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Bascom Hall, located on top of Bascom Hill, is one of the oldest and most famous buildings on the Madison campus of the University of Wisconsin. The building is often considered the "heart of the campus" and is listed on the National Register of Historic Places as a contributing building within the Bascom Hill Historic District. It was originally built in 1857 named Main Hall and later Bascom Hall was named for University president John Bascom (1874). Currently, Bascom Hall houses many administrative offices including the office of the chancellor and vice chancellors. It is also home to the study abroad office, offering students more than 100 different programs on every continent except Antarctica.

(Photo by Caifeng Dai)

Review

Multidisciplinary Team and Team Oncology Medicine research and development in China

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Summary **In the context of transition from "Biomedical Model" to "Biology-Psychology-Society Medical Model", the treatment model of malignant tumors has changed from single-subject treatment to multidisciplinary collaboration treatment led by a Multidisciplinary Team (MDT). On this basis, the concept of "Team Oncology Medicine" strengthens the focus of malignant tumor treatment. This is not only improving cure rate and extending life span, but also paying close attention to patients' actual demands to improve their quality of life. There are many good studies and practices of Multidisciplinary Team and Team Oncology Medicine in the world. China is currently in the exploratory phase of the malignant tumor Multidisciplinary Team treatment model. Many hospitals have investigated and practiced a Multidisciplinary Team treatment model. China is faced with many problems to scientifically construct a malignant tumor treatment model which conforms to national conditions. These conditions include a medical model, a medical care insurance system, public hospitals reform, hospital management approaches, personnel framework, concern with patients' psychosis and psychology, and whether to tell patients their actual condition and how they should express their will, and so on.**

Keywords: Malignant tumor, treatment model, China

1. Introduction

Multidisciplinary Team (MDT) and Team Oncology Medicine are international medical hot topics in recent years. MDT is usually composed of specialists from two or more related disciplines, which work together to discuss some kinds of malignant tumors, and to form a clinical treatment plan (*1*). Team Oncology Medicine is patient-centered, the relevant specialists aim at patients' actual conditions and needs to guide patients with a team advantage, and provide extensive information, resources, and support. This treatment model places prime emphasis on alleviating treatment problems, symptom control, professional nursing care, recovery health care, and psychological intervention. It pays more attention to quality of life and the patients' actual needs as well as extending the patients' life span.

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2. Development of MDT

MDT model was introduced to the field of clinical medicine more than 10 years ago, and has been investigated in many disease treatment situations (*2-5*). Obvious achievements have been made in breast cancer, ovarian cancer, rectal cancer, prostate cancer, and lung cancer using the MDT treatment model (*6-10*). There are many international large-scale cancer centers such as M. D. Anderson Cancer Center (Houston, TX, USA), Philadelphia Veterans Affairs Medical Center (Philadelphia, PA, USA), the Netherlands Cancer Institute of Antoni van Leeuwenhoek Hospital (Amsterdam, Netherlands), and the National Cancer Action Team of St Thomas' Hospital (London, UK) which have set up a MDT treatment model (*11-14*). In this treatment model, the specialists from two or more departments such as oncosurgery, department of tumor medicine, tumor radiotherapy department, medical imaging department, pathology department,

and other related departments get together to discuss a patient's condition and form a treatment plan (Figure 1A). Taken as a whole, MDT lays particular emphasis on cure; it analyzes organism condition, pathologic type, involvement scope, clinical stage, and its development tendency, through applying available therapeutic tools to improve cure rate. A finding from University of Leeds (Leeds, UK) emphasized the importance of MDT in malignant tumor treatment (*15*). This study was a retrospective analysis of 7,602 surgically resected colorectal cancer patients for whom colorectal pathology minimum data sets had been collected. A threshold for an adequate lymphadenectomy was defined as retrieval of 12 nodes. The operating surgeons and reporting pathologists were identified for each tumor. Surgeons and pathologists were then assigned to be team or non-team members according to the results of the National Cancer Peer Review process. The final study data showed that MDT surgeons offered a 40% increase in the odds of retrieving at least 12 nodes, whereas the odds for MDT pathologists was more than twice that of nonspecialist pathologists.

In recent years, cancer morbidity and mortality are not optimistic in a worldwide scope. The International Agency for Research on Cancer evaluated statistical models to estimate incidence and mortality data for 25 cancers in 40 European countries (grouped and individually) in 2008. There were an estimated 3.2 million new cases of cancer and 1.7 million deaths from cancer in 2008 (*16*). The data from The American Cancer Society showed that a total of 1,479,350 new cancer cases and 562,340 deaths from cancer were projected to occur in the United States in 2009 (*17*). China has finished the third national death cause survey and the data show that cancers of lung, liver, stomach and esophagus accounted for nearly 72% of the total cancer deaths in China in 2005 (*18*). During

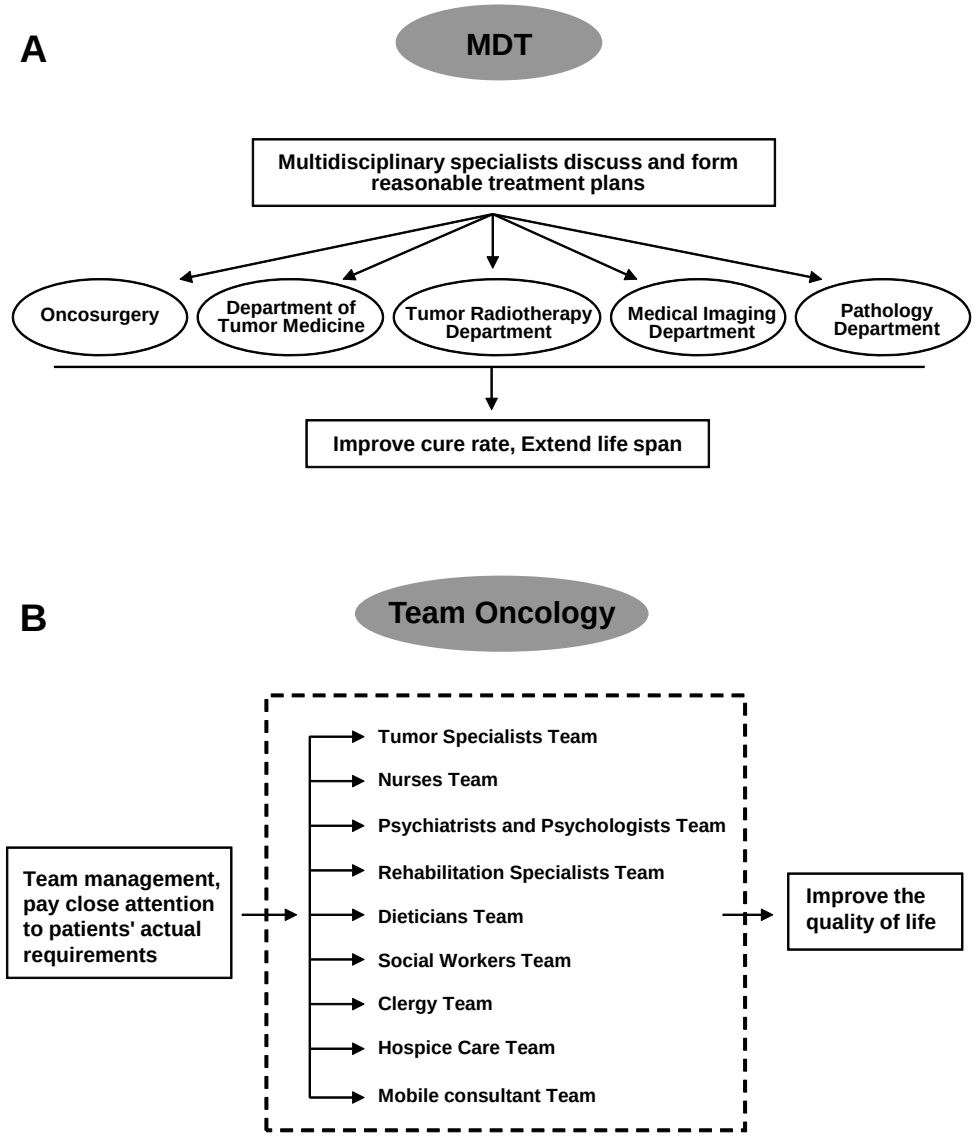

Figure 1. The composition and purpose of MDT (A) and Team Oncology (B).

2004-2005, the top three cancer mortality rates for males were lung cancer (41.34/100,000), liver cancer (37.54/100,000), and stomach cancer (32.46/100,000) in China and for females were lung cancer (19.84/100,000), stomach cancer (16.59/100,000), and liver cancer(14.44/100,000) (*19*).

The malignant tumor prognosis is not ideal. In lung cancer, for example, in the preliminary diagnosis of lung cancer patients, stage IIIB and IV lung cancer account for 50%, and stage I and II lung cancer that could be operable accounted for 30%. The recurrent rate is 30-75% in the surgical resection lung cancer patients and 80% of recurrent patients occur in the first two years after the first operation. The median time from surgical resection to recurrence was 11.5 months (*20*). Many more scholars realize that the concern of malignant tumor treatment is not only extending life span, but also quality of life; patients' requirements are not only simply survival, but also living a quality life with dignity. Therapy should not be at the expense of quality of life. Recognizing this fact, M. D. Anderson Cancer Center set up a consummate malignant tumor treatment – Team Oncology Medicine (Figure 1B). It caused an international research and exploration of Team Oncology Medicine. Many countries lead in the concept of "Team Oncology Medicine" in malignant tumor treatment using their actual conditions. Japan has set up "Japan Team Oncology Program (J-TOP)" and three Team Oncology Work Shops were successfully held. Many scholars have investigated a suitable model for Team Oncology Medicine (*21-24*). The proposed mission of setting up Team Oncology Medicine in Japan was to establish and promote evidence-based multidisciplinary cancer treatment in Japan through outstanding educational and training programs for healthcare providers and the public.

3. Research and practice status of malignant tumor MDT in China

From the view of the medical model, the experience of medical model practice in China in the past 30 years is that although the medical community has accepted the concept of a "Biological-Psychological-Social Medical Model", there are lots of difficulties in practicing this medical model. It does not seem to have made much progress (*25*). In this exploration stage, the international research and practice of MDT brings new ideas to the treatment model in China. The model of multidisciplinary discussion of a single disease was shaped in form by "The Carcinoma of Large Intestine Meeting" held in Shanghai in September 2006 and "The Colorectal Anal Surgery Meeting" held in Zhuhai in November 2006.

3.1. *The first medical institution leading in the concept of MDT in China*

3.1.1. *The whole framework of the MDT model*

West China Hospital of Sichuan University (Chengdu, Sichuan, China) is the first hospital introducing the concept of MDT in China. Combining the characteristics of a large public hospital in China, with a recognized treatment pathway in MDT for colorectal cancer and a medical project construction, the MDT for colorectal cancer project team set up the "multidisciplinary team-working for colorectal cancer of West China Hospital (MDT-CRC-WCH)" with its own characteristics and subject features. The whole constructive concept is forming an effective combination of MDT organizational structure and personnel framework through the guide of the MDT team culture (*26*). In this structure, six directions are determined: *i*) the series functional microinvasive colorectal cancer radical resection based on the MDT model, *ii*) the menu-type colorectal cancer classified operation plan based on a fast-track program, *iii*) the system of new operation types for colorectal cancer based on evidence-based surgery, *iv*) the process of information interactive communication and transmission based on paper clinics, *v*) the database construction of clinical and empirical study based on integrated and shared standards, and *vi*) the sub-professional collaboration platform construction based on the regional network. The six directions laid the basic idea of colorectal carcinoma MDT professional innovation, classified menu-type and comprehensive multi-level interactions.

3.1.2. *Special feature of MDT-CRC-WCH model*

Construction characteristic of MDT-CRC-WCH: MDT for colorectal carcinoma summarized the five basic characteristics of professionalism, classification, interaction, optimization and speed. Concretely speaking, it means a highly specialized technical level, a classified menu-type medical system, doctor-patient communication and interaction at the same time, a most optimum distribution of human resources and medical resources, and a high-quality and efficient fast clinical pathway.

Core competition of MDT-CRC-WCH: With technology as the core and team culture as the driving force, having broken through the traditional concept of colorectal operations, the colorectal carcinoma MDT set up a series of functional microinvasive colorectal cancer radical resections based on the MDT model and the system's new operation types for colorectal cancer based on evidence-based surgery. At the same time, it established the volunteer team composed of residents, interns, nurses, medical students and community volunteers. It also formed the relationship dominated by medical personnel and supported by other personnel.

Branch departments of MDT-CRC-WCH project group:

MDT set up the data-based team, follow-up team, nursing team and public team. Each team had the corresponding functional authority. The data-based team took charge of collecting all the materials and data. The follow-up team took charge of collecting the postoperative patients' prognostic information by telephone, short message service, letter, and email, and the team members gave patients the corresponding follow-up guide. The nursing team carried out nursing care around the clinical model, and it put forward higher requests on the process, system and comprehensiveness of care. The public team took charge of extending the scope and depth of the MDT platform by using cycling and various publicity strategies.

Personnel framework of MDT-CRC-WCH: Based on the main four principles – whole, match, voluntary, and interactive, it constructed reciprocation which is a "concentric circle" with the team of directors, coordinators, colorectal surgeons, related professors, nurses, and other assistants.

Consultation model of MDT-CRC-WCH: The main members are specialists of MDT. The content is identifying diagnosis, establishing treatment processes, making clinical decisions, and getting the feedback message by evaluating the implementation of the decision. The consultation meeting is held weekly, it strictly regulates the consultation time length and the completion time of interdisciplinary discussion of topics and the intervals of consultation. The consultation model divides effectively into preoperative consultation, perioperative consultation, postoperative consultation and follow-up consultation. It arranges chiasmatic clinical rounds by specialists from oncosurgery and tumor medicine departments twice a week. The multidisciplinary specialists communicate with patients and discuss the preoperative basic treatment plan, the postoperative long-term treatment plan and problems in follow-up treatment.

3.1.3. *Therapeutic effect of MDT-CRC-WCH model*

West China Hospital of Sichuan University compared the therapeutic effects between groups of the MDT model (106 cases) and the non-MDT model (129 cases) by retrospective analysis of patients' data diagnosed with colorectal cancer and accepted for in-hospital therapy during December 2006 and May 2007. The results showed that the in-hospital days of the MDT model group during the perioperative period and in the surgical ward were less than that of the non-MDT model group ($p < 0.05$). Also the MDT model group had a higher rate of cancer resection ($p < 0.05$). From the analysis of early postoperative complications, the non-MDT model group encountered more early postoperative ileus ($p < 0.05$). During 5-10 months follow-up, there was a lower cancer recurrence rate in the MDT model group ($p < 0.05$). This retrospective study came to the conclusion that the combined-therapy colorectal cancer strategy should be shown a priority compared to routine methods, not only for the more reasonable time arrangement of therapy, but also for the more satisfactory surgical outcomes (*27*).

3.2. *Beneficial exploration of the malignant tumor MDT model by many medical institutions in China*

Comparing China's actual conditions, there are many beneficial explorations of the malignant tumor MDT model in some cities with better consummate medical conditions. It was represented in Beijing and Tianjin in northern coastal areas, Shanghai in eastern coastal areas, Guangdong in southern coastal areas and Sichuan in the southwest. Some cancer hospitals with extensive experience in treating malignant tumors practiced the MDT model. Generally speaking, these practices absorbed the international advanced experience and explored the malignant tumor MDT model using China's actual conditions. From the view of specific implementation, through a multidisciplinary collaborative approach, all practices carried out multidisciplinary discussions and evaluated patients' conditions. Breaking through the disadvantage of single therapy, these practices formed scientific and reasonable individual treatment plans to improve cure rate and extend life span. Based on these, some hospitals made breakthroughs and innovations using their own conditions (Table 1).

3.3. *Theoretical discussion on the current situation and the challenge of the malignant tumor MDT model*

In 2008, Professor Wu Yilong, the standing director of the Chinese Anti-Cancer Association (Tianjin, China), published an article entitled "The Challenge of Malignant Tumor Combined Therapy". It set off a heated discussion on "The Current Situation and Challenge of the Malignant Tumor MDT Model" in China. According to China's actual conditions, many experts expressed their viewpoints and discussed this topic from various aspects (Table 2).

4. Thinking about the problems faced by the practice of malignant tumor MDT and Team Oncology Medicine in China

China is still in the exploratory phase of the MDT model, it has not yet been developed to the Team Oncology Medicine stage. West China Hospital of Sichuan University is the first hospital introducing the concept of MDT in China. The MDT-CRC-WCH has set up and promoted the clinical research development of the malignant tumor treatment model. Both the heated discussion on "The Current Situation and Challenge of the Malignant Tumor MDT Model" in

Table 1. The distinctive practices of malignant tumor MDT model in representative hospitals

Area	City	Representative Hospitals	The distinctive practices of MDT model
Northern coastal areas	Beijing	Beijing Cancer Hospital	Multidisciplinary collaborative group including department of traditional Chinese medicine and the divisions of basic research (*28*).
	Tianjin	Cancer Hospital of Tianjin Medical University	MDT model with radiotherapy and chemotherapy combined, stratified by group and combined therapy (*29*).
Eastern coastal areas	Shanghai	Fudan University Shanghai Cancer Center	MDT model paying close attention to various factors influencing the prognosis (*30*).
Southern coastal areas	Guangdong	Oncology Center in Guangdong General Hospital	MDT model with excellent team consisting of multidisciplinary talents (*31*,*32*).
		Cancer Center in Sun Yatsen University	MDT model with the regulation of single disease treatment, the single disease diagnosis and treatment management team composed of a chief expert, diseases experts, auxiliary department experts and coordinating secretary (*33*).
Southwest	Sichuan	Cancer Hospital of Sichuan	Developed four kinds of scale to evaluate the quality of life of Chinese cancer patients during the recovery period. Put forward firstly multidisciplinary comprehensive rehabilitation intervention model combined with oncology, psychology and social medicine (*34*,*35*).

Table 2. The discussion and the current situation of malignant tumor MDT model in China

Topics	Viewpoints	Current Situation
Sub-division system	**The advantage of "Division by therapeutic tool"**: Be conducive to the in-depth development of surgery, radiotherapy, chemotherapy and other subjects. **Disadvantage**: Under the single-subject treatment model, the diagnosis and the staging has not been standardized, as well as lack of good communication between disciplines. It is not conducive to patients to receive multiple medical resources. **The advantage of "Division by entity"**: The scientific and reasonable treatment plan developed by the systematic multidisciplinary consultation can make patients receive greatest benefits. **Disadvantage**: It can be divided into dozens of divisions according to tumor entity. It is not conducive to resource centralized management if every division has its own surgeons, physicians and radiologists (*36*).	The Cancer Hospitals make the division by entity and the General Hospitals make the division by therapeutic tool.
Leading talents	The leading talents are requested to master the knowledge of surgery, drugs, and radiation therapy. It means that the leading talents are not only able to develop clinical treatment plan, but also must have the concept of comprehensive treatment, experience in basic research and the practices of transformation research. They could apply a variety of therapeutic tools reasonably and achieve the engagement of the different therapeutic tools (*36*,*37*).	The lack of leading talents conditioned the realization of the details.
The training of tumor specialists	It should strictly guard a pass of the hospitals' qualifications where the tumor specialists are trained. Besides, we should improve the tumor specialists' training institution and the reasonable mechanism of personnel flow. We should train tumor specialists and pay more attention to the concept of MDT model and truly understand the application of surgery, radiotherapy and chemotherapy (*38*,*39*).	Because of the limitations of hospitals' academic level, equipment conditions and the training system, there is a big difference in tumor specialists' level between different hospitals.
The establishment of Cancer Center	It is advocated to establish Cancer Center. At the same time, we should give full play to the role of the Chinese Anti-Cancer Association's professional committees. Within the framework of the Cancer Center, organizing relevant professionals to form a project team is to facilitate communication and collaboration with each other (*39*).	From the 1950s, Chinese government commenced establishing a Cancer Hospital and Research Institute for every province. It has formed many distinctive cancer centers with years of development. However, the MDT treatment model needs to be further improved under the framework of the Cancer Center.

China and the distinctive practices of the malignant tumor MDT model in representative hospitals have played an active role in promoting clinical research development of the malignant tumor treatment model. There is no doubt that the concept of malignant tumor MDT has become the general consensus of the clinical workers in China. However, it has not had a nationwide organization to formulate a development strategy for the MDT treatment model and Team Oncology treatment model. Although the MDT model has been implemented in some hospitals, it still faces many difficulties. How should the MDT model suitable for China's actual conditions be constructed and how should the development of the MDT model for Team Oncology Medicine with Chinese characteristics be further advanced? In-depth reflection on these issues will promote the best development of the malignant tumor treatment model in China (Table 3).

i) With the "Biomedical Model", hospitals' service target is "disease". It carries out tasks as "disease-centric". The service model uses medicine, surgery and other ways to give patients relief. But with the "Biology-Psychology-Society Medical Model", the service target of hospitals is no longer "disease", but is "patient-centered". It requires hospitals and medical workers to change traditional services depending on drugs, surgery, and other treatments, and establish a comprehensive and multi-dimensional service model with psychological treatment and humane care. The aim of increasing the efficiency of treatment and restoring health status is also required to change the physician-patient relationship from "Active-Passive" to scientific integration with "Active-Passive", "Guidance-Cooperative", and "Participation-Consultation". China is still in the transformation stage from "Biomedical Model" to "Biology-Psychology-Society Medical Model". In this transitional period, how can hospitals change the service model to explore the malignant tumor treatment model? How can hospitals build a harmonious and equal relationship between doctor and patient? How can an all-dimensional treatment system combined with medical technology, mental health and human care be created? It requires hospitals, society and patients to make joint efforts to solve these problems.

ii) China's basic medical insurance system is composed of the basic medical insurance system for employees in urban areas, the basic medical insurance

Table 3. Thinking about the problems faced by constructing malignant tumor MDT and Team Oncology Medicine with Chinese characteristics

Problems	Thinking
The service model and the physician-patient relationship under the change of medical model	How to truly achieve "patient-centered"? How to change the traditional services depending on medicine, surgery and other treatments into a comprehensive and multi-dimensional service model with psychological treatment and humane care? How to change the physician-patient relationship from "Active-Passive" into scientific integration with "Active-Passive", "Guidance-Cooperative" and "Participation-Consultation"?
The expense burden under the basic medical insurance system	How to further expand the coverage of the basic medical insurance system to benefit more patients with malignant tumors? How to further increase the input from government and society to reduce the proportion of personal health expenditures for patients with malignant tumors? How to further regulate medical practice and reduce medical expense to relieve the family burden for patients with malignant tumors?
The public hospitals reform in the context of China's medical reform	How to implement the responsibilities of the public hospitals held by government? After the abolishment of the drug price addition, does government financing have sufficient financial resources to compensate public hospitals? Will the additional costs of the pharmaceutical service fee and adjusting technical service fee be passed on to patients? How to transform a part of public hospitals into non-public hospitals? How could government and hospitals overcome the negative impact caused by the choice of therapeutic tools due to financial gain? How to develop and improve the scientific clinical rules about oncology and push tumor specialists to abide by the rules?
The way of hospital administration	How to adjust the department set and the management philosophy according to the demand of patients with malignant tumors and the hospitals' own actual conditions? How to improve and enhance the quality of medical services? How to provide patients a convenient and comfortable medical treatment environment?
Personnel framework under the treatment model	Is it suitable for China's actual conditions to form the team framework composed of team leader, team contact person (tumor coordinator), multidisciplinary experts team for consultations, professional care team, nutrition and recovery guidance team, social workers and so on? What is the requirement for the team members? How to make the members work closely with each other and put forward a rational division of work?
The psychological concerns	How to form a psychological experts team for malignant tumors in accordance with China's actual conditions? How to give full play to the role of social workers? How to mobilize all social strata to give patients with malignant tumors extensive care and psychological support?
The informed consent and willingness expression for patients	Under the influence of traditional Chinese culture and concepts, should doctors tell patients their actual conditions? Which way is better to inform patients? How to choose the opportunity? How to fully respect patients' willingness expression?

system for residents in urban areas, the new-style rural cooperative medical care system, and the medical system for disadvantaged groups in urban and rural areas. This covers the bulk of urban employees, urban non-working population, rural population, and vulnerable groups in urban and rural areas. According to "2009 Chinese Health Statistical Yearbook", in 2008 the total population of China was 13.28 hundred million, the basic medical insurance system for employees in urban areas and residents in urban areas involved 3.18 hundred million people, the new-style rural cooperative medical care system involved 8.15 hundred million people, and the medical system of disadvantaged groups in urban and rural areas benefited 0.46 hundred million people. Thus it can be seen, that nearly 2 million people had not been incorporated into the basic medical insurance system. The total health expenditure of China is composed of government health expenditure, social health expenditure and personal health expenditure, accounting for 20.4%, 34.5%, and 45.2% of the total health expenditure in 2007. Liver cancer led cancer mortality in China. The average per capita health expenditure for liver cancer was 9,402.2 yuan in 2008. The average per capita personal health expenditure for lung cancer patients was 4,249.8 yuan, accounting for 24.9% of urban residents annual per capita income (the annual per capita income of urban residents was 17,067.8 yuan in 2008) and 63.4% of rural residents annual per capita income (the annual per capita income of rural residents was 6,700.7 yuan in 2008) (*19*). Under the current basic medical insurance system of China, the problem is how to further expand the coverage of the basic medical insurance system to benefit more patients with malignant tumors, how to further increase the input from government and society to reduce the proportion of personal health expenditures for patients with malignant tumors and how to further regulate medical practice and reduce medical expense to relieve the family burden for patients with malignant tumors. This requires the Chinese government to exercise macro control over the basic medical insurance system and give full play to commercial health insurance and other forms of supplementary medical insurance to benefit patients with malignant tumors.

iii) China had 19,822 hospitals by the end of November 2009, and the number of public hospitals was 14,086, accounting for 71.1%. The number of outpatients was estimated to reach 18.5 hundred million in 2009, and 17.1 hundred million patients went to public hospitals, accounting for 92.4%. The number of inpatients was estimated to reach 81.2 million, and 75.2 million patients went to public hospitals, accounting for 92.7% (*40*). Thus it could be seen that over 90% of patients went to public hospitals. In reality, there is high expense and low service in public hospitals due to inadequate government input, imperfect hospital management mechanisms and other reasons. On 23 February 2010, the Ministry of Health and five other ministries jointly issued "Guidance on Pilot Reform of Public Hospitals" (*41*), selecting 16 cities (6 cities in the eastern region, 6 cities in the central region and 4 cities in the western region) as a national guide for public hospital reform in some areas. It adheres to the guiding ideology of the public nature of public hospitals to safeguard people's health and first place rights, and it guides the often-criticized system of pharmacies to support doctors' "cut". The idea for reform is changing the compensation mechanism from service charges in public hospitals, medicines plus income and government subsidies into service charges and government grants. The Guidance pointed out that the government will make additional pharmaceutical service fees and adjust part of the technical service fees. The reasonable reduced income for public hospitals will be compensated by the health insurance fund and government investment (*42*). Meanwhile, to promote the pattern of diversified hospital operators and encourage social organizations to run non-profit hospitals, on April 2010, the Chinese government issued "The Major Arrangement about the Five Focal Points of Health System Reform in 2010", putting forward "researching and exploring how to transform a part of public hospitals into non-public hospitals" (*43*). The Public Hospitals Reform involves various aspects such as operational mechanisms, personnel systems, hospital management, compensation mechanisms, and so on. In the existing medical conditions of China, the question is how to implement the responsibilities of the public hospitals held by government and how to form separate management from operations, and gradually realize unified management of public hospitals to establish a coordinated, integrated and efficient public hospital management system. After abolishment of the drug price addition, does government financing have sufficient financial resources to compensate public hospitals? Will the additional costs of the pharmaceutical service fee and the adjustment of the technical service fee be passed on to patients? Also, how to transform a part of public hospitals into non-public hospitals? To solve these issues, support of the basic medical insurance system, the essential drugs system and a series of strong supporting policies and practical measures are needed (*44*). For patients with malignant tumors, the current reform of public hospitals was carried out for the hospital as a whole in terms of reform, and has not yet been refined to specific policies and regulations on disease. In this condition, how could government and hospitals overcome the negative impact caused by the choice of therapeutic tools due to financial gain? How to develop and improve the scientific clinical rules about oncology and to push tumor specialists to abide by the rules is another question. In the context of reform in public hospitals, constructing malignant tumor MDT and Team

Oncology Medicine with Chinese characteristics will face a rare chance for development as well as tough challenges.

iv) In the "patient-centered" system, the malignant tumor MDT and Team Oncology Medicine model is not the simple sum of "surgery + radiotherapy + chemotherapy", but determining the actual treatment according to the patient's life expectancy, treatment tolerance, expectation of life quality, patient's wishes and the tumor's specificity. For hospitals, how to grasp the overall layout, team development, evaluation system, resource allocation and other key factors to adjust the departments set and the management philosophy according to the demand of patients with malignant tumors and the hospitals' own actual conditions is another concern. How to improve and enhance the quality of medical services and how to provide a convenient and comfortable treatment environment to enhance the patients' confidence against resistance? These important issues need to be solved by hospitals' top executives.

v) M. D. Anderson Cancer Center has formed a more complete tumor treatment team framework in practice. It is patient-centered where the relevant specialists such as tumor specialists team, nurses team, psychiatrists and psychologists team, rehabilitation specialists team, dieticians team, social workers team, clergy team, hospice care team, and mobile consultant team get together to guide patients with the team advantage throughout treatment. In China, many hospitals such as West China Hospital of Sichuan University have explored the personnel framework based on absorbing the international advanced experience (*45*). Is it suitable for China's actual conditions to form the team framework composed of a team leader, team contact person (tumor coordinator), multidisciplinary experts team for consultations, professional care team, nutrition and recovery guidance team, social workers, and so on? What are the requirements for the team members? How to make the members work closely with each other and put forward a rational division of work? These issues need to be further explored and practiced.

vi) From the view of psychological concerns of patients with malignant tumors, a psychiatrists and psychologists team in China has not yet been formed. With the malignant tumor MDT model, West China Hospital of Sichuan University established the "Colorectal Cancer MDT Volunteer Team" and "Gastrointestinal Cancer MDT Volunteer Team". The Volunteer Team has about 300 members, and most of them are medical students from different grades. The task emphasis on research, such as data collection and arrangements with patients with malignant tumors, surgery studies, laboratory studies, and so on need to be explored further (*46*). Although the work involves communication between doctors and patients, with the restriction of medical student's own knowledge, they could not carry out scientific psychiatric treatment and psychological persuasion, and they could not compare information with the psychiatrists and psychologists. Under these conditions, how can the teams form a psychological team of experts for malignant tumors consistent with China's actual conditions? How can the teams give full play to the role of social workers to help patients relieve negative emotions? How can the teams mobilize all social strata to give patients with malignant tumors extensive care and psychological support? These are important problems faced by the nation and society medical institutions, and the settlement needs the support of national policies, social concerns and hospital measures.

vii) Knowing the patient's condition is not only a medical problem, but also an ethical issue (*47,48*). In China, most families worry that patients will feel despair and refuse treatment if they know the actual conditions, so the families conceal the truth from the patients with malignant tumors. Many international studies have shown that patients will take the initiative with the treatment if they know the actual conditions. Under the influence of traditional Chinese culture and concepts, should the doctors tell patients their actual condition? Which is the best way to better inform patients, how to choose the opportunity, and how to fully respect patients' willingness of expression? These are not only complex social problems, but also problems that need to be solved by medical personnel, patients and their families.

5. Conclusion

In the "Biology-Psychology-Society Medical Model", with the scientific and technological progress and people's understanding of solid tumors, the malignant tumor treatment model has basically changed from single-subject treatment to multidisciplinary collaboration treatment which was led by a Multidisciplinary Team. On this basis, a more consummate malignant tumor treatment – Team Oncology Medicine has been set up, which pays close attention to patients' actual demand to improve the quality of life. China is in the exploratory phase of the malignant tumor MDT model currently. In the context of international MDT research and practice, many hospitals have made useful explorations of the malignant tumor MDT model and have made some progress. However, China is faced with many problems to scientifically construct the malignant tumor treatment model which conforms to national conditions, such as medical model, medical care insurance system, public hospitals reform, hospital management approach, personnel framework, concern with patients' psychosis and psychology, and whether to tell patients their actual condition and how they can express their will, and so on. In the transitional phase of the medical model and in the context of China's medical reform, how can the

MDT model and Team Oncology Medicine model be constructed with Chinese characteristics? It needs to be further explored and practiced. It is still a long-term and arduous task in China.

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Brief Report

Longitudinal observation of influence of "taspo" on smoking behavior among high school students

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Summary **A system with an adult discrimination IC card "taspo" was introduced in 2008 to prevent minors from purchasing cigarettes in Japan. This study aimed to elucidate the short-term change in smoking behavior among a cohort of high school students through the introduction of the taspo system. We conducted a questionnaire survey in students at one high school in the metropolitan area of Japan in 2008. In this area, the taspo system was introduced on July 1, and the survey was conducted before and after its introduction (June and September). Change in smoking behavior was examined by linking the two questionnaires using a unique identification number for each participant. The questionnaire included basic characteristics, smoking-related behavior, and means of obtaining tobacco. Of 133 students, 123 (response rate 84.7%) completed the before and after questionnaire forms and could be linked. The smoking rate was 22.8% in June and 25.2% in September, with no statistically significant change. Vending machines were the major means of obtaining tobacco in June, while the use of cigarette shops and supermarkets increased after the introduction of taspo. The introduction of taspo hardly influenced underage smoking behavior during the observation period in our study subjects. The only significant change was in the means of obtaining tobacco. To prevent underage smoking, the importance of comprehensive restriction of the procurement route was suggested.**

Keywords: Underage, smoking, vending machine, smoking prevention

1. Introduction

Prevention of underage smoking is one of the most important anti-smoking strategies. A longer smoking duration has been demonstrated to be associated with higher risk of cancer, cardiovascular disease, and other illnesses, and starting to smoke before the age of 20 will increase the level of dependency on nicotine, making smoking cessation more difficult (*1*).

In Japan, vending machines with adult identification functions using the IC card "taspo" were introduced in 2008, to prevent minors purchasing cigarettes. The results of a nationwide survey in 2004 revealed that 82.5% of male students and 77.8% of female students among surveyed high-school students with a smoking habit chose vending machines as the most common place to purchase cigarettes (*2*). The taspo system, therefore, is expected to make cigarette purchase more difficult for minors, leading to a decrease in the smoking rate in this group.

The aim of this study was to elucidate the changes in smoking behavior after taspo introduction among the students of one high school in the metropolitan area, using longitudinal data of a small cohort population.

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2. Methods

2.1. *Survey subjects*

We surveyed 158 underage students (as of September, 2008) at one part-time high school in the metropolitan

area who attended school on the first day of survey (June) and consented to participate in the survey.

2.2. *Methods of survey*

Surveys using anonymous self-administered questionnaires were conducted before and after taspo introduction (June and September, 2008). In this area, the taspo system was introduced on July 1, 2008. To conduct the survey, homeroom teachers distributed the questionnaire forms and instructions after school hours. They explained to the students, following the instructions provided by the investigators, that their responses would remain anonymous and be handled confidentially, and instructed them to answer on their own.

The questionnaires included items of basic demographic characteristics (sex and age), smoking status, age of starting smoking, number of cigarettes smoked per day, and means of obtaining tobacco (vending machines, convenience stores, cigarette shops, given by friends, upperclass students, or other people, and home).

Definitions of smokers were: "ever-smokers" = those who had ever smoked a cigarette; "smokers for the month" = those who had smoked daily or occasionally during the past 30 days; and "daily smokers" = those who had smoked daily for the past 30 days.

Only the students who consented to participate in the survey were included in the study. They were each assigned a unique ID for this study. Data from the baseline survey and the survey after 3 months were linked with each other using these IDs.

The completed questionnaire forms were sealed by the responders, thus securing privacy. This study was reviewed by the ethics committee of the National Institute of Public Health.

2.3. *Analysis*

NcNemar test was used to analyze the relation between taspo introduction and smoking rate. Statistical software, SPSS 15.0J, was used, and the significance level was set at 5% (two-sided).

3. Results

Seventy-four responses from male students and 83 responses from female students were collected, with one uncompleted form in June (collection rate 99.4%). The responses from the two surveys could be linked for 133 students (collection rate 84.7%). Analysis of the survey included a total of 58 male students (15 or 16 years, 29; 17 years, 11; and 18 or 19 years, 18) and a total of 65 female students (15 or 16 years, 32; 17 years, 19; and 18 or 19 years, 14).

Table 1 shows the smoking status by sex and age in June and September. For the male students in June, the proportion of ever-smokers was higher in older age groups, with 61.1% in the 18 to 19-year-old group. Female students did not show the same trend. The overall proportions of ever-smokers in June and September were similar (48.8% and 51.2%, respectively). The overall

Table 1. Proportions of ever-smokers, smokers for the month, and daily smokers before and after taspo introduction (*n* = 123)

		Age	Total	Ever-smokers[a]		Smokers for the month[b]		Daily smokers[c]	
		(years)	*n*	*n*	(%)	*n*	(%)	*n*	(%)
June	Male	15 or 16	29	10	(34.5%)	4	(13.8%)	1	(3.4%)
		17	11	5	(45.5%)	3	(27.3%)	1	(9.1%)
		18 or 19	18	11	(61.1%)	4	(22.2%)	3	(16.7%)
		Total	58	26	(44.8%)	11	(19.0%)	5	(8.6%)
	Female	15 or16	32	17	(53.1%)	9	(28.1%)	6	(18.8%)
		17	19	10	(52.6%)	4	(21.1%)	3	(15.8%)
		18 or 19	14	7	(50.0%)	4	(28.6%)	3	(21.4%)
		Total	65	34	(52.3%)	17	(26.2%)	12	(18.5%)
	Total		123	60	(48.8%)	28	(22.8%)	17	(13.8%)
September	Male	15 or 16	24	10	(41.7%)	7	(29.2%)	0	(0.0%)
		17	14	5	(35.7%)	4	(28.6%)	3	(21.4%)
		18 or 19	20	13	(65.0%)	4	(20.0%)	4	(20.0%)
		Total	58	28	(48.3%)	15	(25.9%)	7	(12.1%)
	Female	15 to 16	24	14	(58.3%)	8	(33.3%)	5	(20.8%)
		17	26	15	(57.7%)	5	(19.2%)	2	(7.7%)
		18 to 19	15	6	(40.0%)	3	(20.0%)	3	(20.0%)
		Total	65	35	(53.8%)	16	(24.6%)	10	(15.4%)
	Total		123	63	(51.2%)	31	(25.2%)	17	(13.8%)

[a] Ever-smokers: Those who had ever smoked a cigarette.
[b] Smokers for the month: Those who had smoked daily or occasionally during the past 30 days.
[c] Daily smokers: Those who had smoked daily for the past 30 days.

proportion of smokers for the month was 22.8% in June, while in September, the proportion was 25.2%. The male proportion of smokers for the month was higher in September (25.9%) than in June (19.0%). The overall proportion of daily smokers was approximately half that of smokers for the month.

Table 2 shows the changes in smoking status after taspo introduction. After taspo introduction, 6 out of 28 ceased smoking, while 9 started smoking. The change in smoking rate after taspo introduction was not statistically significant.

The number of cigarettes per day among smokers for the month in June was 10 or fewer in 15 students (53.6%), 11 to 20 in 11 (39.3%), and 21 or more in 2 (7.1%), while that in September was 10 or fewer in 17 (54.8%), 11 to 20 in 10 (32.3%), and 21 or more in 2 (6.5%).

Table 3 shows the results concerning the means of obtaining tobacco. The preferred means in June was vending machines (82.1%), followed by convenience stores (50.0%), cigarette shops (28.6%), and given by someone (friends, upperclass students, or other people) (17.9%). In September, the ratio of cigarette shops increased to 50.0%, followed by convenience stores (46.7%), and given by someone (26.7%). The ratio of vending machines markedly decreased to 20.0%. Among 19 smokers for the month who had previously purchased cigarettes mainly from vending machines, 16 responded that they had changed the means of obtaining tobacco, with 6 purchasing from cigarette shops, 4 from convenience stores, one from supermarkets, one from shops, one obtaining them at home, and 3 no-response. Among 3 smokers who did not change the means, one used the taspo card of a family member, one asked others to buy tobacco for him/her, and one did both.

4. Discussion

The primary purpose of introducing the taspo system was to prevent underage smoking. In this study, we investigated the short-term impact of taspo introduction at a high school, determining longitudinal changes for each student by linking data on smoking status before and after taspo introduction. The results showed no significant decrease in smoking rate.

There are two factors that may provide reasons for the lack of a decrease in smoking rate. The first is that students have other means of obtaining tobacco, although purchasing cigarettes from vending machines has become more difficult. The most common means in June was vending machines, as in national surveys (*2*,*3*) and other literatures (*4*,*5*), while in September after taspo introduction, the proportion of cigarette shops increased. The unchanged proportion of convenience stores and the increased proportion of cigarette shops may indicate that minors can purchase cigarettes even at shops where age confirmation should be required. The second reason for the lack of decrease in smoking rate is that minors can purchase cigarettes from vending machines merely by using the taspo card of other

Table 2. Smoking status before and after taspo introduction (*n* = 123)

			September						*p*-value
			Smokers[a]		Non-smokers[b]		Total		
			n	(%)	*n*	(%)	*n*	(%)	
June	Male	Smokers	10	(17.2%)	1	(1.7%)	11	(19.0%)	0.22
		Non-smokers	5	(8.6%)	42	(72.4%)	47	(81.0%)	
		Total	15	(25.9%)	43	(74.1%)	58	(100.0%)	
	Female	Smokers	12	(18.5%)	5	(7.7%)	17	(26.2%)	1.00
		Non-smokers	4	(6.2%)	44	(67.7%)	48	(73.8%)	
		Total	16	(24.6%)	49	(75.4%)	65	(100.0%)	
	Total	Smokers	22	(17.9%)	6	(4.9%)	28	(22.8%)	0.61
		Non-smokers	9	(7.3%)	86	(69.9%)	95	(77.2%)	
		Total	31	(25.2%)	92	(74.8%)	123	(100.0%)	

[a] Smokers: Those who smoked daily or occasionally during the past 30 days.
[b] Non-smokers: Those other than smokers.

Table 3. Means of obtaining tobacco before and after taspo introduction (multiple answers by smokers excluding non-respondents) (*n* = 28 in June, *n* = 30 in September)

		Convenience stores		Supermarkets		Vending machines		Cigarette shops		Given by someone		Homes	
		n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
June	Male	3	(27.3%)	0	(0.0%)	8	(72.7%)	2	(18.2%)	3	(27.3%)	0	(0.0%)
	Female	11	(64.7%)	1	(5.9%)	15	(88.2%)	6	(35.3%)	2	(11.8%)	2	(11.8%)
	Total	14	(50.0%)	1	(3.6%)	23	(82.1%)	8	(28.6%)	5	(17.9%)	2	(7.1%)
September	Male	7	(46.7%)	3	(20.0%)	2	(13.3%)	4	(26.7%)	4	(26.7%)	1	(6.7%)
	Female	7	(46.7%)	2	(13.3%)	4	(26.7%)	11	(73.3%)	4	(26.7%)	2	(13.3%)
	Total	14	(46.7%)	5	(16.7%)	6	(20.0%)	15	(50.0%)	8	(26.7%)	3	(10.0%)

people. In our study, some students responded that they had borrowed taspo cards from family members, or asked other people to buy cigarettes for them from vending machines. According to the report by the Tanegashima police department concerning minors taken into custody for smoking during the period of taspo trial operation (*6*), the number of such minors was 39 in 2003 before taspo introduction, while the number gradually decreased to 31 in 2004 when the taspo system was introduced in May, and to 10 in 2005. The number, however, markedly increased to 84 in 2006. As examples of the means of procurement, the police department listed: given by friends, borrowing taspo cards from family members or acquaintances without their permission, and asking someone to let them use their cards.

Our study has a few limitations. First, our results involving only one school may not be generalized. Second, the nature of the study did not allow us to have control samples, thus we failed to take into account a potential increase in smoking rate during long school holidays, suggesting that the effect of taspo to reduce smoking rate may have been underestimated. Third, this study was conducted a few months after taspo introduction, thus only enabling observation of changes in a short period. Since it is obvious that taspo introduction made cigarette purchase by minors difficult, the smoking rate will likely decrease in the middle and long term.

Based on our study, not only the taspo system but also any access to purchasing cigarettes should be limited for minors, with such as stricter age confirmation at convenience stores, cigarette shops, and other places across the nation. Increasing the cigarette price is also expected to be effective to prevent minors purchasing cigarettes. In addition to measures against obtaining tobacco, the following two strategies will be important. The first is to take measures in relation to people around minors. It is suggested that the environment surrounding minors, such as parents' smoking, affects their smoking status (*1*), and household smoking restrictions have been demonstrated to prevent minors from smoking (*7*). Therefore, anti-smoking strategies targeting minors should be wide ranging including those targeting adults. Second, in our study 30.5% of ever-smokers responded that they started smoking before junior high school (data not shown). Consequently, tobacco education should begin in nurseries, kindergartens, and primary schools before minors start smoking and smoking becomes a habit.

In conclusion, our study demonstrated that no significant change was observed in smoking rates of high school students in the metropolitan area after taspo introduction, while the main means of obtaining tobacco changed from vending machines to cigarette shops. The taspo system did not have a major effect in reducing the smoking rate of minors in the short term.

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Brief Report

Bioinformatic analyses and an expression study of a novel gene associated with metabolic syndrome

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Summary **The investigation of novel genes involved in the derangement of glucose and lipid metabolism is of particular importance in understanding the development of metabolic syndrome (MS). In the present study, bioinformatic analyses were carried out to explore the structures and roles of the proteins encoded by the four cDNA sequences identified in our previous studies as associated with MS. Homology analyses demonstrated that the proteins encoded by Sequence 1, Sequence 2, Sequence 3, and Sequence 4 were homologous with fibrinogen gamma polypeptide, liver fibrinogen-like 1, chromosome 10 open reading frame 104, and an unnamed protein product, respectively. Because the structures were well-known for fibrinogen gamma polypeptide and liver fibrinogen-like 1, further analyses were performed only for Sequence 3 and Sequence 4. Analyses of functional domains showed that the predicted proteins encoded by Sequence 3 and Sequence 4 had multiple phosphorylation and myristoylation sites. These results indicated that the two predicted proteins might be intermediate proteins in some signaling pathways. In order to explore the possible association of Sequence 3 with MS, HepG2 cells, a human hepatoma cell line, were treated with different concentrations of glucose (mannitol as osmotic control) for 48 h. Glucose at concentrations of 22 and 33.3 mM significantly increased the mRNA expression of Sequence 3 compared to glucose at 5.6 mM while mannitol had no significant effect on the mRNA expression of Sequence 3. These results indicated that the mRNA expression of Sequence 3 was positively associated with glucose higher than physiological concentrations.**

Keywords: Metabolic syndrome, bioinformatic analysis, mRNA expression, intermediate proteins

1. Introduction

Metabolic syndrome (MS) is a multigenic disorder that encompasses abnormalities such as visceral (abdominal or central variant) obesity, insulin resistance, glucose intolerance, hypertension, and dyslipidemia characterized by elevated triglyceride and decreased HDL concentrations (*1*). This syndrome has been found to be significantly associated with coronary heart disease, stroke, type 2 diabetes mellitus, and increased risk of various cancers (*2-6*). MS has become increasingly common in many populations. A national cross-sectional survey in China indicated that the age-standardized prevalence of MS was 9.8% in men and 17.8% in women (*7*). In the United States, 24% of US adults were reported to have MS in the Third National Health and Nutrition Examination Survey (*8*).

Investigation of novel genes involved in the derangement of glucose and lipid metabolism is of particular importance in understanding the development of MS. In our previous studies, we introduced a rat model having some features of MS using a high-carbohydrate diet and constructed and screened hepatic subtraction cDNA libraries of the model rats (*9*). Four full-length cDNAs were identified by screening a human hepatic cDNA library with a mixture of

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probes of the differentially expressed fragments from the rat hepatic subtraction cDNA libraries (*10*). Sequencing and homology analyses of the four full-length cDNAs demonstrated that Sequence 1, Sequence 2, and Sequence 3 were highly homologous with fibrinogen gamma mRNA, liver fibrinogen-related gene-1 (LFIRE1) mRNA, and chromosome 10 open reading frame 104 mRNA, respectively. Sequence 4 had homology with carbamoyl phosphate synthetase 1 mRNA.

In the present study, bioinformatic analyses of functional domains were carried out for the proteins encoded by Sequence 3 and Sequence 4 because homology analyses of the proteins encoded by the four cDNA sequences showed that the two were novel proteins. Expression studies were also performed using real-time quantitative PCR (RTQ-PCR) to validate the existence of the gene of Sequence 3 in hepatic cells and its possible association with MS.

2. Materials and Methods

2.1. *Reagents*

Glucose-free Dulbecco's modified Eagle's medium (DMEM) was purchased from Sigma-Aldrich, St. Louis, MO, USA. Low-glucose DMEM was from Gibco, Grand Island, NY, USA. Penicillin, L-glutamine, D-glucose, and mannitol were all from Amresco, Solon, OH, USA. Newborn calf serum was from Hangzhou Sijiqing Co., Hangzhou, Zhejiang, China. Bovine serum albumin (BSA) was from Roche Applied Science, Mannheim, Germany. RNA extraction kit and DNase I (RNase-free) were from TaKaRa, Dalian, China. PCR primers and Taqman probes were synthesized by TaKaRa Biotech, Dalian, China. ReverTra Ace reverse transcriptase and Real-time PCR Master Mix were both from Toyobo, Osaka, Japan.

2.2. *Bioinformatic analyses*

Homology analyses of the proteins encoded by the four cDNA sequences were conducted using the Blastp program (*http://www.ncbi.nlm.nih.gov/BLAST*) (*11*). Bioinformatic analyses were performed by Compute pI/Mw, ScanProsite, SignalP, Psort, Motif, and InterPro Scan software in the ExPASy Server (*http://www.expasy.org*) to predict physical and chemical properties, signal peptides, subcellular localization, and functional domains of the novel proteins encoded by Sequence 3 and Sequence 4 (*12*).

2.3. *Cell culture and treatment*

HepG2 cells, a human hepatoma cell line, were maintained in DMEM (5.6 mM glucose) supplemented with 10% heat-inactivated newborn calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1 mM L-glutamine at 37°C and 5% CO_2. After grown to 90% confluence, cells were starved for 24 h in serum-free DMEM, and then incubated with glucose- and serum-free medium with 0.5% BSA at different concentrations of glucose (5.6, 22.0, and 33.3 mM) for 48 h. The cells in the control group were treated with serum-free medium (5.6 mM glucose) with 0.5% BSA at different concentrations of mannitol (0, 16.4, and 27.7 mM) which was used as the osmotic pressure control.

2.4. *Extraction and reverse transcription of total cellular RNA*

Total RNA was isolated from cultured HepG2 cells using the RNA extraction kit and possible remaining DNA was digested using RNase free DNase I. ReverTra Ace reverse transcriptase was used to perform reverse transcription of total RNA.

2.5. *Real-time quantitative PCR*

RTQ-PCR was performed using an ABI 7300 Real-Time PCR System and Sequence Detection Software (version 1.3.1) using the following cycle parameters: 1 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. All data were normalized to *β-actin* expression. For each sample, RTQ-PCR was conducted in triplicate with a reaction volume of 25 μL. The following primers and probes were used for the PCR of Sequence 3: Forward primer, 5'-GGGTCAGTGGA AGTTCTGTCAC-3'; Reverse primer, 5'-TCTCTCAG AGCCATCTTCTAACATC-3'; Oligonucleotide probe, 5'-FAM-CTGGTTTCAGTGTCTCAGACCTTGCCC-TAMRA-3'. The primers and probes used for the PCR of *β-actin* were as follows: Forward primer, 5'-ACCCTGA AGTACCCCATCGAG-3'; Reverse primer, 5'-ACATG ATCTGGGTCATCTTCTCG-3'; Oligonucleotide probe, 5'-FAM-TCACCAACTGGGACGACATGGAGAAA-TAMRA-3'.

2.6. *Statistical analysis*

All quantitative values were expressed as mean ± S.D. The significant differences of the quantitative values among the different concentrations of glucose and mannitol were analyzed by one-factor analysis of variance or rank sum test. Two-sided *p* values below 0.05 were considered to be statistically significant.

3. Results and Discussion

Homology analyses of the proteins showed that the proteins encoded by Sequence 1 and Sequence 2 were respectively highly homologous with fibrinogen gamma polypeptide and liver fibrinogen-like 1 (Table 1). Disturbances in the thrombotic and fibrinolytic

Table 1. Homology analyses of the proteins encoded by MS-associated cDNAs

Sequences	Length	GHH	Length	SH	Gaps (%)	Identities (%)	Scores	E	Description
1	361	> gi|30583001	437	1	0/0 (0)	309/310 (99)	636	0.0	Fibrinogen, gamma polypeptide [Homo sapiens]
2	81	> gb|AAP35281.1	312	1	0/0 (0)	81/81 (100)	191	5e-48	Fibrinogen-like 1 [Homo sapiens]
3	110	> gb|AAH09530.1	110	1	0/0 (0)	110/110 (100)	216	1e-55	Chromosome 10 open reading frame 104 [Homo sapiens]
4	67	> gi|34533908	130	1	2/38 (5)	26/38 (68)	50.8	1e-05	Unnamed protein product [Homo sapiens]

GHH: Known gene of highest homology in GenBank DNA database; SH: Stretch of homology; E: Expect.

systems are features of MS (*13*). The associations have been reported between fibrinogen and other cardiovascular risk factors of MS (*14,15*). Bonora *et al.* (*16*) demonstrated that subjects with MS had disturbances in coagulation (thrombophilia) and showed higher levels of fibrinogen. It was also shown in our preliminary study that alteration in the expression of the fibrinogen gene was associated with MS (*9*). Fibrinogen-like 1 is encoded by LFIRE1 and specifically expressed in the liver (*17*). It is a member of the fibrinogen family. The specific relationship remains unclear between LFIRE1 and MS. The protein encoded by Sequence 3 was highly homologous with chromosome 10 open reading frame 104 and the protein encoded by Sequence 4 had homology with an unnamed protein product (Table 1). These results strongly suggest that for the previously un-annotated chromosome 10 open reading frame 104 and the unnamed protein product, they would likely be functionally related to MS.

Since homology analyses showed that the proteins encoded by Sequence 3 and Sequence 4 were novel proteins, further bioinformatic studies were performed to analyze the functional domains of these two proteins. The results demonstrated that the predicted protein encoded by Sequence 3 contained 110 amino acids with a putative isoelectric point (pI) of 4.91 and molecular weight (MW) of 11,667.04. This protein had no signal peptide and was predicted to localize in cytoplasm. It had four *N*-myristoylation sites at amino acids 11-16 (GGvsGS), 12-17 (GVsgSS), 15-20 (GSsvTG), and 20-25 (GSgfSV), one casein kinase II phosphorylation site at amino acids 24-27 (SvsD), and two protein kinase C phosphorylation sites at amino acids 50-52 (SeR) and 66-68 (TlK). The predicted protein encoded by Sequence 4 contained 67 amino acids with a putative pI of 10.30 and MW of 8,277.72. This protein had signal peptides and was predicted to localize in cytoplasm. It had two protein kinase C phosphorylation sites at amino acids 23-38 (SpK) and 50-52 (SlR), one cAMP and cGMP dependent protein kinase phosphorylation site at amino acids 40-43 (KRfS), and one *N*-myristoylation site at amino acids 55-60 (GLqfCF).

Phosphorylation and acylation of proteins are of great significance in biology. Moreover, phosphorylation and dephosphorylation of proteins are

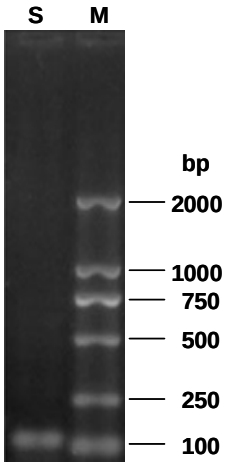

Figure 1. Gel electrophoresis of reverse transcription-PCR products of Sequence 3. S, Sequence 3; M, Marker DL2000.

ubiquitous regulation methods of signal transduction *in vivo* and are involved in almost all of the physiological and pathological processes, such as, cell growth and development, gene expression, and regulation of hepatic glucose and lipid metabolism (*18-21*). Proteins encoded by Sequence 3 and Sequence 4 might be intermediate proteins in some signaling pathway. Both proteins might be phosphorylated by protein kinase C, casein kinase II, or cAMP and cGMP dependent protein kinase, and transmit signals to downstream intermediates.

Total RNA was isolated from cultured HepG2 cells and reverse transcription-PCR for the mRNA of Sequence 3, which we were interested in, was performed to validate the expression of Sequence 3 in hepatic cells. As shown in Figure 1, length of the PCR product of Sequence 3 was 122 bp. This result suggested that Sequence 3, identified by screening a human hepatic cDNA library, was expressed in HepG2 cells.

One of the featured pathophysiologies of MS is its derangement of glucose metabolism. Sequence 3 was screened from a rat model having some characteristics of MS induced by a high-carbohydrate diet. Therefore, we investigated the effect of glucose on the expression of Sequence 3 in HepG2 cells to explore the possible

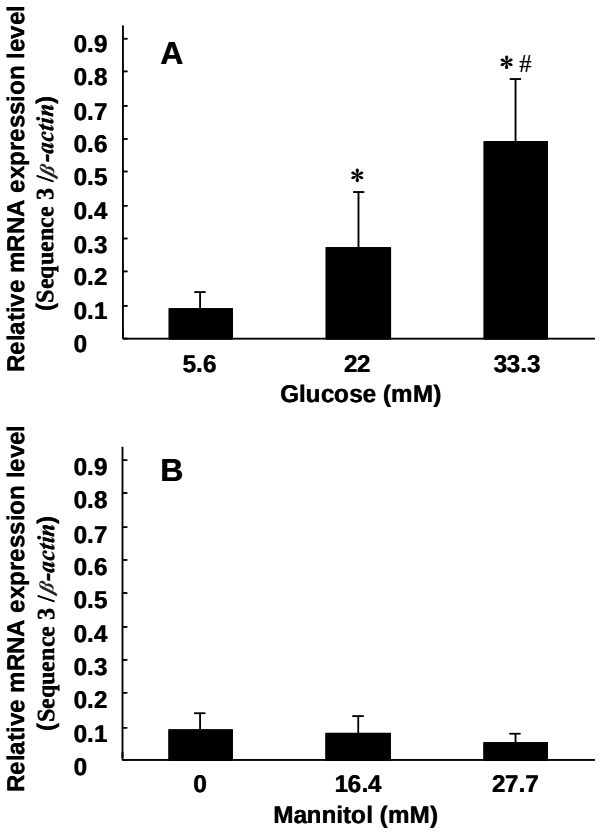

Figure 2. Effects of glucose and mannitol on the mRNA expression of Sequence 3 in HepG2 cells. Values are expressed as mean ± S.D. of four independent experiments. * $p < 0.05$ compared with the concentration of 5.6 mM; # $p < 0.05$ compared with the concentration of 22 mM.

association of Sequence 3 with MS. HepG2 cells possess similar morphology and function compared to freshly isolated hepatic cells (*22*). HepG2 cells were treated with glucose at different concentrations for 48 h. The mRNA expression of Sequence 3 was normalized to *β-actin* expression. There was a significant difference among the cells incubated with glucose at 5.6, 22.0, and 33.3 mM in terms of the mRNA expression of Sequence 3 (Figure 2). mRNA expression of Sequence 3 was increased by glucose above physiological concentrations. Mannitol, an osmotic control, had no effect on mRNA expression of Sequence 3 in HepG2 cells. This result revealed that mRNA expression of Sequence 3 was positively associated with glucose higher than physiological concentrations.

In conclusion, the characteristics of the proteins encoded by cDNA sequences associated with MS were preliminarily obtained by bioinformatic analyses. The proteins encoded by Sequence 3 and Sequence 4 had multiple phosphorylation and myristoylation sites and might play roles in some signaling pathways. mRNA expression of Sequence 3 was positively associated with glucose higher than physiological concentrations. Therefore, Sequence 3 might play roles in some signaling pathways regulating hepatic glucose and lipid metabolism. More studies are needed to validate the existence of Sequence 4 and its possible association with MS.

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Original Article

Translation and cross-cultural adaptation of the Pregnancy Physical Activity Questionnaire (PPAQ) to Japanese

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Summary **The aim of this study was to conduct a translation and cross-cultural adaptation of the Japanese version of the Pregnancy Physical Activity Questionnaire (PPAQ) that consisted of 36 items. We translated and adapted the PPAQ to the Japanese culture. This procedure included a forward step (stages I and II, translations and synthesis), quality control (stage III, back translation, and stage IV, expert committee review), and pre-testing (stage V). In the pre-test, the preliminary Japanese version was tested on ten Japanese pregnant subjects. The content, semantic, technical, conceptual, and experiential equivalents of cultural adaptation were discussed by the research members at each step. In the results section, one new item was added to address "riding a bicycle in order to go to a certain place other than for recreation or exercise", because many Japanese women often use a bicycle. The average age of the pregnant subjects in the pre-test was 32.7 years of age. The response time ranged from 5 to 15 min. Two subjects responded that they rode a bicycle under the new item. The preliminary Japanese version of the questionnaire was revised to reflect the opinions of pregnant subjects for cross-cultural adaptation, including the semantic, experiential, and technical equivalents. The consensus of content and conceptual equivalents of the pre-final version of PPAQ by discussion among the research members was obtained throughout these processes. The original developer approved all revisions. In conclusion, the pre-finalized Japanese version of the PPAQ was indicated to have cross-cultural equivalency with the original English version.**

Keywords: Cultural adaptation, instrument, pregnancy, physical activity, translation

1. Introduction

Physical activity has received significant attention in public health policies. Healthy pregnant women also are encouraged to exercise daily for 30 min at a moderate intensity by the American College of Obstetricians and Gynecologists or least at 120 min a week by the American College of Sports Medicine (*1,2*). A previous study reported that physical activity reduces the risk of maternal complications (*e.g.* gestational diabetes, preeclampsia, and postpartum weight retention) (*3-7*).

However, the prevalence of physical activity participation, which is defined as more than 30 min a time, twice a week and more than one year was found to be 14.6% and 14.0% among subjects of reproductive age, which is defined to be 20-29 and 30-39 years of age in the Japanese population (*8*). Although this prevalence is the lowest among other age groups, encouraging exercising has been not the rule in clinical obstetrics during subjects' gestation period. In addition, the appropriate amount of physical activity required for preventing pregnancy complications remains unknown, and no intervention studies have yet measured the physical activity among pregnant women subjects in

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Japan. Because there were no feasible tools in the form of a validated questionnaire that measures the physical activity of pregnant women in Japan.

The questionnaire is a simple tool for assessing physical activity in large populations for various applications, including epidemiologic research or public health surveillance. It is easy to administer, cost-effective, non-invasive, and allows the accurate estimation of the intensity of physical activity. Physical activities include both occupational, sports and exercise activities, and household and caregiving duties. Most married couples do not equally share the household and caregiving duties in Japan. A previous study showed that 67% of 7,771 wives performed all of the household duties, and 65.2% of 6,991 subjects performed all of the caregiving (*9*). Therefore, an accurate physical activity questionnaire for pregnant Japanese women must include the household and caregiving activities. Although the CARDIA physical activity, Minnesota Leisure-Time Physical activity and YEAL Physical activity questionnaires include household activity, these questionnaires do not include items that address caregiving activity, and they are furthermore not designed to assess the physical activity in pregnant women (*10*).

The pregnancy physical activity questionnaire (PPAQ) is the only widely available tool for assessing a pregnant woman's physical activity (*11*,*12*). The PPAQ is a semi-quantitative questionnaire that asks the respondents to report on the time spent participating in 32 activities, including household/caregiving activities (13 activities), occupational (5 activities), sports/ exercise (8 activities), transportation (3 activities), and inactivity (3 activities) (*11*). The respondents are asked to select a category for each activity to the nearest amount of time spent per day or week. The duration ranges from 0 to 6 or more hours per day, and from 0 to 3 or more hours per week during the subject's current trimester. An open-ended section at the end of the PPAQ allows each respondent to add activities not already listed. Self-administration of the PPAQ in English takes approximately 10 min. The PPAQ is short in length, self-administered, and easily understood by respondents in a variety of settings, making it useful for epidemiologic research and health education during pregnancy. The original English version of PPAQ has been also used in an intervention trial to measure the physical activities among pregnant women for preventing pregnancy complications such as gestational diabetes (*13*). Therefore, the goal of measuring physical activity among pregnant women was to clarify the intensity and amount of physical activity for preventing pregnancy complications in Japan, and to provide initial information for health care providers about the current activity levels in pregnant women.

The aim of the present study was to develop a Japanese version of the PPAQ, which was originally designed to measure the physical activity of pregnant women with a careful cross-cultural adaptation of the assessment content, semantic, technical, conceptual, and experiential equivalents.

2. Methods

2.1. *Translations and cross-cultural adaptation*

Lisa Chasan-Taber, one of the original authors of the PPAQ, granted permission for the development and use of a Japanese version of PPAQ. A discussion of the conceptual equivalence, which ensures that the measuring instrument is the same in each culture and the technical equivalents and that the method of assessment (*e.g.* pencil and paper, interview) is comparable in each culture, was performed by the research members before the forward translation. The research team included an expert in prenatal care, midwifery researchers and graduate students of midwifery (MM, MH, EO, and SY).

Table 1 shows the Japanese version of PPAQ based on the methods proposed by Acquadro (*14*), Guillemin (*15*), Beaton (*16*), and Frayers (*17*) with a slight modification. These included a forward step (stages I and II, translations and synthesis), quality control (stage III, back translation; and stage IV, expert committee review), and pre-test (stage V, pre-testing). The content, semantic, technical, conceptual, and experiential equivalents of cultural adaptation were discussed by the research members at each step (*18*). The recommendations of these steps and stages throughout the cross-cultural adaptations were as follows. The forward step: stage I, which recommends that two Japanese translations be made by informed and uninformed translators. Stage II recommends the merging of the two translations from stage I. In stage II, any discrepancies are resolved with the translators reports. The next step is the quality control stage III, which requires back-translation into English. Two translators whose first language is English created the two back-translations. Stage IV requires an expert committee review. For the committee, a methodologist, developer, language professional, and several translators are recommended. All reports of the translators are reviewed to reach a consensus on discrepancies, and to produce a pre-final version. The final step is the pre-test, or stage V, which indicates the pre-testing. The Japanese version of the PPAQ is completed and tested to obtain a proper understanding of the items. Between 10 and 40 persons are recommended to be tested (*16*,*17*).

2.1.1. *Forward step*

A Japanese graduate student (MM) who was aware of the purpose of study performed one translation in Stage I of the forward step (T1). The translator was provided supervision from an associate professor (SY) in a

Table 1. The step and stage of cross-cultural adaptation recommended and adaptation our process

	Recommendation	Our process
Forward step*	Stage I: Translation**	Stage I: Translation
	Two translations (T1 & T2) into target languege informed + uninformed translator	T1: Japanese graduate student (informed translator) T2: supervised T1 from an associate professor of a university in the United States
	Stage II: Synthesis**	Stage II: Synthesis
	synthesis T1 & T2 into T-12 resolve any discrepancies with translators reports	synthesis T1 & T2 into T-12 resolve any discrepancies with translators reports discussion quality of T-12 by reseach members interview one pregnant woman and two postpartum women about T-12
Quality control*	Stage III: Back translation**	Stage III: Back translation
	two English first-language native to outcome measurement	BT1: one English first-language in translation agency BT2: Japanese graduate student who had stayed for 7 years in America (uninformed translators)
	work from T-12 version create 2 back translations BT1 & BT2	work from T-12 version synthesis BT1 & BT2 into BT-12
	Stage IV: Expert committee review**	Stage IV: Expert committee review
	review all reports methodologist, developer, language professional, translators reach consensus on discrepancies produce pre-final version	review all reports midwives and researchers reach consensus on discrepancies produce pre-final version
Pre-test*	Stage V: Pre-testing**	Stage V: Pre-testing
	n = 10-40 complete questionnaire probe to get at understanding of item	*n* = 10 complete questionnaire probe to get at understanding of item

The recommendations were proposed by * Acquadro (2008), and ** Guillemin (1993), Beaton (2000), and Frayers (2000).

university in the United States (T2).

The research members then discussed the brief, clearly worded, easily understood, unambiguous, and easy responses to the questionnaire, and combined the T1 and T2 into T-12 during Stage II. Thereafter, one Japanese pregnant subject and two postpartum subjects who were not aware of the purposes of the study were interviewed to determine whether they unambiguously understood and could easily respond to T-12, and if the PPAQ addressed their physical activities during pregnancy. The research members reached a consensus on T-12 based on those results. The quality of T-12 on the conceptual equivalence and the semantic equivalence ensured that the meaning of each item was the same in each culture after the translation into the language and idioms (written or oral) of each culture. Proper instruction ensured that the questionnaire was applicable to pregnant women. The included items and the response choices were verified to have maintained equivalent content, ensuring that the content of each questionnaire item was relevant to the practices of each culture.

2.1.2. *Quality control*

Back-translation (BT1) was performed in Stage III by a native English speaking professional in a translation agency. In addition, a Japanese graduate student (CI) who had lived for 7 years as a graduate student in the United States performed another back-translation (BT2). BT1 and BT2 were combined into BT-12. These translators were not aware of the purpose of the study and were blinded from the English version of the PPAQ.

The original version and BT-12 were compared and the content, semantic, technical, conceptual, and experiential equivalents were discussed by the research members during Stage IV. The preliminary Japanese version of the PPAQ was produced by the research members who participated in this study.

2.1.3. *Pre-test*

The preliminary Japanese version of PPAQ was pre-tested by ten Japanese pregnant women in stage V to assess the degree of cultural adaptation and to address any potential problems. Ten pregnant subjects who visited a hospital in Tokyo for a checkup from 17 to 20 October 2006 were recruited. All of the subjects participated in this study. During their hospital visit, participants were asked to complete the preliminary Japanese version of the PPAQ, and the researchers collected it in person.

The researchers interviewed the subjects to document any problem that occurred during the administration of the preliminary Japanese version of the PPAQ. These included any ambiguities or difficult phrasing of the questions and responses, or the layout and flow of the questions. Abstract problems were discussed by the research members and the original developer to produce the pre-final Japanese version of the PPAQ.

2.2. *Ethical considerations*

The study protocol was approved by the Institutional Review Board of the University of Tokyo. Written informed consent was obtained from all participants.

2.3. *Data analysis*

A quantitative and qualitative analysis of the data of the pre-test was conducted, including the subject response time, the distribution of the item responses and the contents of the interviews. A quantitative analysis was used to identify practical equivalents, such as the participants' workload. A qualitative analysis was used to identify the semantic, experiential, technical, and practical equivalents.

3. Results

The original developer and the research team included an expert in prenatal care, midwifery researchers and graduate students of midwifery (MM, MH, EO, and SY), who discussed the results of the forward step, quality control, and the pre-testing.

3.1. *Forward step*

The concepts of the subscale were confirmed by the research members before the forward step. The content and experiential equivalents of T1, T2, and T-12 were discussed. Some of the language was altered to improve the experiential equivalence. The only significant changes involved conversion from English measurements (gallons, pounds) to the metric equivalents (liters, kg) for item 33.

Items 18 and 19 in the original PPAQ refer to the use of a lawnmower, which is not commonly used in Japan. However, it was not deleted or altered, because there are times when a lawnmower is used such as in local regions or in luxury housing. Next, an item (20-2) was added, which addressed "riding a bicycle for reasons other than for recreation or exercise (to catch a bus, to go to work, or to visit a place, *etc.*)", since Japanese women often use a bicycle for basic transportation.

An interview of one Japanese pregnant subject and two postpartum subjects revealed that the wording of questions in T-12 were not ambiguous, difficult or poorly worded and appropriately assessed physical activity. Research members decided that the PPAQ has sufficient content equivalence, because the content of each item of the instrument was relevant to the specific aspects of Japanese culture. PPAQ has sufficient technical equivalents for data collection with the original version which was self-administered, because this method is widely used in Japan.

3.2. *Quality control*

The content, semantic, technical, concept, and experiential equivalents of the back translated Japanese version of PPAQ (BT-12) were discussed by the research members. BT-12 was determined to have sufficient content equivalence, because the items that addressed physical activities were relevant to and consistent with Japanese culture. The responders described the time spent on each activity relevant to household/caregiving tasks, occupational, and sports/exercise in BT-12. BT-12 did not address the feelings or thoughts of pregnant subjects (*19*). Therefore, there were no items that had different meanings in the original and Japanese versions of the PPAQ. BT-12 was determined to have sufficient semantic equivalency. The layout and format of the instruments in the English version were maintained in the BT-12 for the technical equivalent.

Item 10 of the original version read, "Taking care of an older adult". This concept also includes nursing. However, research member asked whether "taking care of" included communication, such as conversations, with the older adult. The concept of "taking care" varied with individual subjects, since Japanese women often live with healthy elderly relatives. Item 10 was determined to have an insufficient conceptual equivalent. This item was modified to "Taking care (nursing) of an older adult".

The instructions were found to have a problem of experiential equivalence. The original instructions referred to "During this trimester", which was difficult to understand. Pregnant subjects did not accurately recognize the separation of each of the trimesters. Therefore, this was changed to "during the last month". These considerations yielded a preliminary Japanese version of the PPAQ.

3.3. *Pre-test*

3.3.1. *Characteristics of pregnant women*

The ten pregnant subjects had a mean age of 32.7 (range: 25-38) years of age, a mean gestational age of 27.3 (range: 16-37) weeks, and a mean pre-pregnancy BMI of 19.5 (range: 16.8-22.2; Table 2). Five pregnant subjects had an educational background beyond a college degree. Five of the subjects had undergone fertility treatment.

Table 2. Patients characteristics (*n* = 10)

	n	Mean ± S.D. (range)
Age		
25-30	4	32.7 ± 4.5 (25-38)
31-35	2	
36-40	4	
Gestational weeks		
< 16	0	27.3 ± 7.2 (16.5-37.0)
16-27	6	
28-37	4	
Pre-pregnancy BMI* (kg/m^2)		
12-20	7	19.5 ± 1.7 (16.8-22.2)
21-25	3	
Parity		
0	9	
2	1	
Regular exercise**		
Yes	3	
No	7	
Residence		
Big city	7	
Urban area	1	
Rural area	1	
No information	1	
Occupation		
Full-time house wife	3	
On maternal leave	1	
Currently employed	6	
Education		
High school graduate	1	
Junior college graduate	3	
College graduate	4	
Post grad or professional degree	1	
No information	1	

* BMI: body mass index; ** Regular exercise: exercise of more than 30 min a time, twice a week.

3.3.2. *Quantitative assessment of the pre-final Japanese version*

Pregnant subjects completed all items of the questionnaire. There were no missing data on any items. All pregnant women responded "not at all" for item 10: Take care (Nursing) of elderly person, item 18: Mowing grass and weeds using a ride-on lawnmower, item 26: Jogging, item 29: Dance, and item 31: an open-ended section which allows each respondent to add activities not already listed. These items were left in the questionnaire by research members to internationally compare the physical activity. Two of ten pregnant women responded that they rode a bicycle other than for recreation or exercise, in order to go somewhere (to catch a bus, to go to work, or to visit a place, *etc.*) as a new item. The remaining eight pregnant women responded "not at all".

The response time for the questionnaire was approximately 5 to 10 min. All pregnant subjects reported that it was easy to complete the responses to the preliminary Japanese version of the PPAQ.

3.3.3. *Qualitative analysis of the pre-final Japanese version*

Several difficulties in answering the items were present in the pre-test. The difficulties may have arisen from the wording of items or other causes, mainly a confusing situation; sitting, standing, running, walking slowly, and walking fast. Table 3 summarizes the difficulties that were identified in the pre-test, and the items that were changed following the committee review.

Item 16 presented a problem in semantic equivalent. The word "Shopping (for food, clothes, or other items)" appeared in item 16. Pregnant women reported that shopping was different every day. They spent a significant amount of time shopping during the holidays. Instructions concerning "average physical activity" were added: "We would like to know some information about your average physical activities during the last month".

Item 2 presented a problem in experiential equivalents. Item 2 in the original version read, "When was the first day of your last period?" This question was included to determine the pregnancy trimester. Japanese pregnant women are aware of gestational weeks. Most pregnant women initially undergo an early dating ultrasound scan at 10 to 12 weeks' gestation to accurately determine gestational dating in Japan. The item was modified to "As of today, how many weeks have you been pregnant?"

The response method presented a problem in the technical equivalent. The original instructions stated "Fill in the circles completely". However, pregnant women found that "Fill in the circles completely" was difficult. The instructions were modified to "Please check the box of the corresponding answer".

Several pregnant women reported that they were confused due to the decision branches, which were a day or a week. Consequently, researchers provided an explanation that each item has a different branch prior to administering the preliminary Japanese version of the PPAQ. Other subjects found some items confusing because of similar wording: walking slowly to go somewhere, walking quickly to go somewhere, and so on. Similar wordings were highlighted by underlining to prevent confusion. The original developer approved all revisions, and the pre-final Japanese version of the PPAQ was completed.

4. Discussion

The present study described the cross-cultural

Table 3. Items in original wording, number of patients who commented on the item, expressed difficulties due to wording of items or other causes in the pretested version, and an indication of whether items were changed after the pre-test

Number	Item with the original wording	Commented on the item	Difficulties due to wording of items	Difficulties due to other causes	Changed after pre-test
Instruction	**It is very important you tell us about yourself honestly. There are no right or wrong answers. We just want to know about the thing you are doing during this trimester.**				Yes
1	Today's date	0	0	0	Yes
2	What was the first day of your last period?	0	0	0	No
3	"When is your baby due? (month/day/year) ○I don't know."	0	0	0	Yes
Instruction	**During this trimester, when you are NOT work, how much time do you usually spend:**	0			Yes
4	Preparing meals (cook, set table, wash dishes)	0	0	0	No
5	Dressing, bathing, feeding children while you are sitting	2	0	0	No
6	Dressing, bathing, feeding children while you are standing	1	0	0	No
7	Playing with children while you are sitting or standing	1	0	0	No
8	Playing with children while you are walking or running	1	0	0	No
9	Carrying children	1	0	0	No
10	Taking care of an older adult	1	0	0	Yes
11	Sitting and using a computer or writing, while not at work	1	0	1	No
12	Watching TV or a video	1	0	1	No
13	Sitting and reading, talking or on the phone, while not at work	1	0	1	No
14	Playing with pets	0	0	0	No
15	Light cleaning (make beds, laundry, iron, put things away)	0	0	0	No
16	Shopping (for food, clothes, or other items)	2	0	2	No
17	Heavier cleaning (vacuum, mop, sweep, wash windows)	0	0	0	No
18	Mowing lawn while on riding mower	0	0	0	No
19	Mowing lawn using a walking mower, raking, gardening	0	0	0	No
Instruction	**Go places (to catch a bus, to go to work, or to visit a place, *etc.*):**				
20-1	Walking, slowly to go places (such as to the bus, work, visiting) Not for fun or exercise	4	4	0	No
20-2	Riding a bicycle for reasons other than for recreation or exercise	4	4	0	No
21	Walking, quickly to go places (such as to the bus, work, or school) Not for fun or exercise	0	0	0	No
22	Driving or riding in a car or bus to go places (such as to the bus, work, or school)	3	0	3	No
Instruction	**For fun or exercise:**				
23	Walking slowly for fun or exercise	1	1	0	No
24	Walking more quickly for fun or exercise	0	0	0	No
25	Walking quickly up hill for fun or exercise	0	0	0	No
26	Jogging	0	0	0	No
27	Prenatal exercise class	1	1	0	No
28	Swimming	0	0	0	No
29	Dance	0	0	0	No
30	Doing other things for fun or exercise? Please tell us what they are.	4	3	1	No
31	Name of activity	0	0	0	No
Instruction	**Please fill out the next section if you work for wages, as a volunteer, or if you are student. If you are a home maker, out of work, unable to work, you do not need to complete this last section. At work….**				
32	Sitting at working or in class	3	0	3	No
33	Standing or slowly walking at work while carrying things (heavier than a 1 gallon milk jug)	4	0	4	No
34	Standing or slowly walking at work not carrying anything	3	0	3	No
35	Walking quickly at work while carrying things (heavier than a 1 gallon milk jug)	3	0	3	Yes
36	Walking quickly at work not carrying anything	3	0	3	No
"Decision branch"	○None	0	0	0	No
	○Less than 1/2 hour per week	0	0	0	No
	○1/2 to almost 1 hour per week	0	0	0	No
	○1 to almost 2 hours per week	0	0	0	No
	○2 to almost 3 hours per week	0	0	0	No
	○3 or more hours per week	0	0	0	No
	○None	0	0	0	No
	○Less than 1/2 hour per week	0	0	0	No
	○1/2 to almost 2 hours per week	0	0	0	No
	○2 to almost 4 hours per week	0	0	0	No
	○4 to almost 6 hours per week	0	0	0	No
	○6 or more hours per week	0	0	0	No
	○None	0	0	0	No
	○Less than 1/2 hour per day	0	0	0	No
	○1/2 to almost 1 hour per day	0	0	0	No
	○1 to almost 2 hours per day	0	0	0	No
	○2 to almost 3 hours per day	0	0	0	No
	○3 or more hours per day	0	0	0	No
	○None	0	0	0	No
	○Less than 1/2 hour per day	0	0	0	No
	○1/2 to almost 2 hours per day	0	0	0	No
	○2 to almost 4 hours per day	0	0	0	No
	○4 to almost 6 hours per day	0	0	0	No

adaptation process of the revised PPAQ from English into Japanese. Experts in prenatal research produced a pre-final Japanese version of the PPAQ after translation and cross-cultural adaptation.

One item (20-2) was added, which addressed "riding a bicycle for reasons other than for recreation or excise (to catch a bus, to go to work, or to visit a place, *etc.*)", since Japanese women often use a bicycle for basic transportation. Two out of ten pregnant women responded that they rode a bicycle. A previous study reported that 50% of 141 Japanese pregnant women responded that they were "not riding a bicycle" and less than 10% responded "easily riding a bicycle" (*20*). Therefore, few pregnant women appeared to regularly ride a bicycle in Japan. In addition, the amount of energy expenditure of bicycling is 8 metabolic equivalents (METs), the same as water jogging. The question on bicycling was included to assess the comprehensive activity of pregnant Japanese women. Item 33 of the original PPAQ was changed from "1 gallon milk jug" to "3 kg bag of rice". This change was appropriate because all pregnant women understood it. There were no missing data in any items in the pre-test, and the response time was very short. These results suggest that the Japanese version of the PPAQ was easily understood and was easy to respond to.

All pregnant women selected identical answers in five items; item 10, 18, 26, 29 and 31. All of these responses were "not at all". The presence of floor effects with an excess of minimum values indicates that the item or scales will have a poor level of discrimination. Therefore, the overall sensitivity and responsiveness is reduced (*17*). However, the cause of these floor effects was thought to be that the participants are more limited. Most of the pregnant women in this study living in an urban area, were primiparous, and had an occupation. A more varied population would have yielded a greater variation in the responses.

The Japanese recommendations for Maternity Safekeeping of exercise or sports during pregnancy is that healthy pregnant women should engage in physical activity less than twice or thrice a week at 60-min intervals at a moderate level of intensity, according to the Japanese Society of Clinical Sports Medicine (*21*). A previous study reported that approximately 90% of 648 pregnant women who had been regularly exercising simply engaged in walking (*22*). Therefore, the floor effects of the jogging and the dance items may reflect the lack of information and evidence about exercise and sports during pregnancy in Japan. Despite the recommendation of exercise during pregnancy in other countries (*1*,*2*), the levels of activity required for favorable pregnancy outcomes remain to be determined in Japan. The use of the Japanese version of the PPAQ in further research may provide evidence for the level of physical activity required during pregnancy in Japan. Several pregnant women confused their responses due to similar wording. Sudman and Bradburn (1982) focused on wording and designing questionnaires. They stated that it was important to use underlining, bold or italics to draw patients' attention to the differences when two or more questions are similar in their wording (*23*). Therefore, using underlining for similar wording is appropriate.

This study had several limitations. First, the sample size of the pre-test was small, and the participants were not representative of the national population. Second, the results of the pre-test showed the presence of floor effects in partial items. Third, to complete the Japanese version of the PPAQ, additional validation study will be necessary. Therefore, further research will establish additional validity to determine the significant associations between the PPAQ physical activity levels and a validated ActiGraph accelerometer (Fort Walton Beach, FL) or a pedometer, measuring the physical activity levels. Our project team is researching the additional validity and reliability study of the Japanese version of PPAQ among Japanese pregnant women.

5. Conclusion

The present study translated and adapted the original PPAQ to the Japanese culture. The pre-final Japanese version of the PPAQ was indicated to be functional in the pre-test. The translated and cross-culturally adapted form of this established instrument of assessing physical activity may provide an important perspective for preventing pregnancy complications and maintaining a healthy life for the fetuses and pregnant women during pregnancy.

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Original Article

Reliability and validity of a Nepalese version of the Kiddo-KINDL in adolescents

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Summary **The objective of this study was to assess the reliability and validity of a Nepalese version of the Kiddo-KINDL to measure Health-Related Quality of Life (HRQOL) in adolescents. We collected data from 204 students between 13 to 16 years old from four secondary schools in Lalitpur district, Nepal. The students answered a Nepalese version of the Kiddo-KINDL and the Center for Epidemiological Studies-Depression Scale (CES-D) with a self-administrated questionnaire. We conducted a test-retest study on the instrument at an interval of 10 days and then compared the Kiddo-KINDL scores between the low CES-D score group and the high CES-D score group students. The instrument showed good reliability and a small response variation. The internal consistency (Cronbach's alpha) of the total score was 0.93. Corrected item-total correlations showed that all items ranged from 0.47 to 0.79. The reproducibility was satisfactory with an Intraclass Correlation Coefficient (ICC) of 0.88-0.95. The Kiddo-KINDL scores in the low CES-D score group were significantly lower than those in the high CES-D score group students. The optimal cut-off score of the Kiddo-KINDL was estimated at 54.7, with an Area Under the Curve (AUC) score of 0.83 and both sensitivity (73.5%) and specificity (71.8%) were acceptably high. We recommended a mean change in Kiddo-KINDL total scores of 4.0 to be used to define a minimal important difference according to two-point CES-D score changes. Our results showed that a Nepalese version of the Kiddo-KINDL has internal consistency, reproducibility, responsiveness, interpretability, and discriminant validity.**

Keywords: School adolescent, quality of life, Nepal, reliability, validity

1. Introduction

The Health-Related Quality of Life (HRQOL) scales for adolescents have been used to assess the influence of a disease in several industrialized countries in the 1980's (*1*). HRQOL scales are used based on a broad range of domains: physical, psychological, social and spiritual focusing on personal life including the concept of the World Health Organization (WHO) definition of health as 'a state of complete physical, mental and social well-being and not merely the absence of disease and infirmity' (*2*,*3*).

In the early 1990s, several disease-specific HRQOL instruments were developed. For example, the Diabetes Quality of Life Instrument (DQOL) measures satisfaction with treatment, impact of treatment, worry about social/vocational issues, and worry related to long-term effects of diabetes for adolescents in USA (*4*). The Childhood Asthma Questionnaires (CAQ) was developed in the UK and is commonly used to measure HRQOL in treatment of chronic childhood asthma (*5*).

The Kiddo-KINDL is a comprehensive HRQOL

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assessment, tapping not only physical and psychological aspects but also social environmental factors such as interpersonal relations with friends and family members, especially parents (*6,7*). This instrument was developed in Germany, and has been translated and validated in many areas including Asian countries and areas such as Singapore (*8*), Taiwan (*9*), and Japan (*10*).

Psychometric properties of these HRQOL instruments were revealed by feasibility with ceiling/floor effects (*6-9*), internal consistency (*4,6-10*), intercorrelations of the scale (*4,6,9,10*), reproducibility in test-retest (*6,9*), construct validity with factor analysis (*5,7,9*), correlation with external criteria (*9,10*), and discriminant validity when comparing healthy and chronically ill children/adolescents (*5,6,8*).

HRQOL instruments are widely used in Asian countries as we have described previously. However, a generic HRQOL instrument which includes school and home environment has not been used for adolescents in Nepal. In Nepal, university students' depressive symptoms, life satisfaction, and the General Health Questionnaire (GHQ) were once measured; in these studies, however, the HRQOL was not used (*11,12*).

The objective of this study was to assess the reliability and validity of a Nepalese version of the Kiddo-KINDL to measure HRQOL in adolescents.

2. Materials and Methods

2.1. *Study design*

This is a cross-sectional and self-administrated, questionnaire-based test-retest study.

2.2. *Instruments and translation procedures*

The Kiddo-KINDL is a self-report questionnaire which has 24-items referring to the past week on a 5-point response scale with the following variables: "1 = never, 2 = seldom, 3 = sometimes, 4 = often, 5 = all the time" (11-items are reverse-coded). It covers the physical, emotional, self-esteem, family, friends, and school domains, targeted for adolescents 13-16 years old (*8-10*). The instrument can be referenced from: *http://www.kindl.org/indexE.html* (*13*).

We measured the depression status of the participants using the Center for Epidemiological Studies-Depression Scale (CES-D). The scale consists of 20-items with a 4-point ordinal scale. The scores range from 0 to 60, where higher scores indicate a higher tendency for depression. This scale has been widely used in measuring depression. It has been applied both as a primary screening tool (*14*) and for clinical and research purposes in general populations (*15,16*). The depressive mood scales are often used for psychometric evaluation as an external criterion for the Kiddo-KINDL (*9,10*). Cronbach's alpha reliability coefficient and construct validity of the CES-D has been described in Nepal from an investigator administrated survey (*17*).

The original version of the Kiddo-KINDL was translated into the Nepali language with permission from the copyright holder (Ravens-Sieberer and Bullinger, 1999). First, two bilingual Nepalese researchers translated the Kiddo-KINDL into Nepali. Then, another Nepali, who was not involved in the forward translation, checked the reconciliation of each translation. Finally, a native Nepali speaker back translated the reconciled Nepali version of the questionnaire. The questionnaire was tested with ten students between 13 to 16 years old and the comprehensiveness of each item was verified.

2.3. *Sample and data collection*

We conducted this study in four schools (two private and two public) in Lalitpur district, the Central Region in Nepal. In Lalitpur district, there were 199 secondary schools with student numbers totalling 14,884 in 2002 (*18*). After receiving approval from the principals, we informed the 320 potential participants about the study in their respective classrooms. We distributed the informed consent forms and asked the students to collect a signature from one of their parents. Of the potential participants, 204 voluntarily participated with parental consent. The questionnaire consisted of the Kiddo-KINDL and the CES-D. After 10 days passed since the initial survey, we administered the same questionnaires to students to evaluate the results using a test-retest study. The study protocol was approved by the Ethical Committees of the University of Tokyo and the Nepal Health Research Council.

2.4. *Statistical analysis*

We first compared the difference of the students who had low depressive symptoms (CES-D < 16) or high depressive symptoms (CES-D ≥ 16); we used this cut-off point as suggested by Radloff (*16*). Of 24 items on the Kiddo-KINDL, we reversed 11 and transformed the raw scores into a linear scale from 0-100. The mean score of the total and all subscales were assessed by gender. We then examined the range and distribution of responses for each item from total scores using ceiling and floor effects.

To check the internal consistency of the Kiddo-KINDL, we used Cronbach's alpha values and the corrected item-total correlation between scores of six dimensions. To examine reproducibility, we calculated the Intraclass Correlation Coefficient (ICC) with 95% Confidence Intervals (CI) from the test-retest study. To examine the discriminate validity, we compared the Kiddo-KINDL scores between the low depressive symptoms group and high depressive symptoms group.

For group comparisons, we used a Student's *t*-test with effect size.

To assess the responsiveness of the Kiddo-KINDL, we measured the sensitivity and specificity of the instrument against an external criterion of the CES-D by suggesting that information be synthesised into Receiver Operating Characteristics (ROC) analysis with an optimal cut-off score.

Score interpretability is defined as the ability of an instrument to detect clinical changes. We estimated the minimal important difference for the Kiddo-KINDL using the deference of CES-D total scores between test and retest at an interval of 10 days. First, we created categories of the CES-D: "improvement" for CES-D scores ≤ -2, "stable" for CES-D scores from -1 to 1 and "worsening" for CES-D scores ≥ 2. Then, we compared the change in test and retest scores of Kiddo-KINDL among these groups.

For all statistical analysis, we used the software package SPSS ver.16.0 (SPSS Inc., Chicago, USA).

3. Results

3.1. *Study participants*

The response rate was 63.8% (n = 204/320). For the retest, 189 of the 204 students participated; its response rate was 92.6%. Table 1 shows the demographic characteristics of the participants by low and high CES-D score and gender. In the low CES-D score group, the mean age of the 92 boys and 74 girls was 14.5 years (S.D. 1.0) and 14.4 years (S.D. 1.1), respectively. In the high CES-D score group, the mean age of the 17 boys and 21 girls was 14.2 years (S.D. 1.0) and 14.4 years (S.D. 1.1), respectively.

3.2. *Score distribution and ceiling/floor effects*

Table 2 shows the score distribution and ceiling and floor effects of the Kiddo-KINDL by gender. The mean total scores were 62.4 (S.D. 16.6) and 59.6 (S.D. 17.3) for boys and girls, respectively. Overall, the total and subscale scores among girls were lower than those among boys. The lowest scores were the "self-esteem" subscale scores for both groups (boys: 56.6, girls: 54.9), followed by "school" (boys: 56.8, girls: 55.3), and "physical well-being" (boys: 58.4, girls: 56.3). The highest scores were in the "family" subscale for both groups (boys: 72.0, girls: 70.9). The differences in scores between the groups were not statistically significant. Ceiling and floor effects of the total scores were not strongly skewed in this study. Though scores on the "family" subscales had slightly higher proportions of ceiling and floor effects (15.1% and 1.4%), other subscales had 10% or fewer scores. Effect of size between boys and girls in the mean of total and subscale scores ranged from 0.05 to 0.22.

3.3. *Reliability*

We evaluated the internal consistency of the Kiddo-KINDL using Cronbach's alpha values (Table 3).

Table 1. Demographic characteristics of the participants

		CES-D low score group (Total score < 16)				CES-D high score group (Total score ≥ 16)			
		Boys (n = 92)		Girls (n = 74)		Boys (n = 17)		Girls (n = 21)	
		n	(%)	n	(%)	n	(%)	n	(%)
Year	13	20	(21.7)	18	(24.3)	5	(29.4)	5	(23.8)
	14	23	(25.0)	22	(29.7)	5	(29.4)	6	(28.6)
	15	33	(35.9)	17	(23.0)	5	(29.4)	6	(28.6)
	16	16	(17.4)	17	(23.0)	2	(11.8)	4	(19.0)
Age	(mean)(S.D.)	14.5	(1.0)	14.4	(1.1)	14.2	(1.0)	14.4	(1.1)

The participants are divided by low (Total score < 16) and high (Total score ≥ 16) CES-D score and gender.

Table 2. Comparisons of score distribution and ceiling and floor effects of the Kiddo-KINDL by gender

	Total (n = 204)		Boys (n = 109)		Girls (n = 95)		Ceiling effect (%)	Floor effect (%)	p	Effect Size[a]
	Mean	S.D.	Mean	S.D.	Mean	S.D.				
Total	61.1	16.9	62.4	16.6	59.6	17.3	0.4	0.0	0.249	0.16
Subscales										
Physical well-being	57.4	20.3	58.4	20.6	56.3	19.9	6.8	0.0	0.457	0.10
Emotional well-being	62.6	22.4	64.9	21.1	59.9	23.6	8.8	0.9	0.109	0.21
Self-esteem	55.8	24.0	56.6	24.0	54.9	24.1	7.8	0.9	0.609	0.07
Family	71.5	22.9	72.0	21.6	70.9	24.1	15.1	1.4	0.718	0.05
Friends	63.2	20.5	65.5	19.7	60.6	24.4	6.3	0.9	0.090	0.22
School	56.1	21.1	56.8	22.7	55.3	19.2	7.3	0.0	0.599	0.07

[a] Effect sizes are calculated from boys and girls.

Subscale alphas ranged from 0.73 to 0.84, and the total alpha score was 0.93. Corrected item-total correlations showed that all items ranged from 0.47 to 0.79 and Cronbach's alpha values if an item was deleted ranged from 0.82 to 0.87. A 10-day test-retest ICC (n = 189) ranged from 0.88 to 0.94 for the subscales, and was 0.95 for the total scale. Internal consistency of CES-D scales was 0.70 (n = 204).

3.4. *Validity*

Table 4 shows the total and subscale scores of the Kiddo-KINDL by gender and the CES-D score group. Except for the "school" subscale, the total and subscale scores of the Kiddo-KINDL were significantly different between the high and low CES-D score groups, for both girls and boys. In the high CES-D score group, the mean scores of "physical well-being", "emotional well-being", and "self-esteem" subscales were lower than those in the other subscales, and ranged from 35.7 to 39.7 in both gender groups. On the other hand, the highest scores in the high CES-D score groups were "school" for boys and "family" for girls. The effect of size for the six subscales ranged from 0.17 to 1.76 for both gender groups, and the total scale effect size for boys and girls were 1.28 and 1.42, respectively. The largest effect size among subscales occurred in the "emotional well-being" subscale for both boys (1.76) and girls (1.41).

3.5. *Responsiveness*

Figure 1 shows the ROC curve of the Kiddo-KINDL mean scores. The optimal cut-off score of the Kiddo-KINDL was estimated at 54.7, with an Area Under the Curve (AUC) score of 0.83 (95% CI 0.76 to 0.90, $p < 0.001$). The sensitivity and specificity at this cut-off score were 73.5% and 71.8%, respectively.

3.6. *Interpretability*

A total of 73 adolescents were classified as improved, 69 as stable, and 47 as worse according to the CES-D total scores between test and retest (Table 5). Among adolescents defined as the "improved" group, an increased difference of the Kiddo-KINDL mean total score was 7.4 and effect size was 0.48. For adolescents defined as the "stable" group, the score was almost the same. Among adolescents defined as the "worse" group, a decreased difference of the Kiddo-KINDL mean total scores was –5.2 and effect size was 0.53.

In addition, we classified these data into nine categories in detail (Figure 2). The mean changes in the Kiddo-KINDL scores according to a two-point

Table 3. Internal consistency and intraclass correlations of the Kiddo-KINDL subscales

Subscales	Cronbach's alpha coefficient	Corrected item-total correlation	Cronbach's alpha coefficient if item deleted	ICC (95% Confidence Intervals)[a] between test-retest
	n = 204			n = 189
Physical well-being	0.81	0.62	0.85	0.90 (0.86-0.92)
Emotional well-being	0.82	0.79	0.82	0.93 (0.91-0.95)
Self-esteem	0.83	0.74	0.83	0.94 (0.92-0.96)
Family	0.84	0.71	0.83	0.94 (0.93-0.96)
Friends	0.81	0.65	0.85	0.88 (0.84-0.91)
School	0.73	0.47	0.87	0.93 (0.91-0.95)

[a] ICC: Intraclass correlation coefficient by test-retest at an interval of 10 days.

Table 4. Comparisons of the Kiddo-KINDL scores between CES-D high score and low score groups

	Boys						Girls					
	CES-D score < 16 (n = 92)		CES-D score ≥ 16 (n = 17)		p	Effect size[a]	CES-D score < 16 (n = 74)		CES-D score ≥ 16 (n = 21)		p	Effect size[b]
	Mean	S.D.	Mean	S.D.			Mean	S.D.	Mean	S.D.		
Total	65.5	14.6	45.3	17.1	< 0.001	1.28	64.3	14.9	43.0	15.1	< 0.001	1.42
Subscales												
Physical well-being	61.8	18.9	39.7	19.8	< 0.001	1.14	61.2	17.4	39.0	19.2	< 0.001	1.21
Emotional well-being	69.8	17.7	38.2	18.1	< 0.001	1.76	66.1	20.9	37.8	19.2	< 0.001	1.41
Self-esteem	60.0	23.2	38.2	19.6	< 0.001	1.02	60.3	23.2	35.7	16.4	< 0.001	1.24
Family	76.0	18.3	50.4	25.8	< 0.001	1.16	76.5	20.5	50.9	26.9	< 0.001	1.08
Friends	68.0	18.5	51.8	21.2	0.002	0.82	64.4	19.5	47.0	22.0	0.001	0.84
School	57.4	22.8	53.7	22.3	0.536	0.17	57.4	18.8	47.6	19.0	0.038	0.52

Total and subscale scores are calculated by CES-D high score and low score groups based on cut-off of 16 points in total CES-D score. [a] Effect sizes are calculated from CES-D score < 16 and CES-D ≥ 16 in boys; [b] Effect sizes are calculated from CES-D score < 16 and CES-D ≥ 16 in girls.

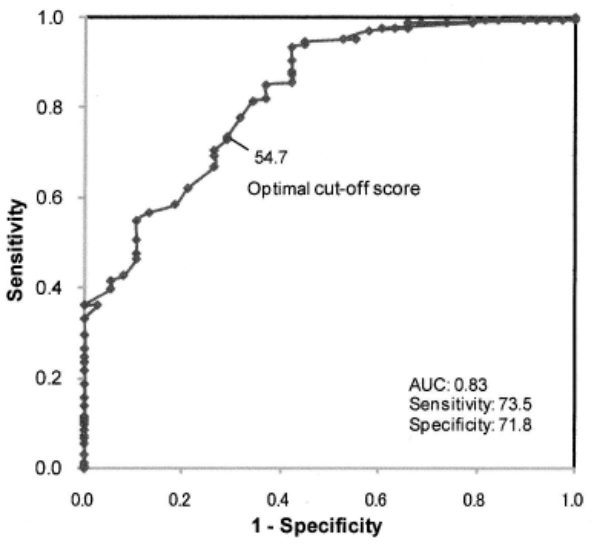

Figure 1. ROC curve of the Kiddo-KINDL mean scores. ROC curve calculated from 204 students. The mean scores of the Kiddo-KINDL were compared between adolescents with and without depressive symptoms.

difference on the CES-D scores are shown for each category. When the score of CES-D was improved (–3 and –2), the mean change in Kiddo-KINDL total scores increased by 4.4 (S.D. 4.6, 95% CI 2.85 to 6.02). Meanwhile, when the score of CES-D was worse (2 and 3), the mean change in Kiddo-KINDL total scores decreased by –4.0 (S.D. 2.9, 95% CI –5.16 to –2.76). We defined that a mean change of 4.0 for two-point CES-D scores was the minimal change.

4. Discussion

Our study results suggest that a Nepalese version of the Kiddo-KINDL could be a reliable and valid assessment tool for measuring HRQOL of Nepalese teenage adolescents. The psychometric properties of the Kiddo-KINDL are sound, and the percent of ceiling and floor effects for the subscales are not regarded to be high because the total scores of the ceiling and floor effects do not exceed 10%. Lamping *et al*. (*19*) have suggested that less than 10% ceiling and floor effects are criteria for acceptability of the scale. The variation

Table 5. Mean change in Kiddo-KINDL total score according to change in CES-D

Change in CES-D score[a]	*n* = 189	Kiddo-KINDL total score				Differences	Effect size[b]
		Test		Retest (10 days)			
		Mean	S.D.	Mean	S.D.		
Improvement	73	52.2	14.7	59.6	14.6	7.4	0.48
Stable	69	63.7	19.6	63.1	19.4	–0.6	0.03
Worsening	47	67.9	10.1	62.7	9.7	–5.2	0.53

[a] Change in health status according to CES-D total scores between test and retest. Improvement for CES-D scores ≤ –2, stable for CES-D scores between –1 and 1, worsening for CES-D scores ≥ 2; [b] Effect sizes are calculated from test and retest at an interval of 10 days.

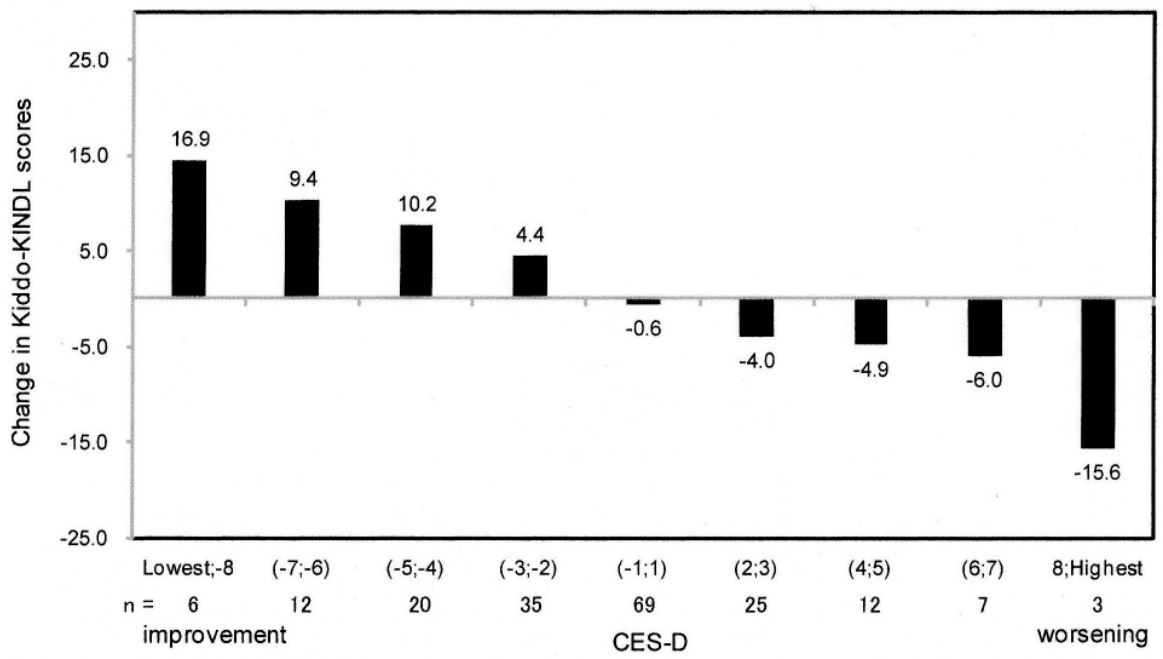

Figure 2. Minimal important difference of the Kiddo-KINDL according to a two-point CES-D score changes. Score between baseline test and 10 days retest. X axis shows extent of change in CES-D score from improvement to worsening between test and retest. Y axis shows mean change in Kiddo-KINDDL total score between test and retest.

of the ceiling and floor effects among the subscales suggests that the instrument is not inclined to distort study findings in one direction or another (*6*,*8*).

"Self-esteem" was the lowest scoring subscale in the overall HRQOL in Nepalese adolescents. Among young people in general, especially during early adolescence, body image and its satisfaction correlates with self-esteem. Girls are more sensitive and dissatisfied with their body (*20*). The "family" subscale score showed a slightly higher ceiling effect compared with the other subscales. This finding was similar to the previous study undertaken using the Kiddo-KINDL in Singapore (*21*). In Nepal, many teenage adolescents, especially girls, remain in the home until their adulthood. Intimacy within domestic relationships and support might have influenced this distributional skew.

Regarding instrument acceptability and usability, the Kiddo-KINDL is short and uses simple grammatical structures with common words, and is therefore easy for teenagers to understand. In our study, there was no missing data and none of the respondents failed to answer any question. Thus, the overall usability and acceptability of the Kiddo-KINDL was considered appropriate.

The Kiddo-KINDL is also reliable, as shown by a high level of Cronbach's alpha coefficient values above 0.70. High values for internal consistency indicates that whole and specific items are mutually consistent (*22*). Corrected item-total correlations indicate the extent to which each item relates to the construct measured by total score. The usual rule of thumb is that an item should correlate with a total score above 0.20 (*23*). A low item-total correlation means the item is slightly correlated with the overall scale and the researcher should consider dropping it. These internal reliability findings refer to the extent to which individual items of the Kiddo-KINDL satisfy scaling criteria as six dimensions in this study.

Assessing reproducibility becomes a defining feature of the precision of the instrument, which assesses the consistency of the repeated measurement. Acceptable test-retest reliability is an ICC of 0.85 (*24*), and the values in this study indicate substantial reproducibility and reliability. According to Marx *et al*. (*25*), a time interval of between two days to two weeks is suitable for test-retest administration. Previous studies on HRQOL have used an interval of one week to 10 days for the test-retest (*26*,*27*).

We used the CES-D in this study to demonstrate the discriminant validity of the Nepalese version of the Kiddo-KINDL. Comparisons based on low and high CES-D score groups showed that the adolescents with depressive symptoms scored lower in HRQOL than adolescents without depressive symptoms. The difference between the mean subscale scores between low and high CES-D score groups was the largest for "emotional well-being". The HRQOL of the adolescents with depressive symptoms tended to be lower in the physical and mental condition domains than for social and human relationship domains. Depression in children and adolescents appears to manifest in somatic symptoms or an emotional disorder in the early phases of the disease; the results from this study are in excellent agreement with this general understanding.

In this study, the subscale of "school" in boys was not statistically different between two groups. The boys with depressive symptoms might have avoided the negative impact of reporting the truth similar to a previous Kiddo-KINDL study among adolescents with diabetes (*8*). This is because parents have higher expectations for the academic performance of their boys than girls in general in Nepal (*28*).

Using the Kiddo-KINDL, we can reflect the features of two different groups appropriately and discriminate those with and without depressive tendencies. The indices are small (d = 0.20), medium (d = 0.50) and large (d = 0.80) (*29*); the results of this analysis in our study show a large effect of size occurring in all subscales except "school". The effect of size is a measure of the strength of the relationship between two variables regardless of sample size. Based on these findings of the Kiddo-KINDL instrument, one can distinguish between groups with and without depressive symptoms by t-test significance. Thus, our result suggests that the Nepalese version of the Kiddo-KINDL has high discriminant validity.

To assess the responsiveness, we first obtained the cut-off score of the Kiddo-KINDL for the CES-D score with ROC analysis. Sensitivity and specificity were acceptably high. The AUC is indexed from 0 to 1, the greater the total AUC from all cut-off points, the greater the instrument's responsiveness (*30*).

Our results indicate that the Kiddo-KINDL is responsive to changes in the CES-D scale. It can discriminate between improvement and worsening in depressive symptoms. We recommended a mean change in Kiddo-KINDL total scores of 4.0 to be used to define a minimal important difference according to two-point CES-D score changes. To assess interpretability in this study, HRQOL changes between test and retest were examined in relation to their benchmark for a minimal important difference, which was the adolescent's depressive tendency in a transition score. Interpretability is concerned with how meaningful are the scores from an instrument (*30*). Therefore, we determined the differences in Kiddo-KINDL scores that may be regarded as the minimal important difference for CES-D scores.

Traditionally, the minimal important difference of HRQOL scales found in patient-reported continuous outcomes, is used to assess chronic disorders (*31*,*32*). Since we did not have sufficient longitudinal data to confirm the minimal important difference in this study, we might go on to an even more detailed examination

of interpretability with clinical intervention followed over time among adolescents with depression.

There are some limitations in this study. First, this is a cross-sectional study based on convenience sampling. Moreover, small sample size and slightly lower response rate (63.8%) of the students is another concern. Therefore, the findings of this study may not be generalized to a larger population of Nepalese adolescents. In particular, one must consider the applicability of HRQOL studies for illiterate young people who do not have opportunities for education and learning, as approximately 19% of children aged between 6 to 10 years old are not in school in Nepal due to extreme poverty (*33*).

Second, the CES-D is a self-rating instrument to identify depressive symptoms during the previous week, and is not a diagnosis tool to identify depression by a suitably trained professional.

Finally, the original Kiddo-KINDL questionnaire included a question for adolescents with long term illness or hospitalization. In this study, we tested the instrument in teenage school adolescents, not in clinical settings. Previous studies have also evaluated the psychometrics of the Kiddo-KINDL with healthy adolescents and adolescents with chronic disease (*6,8,21*).

5. Conclusion

In conclusion, we have validated a Nepalese version of the Kiddo-KINDL to measure the HRQOL of school-attending adolescents. The results of tests of internal consistency, test-retest reproducibility, responsiveness, interpretability, and discriminant validity suggest that the instrument is valid and reliable among school adolescents in Nepal.

Acknowledgements

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Original Article

Pattern and determinants of breast feeding and contraceptive practices among mothers within six months postpartum

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Summary **The present study aims to determine the patterns of breast feeding, return of menstruation, and contraceptive practices in the first six months postpartum in women visiting the outpatient department at a teaching hospital in Lucknow, Northern India. Mothers of infants between six to eight months of age visiting the outpatient department of Era's Lucknow Medical College were interviewed regarding breast feeding practices, return of menstruation, sexual activity, and contraceptive practices within the first six months postpartum using a structured questionnaire. Of all women interviewed only 75.8% practiced exclusive breast feeding with the mean duration of exclusive breast feeding (EBF) being 3.5 months with only 41% practicing EBF for six months, 28% were sexually active within six weeks postpartum, 64.5% women had a return of menstruation within six months. Contraception was practiced by only 54.4% women with a barrier method such as a condom, being the most common. Better education was the only factor significantly affecting EBF ($p < 0.004$) and use of contraception ($p < 0.027$). There were a total of 10 pregnancies within six months postpartum. In conclusion, optimal breast feeding practices are poor in this part of the country and lactational amenorrhoea cannot be effectively and reliably used as a method of contraception. Therefore, optimal breast feeding practices, timely introduction of contraception and institutional delivery need to be encouraged.**

Keywords: Breast feeding practices, postpartum contraception, lactational amenorrhoea

1. Introduction

National family health survey-3 (NFHS-3) from India reports that 96% of children (under the age of 5 years) in India are ever breast-fed. However, the median length of exclusive breast feeding is relatively low *i.e.*, only 2 months (*1*). It is the exclusive and optimal breast feeding practice that has a bearing on the nutrition of the infant and has an added contraceptive advantage for the mother.

A recent ecological risk assessment study concluded that globally there are as many as 1.45 million deaths due to suboptimal breastfeeding in developing countries (*2*). Also, it is recommended that postpartum initiation of contraception should be done in the third postpartum month in fully breast fed and third postpartum week in partial or no breast feeding cases (*3*). The level of effectiveness of contraception by lactational amenorrhoea method will depend on the nutritional status of the mother, the frequency and intensity of suckling and the extent to which supplemental food is introduced (*4*). The present study was undertaken to: (*i*) study prevailing breast feeding practices, along with the timing of initiation of sexual activity and use of any other contraception besides duration of lactational amenorrhoea within the first six months postpartum; (*ii*) define the socio-demographic factors affecting exclusive breast feeding practices and contraception use in the first six months postpartum; and (*iii*) determine whether lactational ammenorhoea can be used as a method of contraception.

2. Materials and Methods

A cross-sectional hospital based survey was conducted

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between January 2009 to October 2009 in Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India. The state of Uttar Pradesh is the second largest state in India with Lucknow being the capital city. Uttar Pradesh has one of the highest total fertility rates (3.8) and the highest infant mortality rate of 73 per thousand live births (*1*).

According to the 2001 census Lucknow has a total population of 3,681,000 with a 63.62% urban population (*5*). Era's Lucknow Medical College and Hospital falls on the outskirts of the city with a local area which caters mostly to a semi-urban population.

Mothers of infants between six to eight months of age visiting the gynecology out patient department were interviewed. Informed consent was obtained prior to the interview. Ethical approval for the study was from the ethical committee of the hospital.

The questionnaire was developed and refined on the basis of peer review and pilot studies. The questionnaire had the following social and demographic variables: age, occupation of head of family, socioeconomic status, educational status of parents, parity, and place of delivery. Data on infant feeding practices included exclusive breast feeding (EBF) practices, time of initiation of breast feeding and method of top feeding were taken. The approximate time of start of sexual activity, return of menstruation and contraceptive practices within the first six months after delivery were also recorded.

3. Results

A total of 272 mothers were interviewed. The overall mean age was 25.56 ± 4.32 years. The age of the respondents ranged between 18 and 45 years. Majority of respondents belonged to the Hindu community, *i.e.*, 60.3% and the rest (39.7%) to the Muslim community. Average per capita income was Rs.1,326.27 ($28.9) (± Rs.2,545.3). The majority belonged to the middle socioeconomic class, *i.e.*, 66.2%. Out of the total women interviewed 22% were illiterate, and 19.5% were graduates (Table 1). All women were housewives except six who were working and 1.5% had unemployed partners. Most (74.3%) had hospital deliveries while 25.2% had delivered at home.

3.1. *Breast feeding*

Only 202 women remembered when they had initiated breast feeding after delivery. Of all the mothers most initiated breast feeding within 1-6 h after delivery for a total of 40.3% (n = 88) while 27% initiated after 24 h (n = 56).

Of all, 97% had breast fed their child. EBF was not practiced by 24.2%. The mean duration of EBF was 3.53 ± 2.51 months. Only 41.3% practiced exclusive breast feeding for 6 months. After applying bivariate analysis (chi-square test) between socio-demographic factors and exclusive breast feeding for six months, only educational status of more than the tenth standard was significantly related to exclusive breast feeding practice (Table 2). Bottle feeding was the most common method of top feeding (80.8%) while bowl and spoon were used by 18%, and 1% of women practiced both methods.

3.2. *Sexual activity*

The mean start of sexual activity after delivery was 2.8 ± 1.7 months with the range being from 12 days to 240 days, 28% woman were sexually active within six weeks postpartum and this rose to 93.3% by the end of 6 months (Table 3). There was a significant difference in mean consummation with mode of delivery. Mean start of sexual activity is much earlier in vaginal than in caesarean section (t = 1.97, p = 0.045).

Table 1. Characteristics of mothers

Characteristics of mothers	*n*	%
Religion		
Hindu	164	60.3
Muslim	108	39.7
Age groups		
< 19	25	9.2
20-25	140	51.4
26-30	81	29.8
> 30	26	9.6
Socioeconomic status		
Lower	63	26.6
Middle	157	66.2
Upper	17	7.2
Educational status of women		
Illiterate	59	21.7
Primary	25	9.2
Middle	43	15.8
High school	41	15.1
Intermediate	30	11.0
Graduate	52	19.1
Postgraduate	16	5.9
Professional	4	1.5
Place of delivery		
Home	70	25.7
Hospital	202	74.3
Parity		
1	117	43.0
2-3	130	47.8
> 3	25	9.2

Table 2. Socio-demographic variables affecting exclusive breast feeding

Variable		Yes	No	*p*
Age	≤ 25	64	61	0.066
	> 25	49	27	
Religion	Hindu	69	55	0.835
	Muslim	44	33	
Parity	< 2	49	46	0.209
	≥ 2	64	42	
Literacy	≤ 10th	57	62	0.004
	> 10th	56	26	
Delivery	Hospital	89	63	0.206
	Home	20	22	

Table 3. Duration of exclusive breast feeding, return of menses, start of sexual activity and contraception

Postpartum (completed) weeks	Exclusive breast feeding *n* (%)	Sexual activity *n* (%)	Return of menstruation *n* (%)	Contraception *n* (%)
Up to 6	187 (68.8)	77 (28.3)	78 (28)	17 (6.2)
Up to 12	170 (62.4)	202 (74.3)	147 (54)	99 (36.3)
Up to 18	133 (48.8)	224 (82.3)	162 (59.5)	109 (40)
Up to 24	112 (41.3)	254 (93.3)	175 (64.2)	148 (54.4)

3.3. *Return of menstruation*

Of the total respondents interviewed 28% had a return of menses within six weeks postpartum which increased to 64.5% at the end of six months while the rest *i.e.*, 35.5% of respondents had lactational amenorrhoea at the end of six months (Table 3).

3.4. *Contraception*

Only 54.4 % (n = 148) of respondents were using some method of contraception. Of these 148 the majority, 85.6% (n = 126) were using condoms. Only 1.8% (n = 3) were using intrauterine contraceptive devices (all 3 insertions were done 4 to 6 months postpartum) and 10% (n = 15) of respondents had undergone sterilization, all were ligations at the time of caesarean section except one which was done along with a medical termination of pregnancy. Oral contraceptives were used by 1.8% (n = 3), and 1% (n = 1) were using coitus interruptus (Table 4). Contraceptive usage was only 14% in six weeks postpartum and rose to 54.4% until the end of six months postpartum (Table 3). On bivariate analysis woman's educational status was the only variable which was related to use of contraception (p = 0.004) (Table 5). There were 10 (3.7%) pregnancies within six months of delivery in the women interviewed.

Table 4. Type of contraception used

Type of contraception	*n* (%)
Pills	2 (1.8%)
IUCD	2 (1.8%)
Injectable/implants	0 (0.0%)
Condom	95 (85.6%)
Permanent	11 (9.9%)
Natural	1 (0.9%)

Table 5. Socio-demographic variables affecting contraception use

Variable		Yes	No	*p*
Age	≤ 25	63	84	0.462
	> 25	35	57	
Religion	Hindu	58	90	0.411
	Muslim	41	51	
Parity	< 2	46	59	0.477
	≥ 2	53	82	
Literacy	≤ 10th	52	94	0.027
	> 10th	47	47	
Delivery	Hospital	76	100	0.209
	Home	20	39	

4. Discussion

Breast feeding initiation should ideally be started within 30 min. Early initiation of breast feeding is important for mother-infant bonding, helps in establishment of longer and more successful breastfeeding and also helps in uterine contractions after delivery by causing release of oxytocin.

In our study there was a poor rate of early initiation (within 1 h) *i.e.,* only a total of 19% and approximately 28% initiated after 24 h. This is quite a contrast compared to other studies conducted in India which show a much higher early initiation of breast feeding (*7*). It is a well-known fact that exclusive breast feeding protects the child from malnutrition and infection. A Dhaka study showed that when EBF rates at 6 months were increased from 39% to 70% there was a reduction in infant mortality by 32% which is quite significant (*8*). The Bellagio Child Survival Study Group also stressed the advantages of exclusive breast feeding and said that universal exclusive breast feeding for the first six months could reduce infant mortality rate by 13% (*9*). Our study showed that only 41.3% practiced EBF for six months. This is even below the national average of 46.4% (*1*) and well below the rate of 70% from a study from Nigeria (*10*).

A striking fact here is the use of bottle feeding by 80% of mothers even though the WHO discourages bottle feeding because it is difficult to sterilize the nipple properly (*11*).

Postpartum sexual abstinence is traditionally practiced especially in some African societies. Although there is no published data for a period of postpartum sexual abstinence it is commonly believed in India that abstinence should be practiced for a period of about the first six weeks after delivery. In our study the mean start of sexual activity after delivery was 2.8 months with 28% having intercourse within puerperium. This was late as compared to a study from Thailand in which 35% of women had resumed sexual activity within six weeks postpartum (*12*) and a Uganda study in which 49.3% had resumed intercourse within this period (*13*).

Breast feeding has a positive influence on duration of lactational amenorrhoea. Though 68.8% were still exclusively breast feeding by the end of the first six weeks, 28% had resumed menstruation; this could be

the result of poor exclusive breast feeding practice and reduced intensity of breast feeding. In our study 64.5% of women had a return of menses within six months as compared to 70.2% in a similar study from Africa (*14*).

Contraceptive demand is not constant throughout the reproductive life of a woman, postpartum period being the most crucial as appropriate birth spacing can improve the maternal and infant mortality rates (*15*). The contraceptive practice in our study was comparable to NFHS-3 national data (56.3%) (*1*), and higher than a Sri Lankan study published in 2009 which found contraceptive practice to be only 41.1% among 129 mothers interviewed (*16*). However, a study from Turkey showed a much higher (76%) contraceptive use in postpartum women with an intrauterine contraceptive device being the most common method of contraception (*17*), but in our study the most commonly used method was condoms which have a much higher failure rate.

Not only increasing the use of contraception, but also timely introduction of contraception is important. Because only 6.2% of the women were using contraception within six weeks postpartum while 28% had resumed menstruation and the same percentage were sexually active within the same time frame and therefore were unprotected and at risk of conception (Table 4).

Since this is a retrospective study there could be a recall bias especially for breast feeding initiation and resumption of sexual activity. As most women were using a barrier method and its use was irregular it was difficult to ascertain the timing of initiation of contraception.

5. Conclusion

The first and foremost inference of this study is that optimal breast feeding rates and contraceptive practice rates are poor in India and even worse in this part of the country. Increasing awareness regarding use of "exclusive" breast feeding and not only merely stating benefits of breast feeding is required to be incorporated in breast feeding awareness campaigns. Secondly, emphasis on institutional delivery will go a long way to bring down maternal and infant mortality rates. Lastly, contraception counselling should start early, preferably during antenatal or the immediate postpartum period because lactational ammenorhoea is not a very reliable method for contraception and especially so for this part of the world.

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Original Article

Anti-aggressive activity of a standardized extract of *Marsilea minuta* Linn. in rodent models of aggression

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Summary **The present study was undertaken to evaluate *in vivo* anti-aggressive potential of a standardized extract of *Marsilea minuta* Linn. (Marsileaceae). The standardized extract of *Marsilea minuta* was evaluated for its potential effects against defensive and offensive aggressive behavior models of rodents. *Marsilea minuta* extract was orally administered at three dose levels (100, 200, and 400 mg/kg BW) once daily for 14 consecutive days as a suspension in polyethylene glycol (PEG), diazepam (2.5 mg/kg, *p.o.*) was used as a standard anti-aggressive agent. Control group animals were given an equal volume of vehicle (10%, v/v, PEG suspension). Anti-aggressive activity was evaluated using the following validated models of aggression, *viz.*: foot shock-induced aggression, isolation-induced aggression and resident-intruder aggression, in rodents. As a result, *Marsilea minuta* extract showed dose dependant anti-aggressive activity in the aforementioned, validated models of aggression. This suggests that the extract from *Marsilea minuta* has a promising anti-aggressive activity qualitatively comparable to that of diazepam.**

Keywords: *Marsilea minuta*, aggression, stress, foot shock, isolation

1. Introduction

Marsilea minuta Linn. (Marsileaceae), a common species of water fern, is widely found in wet and humid places (*1*). In Ayurveda, the plant is recommended for treatment of psycopathy, diarrhea, cough, bronchitis, and skin diseases (*2*). A standardized extract of *Marsilea minuta* has been reported to possess anti-amnestic (*3*), anxiolytic (*4*), and antidepressant activities (*5*). Marceline, an ester of 1-triacontanol and hexacosanoic acid, isolated from *Marsilea minuta* is known to have sedative and anticonvulsant activity (*6*). Gupta *et al*. (*7*) reported hypocholesterolemic activity of the methanolic extract of the plant in gerbils. Other reported activities include antifertility activity (*8*), tranquilizing activity (*9*), antibacterial (*10*), and antifungal activity (*11*). In our earlier study (*12*), we reported adaptogenic and anti-stress activity of the standardized extract of *Marsilea minuta*. Aggression is now a significant public health problem and association between mental illness and aggression is well established (*13,14*). Besides this, stress is another major factor promoting aggression and violence in humans (*15,16*). Keeping in view the beneficial effect of *Marsilea minuta* Linn. in neurological disorders such as amnesia, depression, anxiety and antistress activity we decided to investigate the anti-aggressive activity of *Marsilea minuta* Linn.

2. Materials and Methods

2.1. *Materials*

Whole plants of *Marsilea minuta* were collected during the month of July 2004 from Berhampur, Orissa, India. *Marsilea minuta* Linn. (Marsileaceae) was authenticated by Prof. N. K. Dubey, Incharge herbarium, Department of Botany, Banaras Hindu University, Varanasi, India.

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A specimen copy of the same (Sept-2004-1) was deposited in the herbarium, Department of Botany, Banaras Hindu University. All other reagents used were of analytical grade.

2.2. *Preparation of extract*

The whole plant of *Marsilea minuta* was dried under shade in a drying room with a relative humidity of 40%. The room temperature was maintained between 37 and 40°C. The drying process was carried out for 5-7 days. The shade-dried plant was reduced to coarse powder in a roller grinder and was finely powdered further. The fine powder was then passed through a No. 40 sieve. About 500 g of plant powder was thoroughly extracted with 2.5 liters of 90% ethanol in a soxhlet apparatus for 48 h. The extract was concentrated under vacuum at 50°C and then lyophilized (yield 16.3%, w/w), and was stored at −20°C until required. The presence of steroids, flavonoids, alkaloids, and saponins was confirmed in a preliminary phytochemical investigation of the ethanolic extract of *Marsilea minuta* (*17*). Marsiline was isolated as described previously (*6*) and characterized. The extract was standardized for marsiline (purity, 94.32%) using a Perkin Elmer HPLC with a diode array detector. The method was standardized and validated with an initial sample of 5 μg/mL. Eight replicates of this concentration (5 μg/mL) were prepared and analyzed. The limit of detection and limit of quantification obtained was 1.53 and 5.11 μg/mL, respectively. The average percent recovery and coefficient of variation was found to be 91.75 and 1.11%, respectively. A standard curve was prepared using five standards at 10, 20, 50, 100, and 200 μg/mL. The curve showed good linearity with an r^2 value of 0.942. The standardized ethanolic extract of *Marsilea minuta* (Mm) (1.15%, w/w of marsiline) was used for the pharmacological evaluations.

2.3. *Animals*

Swiss albino mice (20 ± 2 g) and Wistar rats (200-250 g) of either sex were obtained from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University (Regd. No. 542/02/ab/CPCSEA). Animals were randomly housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1°C and 45-55% relative humidity, with a 12 h light/dark cycle (lights on at 7 am). The animals had free access to standard pellet (Hindustan Lever, India) and water *ad libitum*. Experiments were conducted between 8:00 and 14:00. The experiments were conducted according to the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Prior permission was obtained from the Institutional Animal Ethics Committee (IAEC) to carry out the experiments.

2.4. *Drug treatments*

Based on our earlier studies, the standardized ethanolic extract (1.15%, w/w of marsiline, HPLC) of Mm was administered orally, as a polyethylene glycol (PEG) suspension in doses of 100, 200, and 400 mg/kg of body weight, once daily for 14 consecutive days. Experiments were conducted on day 14, 1 h after the last oral treatment. Diazepam (2.5 mg/kg, *p.o.*) was used as the standard anti-aggressive agent for comparison. Control animals were treated with an equal volume of vehicle (10%, v/v, PEG suspension).

2.5. *Experimental methods*

The three most widely used rodent models, often used to detect potential effects of a therapeutically used anxiolytic drug on aggression were chosen to evaluate the effect of Mm on aggressive behavior, *viz.*: foot shock-induced aggression, isolation-induced aggression, and resident-intruder aggression.

2.5.1. *Foot shock-induced aggression*

Weight matched Swiss mice were divided into five groups (each containing 6 pairs), treated with vehicle, Mm (100, 200, and 400 mg/kg BW) or diazepam respectively, once daily for 14 consecutive days. On the 14th day, 1 h after the last oral treatment, all pairs of mice were subjected to foot shock by placing them in an aggressometer (Techno) for 3 min. During a 3 min observation period, every 5 sec a 60-Hz current was delivered for 5 sec. Each pair of mice was dosed and tested without previous exposure. The total number of fights were recorded for each pair (*18,19*).

2.5.2. *Isolation-induced aggression*

Male Swiss mice (body weight of 25 ± 5 g) were kept isolated in small cages for two months. Prior to the drug treatment, the aggressive behavior of the isolated mouse was assessed against a male mouse (similar in weight to that of the isolated mouse, and accustomed to living in a group and put into the cage of an isolated mouse for 5 min). Immediately, the isolated mouse started to attack the "intruder". The aggressive behavior of the isolated mouse was characterized by hitting the tail on the bottom of the cage, screaming and biting. Isolated mice not exhibiting aggressive behavior were excluded from the test. One day after the initial trial, isolated animals were distributed into five groups (n = 6) and were treated with vehicle, Mm (100, 200, and 400 mg/kg BW) or diazepam for 14 consecutive days. One hour after the last dose, aggressive behavior of the isolated mouse against a male mouse was evaluated for 5 min (*19-21*). Aggressive behavior related parameters assessed during this test were latency to first attack, screaming, pursuit frequency,

tail rattle, aggressive posture, and total number of fights.

2.5.3. *Resident-intruder aggression*

Male rats (400 ± 20 g) were tested in their home cages for aggression against a smaller (200 ± 20 g) male intruder. Before the start of the experiments, each resident male rat was kept in a pair with one female rat in a polypropylene cage for 15 days, and they were randomly divided into 5 groups (n = 6). Drug treatment was started from the 16th day onward, and only male rats of each pair were administered with vehicle, Mm (100, 200, and 400 mg/kg BW) or diazepam for 14 consecutive days. The resident female was removed from the cage 30 min prior to the start of the test. One hour after the last oral treatment, a male intruder (~ 200 g) was placed in the territorial cage of the resident male, and behavior of the resident male was observed for the next 15 min. During this period, the time until the first attack (in seconds), number of attacks, and duration of each attack (in seconds) were recorded by a blind observer (*19*).

3. Results

3.1. *Foot shock-induced aggression*

All three doses of Mm (100, 200, and 400 mg/kg) significantly reduced the total number of fights as compared to controls. Diazepam treatment also significantly reduced foot shock-induced fighting behavior in mice (Figure 1).

3.2. *Isolation-induced aggression*

All three doses of Mm (100, 200, and 400 mg/kg) significantly increased latency time to first attack (Figure 2) while the number of aggressive postures, aggressive pursuit, tail rattle frequency and attacks were significantly reduced by all three doses of Mm. These effects of Mm (100, 200, and 400 mg/kg) were identical to that of diazepam (2.5 mg/kg) (Figure 3).

3.3. *Resident-intruder aggression*

All three doses of Mm (100, 200, and 400 mg/kg) significantly prolonged the latency period of first attack (Figure 4) and significantly reduced the frequency of aggressive posture, aggressive grooming and total number of attacks (Figure 5). The total duration of fighting was also reduced significantly by all three doses (100, 200, and 400 mg/kg) of Mm (Figure 6). The observed effects of diazepam in this model were qualitatively similar to those of Mm.

4. Discussion

The present anti-aggressive study was carried out to explore knowledge about the beneficial effect of Mm in

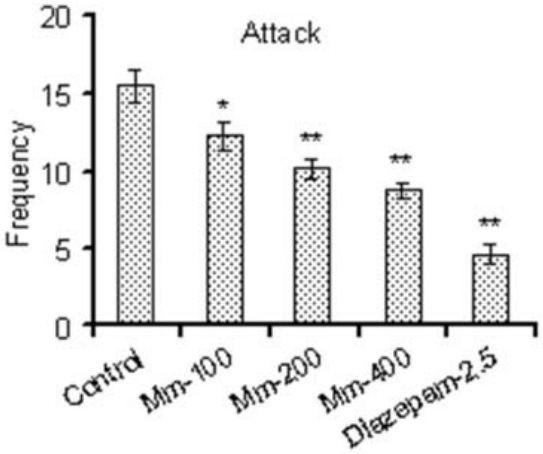

Figure 1. Effect of *Marsilea minuta* on foot shock-induced aggressive behavior. Values are given as mean ± S.E.M. (n = 6). * $p < 0.05$, ** $p < 0.01$ compared to control.

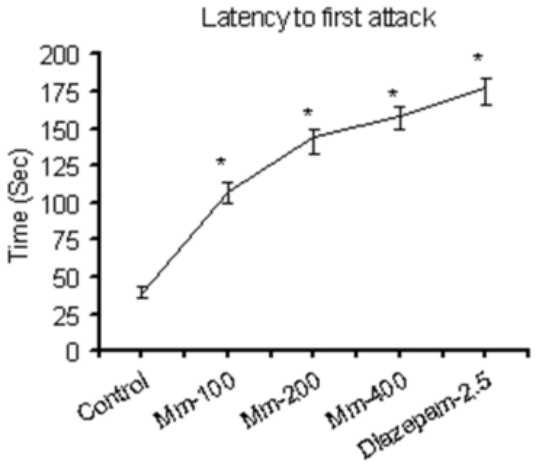

Figure 2. Effect of *Marsilea minuta* on latency time to isolation-induced first attack. Data are given as mean ± S.E.M. (n = 6). * $p < 0.01$ compared to control.

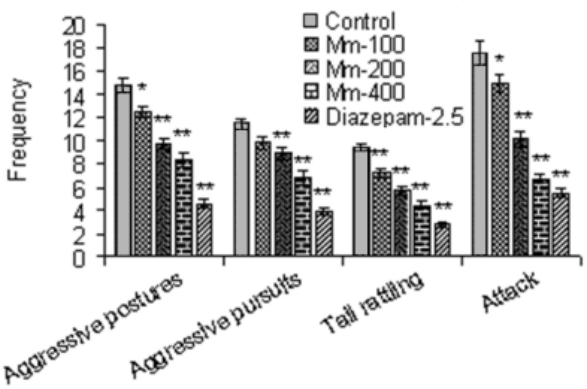

Figure 3. Effect of *Marsilea minuta* on frequency of various isolation-induced aggressive behavior. Data are given as mean ± S.E.M. (n = 6). * $p < 0.05$, ** $p < 0.01$ compared to control.

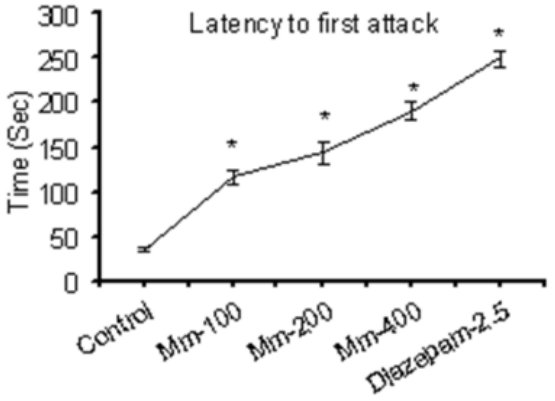

Figure 4. Effect of *Marsilea minuta* on latency period of first attack against resident intruder. Data are given as mean ± S.E.M. ($n = 6$). * $p < 0.01$ compared to control.

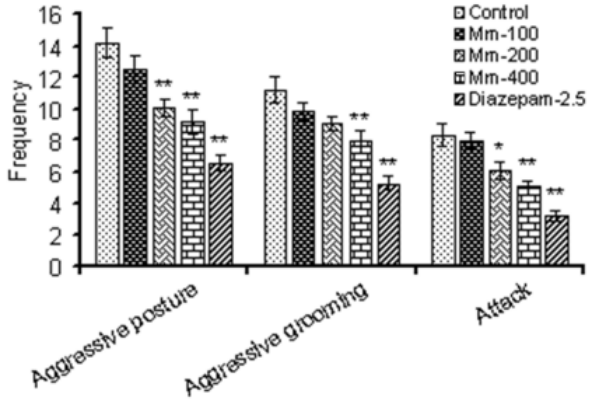

Figure 5. Effect of *Marsilea minuta* on frequency of various resident intruder aggressive behaviors. Data are given as mean ± S.E.M. ($n = 6$). * $p < 0.05$, ** $p < 0.01$ compared to control.

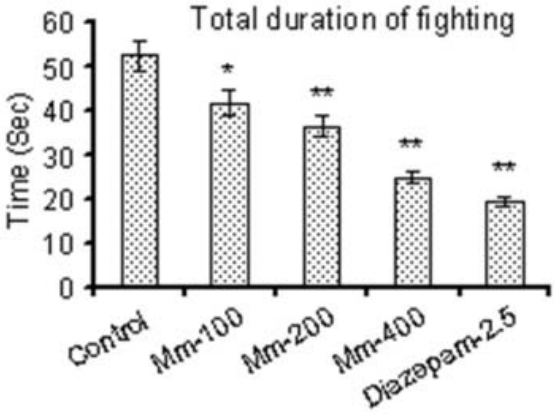

Figure 6. Effect of *Marsilea minuta* on total duration of fighting against resident intruder. Data are given as mean ± S.E.M. ($n = 6$). * $p < 0.05$, ** $p < 0.01$ compared to control.

neurological disorders as already established in anxiety (*4*), depression (*5*), amnesia (*3*), and convulsions (*6*). The result of the study indicates that Mm has a dose dependent significant anti-aggressive activity which is comparable to diazepam. All three doses of Mm (100, 200, and 400 mg/kg, *p.o.*) significantly reversed the parameters of aggression in all three models of aggression used, *viz.*: foot shock-induced aggression, isolation-induced aggression, and resident-intruder aggression.

The term aggression is widely employed to indicate various patterns of psychological or sociological behavior resulting from pathological, biochemical or physiological alteration of central nervous system constituents. There are many psychiatric disorders such as schizophrenia and Alzheimer's disease which show close association with aggression (*14*).

Like any other behavior, aggression is also controlled and modulated by neurotransmitters. The agonist of 5-HT_{1A}/5-HT_{1B} and antagonist of 5-HT_{2A}/5-HT_{2C} receptors have been reported to possess anti-aggressive properties (*22,23*). Bernard *et al*. (*24*) showed that dopamine levels and measurement of dopamine synthesis and turnover in the whole brain have increased in aggressive strains of mice and in mice that have just engaged in aggressive behavior. In the isolation-induced aggressive behavior model the level of dopamine increases in the striatum (*25*). In a postmortem study, Clement *et al*. (*26*) showed that the levels of GABA and glutamic acid decarboxylase, are low in brain areas such as the striatum and olfactory lobes of mice and rats which exhibited aggressive behavior. Initial studies targeting the α subunit of the $GABA_A$ receptor point to their significant role in the aggression-heightening effect of alcohol, benzodiazepines, and neurosteroides (*27*). Tsuda *et al*. (*28*) and Tanaka *et al*. (*29*) have reported that expression of aggression is an alternative mechanism to decrease the stress related increase of noradrenaline.

Antidepressants, anxiolytics, cognitive function modulators, anticonvulsants, and other psychoactive agents are now identified as potential anti-aggressive therapeutics because of their neurotransmitter modulator properties. Mm has been investigated in various experimental models of depression, anxiety and memory and learning to reveal its modulator action on a variety of neurotransmitters. In this regard the effect of Mm on serotonin levels is of particular interest. Bhattamisra *et al*. (*3*) showed that Mm significantly decreases the serotonin level in the whole brain region of mice. That activation of 5-HT_{1A} receptors which results in decreased release of serotonin is accompanied by anti-aggressive behavior after administration of a 5-HT_{1A} agonist is a well established fact (*30,31*). Based on this premise, it can be concluded that the observed anti-aggressive property of Mm is due to its serotonin inhibitory action in whole brain. Besides the possible role of neurotransmitters in mediating aggression, stress has also been implicated to promote aggression and violence in humans (*15,16*). An earlier study (*12*) in the author's laboratory reported anti-stress activity of the standardized extract of Mm. Thus, it is concluded that the anti-aggressive activity of Mm may be

supplemented by its anti-stress property along with its neuromodulatory action.

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Original Article

Identification of mouse mutant cells exhibiting the plastic mutant phenotype

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Summary **The initial processes involved in radiation carcinogenesis have not been clearly elucidated. We isolated mouse mutant cells exhibiting plasticity in their mutation phenotypes. These mutant cells were originally isolated from an irradiated cell population as 6-thioguanine resistant ($6TG^R$) mutants that were deficient in hypoxanthine phosphoribosyl transferase (Hprt, E.C.2.4.2.8) activity at the frequency of approximately 6.2×10^{-5}. Approximately 10% of $6TG^R$ cells showed plasticity in their mutant phenotypes and reverted to HAT-resistant (HAT^R), which is Hprt-proficient, wild type phenotype. Eventually we identified the plastic mutants in the un-irradiated wild type cell population as well and found that ionizing irradiation enhanced the frequency of the plastic mutation approximately 24 times. Treatment with 5-aza-cytidine did not affect the plasticity of mutant phenotypes identified in this study, suggesting that DNA methylation was not involved in the plastic changes of the mutant phenotypes. The plastic mutant phenotype identified in our study is a new type of genomic instability induced by ionizing irradiation, and it is likely to be involved in one of the primary changes that occur in the process of radiation carcinogenesis, and may explain one element of carcinogenesis, which is composed of multi-stages.**

Keywords: Plastic mutation, genomic instability, ionizing radiation, mouse FM3A cells, hypoxanthine phosphoribosyl transferase (Hprt)

1. Introduction

The carcinogenic potential of ionizing radiation (IR) was recognized very soon after its discovery in 1895. Frieben reported the first tumor induced by IR in 1902. IR was also the first mutagen shown to increase the mutation rate in an organism (*1*). IR exerts its effects through the deposition of energy in the cells and the subsequent generation of hydroxyl radicals, leading to damage on DNA strands. The comparative importance of the base alterations caused by IR in mutagenesis has been demonstrated in bacteria (*2*), bacteriophages (*3*) and lower eukaryotes (*4*). In mammalian cells, the majority of mutations induced by IR have been shown to be deleterious (*5*). More recently, genomic instability has been shown to play an important role in mutagenesis and carcinogenesis in mammalian cells. However, genomic instability is not a specific phenomenon observed in irradiated cells. Un-irradiated normal cells also exhibit the same characteristics, such as chromosome aberration and microsatellite/minisatellite instability, at lower frequencies. IR increases the genomic instability in the irradiated cells, as well as neighboring cells. Genomic instability induced by IR has been characterized by an increased rate of alteration acquisition in the genome, such as chromosomal aberrations, micro-nucleation, mutations, microsatellite instability, and cell death (*6*). Increased genomic instability caused by IR is of great concern in the age of advanced medical technologies

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using IR, not only chest X-ray and mammography, but also computed tomography (CT) and positron emission tomography (PET), in which higher doses of X-rays are often employed. Although IR is currently recognized as a relatively ineffective carcinogen, Berrington de Gonzalez and Darby reported that a significant proportion of cancer incidence is attributable to the recent extensive use of these X-ray diagnostic apparatuses in medical procedures (*7*).

Through our investigation on the genomic instability induced by X-ray irradiation in mouse cells, we identified mutant cell clones that exhibited plasticity in their gene regulation. This genomic instability was manifested as reversible drug resistant phenotypes with increased mutation frequencies concerning hypoxanthine phosphoribosyl transferase (Hprt, E.C.2.4.2.8) activity. It has been shown that the resistance to the cytotoxic drug 6-thioguanine (6TG) could be achieved by the loss of Hprt activity (*8,9*). Involvement of DNA methylation at the promoter region has been reported in the event of suppressing Hprt activity (*10,11*); however, the genomic instability identified in this study seemed to involve different mechanisms. Here, we report the isolation and preliminary molecular characterization of the mutant cells exhibiting the plastic mutant phenotype.

2. Materials and Methods

2.1. *Cell culture*

Mouse FM3A cells were maintained in ES medium (Nissui, Tokyo, Japan) containing 2% fetal bovine serum (FBS) (Nichirei, Tokyo, Japan), as described previously (*12*).

2.2. *X-ray exposure*

Cells were exposed to 5 Gy of 250 keV X-ray using the Shimadzu Pantak model HF-250 (Shimadzu, Kyoto, Japan) at a dose of 0.5 Gy/min. After X-ray exposure, cells were allowed to recover for 24-48 h prior to further treatment.

2.3. *Isolation of 6TG-resistant ($6TG^R$) mutants*

Prior to the selection experiment using 6TG, cells were cultured in HAT medium (*13*) containing 10^{-4} M hypoxanthine, 10^{-6} M aminopterin (also known as methotrexate, MTX), and 10^{-5} M thymidine for 48 h, and then in HT medium containing 10^{-4} M hypoxanthine and 10^{-5} M thymidine for 24 h. Cells were plated onto ES plates containing 5% FBS, 0.5% agarose, and 10^{-5} M 6TG; 0.05 mM of 5-aza-cytidine was also included in the selection plates in the experiments examining DNA methylation (*14*). The number of the cells employed in the drug selection experiment was estimated by the number of colonies formed on the ES plates without 6TG using the appropriate dilution of the cell suspension. Colonies formed on the selection plates containing 6TG were independently isolated and cultured for further analyses. All chemicals were obtained from Wako Chemical (Osaka, Japan), unless otherwise specified.

2.4. *Isolation of HAT-resistant (HAT^R) revertants*

Prior to HAT selection, $6TG^R$ clones were transferred to normal growth medium without 6TG for 24 h. HAT^R clones were selected using ES liquid medium containing 2% FBS and the HAT contents described above or ES plates containing 5% FBS, 0.5% agarose, and the HAT contents; 0.05 mM of 5-aza-cytidine was included in the selection plates in the experiments examining DNA methylation. The number of cells employed in the HAT selection was estimated using the ES plates without HAT.

2.5. *Loss-of-heterozygosity (LOH) analysis*

Genomic DNA was extracted from the cells by proteinase K-SDS treatment and purified by the phenol-chloroform extraction method, as described previously (*15*). LOH at the *Hprt* locus coding the *hypoxanthine phosphoribosyl transferase (Hprt)* gene was examined by polymerase chain reaction (PCR) with the oligonucleotide primers UniSTS178186 *Hprt* F 5'-GAA ATG TCA GTT GCT GCG TC-3' and UniSTS178186 *Hprt* R 5'-GCC AAC ACT GCT GAA ACA TG-3' (*16*). The PCR mixture was prepared as recommended by the manufacturer (Takara, Shiga, Japan). The reaction was started with 5 min at 94°C, which was followed by 40 cycles of 30 sec at 94°C, 30 sec at 55°C, and 30 sec at 72°C using the GeneAmp PCR System 9700 (Applied Biosystems Inc., Carlsbad, CA, USA). PCR products were analyzed by 3% agarose gel electrophoresis.

3. Results

3.1. *Isolation of $6TG^R$ mutants*

Prior to the drug selection experiments using 6TG, mouse FM3A cells were cultured in HAT medium for 48 h to eliminate naturally ocurring Hprt-deficient cells. Since Hprt activity is not an absolute requirement for survival, cells that lack Hprt activity will grow in normal growth medium and affect the results of experiments examining the mutation frequency.

Cells were allowed to recover from the toxic effects of MTX in HT medium for 24 h. MTX is a drug that inhibits dihydrofolate reductase activity, leading to the deprivation of *de novo* biosynthesis of both purine and pyrimidine nucleotides. If the cells grown in HAT medium were transferred immediately to the medium

containing 6TG without recovery of the *de novo* syntheses of nucleotides, not only Hprt-proficient wild-type cells, but also Hprt-deficient mutant cells would not survive.

Cells were then exposed to 5 Gy X-ray to induce the Hprt-deficient mutations. Hprt-deficient mutants were selected as the $6TG^R$ phenotype. 6TG is a toxic nucleotide analogue that is incorporated into the cell metabolism through Hprt enzymatic activity, leading to cell death. The mutant cells deficient in Hprt activity do not incorporate the toxic analogue into the nucleotide metabolism and thus will survive and grow to form colonies in the presence of 6TG.

In our experiments, X-ray exposure induced 192 $6TG^R$ mutants from 3.1×10^6 cells at a mutation frequency of 6.2×10^{-5}, as shown in Table 1. Additionally, 187 spontaneous $6TG^R$ mutants were obtained from the un-irradiated cell population at a frequency of 1.2×10^{-5}. Thus, 5 Gy X-ray exposure enhanced the frequency of Hprt-deficient mutations about 5 times.

3.2. *LOH analysis at the Hprt locus*

The genomic structure at the *Hprt* locus in $6TG^R$ cells was examined by PCR using a set of UniSTS primers. The *Hprt* locus was not detected in 94 clones of the 187 spontaneous mutants and in 138 clones of the 192 irradiated mutants. DNA sequencing analysis of the genomic *Hprt* gene was not carried out. The cells that did not provide PCR products were regarded as having deleterious mutations in the *Hprt* allele and were not employed in further experiments.

3.3. *Isolation of HAT-resistant reversion mutants*

Using the $6TG^R$ mutant cells without LOH, namely, 93 spontaneous mutants and 54 irradiated mutants, we attempted to isolate revertants by culturing $6TG^R$ cells in HAT medium. MTX inhibits the biosynthetic pathways of both types of nucleotides, purine and pyrimidine, and the cells growing in HAT medium must incorporate hypoxanthine as a substrate for purine nucleotides through the salvage pathway catalyzed by Hprt activity and thymidine for pyrimidine nucleotides through thymidine kinase activity. The cells deficient in Hprt activity do not incorporate hypoxanthine for purine biosynthesis and will not survive in HAT medium. Thus, revertant cells that reactivated Hprt activity will survive in HAT medium.

As summarized in Table 1, we isolated 4 reversion mutant clones from 93 spontaneous $6TG^R$ mutants without the deletion and 19 from 54 irradiated mutants. The frequency of reversible mutants was 4.3% among spontaneous mutants and 35.2% among irradiated mutants. As a result, the irradiated $6TG^R$ mutants contained approximately 10 times more reversible mutant cell clones than the spontaneous mutants. The frequency of the plastic mutant was approximately 2.5×10^{-7} in the normal cell population and approximately 6.1×10^{-6} in the irradiated population, indicating that IR induced approximately 24 times more phenotypic plasticity in mouse FM3A cells. Primary screening of reversion mutants was carried out in ES liquid culture medium containing 2% FBS and the HAT contents. For calculation of the reversion frequencies, the cells were plated on ES agarose plates containing 5% FBS and the HAT contents, and the number of colonies formed was counted. The plastic mutants changed their phenotype at a frequency of approximately 10^{-2} on average, as summarized in Figure 1. The remaining $6TG^R$ mutants exhibited the stable 6TG-resistant phenotype and did not grow in HAT medium.

3.4. *Effect of X-ray exposure and 5-aza-cytidine treatment on plastic mutation*

We examined the effect of X-ray exposure on the induction of reversion mutation from the plastic mutants isolated in this study. A total of 93 clones of the spontaneous $6TG^R$ mutants without the deletion and 54 clones from the irradiated mutants were exposed to 5 Gy X-ray and cultured in liquid ES medium containing 2% FBS and the HAT contents. As a result, the HAT^R revertants appeared from the same $6TG^R$ clones, but no enhancements by X-ray irradiation were observed in the number of HAT^R clones or in the frequency of HAT^R clones obtained. The inclusion of 5-aza-cytidine also did not affect the plasticity of the mutant phenotypes. The plastic mutants isolated in our experiments changed their phenotypes from HAT^R wild-type to $6TG^R$ mutant phenotype, as well as from $6TG^R$ mutant to HAT^R wild-

Table 1. Summary of the cell culture experiments isolating the plastic mutants from the un-irradiated or irradiated cell population

	0 Gy	5 Gy X-ray
No. of cells selected	1.6×10^7	3.1×10^6
No. of 6TG-resistant clones	187	192
Frequency of 6TG-resistant mutation	1.2×10^{-5}	6.2×10^{-5}
No. of clones with/without LOH in *Hprt* allele	94/93	138/54
No. of HAT-resistant clones	4	19
Frequency of plastic mutants	2.5×10^{-7}	6.1×10^{-6}

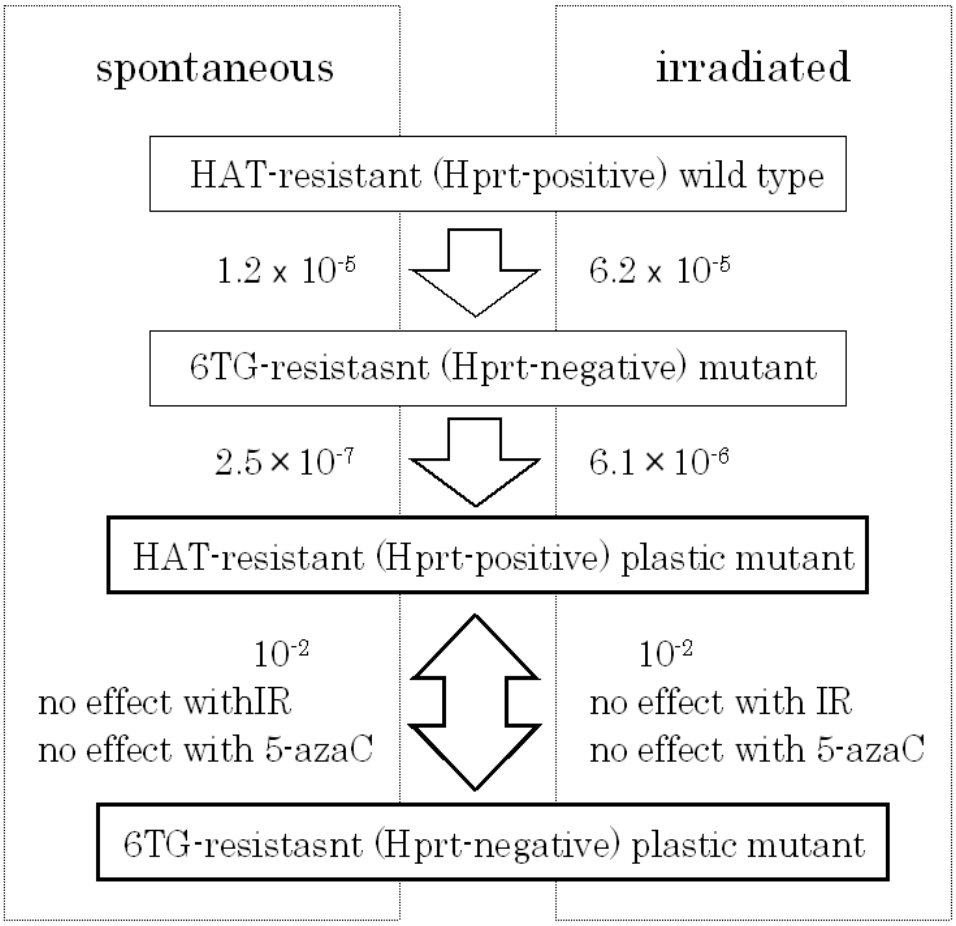

Figure 1. Characteristics of the plastic mutation. The frequencies leading to isolation of plastic mutants are given. Note the direction of the arrows, one directional or bi-directional. Hprt-positive means Hprt-proficient and Hprt-negative means Hprt-deficient.

type in the presence of 5-aza-cytidine at a frequency of approximately 10^{-2} on average, as shown in Figure 1.

4. Discussion

In this manuscript, we described the identification of a new class of genomic instability in cultured mouse FM3A cells, which shows plasticity in its mutant phenotype. The forward mutation from HAT^R wild-type to $6TG^R$ mutant phenotype occurred at a rate of 10^{-5} and the mutation frequency was increased approximately 5 times by irradiation with 5 Gy X-ray. These results are consistent with previous observations (*17-19*).

We then isolated the mutant clones that reverted from $6TG^R$ mutant phenotype to HAT^R wild-type phenotype. We first identified such plastic mutants only among the $6TG^R$ clones isolated from the irradiated cell populations; however, we eventually also succeeded in isolating the $6TG^R$ mutants from un-irradiated cell populations, showing the plasticity of their $6TG^R$ mutant phenotype.

The frequency of the plastic mutant in the spontaneous cell population was calculated to be 2.5×10^{-7}, whereas it was 6.1×10^{-6} in the irradiated cell population, as presented in Table 1 and Figure 1. Thus, the frequency of $6TG^R$ clones showing plasticity of the mutant phenotype was increased by X-ray irradiation approximately 24 times. In other words, IR induced the plasticity of gene regulation in mouse FM3A cells. Approximately 35% of the $6TG^R$ mutants without LOH derived from the irradiated cell population exhibited the plasticity in their mutant phenotype.

The reversion frequency from $6TG^R$ to HAT^R of each clone was approximately 10^{-2}. This frequency is almost equivalent to the one observed in the germline mutation of hyper-variable minisatellite repeats, such as Ms6-hm (*15*). However, the molecular mechanism involved in the hyper-variable repeat instability appears to differ from the plastic mutation observed here. Repeat instability through cell division has been explained by replication slippage. Additionally, instability of hyper-variable repeats has been observed in cultured cell lines at much lower frequencies, ranging from 10^{-5} to 10^{-8} (*20*).

The plastic mutation phenotype identified here appeared to be stable. Once the genomic instability was acquired by the cell lines, it was transmitted stably to the daughter cells for at least three months through more than 100 cell divisions (data not shown). Interestingly, additional radiation exposure of the

plastic mutant cells derived from both spontaneous and irradiated cell populations did not affect the frequency of phenotypic changes in either direction.

We speculated that these phenotypic changes could be attributable to the change in transcriptional level of the *Hprt* gene through DNA methylation. DNA methylation is one of the most common mechanisms involved in the regulation of gene transcription, especially in the gene suppression often observed in the inactivation of the X chromosome (*10*,*11*). As observed in this study, 5-aza-cytidine treatment did not affect the frequency of plastic mutations in either direction, implying that DNA methylation was not involved in the plasticity of the mutant phenotypes we examined. Histone modification may be another mechanism involved in the transcriptional regulation of these genes (*21*-*23*). However, histone modifications have been reported to be frequently associated with DNA methylation (*24*,*25*). A detailed examination of the methylation status at the promoter region of the *Hprt* gene may provide useful information for the understanding of the molecular mechanisms involved in the plasticity of the mutant phenotype identified in our study. An examination of the DNase I sensitivity of the *Hprt* gene promoter region (*26*,*27*) may also provide useful information for understanding the underlying molecular mechanisms.

In this report, we demonstrated the induction of plasticity in the regulation of Hprt activity by IR. Examination of this new type of genomic instability in other cell lines, as well as primary culture cells, may provide useful information on genomic plasticity. The plastic mutant phenotype identified in our study appeared to be a new type of genomic instability induced by IR. Involvement of this plastic mutation in the initial processes of radiation carcinogenesis, which is composed of multiple stages, would be of great concern in the age of advanced medical technologies using IR, assuming that the linear-non-threshold (LNT) model (*28*-*30*) could also be applicable for the induction of plastic mutation by IR. The LNT model basically says that there is no safe dose of radiation, and it is the current basis of radiation protection rules imposed by the United Nations Scientific Committee on the Effect of Atomic Radiation (UNSCEAR). We employed a relatively acute dosage of X-ray irradiation in the primary induction of the plastic mutation phenotype. The effect of irradiation dose, fractionated irradiation, and the type of radiation in the induction of the plastic mutation phenotype should be examined in future experiments.

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Case Report

Case report: Huge amoebic liver abscesses in both lobes

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Summary **We describe the case of a patient who returned to China from Africa and underwent emergency open surgical drainage with evacuation of 600 mL of anchovy sauce-like fluid from hepatic lesions. Computed tomography scans and surgical findings indicated abscesses in both hemilivers and communication between them. Bacteriological investigation of the fluid yielded negative results, but DNA assay of the pus detected 18S rRNA genes of *Entamoeba histolytica*. Serum anti-amoebic antibodies were detected using an indirect fluorescent-antibody test. Consequently, anti-amoebic drugs were administered and drainage was performed, leading to improvement in the patient's condition. As is evident from this case, an amoebic liver abscess in the left hepatic lobe is rare but treatable.**

Keywords: *Entamoeba histolytica*, amoebiasis, amoebic liver abscess

1. Introduction

Entamoeba histolytica is a causative agent of amoebic dysentery and extra-intestinal abscesses. It is prevalent in developing countries where its fecal-oral spread is difficult to control. *E. histolytica* is responsible for approximately 50 million cases of invasive amoebiasis annually with a mortality of 40,000 to 110,000 (*1*). Invasive amoebiasis is a major health problem worldwide and is second to malaria among protozoan causes of death (*2*).

The prevalence of *E. histolytica* infection in China has not been definitively ascertained. Recent data have revealed a higher seroprevalence of *E. histolytica* infection in HIV/AIDS patients in China (*3*) and approximately 0.7-2.7% of the Chinese population is reported to suffer from the amoebiasis (*4*). Liver abscesses are the most common non-enteric complication of amoebiasis. Presented here is a case of amoebic liver abscesses in both lobes in a patient with high fever and continuous abdominal pain.

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2. Case report

This case involved a 57-year-old Chinese man who served as a doctor for ten years in the Republic of Cote d'Ivoire. He had fever, anorexia, and dull and continuous epigastric pain. He had been hospitalized at a local clinic in Cote d'Ivoire for three weeks. He presented with chills, a temperature of up to 39°C, and epigastric pain upon hospitalization. The fever and abdominal pain persisted and edema and respiratory distress developed during the final ten days of treatment. The patient had no history of diarrhea or vomiting. At the local clinic, he was diagnosed with malaria and treated with empiric antimalarial and antityphoid drugs to no effect. He was then sent back to China and admitted to the hospital.

Upon examination, he was febrile (38.5°C) and presented with hepatomegaly and pitting edema. Ultrasonography of the abdomen revealed multiple hypoechoic lesions in both hemilivers. Computed tomography (CT) scans revealed these to be multiple lesions. Results indicated pleural effusion on both sides and two hypodense lesions in the liver, 9.9 × 9.5 × 10 cm on the right and 13 × 9 × 9 cm on the left (Figure 1). Whole blood analysis revealed a leukocyte count of 13,620/mm^3, mild normochromic normocytic anaemia (96 g/L), thrombocytosis (40,100/mm^3),

and high erythrocyte sedimentation rate (82 mm/h). The patient's renal function was normal. Data on the patient's liver function revealed slightly decreased liver function indicating hypoglycemia and hypoproteinemia. Liver biochemistry results were abnormal. The patient tested positive for hepatitis B surface antigen and anti-hepatitis B core IgG and anti-hepatitis B eAg antibodies. However, the patient tested negative for hepatitis B eAg and anti-hepatitis B surface antigen antibodies. PCR was performed to confirm the HBV viral load. The patient tested negative for anti-HIV and anti-hepatitis C virus antibodies. Sera tests for infection with *Schistosoma japonicum*, *Echinococcus granulosus*, and *Fasciola hepatica* were negative.

The patient was heterosexual with no history of intravenous drug abuse and was not an active smoker or drinker. He had no changes in toilet habits and no history of yellow fever and tuberculosis. He had malaria 12 years ago.

A serum indirect fluorescent-antibody test (IFA) for *E. histolytica* was performed (*5*). The patient's anti-*E. histolytica* antibody titer was 1:1,024 (Figure 2). Ornidazole and levofloxacin were not effective. Two weeks of subsequent treatment with chloroquine caused the patient's fever to go down. Pleural effusion and edema gradually decreased. However, abdominal pain still persisted. Open surgical drainage was performed. Two pigtail catheters were placed into the lesions, and 600 mL of thick anchovy sauce-like pus was drained from the lesions. The diagnosis of an amoebic liver abscess was confirmed by DNA assay by detecting 18S rRNA genes (*6*) (Figure 3). Histopathological examination of necrotic inflammatory exudates revealed multiple trophozoite-like cells of *E. histolytica* (Figure 4). After aspiration and pigtail catheter drainage of the abscesses, cultures of the pus were bacteriologically sterile. A CT examination 3 weeks after drainage revealed that the abscesses had decreased markedly in size (Figure 5). The pigtail catheters were removed and the patient was discharged.

3. Discussion

Hepatic amoebiasis is the most serious consequence

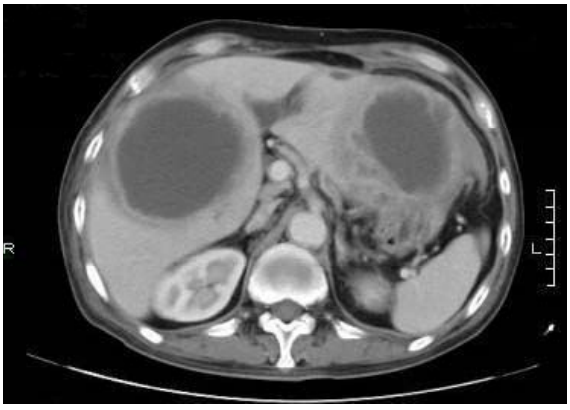

Figure 1. Abdominal computed tomography scan showing lesions of 9.9 × 9.5 × 10 cm in the right hemiliver and 13 × 9 × 9 cm in the left. Lesions were hypodense with rim enhancement.

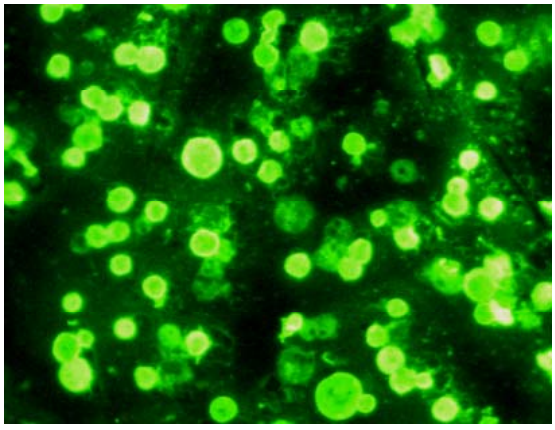

Figure 2. Detection of serum anti-*E. histolytica* antibodies using IFA. Original magnification: ×100.

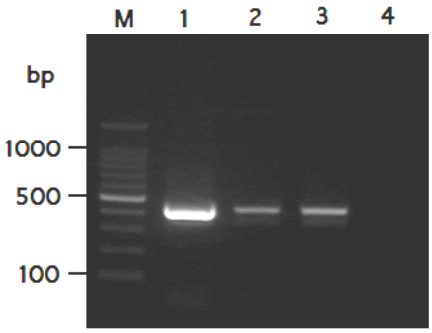

Figure 3. PCR amplification of 18S rRNA genes from liver pus DNA. The *E. histolytica* 18S primer was used. Templates are genomic DNA from *E. histolytica* HK9 (*lane* 1), liver pus from the patient (*lanes* 2 and 3), and a negative control (*lane* 4). M, DNA size marker (100 bp ladder).

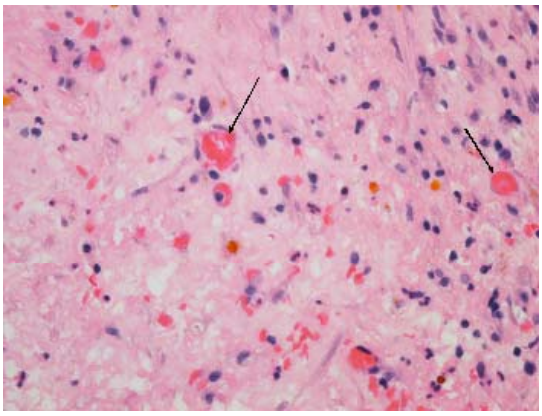

Figure 4. Hematoxylin/eosin-stained section from the patient's liver. Numerous trophozoite-like objects (*arrows*) are present in the peripheral region of the abscess.

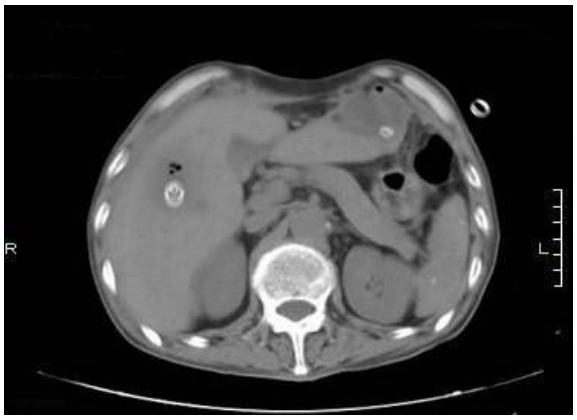

Figure 5. Abdominal computed tomography scan after surgery showing insertion of two catheters into the hepatic lesions.

of invasive amoebiasis since various complications associated with amoebic liver abscesses include rupture of the abscess into the pleural, pericardial, and peritoneal cavities and the bile ducts. The early detection of *E. histolytica* is crucial to reducing morbidity and mortality (*7*). The current patient lived in the Republic of Cote d'Ivoire for over ten years, which may be a major factor for infection with *E. histolytica* (*8*). Hepatic amoebiasis is a result of trophozoites entering mesenteric venules and traveling to the liver through the hepatoportal system. Amoebic liver abscesses are often seen in young men and more often involve the right hemiliver than the left.

Amoebic liver abscesses are difficult to distinguish from bacterial abscesses or other liver diseases. Although epidemiological information may indicate that the patient has come from an area where amoebiasis is endemic, acute onset of fever, abdominal pain, and hepatomegaly are common to both amoebic and bacterial abscesses. Ultrasonography, abdominal CT, and magnetic resonance imaging are not specific for the differentiation of an amoebic liver abscess from a pyogenic liver abscess, necrotic hepatoma, or echinococcal cyst. Helpful clues to an accurate diagnosis include the presence of epidemiologic risk factors for amoebiasis and the presence of serum anti-amoebic antibodies.

In general, nitroimidazoles, and metronidazole in particular, are the mainstay of therapy for invasive amoebiasis. Nitroimidazoles with longer half-lives (namely, tinidazole, secnidazole, and ornidazole) are better tolerated and cause fewer side effects, allowing shorter periods of treatment. That said, complicated amoebic abscesses may require drugs with drainage according to the principles for treatment of extraintestinal amoebiasis (*9*).

A review of the current case suggests that a primary diagnosis of amoebiasis would have led to prompt management of the condition with minimal morbidity. The combination of serological tests with target gene detection by PCR amplification of the parasite offers the best approach to diagnosis. Absence of diarrhea and parasites in the stool should not exclude the possibility of amoebiasis. Amoebiasis should be considered in patients from a population with a high prevalence of the condition should they present with a high fever and abdominal pain.

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