnosis of rare diseases in the Middle East

Orphan diseases are so rare that a physician will not observe a case often. In order to diagnose accurately a rare disease, doctors rely on the published literature and rare-disease registries, which vary considerably in volume and availability across rare diseases. Misdiagnosis or delayed diagnosis is very risky for rare-disease patients. For example, delaying the treatment for infantile-onset Pompe disease until the patient is 6 months old is already too late (19). Another problem with diagnosis is that there is no special coding system for rare diseases. The International-Classification-of-Diseases system that is used in most countries is not suitable for rare diseases. The absence of a universally recognized coding system is an obstacle for reliable registration of patients in national or international databases (20).

On top of the worldwide difficulty in diagnosing rare diseases, Middle Eastern countries generally have a shortage of trained medical professionals, partly due to the lack of medical schools in some of the countries and partly due to limited training in certain medical specialties including diagnostic medicine. The growing demand for physicians and other medical workers is currently being met in the Middle East partly by expatriates from the West, as well as from the Indian subcontinent and the Philippines, all of whom are unlikely to be trained in Arab rare diseases (8). However, Bahrain employs a relatively high proportion of nationals in healthcare; Dubai has attracted Harvard Medical School to Dubai Healthcare City where nationals will be trained; and Qatar is building a specialty teaching hospital run in association with Weill Cornell Medical College. Saudi Arabia is sending nationals abroad for training while it builds more teaching hospitals with the help of private investment (8). Basic training and continuing professional development are needed to ensure that all doctors have the ability to detect a rare disease, especially one more likely to occur in the Arab countries.

Meanwhile, efforts are being organized to keep track of patients with rare diseases. The Centre for Arab Genomic Studies (CAGS) launched a pilot project to construct the Catalogue of Transmission Genetics in Arabs (CTGA) database. This database helps Middle Eastern governments educate the medical community and raise public awareness in at-risk populations (21). In addition, Kuwait University established the Molecular Genetics Diagnostic Service Division, within the Faculty of Medicine, Department of Pathology, which focuses on delivering state-of-the-art genetic analysis for the Kuwaiti population. This service includes autozygosity (homozygosity in which two alleles are identical by descent) mapping in families with consanguineous marriages (22). Pre-marital genetic screening is offered in a number of countries, including Saudi Arabia, Bahrain, the UAE, and Jordan (10). A comprehensive program for thalassaemia screening and genetic counseling was started in Iran in 1996 (10).

4. Availability of orphan drugs in the Middle East

Orphan drugs are very expensive. Insurers in the United States have traditionally covered these therapies because only a small number of patients have needed them. However, as more new products are launched, payers will become more and more sensitive to cost, potentially affecting utilization (23). Some countries in the Middle East with per-capita income approaching or exceeding that of the United States should be able to pay for the drugs through public or private health insurance, though they will eventually face the same problems that United States payers are facing. Patients in other lower-income Arab countries, however, may have to rely on charitable organizations. Several of the countries in the region, including Qatar, Saudia Arabia, and Bahrain, are committed to increasing the role of the private sector in the public-private mix. The first private hospital in Qatar opened in 1999 (8). In 2008, expatriate health insurance became mandatory in Saudia Arabia for firms employing foreigners (8).

Orphan Europe, established in 1990, is a pharmaceutical company that develops and distributes orphan drugs. Today, the company provides 9 orphan products to patients all over the world. One of its products, Cystadane (betaine anhydrous), has marketing authorization in the United States, Canada, Australia, and Israel (24). It treats homocystinuria (an inherited rare condition where the body is unable to metabolize certain amino acids properly). This condition seems to be more common in some countries, including Qatar, where it is estimated that 1 in 1,800 people is affected (25). Orphan Europe has an office in Dubai Healthcare City. This move should help to increase awareness of rare diseases and orphan drugs in the Middle East.

Taiba is a leading regional specialty healthcare company, focused on marketing and distribution of pharmaceutical products for rare diseases. It acquires
and licenses innovative orphan drugs by building a strong network with international partners (26). The head office is in Muscat, Oman, and there is a regional office in Dubai. Indeed, leading pharmaceutical companies are turning to Taiba to help grow their business. For example, Dyax Corporation has given exclusive distribution rights to Taiba for the distribution of Kalfitor (ecallantide) in the Middle East (27). Kalfitor is used for the treatment of hereditary angioedema (a genetic defect that results in episodes of swelling in various parts of the body).

5. Development of orphan drugs in the Middle East

Orphan drug development in the United States took off after the passage of the Orphan Drug Act (ODA) of 1983. Under the ODA, drugs, vaccines, and diagnostic agents would qualify for orphan status if they were intended to treat a disease affecting fewer than 200,000 American citizens (28). Orphan drug designation means that the drug-company sponsor qualifies for certain benefits, including 7-year market exclusivity, tax incentives, and grants for drug development (28). Furthermore, the Food and Drug Administration (FDA) has been expediting the marketing approval for many orphan drugs (29). In 1982, only 34 drugs were marketed in the United States to treat orphan diseases (30). From the passage of the ODA until May 2010, the FDA approved 353 orphan drugs and granted orphan designations to 2,116 compounds (31). The European Union enacted legislation similar to the ODA in 1999. In Europe, an orphan designation is granted only if “… it is unlikely that the revenue after marketing of the medicinal product would cover the investment in its development” (32). Because of the nature of orphan drugs, no country can expect private-sector firms to embrace their development without public-sector legislation offering companies a potential return on their investment. It is simply much more profitable for them to develop drugs that can benefit large numbers of people globally.

Short of encouraging the growth of a domestic pharmaceutical industry, of course, attracting foreign investment is another option for countries in the Middle East. Indeed, the Dubai Biotechnology & Research Park (DuBiotech) provides an environment for life sciences companies to set up operations in the Middle East and to collaborate in productive partnerships, potentially with local firms (33). Pharmaceutical companies in DuBiotech include Pfizer, Genzyme, Merck-Serono, Amgen, Maquet, and Fimenich. Pharmax Pharmaceuticals, a “home-grown” pharmaceutical company in the United Arab Emirates that manufactures oral solid dosage products including tablets and capsules, will develop a 90,000-square-foot manufacturing facility, becoming the first pharmaceutical production unit at DuBiotech (33). Eventually, a successful development effort will require education and training in pharmaceutical sciences at Middle Eastern or foreign universities. The size of the global orphan-drugs market was about $84.9 billion in 2009, and the market is expected to reach $112.1 billion by 2014 (34). There will be profit-making opportunities for firms from all over the world, including from the Middle East, provided that some governmental incentives are in place.

Not all orphan drug development has to be executed by private pharmaceutical companies, with incentives from the government, although this has been the development model in the West. In the Middle East, public-private partnerships (PPPs) are public-health-driven, not-for-profit organizations that encourage pharmaceutical companies to develop new orphan drugs for rare diseases in partnership with them. Even large, multinational pharmaceutical companies may find it in their long-term interest to participate in the neglected-disease market provided that they can partner with experts in distribution and patient needs in developing countries. As of 2004, eight neglected-disease projects (Artemotil, Paluther, Coartem tablets pediatric label extension, Lapdap, Biltricide, Impavidio, Ornidyl, and Mectizan) had been conducted in public-industry collaboration (35). One of the resulting products that had a major impact in the Middle East is Biltricide (praziquantel), which helps to control schistosomiasis (a parasitic disease). The PPPs model may, in at least some situations, be able to deliver better health care, both more efficiently and less expensively, than either the (primarily Western-based) private drug companies or the not-for-profit or public sector acting alone (35).

6. Conclusion

As healthcare improvement rises in priority in Middle Eastern countries, as a natural consequence of economic development, a focus on rare diseases and orphan drugs is to be expected. Although some diseases, cultural environment, and health-system features are uniquely Arab, ideas for government legislation and an optimal public-sector-private-sector mix in orphan-drug distribution and development can come from other countries that are currently paving the way. Ultimately, the rare-disease-and-orphan-drug problem is global. International discourse and cooperation should be at the top of every country’s list of relevant policies.

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Peripheral stimulation in treating Parkinson's disease: Is it a realistic idea or a romantic whimsicality?

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Summary Parkinson's disease (PD) is a common, however, intractable neurodegenerative disorder in the aging population. Levodopa (L-dopa) administration is regarded as the most effective strategy in treating PD with prominent motor side-effects after undergoing long-term treatment. Surgical therapies such as deep brain stimulation (DBS) show certain efficacy, yet there are several limitations in adopting such surgical procedures. Therefore, performing electrical stimulation out of the brain, namely peripheral stimulation for PD has been a dream of many clinicians. Recently, the efficacy of dorsal column stimulation was verified in animal PD models; on the other hand, tons of acupunctural studies from East Asia claim good efficacy in treating PD both in bench and clinical studies. This review will introduce the progress of peripheral stimulation for PD, and will discuss the potential mechanisms involved in these strategies.

Keywords: Parkinson's disease, deep brain stimulation, peripheral stimulation, dorsal column, acupuncture, somatosensory system

1. Introduction

Parkinson's disease (PD), first reported by James Parkinson (1817), is a progressive neurodegenerative disorder which is common in the elder population with an unclear pathogenesis (1). The pathophysiological hallmark is the progressive degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc), and the main symptoms are static tremor, rigidity, bradykinesia, gait dysfunction and postural instability (2,3). The detailed pathogenesis remains unclear. It is believed that PD is a comprehensive result of genetic factors and environmental toxins (Figure 1) (4).

There is no perfect strategy in treating PD. Medical therapy is still playing the most important role for PD. Currently, levodopa (L-dopa) is certainly the best medicine for idiopathic PD. Other medicines such as dopamine receptor agonists, monoamine oxidase-B (MAO-B) inhibitors, amantadine and anticholinergic medications are also used as adjuvant drugs for L-dopa with functions of reducing the dose of L-dopa, or prolonging L-dopa's effective time, or releasing the side-effects of L-dopa (4). Traditional surgical processes include old surgical ablation (pallidotomy or thalamotomy) and newer high-frequency deep brain stimulation (DBS) of certain structures such as the subthalamic nucleus (STN). STN-DBS has been proved as an effective therapy both in clinical reports (5) and animal studies (6-8). The mechanisms of such traditional surgical therapies are unclear. It is believed that the surgical ablation or STN-DBS breaks the motor controlling circuits concerning the basal ganglia, while DBS corrects the overactive state of STN in the PD state (9-11).

If we define the medical and surgical treatments...
as "classic treatments", the next generation treatments for PD should include gene therapy and stem cell transplantation. At present, the main solutions of gene therapy include two directions: one is to improve the cerebral neurotrophic factors, including the brain derived neurotrophic factor (BDNF) (12-14) and glial cell line-derived neurotrophic factor (GDNF) (15,16). However, some clinical trials regarding GDNF produced incompatible results, and thus the efficacy of improving cerebral GDNF needs more evidence (17-19). Another direction of gene therapy is to enhance GABA expression of STN by transfer of the glutamic acid decarboxylase (GAD) gene using adeno-associated virus (AAV) (20). It is hopeful that the method will be acceptable as a new treatment. The most challenging/dramatic next generation therapy is stem cell transplantation. The development of induced pluripotent stem cells (iPSCs) resolved the derivation of stem cells, and has allowed using stem cells to "make" DA neurons which is a promising strategy for DA. However, technical problems such as a low success rate in making DA neurons in vivo and a high cancer rate hold back the clinical application of stem cells.

2. The limitations of the classic therapies currently

All the classic treatments, including medical and surgical, are symptomatic therapies, which contribute little to stop/ameliorate neuron degeneration progression. Such symptomatic therapies have many weaknesses, and are far from satisfactory therapies. In this regard, PD is always thought of as an intractable disease.

L-dopa administration is regarded as the most effective therapy currently. Most of the patients experience a dramatic improvement during the early stage of treatment. Unfortunately, with the progress of PD, the dose of L-dopa has to be enhanced to achieve the same efficacy (wearing off sign). At the advanced stage, the efficacy becomes weaker, and some motor side-effects appear. Such motor side-effects of L-dopa always emerge along with the motor symptoms, which make the patients always suffer from severe motor dysfunction (4).

As to the surgical processes, several limitations are reported in the previous studies: i) The mechanisms of surgical treatments remain unclear, which will influence clinical practice using such therapies. For instance, parameter selection is a tough problem faced by the clinicians and patients undergoing DBS. Albeit the high frequency, experiential pulse (about 60 μs) is accepted by most of the researchers (11,21,22), the stimulation current intensity is a difficult problem. Recently, several animal studies revealed that the best parameters to ameliorate contrasting symptoms are quite different (7,8). The parameter selection is individualized and experience-based in different patients. Moreover, the problem of the battery life of the stimulator embedded under the skin can not be ignored. This problem usually forces the patients to make a tough decision: either to undergo another surgical operation to change the battery, or adopt a palliative pattern by reducing the stimulation current to save the battery (4). ii) DBS is an invasive therapy with a high surgical risk, and the long-term efficacy is also uncertain. Although several
clinical studies claimed a good efficacy for DBS after long-term observation (23,24), the significant adverse events reported and the battery life of the stimulator can not be ignored. iii) DBS is an expensive process, which can not be popularized in some developing countries without a good health insurance system.

There are so many limitations of the classic medical and surgical therapies currently, and there is still a long way to go to apply the next generation treatments clinically. To explore a safe (less invasive), low-cost, however effective therapy is a dream of all PD clinicians. In this regard, peripheral stimulation is taken into account.

3. Overview of peripheral stimulation

DBS is an invasion strategy with high surgical risk for PD. If the electrical stimulation can be performed out of the brain, the invasiveness, risk and cost will be profoundly reduced, while the operation process can be dramatically simplified. We defined such performing electrical stimulation at structures out of the brain, such as spinal cord, peripheral nerve, muscle, skin, etc., as peripheral stimulation. Currently, mainly two sorts of peripheral stimulations are reported, namely spinal cord electrical stimulation (25) and acupuncture (4).

Fuentes in 2009 first reported that epidural electrical stimulation of dorsal columns in the spinal cord improves motor impairments in both rat and mouse PD models (25). They used acute pharmacologically induced DA-depleted mice and chronic 6-hydroxydopamine (6-OHDA)-lesioned rats. Dorsal column stimulation (DCS) was performed and evaluated in these models. They found 300 Hz stimulation dramatically enhanced the amount of locomotion during the stimulation period compared to the control (Figure 2A). DCS also contributed to the alleviation of bradykinesia since fast-movement components are significantly ameliorated (Figure 2B). They also found that DCS affected the firing patterns of individual neurons. When performing DCS in combination with L-dopa administration, they found DCS achieved a 4/5 dose reduction of L-dopa to reach the same efficacy (Figure 3A). Such results were repeated in 6-OHDA-lesioned rat models (Figures 3B and 3C). However, the subsequent clinical study produced incompatible results (26,27). High-frequency epidural cervical spinal cord stimulation was performed for two PD patients using different frequencies and current intensities. Unfortunately, they did not find any significant difference (Table 1). DCS stimulation is a total new approach for PD, and the mechanisms are unknown (we will discuss it in the next section). More bench and clinical studies should be involved since the dorsal column may be a potential target for peripheral stimulation.

Acupuncture is another method reported to claim "good efficacy" for treatment of PD by stimulation out of the brain (4). Acupuncture is an alternative therapy which achieves improvement of certain diseases by stimulation of acupoints at the body surface, based on the theories of traditional Chinese medicine (TCM). Acupuncture is popular in east Asia. Tons of papers published in China and Korea claimed good efficacy of acupuncture in treating PD. However, most of these studies are poorly designed, and therefore we can not get rigorous evidence to evaluate the efficacy of acupuncture. Lee in 2008 investigated the acupuncture studies and found only 11 of the 103 studies reached a level of randomized controlled trials (RCTs) with subjective outcome measures, while only 1 study described a double-blind method. This rigorously designed study by Cristian et al. could not find any efficacy for acupuncture in treating PD (28,29). Lam in 2008 evaluated acupuncture studies available in the database, and
found only 10 of 784 can be attributed to RCTs, however, there are still flaws in the experimental design in these 10 studies (30). Asakawa in 2012 reviewed 2,354 original studies using acupuncture to treat PD, and could not find even one paper providing believable evidence to prove acupuncture's efficacy (Table 2). He summarized the main flaws involved in the acupuncture studies and aroused large, well-designed and multicenter clinical trials to evaluate the efficacy and safety of acupuncture (4,31).

Albeit peripheral stimulation is an attractive and hopeful approach for PD, unfortunately at present there is no powerful evidence to prove efficacy clinically, either in spinal cord stimulation, or in acupuncture. More studies should be engaged in these two directions since peripheral stimulation is a good clue for developing low-invasion PD treatment.

4. The potential mechanisms involved in peripheral stimulation

Until now, we still do not know whether peripheral stimulation can be employed as a candidate new treatment for PD. If the potential mechanisms can be clarified, it will be helpful to develop effective peripheral stimulation.

4.1. The somatosensory system, a bridge between peripheral stimulation and the dopaminergic system?

The essence of this problem is that if stimulation of peripheral structures can affect the cerebral dopaminergic system. Several reports revealed that peripheral electrical stimulation is able to affect cerebral DA release. As far back as 1977, Nieoullon found electrical stimulation of the cats' forepaw resulted in DA release which was reduced in the ipsilateral substantia nigra and enhanced in the caudate nucleus (32). Subsequently, several studies found stimulation of the somatosensory system affects the dopaminergic system, which is related to motor function (Figure 4) (33-35). These findings all indicated a close connection between the somatosensory system and DA system. However, the anatomical structure, distribution and circuits of the somatosensory system are poorly understood. Inoue's 2004 paper deduced the possible anatomical pathways which exist between the mesencephalic DA-ergic nuclei and the sensory system causing the observed modulation of DA release in the basal ganglia. One important plausible connection is from the sensory areas in the contralateral neocortex which projects back into the ipsilateral striatum, and then activates ipsilateral DA release from mesencephalic DA-ergic nuclei.

Table 1. DCS did not conduct any significant amelioration in two PD patients (Thevathan et al., 2010)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Motor UPDRS (score/104)</th>
<th>Timed 10-meter walk (s)</th>
<th>Timed hand-arm movements (n/30s)</th>
<th>Timed lower limb tapping (n/30s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline (off stimulation)</td>
<td>37.8 (11.5)</td>
<td>5.5 (1.2)</td>
<td>30.3 (15.9)</td>
<td>54.2 (21.9)</td>
</tr>
<tr>
<td>subthreshold stimulation</td>
<td>35.4 (12.5)</td>
<td>5.4 (0.4)</td>
<td>32.7 (18.0)</td>
<td>54.2 (22.8)</td>
</tr>
<tr>
<td>suprathreshold stimulation</td>
<td>37.3 (10.5)</td>
<td>5.6 (1.0)</td>
<td>31.2 (16.3)</td>
<td>52.0 (24.7)</td>
</tr>
<tr>
<td>friedman (p value)</td>
<td>0.44</td>
<td>0.72</td>
<td>0.32</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Figure 3. DCS improved locomotion in severely DA-depleted mice and in chronically lesioned rats (Fuentes et al., 2009). (A), DCS established better efficacy in the group undergoing DCS in combination with successive L-dopa injections (black) than the group only receiving L-dopa (gray); (B), DCS (yellow shaded area) caused significant improvements of locomotion in 6-OHDA-lesioned rats (shaded area around trace is SEM); (C), (Left) DCS specifically improved locomotion in 6-OHDA-lesioned rats, (Right) Faster movement components of locomotion were also ameliorated by DCS in the 6-OHDA-lesioned rats.
Another possible anatomical connection could be from the projecting fibers between the nuclei intralaminas thalami and the SNpc through the striatum on the contralateral side. Furthermore, there is another potential pathway between the ventral tegmental area and the mesencephalic central gray area, which is innervated collaterally by the spinothalamic tract (4,33).

The mysterious somatosensory pathways may play a role in the connection between peripheral stimulation and the DA system. We hypothesize that the dorsal column and the effective acupoints (if the efficacy can be strictly verified) should be the "stations" of the somatosensory pathways. The motor functions related to the dopaminergic system are then affected through the somatosensory pathways when the "stations" are undergoing electrical stimulation.

4.2. DCS unlocks the basal ganglia-cortical circuits?

Besides the dopaminergic system, another important possibility is that DCS (or effective acupuncture) activates the locked basal ganglia-cortical circuits in the PD state. In a later paper to explain the mechanisms of DCS, Fuentes pointed out that the improvement of motor function might be the result of basal ganglia-cortical circuits being unlocked and conducted by synchronous stimulation of a number of tactile afferent fibers terminating in the dorsal column nuclei and ascending through the lemniscal pathway to cortical areas through the thalamus, and the thalamic nuclei most directly activated by DCS differ from those primarily affected by STN-/GPi-DBS. In addition, activating the pedunculopontine nucleus (PPN) through some ascending and descending anatomical tracers from the cervical and thoracic spinal cord dorsal horns projects directly to PPN and may play a role in the mechanisms of DCS (36). It has been well investigated that activation of PPN contributes to improvement of the initiation of movement through a descending drive to locomotor circuits directly; and activation/desynchronization of the motor cortex along with certain structures within the basal ganglia through ascending thalamocortical pathways indirectly (36-38).

No matter what the mechanisms are concerning the dopaminergic system, or concerning the basal ganglia circuits, achieving deeper understanding of the potential anatomical connections is crucial. It may be a key to uncover the secrets of peripheral stimulation.
5. Conclusion

Although only one rigorously designed bench study (25) verified the efficacy of peripheral stimulation in treating PD, we can expect the possibility of treating PD by stimulation outside of the brain. More bench and clinical studies should be designed and verified for peripheral stimulation. Certainly the efficacy of acupuncture should be also strictly verified. The "effective" acupoints may be employed in affording hints of stimulating targets or finding the "stations" of unknown anatomical connections.

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Classification and management of hepatolithiasis: A high-volume, single-center's experience

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1. Introduction

Hepatolithiasis is endemic to East Asia, which includes China, South Korea, Japan, the Philippines, Vietnam, Thailand, Malaysia, and Indonesia, and its prevalence there can range as high as 30-50% (1). This disease involves gallstones in the bile ducts proximal to the confluence of the right and left hepatic ducts, irrespective of the co-existence of gallstones in the common bile duct (CBD) and/or gallbladder (2). In the past, this disease was rare, with a prevalence of 0.6-1.3% (3) in the West, but it is increasingly encountered in the West because of greater immigration from Asia (4-7).

The etiology of hepatolithiasis has yet to be fully elucidated, although genetic, dietary, and environmental factors are thought to contribute to the disease. Hence, curative management of hepatolithiasis is difficult since treatment depends greatly on fully understanding the mechanism of stone formation. The goal of hepatolithiasis treatment is to resolve ongoing infections, prevent recurrent cholangitis and subsequent hepatic fibrosis, decrease the need for recurrent instrumentation, and prevent progression to cholangiocarcinoma (2). Available treatments include medication and surgery. Surgery, including removal of the affected liver segment(s), has been the best treatment thus far. Hepatectomy can remove stones and focal lesions to eliminate the risk of cholangiocarcinoma, strictures, and subsequent bile stasis to provide effective drainage of biliary tract. Complete removal of the diseased lobe or segment is crucial to preventing recurrence and progressive liver disease. The current report describes a system of classifying hepatolithiasis for surgery and firsthand experience managing the disease.

2. Classification of hepatolithiasis for surgery

Classifying surgical candidates or indications for surgery is crucial for a hepatectomy to treat hepatolithiasis to result in the best outcome. Normally, the indications for hepatectomy to treat hepatolithiasis are as follows: i) unilobar hepatolithiasis, particularly left-sided; ii) atrophy, fibrosis, and multiple abscesses secondary to cholangitis; iii) suspicion of concomitant intrahepatic cholangiocarcinoma; and iv) multiple intrahepatic stones with biliary strictures that cannot be treated percutaneously or endoscopically (8).
The criteria for classification should be based on the pathological characteristics of the biliary tree and hepatic parenchyma. Classification should help to determine treatment strategies and it should be simple and easy to apply. However, there has been no universal classification of hepatolithiasis until now.

Based on previous experience managing hepatolithiasis, the current authors propose a system of classification, designated "Dong's Classification" to determine reasonable surgical approaches to treating hepatolithiasis (Table 1). In this classification, hepatolithiasis is divided into two types, type I and type II. Type I is a localized stone disease with stones located in one (Figure 1A, Type Ia) or both lobes (Figure 1B, Type Ib). Type II is a diffuse stone disease, which is divided into three subtypes: type IIa involves no atrophy of the hepatic parenchyma or stricture of the intrahepatic bile ducts (Figure 2A); type IIb involves segmental atrophy or/and stricture of the intrahepatic bile ducts (Figure 2B); and type IIc involves biliary cirrhosis and portal hypertension (Figure 2C).

The letter "E" represents an additional type of hepatolithiasis with extrahepatic stones. This type is divided into three subtypes: "Ea" representing normal functioning of the sphincter of Oddi; "Eb" representing relaxation of the sphincter of Oddi, and "Ec" representing stricture of the sphincter of Oddi (Figure 3).

### 3. Hepatectomy to treat hepatolithiasis

Data were collected on patients at the Institute of Hepatobiliary Surgery, Southwest Hospital, and Chinese PLA General Hospital, both of which are leading facilities for biliary surgery in China. Hepatectomy to treat hepatolithiasis was initiated at Southwest Hospital by Prof. Zhiqiang Huang in 1957 and was reported by him in a Chinese medical journal in 1959 (9).

Hepatectomy is the best approach to treating hepatolithiasis because it removes stones and also because it removes the strictured bile duct, resects the atrophic portion of the liver, and eliminates the potential presence of cholangiocarcinoma, thus reducing the risk of recurrent stones.

<table>
<thead>
<tr>
<th>Type</th>
<th>Definition or content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Localized stone disease: unilobar or bilobar.</td>
</tr>
<tr>
<td>Type II</td>
<td>Diffuse stone disease.</td>
</tr>
<tr>
<td>IIa</td>
<td>No atrophy of the hepatic parenchyma or stricture of the intrahepatic bile ducts.</td>
</tr>
<tr>
<td>IIb</td>
<td>Segmental atrophy or/and stricture of the intrahepatic bile ducts.</td>
</tr>
<tr>
<td>IIc</td>
<td>Biliary cirrhosis and portal hypertension.</td>
</tr>
<tr>
<td>Additional Type E</td>
<td>Extrahepatic stones.</td>
</tr>
<tr>
<td>Ea</td>
<td>Normal sphincter of Oddi.</td>
</tr>
<tr>
<td>Eb</td>
<td>Relaxation of the sphincter of Oddi.</td>
</tr>
<tr>
<td>Ec</td>
<td>Stricture of the sphincter of Oddi.</td>
</tr>
</tbody>
</table>

**Figure 1. Type I hepatolithiasis.** (A), Type Ia. Localized stone disease with stones located in only one lobe. In this case, stones were localized in the atrophic right anterior portion of the liver. Segmentectomy of S5 and S8 was performed; (B), Type Ib. Localized stone disease with stones located in both lobes. In this case, stones were localized in the atrophic right anterior portion and left lateral portion of the liver.
Based on experience, patients with type I and type IIb hepatolithiasis are the best candidates for hepatectomy. In type I localized stone disease, surgery resects the stone-bearing segments, regardless of where atrophy or a stricture is found (Figure 4). Type II involves a high risk of stone recurrence, so all patients with type II should undergo stone removal along with a Roux-en-Y hepaticojejunostomy (10,11) or hepaticocutaneous jejunostomy (12-15). Hepatectomy is the best way to resect lesions in patients with type IIb hepatolithiasis and hepatic lesions (e.g. segmental atrophy hepatic abscess or cholangiocarcinoma). Patients with type IIc hepatolithiasis consistently have biliary cirrhosis, portal hypertension, and liver failure as well, indicating the need for a liver transplant. Additionally, a hepaticojejunostomy should also be performed to treat "Eb" and "Ec" hepatolithiasis.

3.2. Outcomes of hepatolithiasis treated by surgery

From June 1976 to June 2009, 1,930 patients underwent surgery for hepatolithiasis at the Institute of Hepatobiliary Surgery. Of these, 1,175 patients underwent hepatectomy and 755 patients primarily underwent liver-preserving surgery and stone removal.

Perioperative outcomes showed that patients who underwent a hepatectomy had much greater intraoperative bleeding than patients who had stones removed. Hepatectomy was associated with a longer operating time than stone removal. There were no significant differences between the patients in terms of mortality and morbidity. However, the postoperative rate of residual stones was much lower after hepatectomy than after stone removal (Table 2).

The operative morbidity rate was 13.3% for 1,175 patients who underwent a hepatectomy. The most common complication was wound infection, followed by biliary leakage, pleural effusion, pneumonia, stress ulcer, intraabdominal bleeding, and hepatic failure (Table 3).

3.3. Typical cases where a hepatectomy was used to treat hepatolithiasis
3.3.1. Anatomic left lobectomy, segmentectomy, and sectionectomy for type I hepatolithiasis

In a case of stones localized in the atrophic left lobe and extrahepatic bile duct, the hepatolithiasis was classified as type I plus Ea, and anatomic left lobectomy was performed and extrahepatic stones were removed (Figure 4A). In a case of a stone localized in atrophic segment VIII, hepatolithiasis was classified as type I, and segmentectomy of S8 was performed (Figure 4B). In a case of stones localized in the atrophic right posterior portion of the liver, anatomic right posterior sectionectomy was performed (Figure 4C).

Table 2. Perioperative outcomes for 1,930 patients with hepatolithiasis (June, 1975 – June, 2008)

<table>
<thead>
<tr>
<th>Types of Complications</th>
<th>HT group (n = 1,175)</th>
<th>SR group (n = 755)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating time (min)</td>
<td>332 ± 123</td>
<td>289 ± 106</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Blood loss (mL)</td>
<td>717 ± 712</td>
<td>443 ± 510</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Postoperative mortality</td>
<td>3 (2.6%)</td>
<td>1 (1.3%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Residual stones</td>
<td>224 (19.1%)</td>
<td>332 (44.0%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Perioperative complications</td>
<td>156 (13.3%)</td>
<td>93 (12.3%)</td>
<td>0.157</td>
</tr>
</tbody>
</table>

HT, Hepatectomy; SR, Stone Removal; data are expressed as average ± S.D.; data represent cases (ratio).

Table 3. Postoperative complications in 1,175 patients who underwent a hepatectomy (June, 1975 – June, 2008)

<table>
<thead>
<tr>
<th>Types of Complications</th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound infection</td>
<td>81 (6.9%)</td>
</tr>
<tr>
<td>Biliary leakage</td>
<td>26 (2.2%)</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>21 (1.8%)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>14 (1.2%)</td>
</tr>
<tr>
<td>Stress ulcer</td>
<td>13 (1.1%)</td>
</tr>
<tr>
<td>Abdominal bleeding</td>
<td>6 (0.5%)</td>
</tr>
<tr>
<td>Hepatic failure</td>
<td>3 (0.3%)</td>
</tr>
</tbody>
</table>

3.3.2. Bilateral lobectomy excluding the caudate lobe for type II hepatolithiasis

From March 2003 to July 2011, 252 patients with type II hepatolithiasis at this Institute underwent surgery. Of these, 12 (4.8%) underwent bilateral lobectomy excluding the caudate lobe. In a typical case, a 39-year-old female had diffusely distributed stones with atrophy in all segments except for the caudate lobe (Figure 5). All 12 of the patients successfully underwent bilateral lobectomy excluding the caudate lobe.

4. Conclusion

Economic development and a more Western lifestyle are associated with a decline in the incidence of...
hepatolithiasis, but the same is not true for East Asia. Moreover, immigration from the Asian-Pacific region means that this rare but emerging disease will pose a therapeutic challenge to doctors in the West as well.

The optimal management of complex hepatolithiasis remains a very difficult and challenging task for hepatobiliary surgeons. Surgery should be indicated for all cases of hepatolithiasis. The current report describes the largest number of patients with hepatolithiasis treated with a hepatectomy (Table 4) and a novel classification to help in determining surgical strategies. Segmentectomy is an effective treatment for type I and type IIb hepatolithiasis. The caudate lobe is a unique segment that is anatomically separate from the rest of the liver; if the liver were a car, the caudate lobe would be its "spare tire". Finally, better selection of which patients should undergo a hepatectomy will lead to better mid-to long-term outcomes.

Acknowledgements

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The use of cffDNA in fetal sex determination during the first trimester of pregnancy of female DMD carriers

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Summary

Chorionic villus sampling (CVS) or amniocentesis for fetal sex determination is generally the first step in the prenatal diagnosis of X-linked genetic disorders such as Duchenne muscular dystrophy (DMD). However, non-invasive prenatal diagnostic (NIPD) techniques such as measurement of cell-free fetal DNA (cffDNA) in maternal plasma are preferable given the procedure-related miscarriage rate of CVS. We determined fetal sex during the first trimester using a quantitative real-time polymerase chain reaction (PCR) assay of cffDNA in pregnant carriers of DMD. The fetal sex was confirmed by amniocentesis karyotype analysis and multiplex ligation-dependent probe amplification (MLPA) at 16 weeks. This procedure may avoid unnecessary CVS or amniocentesis of female fetuses.

**Keywords:** cell-free fetal DNA (cffDNA), non-invasive prenatal diagnostic (NIPD), Duchenne muscular dystrophy (DMD), fetal sex determination

1. Introduction

The first step in the prenatal diagnosis of X-linked genetic disorders like Duchenne muscular dystrophy (DMD) or hemophilia is the determination of fetal sex. Chorionic villus sampling (CVS) and amniocentesis have long been used to determine sex. A female fetus may have a wild-type genotype or be a carrier of DMD, but further genetic analysis is crucial for a male fetus because a male fetus has a 50% chance of having DMD. Pregnant carriers risk miscarriage when undergoing an invasive prenatal diagnosis (IPD). Non-invasive prenatal diagnosis (NIPD) is preferable for fetal sex determination during the first trimester since it avoids the unnecessary risks of IPD in pregnant female DMD carriers.

Cell-free fetal DNA (cffDNA) was found in maternal plasma in 1997 (1) and its measurement represents a potential form of NIPD. The cffDNA in maternal plasma can be detected as early as 7 weeks. cffDNA comprises about 3-6% of the total cell-free DNA in maternal plasma (2). Detecting the sex-determining region on the Y chromosome (SRY) or other Y chromosome-specific sequences based on cffDNA from maternal plasma is one technique for non-invasive fetal sex determination during the early trimester of pregnancy (3-5). Fetal sex can be diagnosed before CVS can be performed. In the current study, cffDNA was measured for fetal sex determination during prenatal diagnosis of DMD. The fetal sex was determined at about 9 weeks of gestation by means of quantitative real-time polymerase chain reaction (PCR) with a taqman probe to detect SRY with cffDNA in maternal plasma. If the fetus was male, CVS was performed at 12 weeks followed by DMD and multiplex ligation-dependent probe amplification (MLPA) analysis. If the fetus was female, CVS was avoided. Fetal sex was later confirmed by ultrasound at 16 weeks. Whether a female fetus is a carrier or not can be determined after delivery.

2. Materials and Methods

2.1. Materials

All study protocols were approved by the Ethics Committee of He'nan Provincial People's Hospital. All of the pregnant women and their partners gave written informed consent and received genetic counseling. MLPA analysis was performed on the fetuses of 15 pregnant women who were DMD carriers.
2.2. Sampling and extraction of cffDNA in maternal plasma

EDTA blood samples (8 mL) were taken at about 9 weeks of gestation. The blood samples were centrifuged twice at 3,000 g and then at 12,000 g to obtain cell-free plasma. Cell-free DNA was extracted from 2 mL of maternal plasma using the QIAamp blood mini kit (Qiagen, Hilden, Germany). DNA was eluted into 40 μL of solution buffer.

2.3. Sex determination using quantitative real-time PCR

Quantitative real-time PCR analysis was performed using an Applied Biosystems 7500 Fast Real-Time PCR System. TaqMan amplification reactions were set up in a reaction volume of 20 μL by use of components (except TaqMan probes and amplification primers) supplied in the TaqMan® Fast Universal PCR Master Mix (2×) (Applied biosystems). TaqMan probes and PCR primers were synthesized by Sangon Biotech (Shanghai, China) Co., Ltd. (Primer1: 5’-TGGCGATT AAGTCAAATTCGC-3’; Primer2: 5’-CCCCCTAGTACCTGACATATGTTATT-3’; Probe: 5’-(FAM)AGCAGTAGCAGGGAAGGCCAGAT(TAMRA)-3’). Each reaction included 12.5 μL of TaqMan® Fast Universal PCR Master Mix (2×), 300 nM of each amplification primer, and 200 nM of the TaqMan probe. Eight μL of the extracted plasma DNA was used for amplification. Each sample was analyzed twice. The 500 genome equivalent (GE, 6.6 pg of DNA per genome equivalent), 100 GE, and 10 GE were used as positive controls to confirm the sensitivity of the PCR assay. Thermal cycling was initiated with a first denaturation step of 20 s at 95°C and then 40 cycles of 95°C for 3 s and 60°C for 30 s.

2.4. MLPA analysis of male fetuses

For male fetuses, DNA was isolated from amniotic fluid cells using the TIANamp Genomic DNA kit (TIANGEN, Beijing, China), and the quality and quantity of DNA was checked using a Nano-Drop 2000 Spectrophotometer. Salsa MLPA kits (P034A2 and P035A2, MRC Holland, Amsterdam, Netherlands) were used to determine whether the fetus had DMD or not. In accordance with the manufacturer’s instructions, amplification products were electrophoresed on an ABI 3130 Genetic Analyzer (6). Products were then analyzed using Coffalyser v9.2 software.

3. Results and Discussion

3.1. Fetal sex determination using cffDNA in maternal plasma

The 15 fetuses studied included 6 males and 9 females, and sex was later confirmed by CVS or ultrasound. Fetal sex was determined by means of quantitative real-time PCR after 9 weeks (Figure 1).

3.2. MLPA analysis of male fetuses

As shown in Figure 2, MLPA revealed deletion of exons 3-20 (A, B) and duplication of exons 13-43 (C, D) in male fetuses. Details on the mutations identified are shown in the figure.

The main advantage of measuring cffDNA as part of prenatal diagnostic is that sampling can avoid unnecessary risks associated with conventional techniques of prenatal diagnosis (including CVS and amniocentesis). The mean concentration of cffDNA in maternal plasma was more than 20 times higher than that in the cellular fraction of maternal blood at the same gestational stage. The cellular fraction may be present in maternal plasma for years. cffDNA cannot be detected by enzymolysis a few hours after delivery (1), so false-positive results from women who had previously carried a fetus with DMD can be avoided.

cffDNA can be detected in the first trimester of pregnancy. Its measurement is necessary for fetal sex determination since a positive result can allow pregnant women to avoid suffering by terminating a pregnancy early. Non-invasive fetal sex determination using cffDNA can avoid unnecessary CVS and amniocentesis of female fetuses for the prenatal diagnosis of X-linked genetic disorders (4,7-9). DMD is one of the most common genetic muscular dystrophies. The onset of symptoms in affected individuals is generally before the age of 5 and most die in the course of the second or third decade of life due to respiratory or heart failure (10). Thus, prenatal diagnosis is crucial for families of
carriers.

Fetal DNA analysis using maternal plasma would be most useful in the detection of paternally-inherited fetal mutations or autosomal recessive genetic disorders where the father and mother carry different mutations (11-14). Increased amounts of fetal DNA may also be found in instances of conditions associated with placental damage, such as pre-eclampsia (15,16). Recent studies showed that cfDNA could be used in prenatal screening for fetal chromosomal disorders (trisomy 18, trisomy 13, and Down syndrome) (17,18).

The main difficulty of using cfDNA in NIPD lies in the low concentration of cfDNA and presence of a larger quantity of background maternal DNA in plasma, so sensitive and specific techniques like the use of a real-time TaqMan system are needed. In conclusion, cfDNA could be used for fetal sex determination in the first trimester of pregnancy to screen for gender-specific inherited disorders while avoiding an unnecessary CVS.

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References


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Study and analysis of the state of rare disease research in Shandong Province, China

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Summary As the world's most populous country, China has the world's largest number of rare disease groups in terms of prevalence. However, the country has no system of registering cases of most rare diseases, so there is very little documented information on the epidemiology of those diseases. The purpose of this study was to study the state of rare disease research and survey doctors in Shandong Province regarding their level of awareness of rare diseases. Types of rare diseases and numbers of cases were tallied and their geographical distribution over the decades was analyzed. Eight hundred and twenty-four doctors in tertiary hospitals and maternity and child care hospitals were surveyed by questionnaire. Data were descriptively analyzed and a map of disease distribution was created. Articles about rare diseases were retrieved from the Chinese Biomedical Literature Database to provide pertinent data. This study yielded 5,749 cases of 323 different types of rare diseases. The survey found that doctors lack awareness of research on rare diseases. An authoritative and information-rich platform for rare disease research is urgently needed. Key steps are to study epidemiological and statistical techniques and then obtain available data to provide a basis for the definition and regulation of rare diseases in China.

Keywords: Rare diseases, awareness survey, descriptive analysis

1. Introduction

Rare diseases are also known as "orphan diseases", but there is no satisfactory definition of rare diseases around the world. In the United States of America (USA), a rare disease is defined as a disease that affects fewer than 200,000 individuals, but in Japan the number is 50,000 and in Australia it is 2,000. The European Union (EU) definition is less than 5 in 10,000. The World Health Organization (WHO) defines a rare disease as all pathological conditions affecting 0.65-1 out of every 1,000 inhabitants (1). These numbers clearly relate to the population sizes of these countries, but even adjusting for that, the definitions vary from about 1 to 8 in 10,000 (2). Data on rare diseases are constantly collected and updated through the combined efforts of government, patient organizations, and medical and scientific institutions. Many organizations, such as orphanet (http://www.orpha.net) in the EU, have showed that these data play an important role in areas such as the prevention and treatment of rare diseases, policy-making, medical research, and social welfare.

Study and regulation of rare diseases has progressed worldwide, and this is especially true in the USA and EU. The USA adopted important legislation on rare disease and orphan drugs in 1983 that has successfully promoted investment in research and development (R&D) of new pharmaceutical products to treat rare diseases. Similar legislation was also enacted in Australia in 1997 and in the EU in 1999. This legislation explicitly recognized the unmet need for targeted treatments for rare diseases and it created
regulatory pathways and incentives for manufacturers to develop orphan drugs (3-5). In Asia, Japan, South Korea, and Taiwan have established systematic economic and regulatory incentives to encourage R&D of drugs for rare diseases.

China is also actively promoting regulation of rare diseases, but these diseases have not been covered by the national health system and special legislation on orphan drugs was only recently enacted (6). Given its large population, China probably has a large number of patients with rare diseases according to the definition of the WHO. However, there are still no official data on "the prevalence of rare diseases, their variety, and the number of cases of rare diseases". A crucial step is to collect data on rare diseases in China.

Shandong Province is one of China’s most populous provinces, the sixth census recorded its population as 95,793,065, which represents 7.2% of China’s total population. The province started researching rare diseases early on and it created a rare disease prevention and control association. At present, the goal of that association is to establish a platform for rare disease diagnosis and information. This project is also supported by the provincial government and has a good research foundation. Shandong has a varying terrain, rich mineral resources, traditional, historical, and cultural backgrounds, both an industrial and an agricultural economy, various occupations and socioeconomic levels, and relatively developed medical technology. It epitomizes China. Data on rare diseases from Shandong Province can be used as a national analogue to a certain extent and will help to study rare diseases and formulate responses nationally. Thus, the current study examined the state of rare disease research and level of awareness of rare diseases among doctors in Shandong. This study also tallied the types of rare diseases and number of cases and analyzed their geographical distribution over the decades.

2. Methods

2.1. Data collection

A questionnaire was sent to 824 doctors in a total of 103 tertiary hospitals and municipal maternity and child care hospitals in 17 cities of Shandong Province. The questionnaire (Table 1) consisted of three parts. The first dealt with background information, including name, specialty, phone number, e-mail, position, title, education, years of experience, and department. The second part was a survey on awareness and recommendations for prevention of rare diseases. The third part dealt with information on which types of rare diseases were present and the number of cases encountered. There were 472 responses from doctors at 59 hospitals (219 doctors at 3 hospitals has never encountered a rare disease and thus did not answer the questionnaire), for a total response rate of 57.28% (472/824). Here, the 253 responses (53.60% of valid responses) from doctors who had encountered a rare disease are analyzed.

Also studied were articles on rare diseases from the Database of Chinese Biomedicine Literature (http://

Table 1. Portions of the questionnaire

<table>
<thead>
<tr>
<th>Topic</th>
<th>Item</th>
<th>Control</th>
</tr>
</thead>
</table>
| Survey on awareness of rare diseases among doctors and recommendations for their prevention | 1. How did you learn of rare diseases? | a) Newspapers and the Internet  
b) Professional lectures  
c) Related training  
d) Other |
| | 2. If you encounter a rare disease that you are unfamiliar with, what will you do? | a) Treat in accordance with clinical experience  
b) Ask a veteran physician  
c) Look for further information  
d) Refer to another department or hospital |
| | 3. What is the best way to improve the diagnosis and treatment of rare diseases? | a) Establish a network and create a platform at the provincial level to diagnose, treat, and study rare diseases  
b) Provide consultations and improve the efficiency of diagnosis and treatment of rare diseases at the provincial level |
| | 4. Which step is the most important in setting up a network to study rare diseases? | a) Assemble cases and conduct scientific research  
b) Participate in joint consultations and improve diagnosis and treatment  
c) Establish molecular diagnostic techniques  
d) Carry out collaborative research and joint studies on rare diseases |
| | 5. Have you ever conducted research on rare diseases? | a) Yes  
b) No |
| | 6. Have you ever published papers about rare diseases? | a) Yes  
b) No |
| | 7. Have you ever cared for patients with rare diseases? | a) Yes  
b) No |

Disease information

1. Disease  
2. Number of cases
2.2. Data processing and analysis

SPSS 20.0 was used to input and manage data, and then random sampling was used to ensure the accuracy of data. If there were abnormal data or missing values, they were corrected in accordance with the original data.

Descriptive statistical analysis in the form of frequency distribution analysis was used and data were summarized to describe the data characteristics. Charts were then drawn with SPSS and Excel.

Maps with shades of color reflecting the number of cases of rare diseases and number of articles published in each city were created with ArcMap 10.0 and SPSS 20.0.

3. Results

This study found 5,749 cases of 323 different types of rare diseases in Shandong Province. The survey of doctors yielded 293 types and 4,068 cases and the Chinese Biomedical Literature Database yielded 50 types and 1,681 cases. Figure 1 shows data on rare diseases according to descriptive analysis. The ten most prevalent rare diseases were fibrous dysplasia of bone, congenital hypothyroidism, Marfan’s syndrome, infectious mononucleosis, hemophilia, osteogenesis imperfecta, multiple osteochondroma, synpolydactyly, phenylketonuria, and neurofibromatosis.

Figure 2 displays the geographic distribution of rare disease cases and articles in shades of color. Binzhou, Ji’nan, Qingdao, and Linyi had more cases than other locations (Figure 2A). Cities with the higher number of articles on rare diseases published in the Chinese Biomedical Literature Database were Ji’nan, Qingdao, and Weifang (Figure 2B). Dezhou, Laiwu, and Rizhao had few cases of rare disease and few articles on those diseases in the database.

The data in Figure 3 shows the awareness of rare diseases among doctors and the recommendations to prevent those diseases. As shown in the figure, doctors learned about rare diseases through professional seminars, clinical consultation, magazines, newspapers, the Internet, and related training, with professional seminars being the most frequent method of learning (Figure 3A). When doctors encountered a patient with a rare disease, 46% choose to seek more information, 27% referred the patient, 20% consulted a veteran physician and the remaining 7% treated the patient based on their clinical experience (Figure 3B). Seventy percent of respondents felt that the best way to improve the diagnosis and treatment of rare diseases was to establish a network and platform to diagnose, treat, and study rare diseases (Figure 3C). According to respondents, important steps in establishing such a network are assembling cases and conducting scientific research, participating in joint consultations and improving diagnosis and treatment, establishing molecular diagnostic techniques, and carrying out...
collaborative research and joint studies on rare diseases (Figure 3D).

Figure 4 shows the low degree of emphasis on rare disease research among doctors. Forty-four of 247 doctors had carried out research on rare diseases, 33 of 250 had published an article on a rare disease, and 10 of 249 had participated in a project on a rare disease.

4. Discussion

As the world's most populous country, China has the world's largest number of rare disease groups in terms of prevalence. However, the country has no system of registering cases of most rare diseases, so there is very little documented information on the epidemiology of those diseases (7,8). The current study tallied the ten most prevalent rare diseases in Shandong Province, but this list did not match the diseases described in other articles. Bibliographic data published in 2011 by Orphanet (http://www.orpha.net) in Europe showed that the ten most prevalent rare diseases were Klippel-Trenaunay-Weber syndrome, Whipple disease, incontinentia pigmenti, Aicardi syndrome, CADASIL, Li-Fraumeni syndrome, Silver-Russell syndrome,
Castleman disease, cutis marmorata telangiectatica congenital, and Mobius syndrome. The prevalence of rare diseases may differ in different countries or different areas, so a database specific to China must be created for future research.

A survey of rare disease awareness among doctors found that they learn about rare diseases in many ways, though none is authoritative and standards and effective programs for diagnosis and treatment of rare diseases are lacking. A similar survey was conducted in Europe, but patients were surveyed. The survey was part of a long-term study that began in March 2004 by sending a questionnaire to 18,000 patients to ask about experience being diagnosed with a rare disease in 17 countries. The survey results highlighted the dilemma of rare diseases: lack of information, lack of appropriate medical training, difficulties with access to care, and subsequent loss of patient confidence in the health care system and the medical profession. The survey authors put forward solutions including reference centers, databases for the exchange of information, DNA and tissues banks, and networks of professionals (9). The establishment of a rare disease network and information exchange platform is a pressing matter. At the same time, work must also be done to assemble cases and conduct scientific research, participate in joint consultations and treatment, establish molecular diagnostic techniques, and carry out collaborative research and joint studies on rare diseases.

The current study noted a very serious problem: the low degree of emphasis on rare disease research among doctors. Rare disease research received little attention and also involved a low degree of cooperation. The current survey had a response rate below 60%, and fewer than 55% of respondents had ever encountered a rare disease. The problem may be due to doctors' specialties, interests, or level of knowledge, but definitive conclusions cannot be reached due to the lack of data. Further study and analysis will be done to determine the factors influencing attention to rare diseases among doctors and increase that attention.

The study of rare diseases in China is still in its infancy. China is also actively promoting regulation of intractable and rare diseases. Some rare disease websites and online databases to register cases have been set up. A number of centers have been established to offering counseling on rare diseases in major Chinese cities like Beijing and Shanghai. Moreover, in Shanghai patients with 12 rare diseases recently became eligible for partial reimbursement, and some special orphan drugs for children are now covered by insurance, but these diseases have not been covered by the national health system and special legislation on orphan drugs was only recently enacted. China still lags far behind the US, EU, Japan, and other countries and regions in terms of orphan drug legislation (6,10,11). Key steps are to examine epidemiological and statistical techniques and then obtain available data to provide a basis for the definition and regulation of rare diseases in China.

The current study has several limitations. There was, for example, substantial bias in the data collection process and the survey was conducted using only descriptive analysis. Rare disease cases and articles have different geographic distributions, but definitive reasons for these differences were not apparent, so data need to be collected and in-depth analysis needs to be performed to determine whether there are significant differences in rare diseases seen in different cities and hospital departments.

5. Conclusion

Shandong Province had at least 5,749 cases of 323 different types of rare diseases, and the distribution of these diseases differed in different cities. Few doctors in the province had ample knowledge about rare diseases and there is insufficient emphasis on the treatment and research of rare diseases. An authoritative and information-rich platform for rare disease research is urgently needed, and the low degree of emphasis on rare disease research among doctors must soon be improved. Key steps are to examine epidemiological and statistical techniques and then obtain available data to provide a basis for the definition and regulation of rare diseases in China.

Acknowledgements

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Henoch-Schönlein purpura associated with a neuroblastoma: Report of one case and a review of the literature

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Summary Malignancies such as solid tumors and hematologic malignancies can often induce or be associated with Henoch-Schönlein purpura (HSP) in older males but not in children. Described here is the case of a 5-year-old boy who clinically presented with HSP. An imaging study of the abdomen revealed a right retroperitoneal neoplasm that histopathology postoperatively confirmed to be a neuroblastoma. Malignancies are sometimes associated with HSP mostly in older males, though children are affected, albeit rarely. Thus, all patients with HSP must be carefully examined to identify or exclude an underlying disease.

Keywords: Henoch-Schönlein purpura (HSP), neuroblastoma, malignancy

1. Introduction

Henoch-Schönlein purpura (HSP) is the most common vasculitic disease affecting children. HSP is a multisystem immunoglobulin A-mediated vasculitis with a self-limited course that affects the skin, joints, gastrointestinal tract, and kidneys (1, 2). HSP has many causes, including infections, drugs, foods, and malignant tumors. Many malignancies, such as solid tumors and hematologic malignancies, are reported to induce or be associated with HSP. Such tumors include carcinoma of the lung, bronchus, esophagus, stomach, intestine, breast, kidney, prostate, and thyroid (3-12) while hematologic malignancies include non-Hodgkin lymphoma, Hodgkin disease, multiple myeloma, myeloproliferative disease, and myelodysplastic syndrome (10,11,13-17).

However, patients with HSP associated with a malignancy are mostly older males with a mean age of 60 years (7,8,10,11). Neuroblastomas are the most common extracranial solid tumors in children, but there are no reports of children with HSP associated with a neuroblastoma as has been reported here.

2. Case report

A 5-year-old boy with a weight of 19.5 kg (75 percentile), height of 110.0 cm (50 percentile), and blood pressure of 100/75 mmHg presented with numerous purpuras, bilateral knee joint pain, and abdominal pain without bloody diarrhea at almost the same time. The boy had no upper respiratory tract infections or other precipitating factors prior to the onset of those symptoms. A physical examination on admission found numerous flat or palpable purpuras that were typical of HSP in both lower extremities. No lymphadenopathy and hepatosplenomegaly was evident. The boy was diagnosed with HSP.

Laboratory results such as urine output, renal function, prothrombin time, complements C3 and C4, anti-double stranded DNA antibodies, antinuclear antibodies, and anti-smooth muscle antibodies, C-reaction protein, and antistreptolysin O were all normal. The boy was treated with vitamin C, calcium gluconate, cimetidine, and dipyramidole. Joint pain and abdominal pain improved but purpuras did not change. A routine abdomen ultrasound two weeks after the diagnosis of HSP revealed a right adrenal-occupying lesion (4 cm × 6 cm) and magnetic resonance imaging suggested a neoplasm. Further laboratory tests revealed higher levels of vanillylmandelic acid (VMA) and...
homovanillic acid (HVA) in the urine. Histopathology confirmed the neoplasm to be an adrenal neuroblastoma (stage II, American Children's Oncology Group staging system, CCSG). HSP completely resolved soon after surgery, and continued chemotherapy was given using the OPEC schedule (vincristine [O], cisplatin [P], etoposide [E], and cyclophosphamide [C]). HSP did not recur during a follow-up of 12 months.

3. Discussion

The patient experienced purpura and joint and abdominal pain without kidney involvement, fulfilling the diagnostic criteria for HSP (18). Abdominal ultrasound and magnetic resonance imaging revealed a right retroperitoneal neoplasm that histopathology postoperatively confirmed to be a neuroblastoma. HSP was associated with a neuroblastoma in this 5-year-old boy. Moreover, neuroblastoma was an incidental finding diagnosed by the routine abdomen ultrasound; the patient had no abdominal signs or abnormal physical findings on admission.

Malignancies are known to cause vasculitis like HSP. In 2006, Zurada et al. (11) reviewed literature on adult malignancy-associated HSP from around the world, and they found a total of 31 cases. Patients were overwhelmingly male (94%) with a mean age of 60 years and presented predominantly with solid tumors (61%) and secondly with hematologic malignancies (39%). The most frequent tumors were lung cancer (n = 8), multiple myeloma (n = 5), prostate cancer (n = 5), and non-Hodgkin lymphoma (n = 3). The majority of patients (55%) developed HSP within 1 month of cancer diagnosis or detection of metastases. Their findings were similar to those in a report by Pertuiset et al. (8).

In 2009, Mitsui et al. (10) reported 23 cases of HSP in patients with underlying malignant tumors. HSP was thought to be closely associated with a tumor in nine patients, and seven of the nine exhibited new metastatic lesions or died due to underlying cancer within 1-32 months. Based on these reports, HSP associated with a malignancy is characterized by: i) patients who are mainly older males (over 40-60 years; over 85%); ii) development within 1-3 months of diagnosis or metastasis of a neoplasm; iii) causes are mostly solid tumors (over 60%), and especially carcinoma of the lung, followed by hematologic malignancies (about 40%); and iv) development in the absence of a precipitating factor. This suggests that adults, and especially older men who present with unexplained HSP, should be evaluated for an occult neoplasm (5,6), while patients with a known history of malignancy who present with HSP should be evaluated for metastatic disease (11). A skin biopsy is an important way to determine the underlying pathology in adult HSP (17). Malignancies induce or are associated with HSP mostly in older males, but children can also be affected, albeit rarely. Funato et al. (19) reported acute lymphoblastic leukemia mimicking HSP in a 3-year-old boy.

Neuroblastomas are the most common extracranial solid tumors in children, accounting for about 8%-10% of all pediatric tumors (20,21). That said, there are no reports of HSP associated with a neuroblastoma or other malignancy in children. The current case is the world's first case of HSP associated with a neuroblastoma.

Tumors are known to be one of the causes of vasculitis (8,10,22). Vasculitis is reported to occur during the course of malignancies in 2.3%-8% of patients (23). The incidence of vasculitis in cancer is estimated to be 1 in 1,800 for hemopathies and 1 in 80,800 for solid tumors (24). The relationship between vasculitis and malignancy remains unclear: e.g. fortuitous association, paraneoplastic syndrome, or neoplasms induced by immunosuppressive drugs prescribed to treat vasculitis, and so on (25). HSP is an allergic vasculitis disease caused by an immunologic mechanism (26-29). Neoplasm antigens such as paraneoplastic antibodies or abnormally produced IgA lead to the formation of immune complexes that induce the lesions of HSP (10).

The development of HSP and a neuroblastoma at the same time in the current patient is curious. Maybe both diseases developed independently or maybe HSP was a paraneoplastic syndrome of the neuroblastoma. However, neuroblastomas are known to induce other forms of vasculitis such as Kawasaki disease (30). In the current patient, HSP was likely to be induced by the neuroblastoma. The first reason for this conjecture is because the neuroblastoma appeared to develop prior to HSP given to the size of the neuroblastoma and the course of HSP. Second, there were no precipitating factors before the onset of HSP and no relapse during 12 months of follow-up. However, genetic studies of the neuroblastoma, e.g. studies of the N-Myc gene and paraneoplastic antibodies, were not performed, and neither was a skin biopsy.

In conclusion, many malignancies may cause HSP, but in older males HSP is mostly caused by solid tumors. Reported here is the first case of HSP associated with a neuroblastoma in a 5-year-old boy. Epidemiological studies are needed to determine the association between HSP and malignancy in children.

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Policy Forum articles discuss research and policy issues in areas related to life science such as public health, the medical care system, and social science and may address governmental issues at district, national, and international levels of discourse. Policy Forum articles should not exceed 2,000 words in length (excluding references).

Case Reports should be detailed reports of the symptoms, signs, diagnosis, treatment, and follow-up of an individual patient. Case reports may contain a demographic profile of the patient but usually describe an unusual or novel occurrence. Unreported or unusual side effects or adverse interactions involving medications will also be considered. Case Reports should not exceed 3,000 words in length (excluding references).

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