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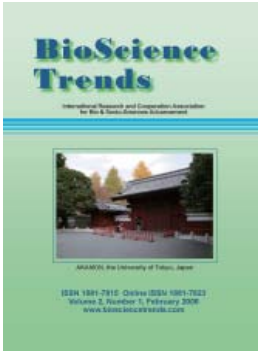

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Cover Photo of this issue

AKAMON, the University of Tokyo, Japan

The University of Tokyo is the highest educational institution in Japan. The Red Gate (赤門, Akamon) is the best known landmark of the university's Hongo campus. The gate was built in 1827 for Yoh-Hime, the 21st daughter of the 11th Tokugawa Shogun, Ienari, when she married Maeda family, one of the most famous feudal lords in the Edo period. The vivid vermilion of the gate recalls her youth and beauty. (*Photo by Hui Tang*)

News

Newly clarified target may shed light on the anti-viral treatment of hepatitis C

Yoshinori Inagaki[1,*], Huanli Xu[1, 2], Fengshan Wang[2]

Keywords: Hepatitis C, Anti-viral treatment, Japan, China

Viral hepatitis B and C are diseases known as ‘‘silent killers.’’ Compared to other types of viral hepatitis, these types frequently become chronic, and chronic hepatitis tends to progress to liver cirrhosis and hepatocellular carcinoma (HCC). Because the progression lacks symptoms, many patients are diagnosed with these advanced diseases along with viral hepatitis when they receive medical treatment. In particular, hepatitis C is likely to develop into HCC and there is no vaccine to prevent this infection, while hepatitis B can be prevented by vaccination. Thus, developing medical treatment for hepatitis C is crucial. Researchers have investigated hepatitis C to clarify its biological mechanism and to develop new targets for therapy, and one of the pioneers in such hepatitis C investigation is Prof. Kazuhiko Koike (*the University of Tokyo, Japan*).

In collaboration with a research group in Osaka University, Japan, Koike found that a kind of protein known as ‘‘proteasome activator PA28γ’’ is involved in the mechanism of the progression of viral hepatitis C to liver cancer. Their work suggested that this protein binds to hepatitis C virus (HCV) core protein and that this reaction is related to oncogenesis *in vivo*. The researchers hope their results will lead to the development of novel therapies for viral hepatitis C.

The research group focused on the HCV core protein. They previously found that degradation of HCV core protein depended on PA28γ inducing the progression of hepatitis C. To clarify the function of PA28γ *in vivo*, they recently created PA28γ knockout HCV core gene transgenic ($PA28\gamma^{-/-}$CoreTg) mice and analyzed this phenomenon using this animal model. They found that the accumulation of fat (steatosis) in the liver, which is known to be induced by HCV and enhance oncogenesis, was induced in $PA28\gamma^{+/+}$CoreTg mice but not in other genotypes including $PA28\gamma^{-/-}$CoreTg mice. HCC also developed only in $PA28\gamma^{+/+}$CoreTg mice but not in $PA28\gamma^{-/-}$CoreTg mice. Furthermore, analyses of gene expression in these genetically-modified mice showed that the transcription of some genes related to lipid biosynthesis in the liver was up-regulated in $PA28\gamma^{+/+}$CoreTg mice in comparison to $PA28\gamma^{-/-}$CoreTg mice and other genotypes. The molecular mechanism of activation of lipid biosynthesis in $PA28\gamma^{+/+}$CoreTg mice was also found to depend on the existence of PA28γ. Results indicated that the up-regulation of lipid biosynthesis in the liver requires the existence of both HCV core protein and PA28γ and that these phenomena might lead to the pathogenesis of steatosis and the development of HCC. Thus, PA28γ may have a crucial role in the progression of hepatitis C and may represent a strong candidate for development of new anti-viral treatments (*Moriishi K et al. Proc Natl Acad Sci USA, 2007; 104:1661-1666.*).

The number of viral hepatitis patients is increasing worldwide. This situation is particularly severe in eastern Asian countries such as Japan and China. In Japan, approximately 70 to 80% of HCC is caused by hepatitis C, and there may be many HCV carriers who are unaware of their situation, especially among the elderly, because they were injected with shared needles during childhood. According to reports in the forum of prevention and treatment for hepatitis C in China (*November 16, 2007; Beijing, China*), the number of hepatitis C patients in China also rapidly increased in 2007 in addition to the continuous spread of hepatitis B, and concerns are that this trend will continue. Combination therapy with pegylated interferon α and ribavirin has been developed as a therapy for chronic hepatitis C. However, estimates are that approximately 70% of hepatitis C patients in Japan are infected with HCV genotype Ib, which research suggests is refractory to interferon therapy. Bio-science researchers must scientifically elucidate hepatitis C and develop an effective medical treatment for this disease.

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News

Work engagement: An emerging concept in occupational health psychology

Akihito Shimazu[1], Wilmar B. Schaufeli[2]

Keywords: Occupational health psychology, Work engagement

Occupational Health Psychology concerns the application of psychology to improving the quality of work life and to protecting and promoting the safety, health, and well-being of workers. Contrary to what its name suggests, Occupational Health Psychology has almost exclusively dealt with ill health and poor well-being. For instance, a simple count reveals that about 95% of all articles that have been published so far in the leading Journal of Occupational Health Psychology have dealt with negative aspects of workers' health and well-being, such as cardiovascular disease, repetitive strain injury, and burnout. In contrast, only about 5% of the articles have dealt with positive aspects such as job satisfaction, commitment, and motivation.

However, times appear to be changing. Since the beginning of this century, more attention has been paid to what has been coined positive psychology: the scientific study of human strength and optimal functioning. This approach is considered to supplement the traditional focus of psychology on psychopathology, disease, illness, disturbance, and malfunctioning. The emergence of positive (organizational) psychology has naturally led to the increasing popularity of positive aspects of health and well-being in Occupational Health Psychology. One of these positive aspects is work engagement, which is considered to be the antithesis of burnout.

While burnout is usually defined as a syndrome of exhaustion, cynicism, and reduced professional efficacy, engagement is defined as a positive, fulfilling, work-related state of mind that is characterized by vigor, dedication, and absorption. Engaged employees have a sense of energetic and effective connection with their work activities. Since this new concept was proposed by Wilmar Schaufeli (*Utrecht University, the Netherlands*) in 2001, 93 academic articles mainly focusing on the measurement of work engagement and its possible antecedents and consequences have been published (see *www.schaufeli.com*). In addition, major international academic conferences organized by the International Commission on Occupational Health (ICOH) and the European Association of Work and Organizational Psychology (EAWOP) include symposiums and workshops on work engagement, as does the APA-NIOSH interdisciplinary conference on work, stress, and health.

Although work engagement is currently a buzzword in consultancy, its academic pedigree is still rather limited. Thus, the international journal "Work & Stress" is now publishing a special issue on work engagement with guest editors including Arnold Bakker (*Erasmus University Rotterdam, The Netherlands*), Wilmar Schaufeli, Michael Leiter, (*Acadia University, Wolfville, Canada*), and Toon Taris (*Radboud University Nijmegen, The Netherlands*). The issue called for papers that dealt with the following questions in particular:

1. Which personal and organizational resources contribute to work engagement?
2. Do engaged employees have better performance than unengaged employees?
3. Is work engagement a risk factor for burnout?
4. How does work engagement vary from day to day?
5. Does work engagement exhibited by leaders continue on to team members?
6. What is the difference between work engagement and workaholism?
7. Are there cultural differences regarding work engagement?

Because work engagement is an emerging concept, these questions and more must be resolved in future research. For instance, is there a relationship between engagement and biological factors such as gene, immune, and endocrine functions? What is intervention strategy is appropriate at improving engagement? To answer these questions and to improve workers' well-being, interdisciplinary and international collaboration are needed.

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News

Bangladesh: Surveying the post-Sidr situation

Sadequa Shahrook, Chushi Kuroiwa*

Keywords: Bangladesh, Cyclone Sidr, The poorest of the poor, Japan-Bangladesh Cultural Foundation

In November 2007, Cyclone Sidr (category 4) struck the south and south-western coastal areas of Bangladesh. In the span of mere moments, the 4.57M tidal wave washed away thousands of lives, paddy fields and millions of houses. As usual, the most affected were the poorest of the poor. The devastation was extensive and will take a long time to tackle. About 2.6 million Bangladeshis across nine districts are still in need of emergency assistance. The estimate for damaged livestock could reach at least 1.25 million, and the area of damaged crop land is 810,000 hectares. At this stage, loans for rehabilitation and fertilizers for the farmers are crucial.

According to the UN, food, shelter and cash are the top priorities at this time, along with better sanitation, drinking water, prevention of water-borne diseases and electricity and livelihood assistance. The government is prioritizing the obtainment of budget support for food imports, rebuilding houses and other long-term needs.

Many international and national aid agencies have extended helping hands in support of the victims. Although Bangladesh's chief adviser claims that the situation is under control and that disaster management has been tackled successfully, field-level reports and data yield contradictory statements. The Japanese Government has already declared itself united with Bangladesh in efforts to tackle the catastrophe by offering assurances of assistance in rebuilding houses in some of the worst-affected areas. Recently, the Japan-based NPO Japan-Bangladesh Cultural Foundation (JBCF) also distributed relief materials in Sidr-affected areas. JBCF distributed rice to the affected people in the Mongla seaport and monetary aid to the schools in the Shoronkhola village of Bagerhat, the region that suffered the brunt of the impact. Millions of dollars have been donated from different countries, which is being handled primarily by the Army, the TNO (Thana Nirbahi Officers), and local leaders. While the army is allocating funds properly, JBCF and other activist organizations have reported, on the basis of field observations, that mishandling in fund allocation exists on the part of other groups. The rehabilitation progress is sluggish as well. Given the prevailing circumstances, the distribution of a handsome sum to each affected family seems more practical than awarding just small incentives.

Figure 1. The area of the poorest of the poor affected by Cyclone Sidr.

Sidr caused much harm in various ways, *e.g.*:

1. Due to this cyclone, the sea temperature in the Bay of Bengal has changed, triggering a decline in the fish population and concomitant damage to the economy.
2. It washed away many trees, endangering the forest resources and, in the long run, the economy.
3. The dams are broken. Hence, if disaster strikes again (a yearly routine during the monsoon season), the situation will be yet more dire.
4. The sea water has been mixed with the ground water, resulting in a lack of drinking water.

The government already exhibited a distinct lack of foresight in refraining from importing foods in the wake of the last flood, and in its failure to curb the price-hiking of daily essentials. Moreover, the government and aid agencies have been criticized for not delivering funds to the worst-affected areas. Therefore, careful and more unified and harmonized political movements are now necessary for the effective management of disaster relief and economic reforms.

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Policy Forum

The reality of health information systems: Challenges for standardization

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Summary **Health information systems (HISs) serve as an indispensable foundation for developing health policy and strategies and improving delivery of routine health services in an evidence-based manner. In developing countries, HISs are not adequately functioning in spite of their important role such as monitoring tools for the progress of the Millennium Development Goals. This paper attempts to classify the HISs into four types according to their data sources. Information requirement by the diseases-specific funding partnerships (*e.g.* Global Fund to Fight AIDS, Tuberculosis and Malaria) and projects implemented by development agencies increase the workloads of health professionals at facility level and subsequently compromise data quality. For the data quality assurance and comparability of data across countries overtime of major health indicators, standardization of HISs is the urgent task.**

Keywords: Health information system, Health system, Developing countries

Introduction

To achieve better health status in developing countries, vertical and horizontal types of interventions have been tested and practically employed (*1*). A series of applications of both types provide us with the insight that a vertical national program is more effective in reducing the prevalence and burden of specific infectious diseases (*e.g.* HIV/AIDS, tuberculosis, and poliomyelitis) (*2*,*3*). Therefore, tremendous amounts of funds have been made available to target those diseases, *i.e.* Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), Global Alliance for Vaccines and Immunization (GAVI), and President's Emergency Plan for AIDS Relief (PEPFAR). However, noted that two previous studies reported that largely funded mass vaccination campaigns for poliomyelitis eradication and measles elimination, a typical vertical approach, could damage the health system of routine immunization (*4*,*5*). When addressing other diseases and the issues related to preventive health services, a horizontal or comprehensive approach is likely to be more effective and sustainable (*2*). Therefore, the Primary Health Care has been relevant, as a minimum package of community-centered horizontal health system, since the Alma-Ata declaration in 1978. Yet, it is reality that the global debate is continuously taking place on which of two types is more effective and efficient for addressing respective health-related issues (*1*,*6*).

Given these lessons learned, health systems are currently required to play an increasingly important role by combining vertical and horizontal approaches in a complementary manner. For this reason, major development agencies have recently been emphasizing the importance of health systems and raising it as a crucial agenda (*7*). In 2000, WHO evaluated the health systems performance of all the member states (*8*) and publication of the results of its ranking brought about a series of technical and even political debates (*9-14*). This argument, however, rather fostered the foundation for mainstreaming health systems as a global agenda. For instance, World Bank employed health systems as

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one of the top priorities in its development assistance strategies in 2007 (*15*). WHO keeps health systems prioritized and further has innovated a new concept and model of health systems in 2007 (*7*). Note that, also, there is the global consensus that strengthening health systems is a crucial step for achieving Millennium Development Goals (MDGs) by 2015 (*16*) .

Health information systems (HISs) should serve as a core foundation which enables health planners and health administrators to make reasonable and accountable decisions in an evidence-based manner for building better health systems (*17*), as well as it is one of the components of the health systems, per se. However, it is highly questionable whether we have built consensus and are sharing common understandings and perception on HISs. This is most probably due to lack of recognition of the importance of HISs (*18*) and thereby lack of needs for clear definition and classification of HISs. This paper attempts to suggest one way of classifying HISs and further highlights the major challenges we are encountering.

Raison D'etre of HISs

The *raison d'etre* of HISs, particularly in context of developing countries, is summarized into three dimensions.

First, HISs are essential for accurate monitoring of the progress towards the MDGs by 2015 (*19*). Of a total of eight MDGs, three are directly health-related, *i.e.* (i) reduce by two thirds the under-five mortality rate; (ii) reduce by three quarters the maternal mortality ratio; and (iii) halt and begin to reverse the spread of HIV/AIDS and reverse the incidence of malaria and other major diseases (*20*). Ideally, these basic health and demographic indicators should be measured through the national HISs operated by governments. However, the governments of many developing countries are not capable enough to undertake data collection and analysis on a sustainable basis. It is reality that they are dependent on the surveys conducted with external assistance, *e.g.* the Demographic and Health Survey (DHS) which have been conducted in over 75 developing countries through support from the United States Agency for International Development (*21*). Needless to say, developing countries receive tremendous assistance from developed countries also in health interventions in parallel. Even though we admit the needs for assistance in achieving the MDGs, data collection and analysis for monitoring the progress towards the MDGs should, ideally or in the long run if not, be undertaken by the governments of developing countries. Therefore, the governments are currently required to establish reliable HISs and equip themselves with operational capacity of HISs.

Second, HISs are expected to play a core role in performance-based disbursement of donor-pooled funds (*i.e.* common basket funds) which a ministry of health (MOH) in principle manages by their initiative (*19*). In 1970s-1990s, a number of health projects were implemented with little coordination in a fragmented manner. As a result, a number of inefficient development-agency-driven implementations have been identified and criticized. Also, the workloads of health administrators and health practitioners in many developing countries had significantly increased. To address these issues, common basket funds have been created as one of the solutions in many developing countries since late 1990s. In other words, development agencies have rapidly shifted their primary roles from implementation of the projects to monitoring and evaluation of the projects being implemented by the MOH. In order for a MOH to justify the use of common basket funds and ensure smoother project implementation, performance-based disbursement of the common basket funds is required by development agencies who contribute to the funds. Under these circumstances, HISs are essential sources of information for gauging the performance.

Third, HISs are an indispensable foundation for continuous improvement of health services. A health management information system (HMIS), one of the representative HISs, collects data of daily operation of health facilities (*e.g.* the number of outpatients, type of health problems diagnosed, bed occupancy rate, drug stock, and users fee account). The information should flow in smaller cycle, *i.e.* (i) health facility to collect data and submit them to district health administration; (ii) district health administration to compile and analyze the data; (iii) district health administration to utilize the results of analysis for solving health facilities' operational problems to improve quality of health services; and (iv) provincial health administration office and MOH headquarters to analyze and utilize the data for developing provincial and national health policies and strategies by referring to the database from district health administration (Figure 1b). This is precisely in line with on-going decentralization and devolution process in many developing countries (*22*). In those countries, district health administration offices are required to prepare budget proposals and submit them not to provincial and MOH headquarters but to district councils or city offices. Then, budget competition with other sectors (*e.g.* public works, agriculture, and education) takes place at district level. In order for the health budget proposals to be justified and approved at district parliaments, they need to be prepared in an evidence-based manner by maximizing well-functioning HISs. This will also contribute to the increase in transparency and accountability of local governance in developing countries. Note that it is reality that the HMIS in many developing countries was or is still utilized primarily for preparation of the MOH annual report at central level without appropriate and

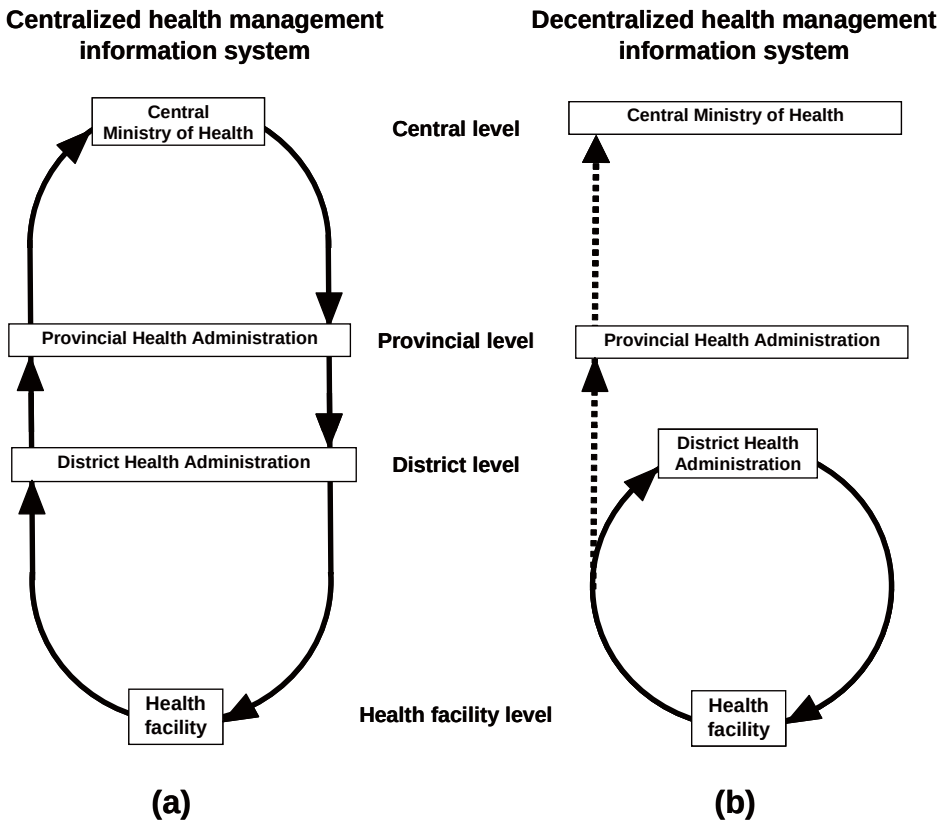


Figure 1. Two types of information flow in health management information system.

timely feedback to health facilities and district health administration offices. The information flow of this type of centralized HMIS created larger information cycle which is unable to provide appropriate and timely feedback to lower levels for maintaining and improving the quality of health services (Figure 1a). Clear shifting from centralized HMIS model (Figure 1a) to decentralized one (Figure 1b) will be conducive to quicker realization of delivery of better quality of health services and higher ownership of the data at district level.

Type of HISs

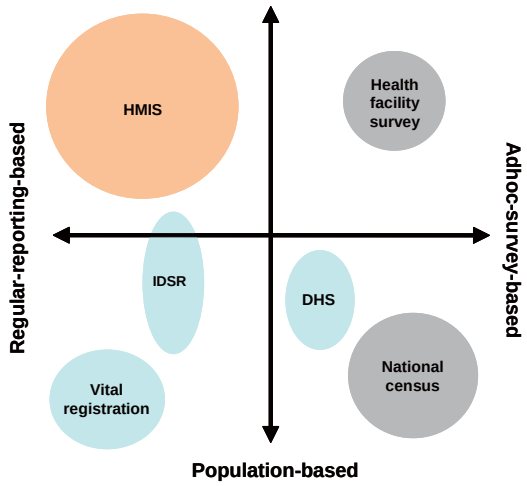
HISs are meant to be literally a package of various HISs and cannot be integrated into the single system. This is because several HISs employ the same indicators but their figures can be significantly different due to difference in the measurement methods. For instance, incidence of diarrhea at health centers (*e.g.* proportion of patients diagnosed as diarrhea cases to total number of patients) should differ from that at community level (*e.g.* proportion of persons with diarrhea to total number of residents in the community). Thus, facility-based and population-based incidences of diarrhea need to be independently measured and utilized for different purposes. Facility-based incidence of diarrhea should serve as the evidence for the measures for improving curative services (*e.g.* increase in availability of oral rehydration salt at health centers), while population-based one should serve as the evidence for the measures for improving preventive services (*e.g.* environmental and behavioral interventions for diarrhea prevention in communities).

Thus, clear definition and classification of each HIS type should be readily available and commonly understood. We suggest that HISs be defined and classified into four categories as shown in Table 1. Figure 2 shows the characteristics of HISs according to their data collection approaches, *i.e.* (i) data collection regularity; and (ii) data source. Figure 3 shows the characteristics of HISs according to their data utility, *i.e.* (i) the level of primary data user(s), and (ii) objectives of data utilization. There should be several other ways of classifying HISs. One of the examples is a classification of HISs by the length of period during and after which the data are most frequently and effectively utilized, *i.e.* (i) HISs for long-term policy and strategies at national level; (ii) HISs for long-term or medium-term capital investment plan; (iii) HISs for short-term annual resource allocation planning; and (iv) HISs for quick or timely response to disasters and disease outbreaks.

Thus, HISs are again a package of various HISs which should coexist in a complimentary manner. The MOH of each developing country should strategically prioritize which HISs must most urgently be improved

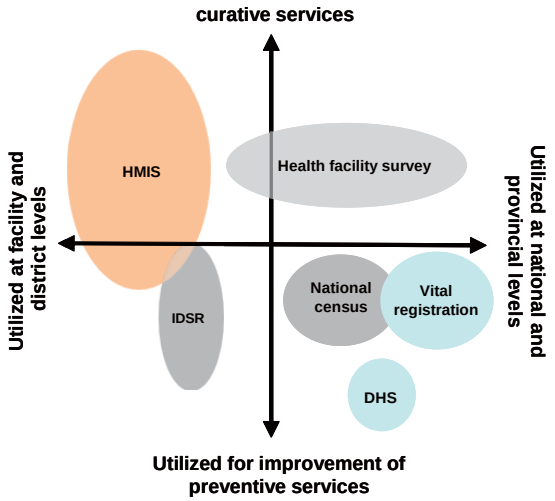
Table 1. Type of health information systems

	Definition of HIS	Examples
Type I	HISs which are designed to improve and maintain smooth and sustainable operation of curative and preventive health services at health facilities such as health centers and district hospitals	■ Integrated Disease Surveillance and Response (IDSR) ■ Disease Early Warning System (DEWS)
Type II	HISs which are designed to measure the locations and quantities of health resources (facility building, health professionals, medical equipment, *etc.*)	■ Health facility survey ■ Health workforce survey
Type III	HISs which are designed to estimate birth and mortality rates using population-based datasets	■ Vital registration ■ Demographic and Health Survey (DHS) ■ Multi-Indicators Cluster Survey (MICS) ■ World Health Survey (WHS)
Type IV	HISs which are designed to propose immediate necessary measure to be taken to address specific disease (particularly, to cope with an outbreak of an infectious disease)	■ Integrated Disease Surveillance and Response (IDSR) ■ Disease Early Warning System (DEWS)



[Note] HMIS: Health Management Information System
IDSR: Integrated Disease Surveillance and Response
DHS: Demographic and Health Survey

Figure 2. Data collection characteristics of respective HISs.



[Note] HMIS: Health Management Information System
IDSR: Integrated Disease Surveillance and Response
DHS: Demographic and Health Survey

Figure 3. Data utilization characteristics of respective HISs.

and adjusted in order to meet their data requirement, given the national health policy and strategies.

Challenges for Standardization of HISs

The most significant challenge on HISs is to, in principle, integrate and standardize the same or similar HISs at health facility and district health administration level into one (micro-level standardization). Some development agencies and NGOs still tend to develop and introduce a HIS exclusively for the purpose of monitoring and evaluation of their projects. After a project is completed, its HIS is expected to be kept functioning and its reporting forms and database remain at health facilities and district health administration offices. Yet, the same information (*e.g.* number of diarrhea cases among children under five years of age) often needs to be recorded in the existing MOH's HIS reporting forms as well as a newly introduced project-specific HIS reporting forms. As a result, the workloads of health workers significantly increase. This situation is likely to compromise the quality of both data and health services. Thus, it is suggested that development agencies and NGOs employ the existing HISs in the country as the major sources of information for monitoring and evaluation without building up a new HIS exclusively for their projects. If the existing HISs do not include indicators the project requires, development agencies ideally should either select any proxy indicators from the existing HISs or request the MOH to add some key indicators to the existing HISs. However, note that it might be necessary for a project aiming at piloting an intervention model to tentatively set up an additional HIS specific to the project.

Another major challenge on HISs is to harmonize and standardize the HIS overall framework at global level (macro-level standardization). As earlier described, a number of global health partnerships are playing an increasingly important and dominant role in combating major diseases in developing

countries (*e.g.* GFATM, GAVI, and PEPFAR). For accountability and result-orientation reasons, these disease-specific partnerships tend to require beneficiary countries to report the progress of interventions they are financing by employing specific recommended indicators. However, the efforts have been made by the partnerships to promote the employment of indicators available in existing HIS in each country. Despite their flexibility on choice of indicators, the MOH of developing countries tends to create or maintain ad-hoc disease specific HISs in little coordination with existing routine national HISs, in order to attract the partnerships by increasing more indicators in the reports. This situation often leads the MOH to manage two major HIS channels (*i.e.* integrated routine HIS and ad-hoc disease-specific HIS channels) and subsequently causes the compromise in data accuracy and the significant loss of opportunity costs spent by health practitioners and health administrators. Moreover, there could be different figures for the same indicators if there are any different measurement methods and timing between the two HIS channels (*23*). In order to avoid unnecessary confusion that stems from several HIS channels being functioning in parallel, it is essential to develop the globally standardized HIS framework and agree on it among those major disease-specific funding partnerships and other stakeholders. Note that, also, this helps to ensure comparability of data across the countries over time.

Health Metrics Network, being hosted in WHO headquarters, is ardently addressing these issues related to HISs (*24*). To accelerate the process, more serious international attention should be drawn. It is the global urgent task that harmonization and standardization of HISs in developing countries are undertaken and completed.

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Brief Report

Risk factors for injury in Pakistani children

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Summary **Over 1 million fatal child injuries occur per year, the bulk of them in less-developed countries. There remains a need, from country to country, to identify personal and environmental risk factors correlated with this mortality. The present study focused on Pakistan, home to a sizeable pediatric population of 60 million. The study employed case-control methods (i) to identify situational risk factors and (ii) to test when children were most at risk, and whether these patterns differed for preschool versus school children. For two months the families of 300 consecutive inpatients were interviewed at Children's Hospital in Islamabad. Most children (79%) were unsupervised at the time of the injury. The case-control study found risk factors in the mother's level of education, size of the home, and number of children in the home (all $p < 0.05$). With respect to temporal patterns, the time period of greatest risk was 3-6 PM. Compared to preschoolers, school children showed elevated risk on weekends (odds ratio 4.0, $p < 0.001$) and reduced risk during school (odds ratio 0.2, $p < 0.0001$); in contrast, the risk of injury for preschool children remained constant throughout the week. The results support the conclusion that overall, poor supervision, domestic crowding, and low maternal education were risk factors for injury in our sample of Pakistani children. Based on this conclusion, we recommend further efforts to keep children off roofs, isolate them from hazards, promote supervision, educate parents, and provide safer play.**

Keywords: Pediatric, Trauma, Case-control study, Pakistan

Introduction

Worldwide, over 1 million children die from injuries annually (*1,2*). This mortality is compounded by considerable morbidity, disability, and economic cost (*1-5*).

Of the 1 million fatalities per year, the bulk occurs in the developing world (*2*). There remains a need to identify personal and environmental risk factors that correlate with this mortality. We wished to lay a foundation for this work in Pakistan, where studies have already examined injuries in adult and general populations (*6-10*), but not specifically in pediatric populations.

We conducted a case-control study of children at one of Pakistan's leading public children's hospitals. Our objectives were (i) to identify situational risk factors for child injury and (ii) to test when, in the context of their schedules, children were more likely to be injured, and whether these patterns differed for preschool versus school children.

Methods

The study enrolled 150 consecutive cases and 150 consecutive controls at Children's Hospital of the Pakistan Institute of Medical Sciences (PIMS), Islamabad. This hospital is the principal public children's hospital in the capital of Pakistan, and was chosen as the one of the best available sites in Islamabad to obtain a representative cross-section of the Pakistani pediatric population. PIMS serves a complex catchment area in northern Pakistan, where several other hospitals overlap. The hospital includes both public and private inpatient wards and serves people from all points on the socioeconomic spectrum. We interviewed eligible patients for 60 consecutive days in

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the months of November and December.

Cases were children up to 12 years old admitted to PIMS due to an injury. Injury was defined prospectively as any fracture, burn, foreign body, internal injury, blunt injury, crush, laceration, penetration, animal attack, poisoning, or drowning. Controls were children up to 12 years old admitted for any other reason. Data from the control group was used for other research, but not in a way that duplicates any of the work described in this paper.

The study was carried out under a protocol approved by PIMS and the Human Investigations Committee (HIC) at the Yale University School of Medicine (USA). The parent or guardian of each subject was approached, and informed consent was obtained. The parent or guardian received an information sheet describing the purpose of the study, the subject's rights, and contact information for the investigators. The investigator collected all information verbally from the family.

SAS software was used for all statistical analysis (SAS, Cary, North Carolina, USA), which included chi-square analysis and logistic regression with a forward conditional model using as variables age, sex, family income, maternal education, number of rooms in home, number of children in home, and use of tent as a home.

For analysis of time-related risk, we surveyed children on their typical daily schedule and then divided the schedule as follows: "Sleep Time," Monday through Sunday 22:00-06:00; "Before School," Monday through Saturday 06:00-08:00; "School Time," Monday through Thursday and Saturday 08:00-14:00, Friday 08:00-12:00; "After School," Monday through Thursday 14:00-22:00; "Friday PM" (when students are dismissed from school early for prayers), 12:00-22:00, and "Holiday Time," Saturday 14:00-22:00, Sunday 06:00-22:00, and national holidays 06:00-22:00.

From the schedule we developed a model to predict the distribution of injuries expected by chance. The model made two assumptions: (i) no injuries occur during "Sleep Time" and (ii) injuries occur with equal frequency during all other periods. The proportion of injuries expected for each period was calculated as the proportion of hours occupied by each period, with "Sleep Time" excluded.

Results and Discussion

Of 190 cases who presented, 39 did not qualify because a parent could not be reached for consent, and one other family chose not to participate. Of the 150 cases recruited, 59% percent had suffered a fall, 16% a road collision, 13% a burn, and 12% other (foreign body ingestion or aspiration, animal attack, toxic ingestion, assault). Overall, only 21% of children were accompanied by an adult when the injury occurred. For cases, 36% were from Islamabad, 36% from Rawalpindi, 15% from other parts of Punjab Province, 6% from Kashmir, and 6% from the Northern Areas and NWFP (5% were Afghan refugees).

Of 150 controls whose families were invited to participate, all provided their consent. Recorded reasons for admission, from more common to less common, included pneumonia, gastroenteritis, tonsillitis, pharyngitis, cleft palate, club foot deformity, meningitis, sepsis, Hirschsprung's disease, asthma, hydrocephalus, renal failure, malnutrition, thalassemia, and neural tube defect. For controls, 28% were from Islamabad, 27% from Rawalpindi, 29% from other parts of Punjab Province, 6% from Kashmir, 10% from the Northern Areas and NWFP, and 1% from Afghanistan (3% were Afghan refugees).

The 88 children with a history of fall presented with head injury or serious fractures. Only 15% were accompanied by an adult when they fell (below the overall mean supervision rate of 21%). Most (70%) fell from a rooftop, typically at home from an unprotected roof. Only 12% of roofs were protected by rails or walls. Kite flying and cricket were common activities. The male:female ratio was 71:29, similar to the overall ratio for cases and controls. Half of the children were under 5 years old.

Most of the 24 children with a history of road collision were admitted due to head injury or complicated fractures. The male:female ratio of 71:29 was similar to the overall mean. Of the 20 children admitted with burns, 32% were accompanied by an adult when the burn occurred (above the overall mean). Many burns occurred near dinner time: 60% between 3 and 9 pm; 35% between 5 and 7 pm. In 85% of cases the burn source was at ground level. Burn sources included stoves and cooking fires (60%), tea or hot water (30%), and outdoor fires (10%). In contrast to other types of injury, the male:female ratio was 50:50. Half of the children were 2 years or under.

Table 1 compares cases and controls for different descriptors. Cases tended to be older than controls; however, logistic regression and pairwise linear correlations showed that age did not cause an interaction effect with any of the other variables. Case mothers reported significantly less education than control mothers ($p = 0.016$). Cases reported significantly more children in the home ($p = 0.047$). They also reported significantly fewer rooms in the home ($p = 0.023$). Cases were more likely to live in tents than controls: 6% for cases and 1% for controls ($p = 0.023$).

When we analyzed the results by logistic regression (by a number of forward conditional models), results compared favorably with the chi-square analyses reported above: most notably, maternal education, rooms in home, and type of shelter emerged as significant independent factors (all $p < 0.05$). The number of children in the home, however, did not emerge as an independent variable.

A child's risk depended on the time of day. For

Table 1. Descriptors for cases versus controls

Factor	Cases (n = 150) Number	%	Controls (n = 150) Number	%	Odds ratio	p value
SEX						
Male	100	67	101	67	1.00	
Female	50	33	49	33	1.00	0.902
AGE (years)						
Under 2	21	14	89	59	0.24	
2-5	63	42	32	21	2.00	
Over 5	66	44	29	19	2.32	< 0.001
INCOME (Rp. per month)						
Up to 5,000	92	61	117	78	0.78	
Over 5,000	38	25	26	17	1.47	0.045
Not reported	20	13	7	5		
MATERNAL EDUCAT.						
No school	79	53	67	45	1.18	
1-8 years	32	21	32	21	1.00	
> 8 years	25	17	49	33	0.52	0.016
Not reported	14	9	2	1		
CHILDREN IN HOME						
1-2	37	25	58	39	0.64	
3-4	52	35	49	33	1.06	
> 4	54	36	42	28	1.29	0.047
Not reported	7	5	1	1		
ROOMS IN HOME						
1-3	112	75	99	66	1.14	
> 3	31	21	50	33	0.64	0.023
Not reported	7	5	1	1		
TYPE OF SHELTER						
Tent	9	6	2	1	6.00	
House	134	89	144	96	0.93	0.023
Not reported	7	5	4	3		

Table 2. Injury risk in the context of children's daily routine

Time period	Odds Ratios (*95% CI*), p value		
	ST *vs*. PS[a]	PS *vs*. Model	ST *vs*. Model
Before school	2.1 (0.4 - 11.4), $p = 0.538$	0.4	0.7
School	0.1 (0.03 - 0.4), $p < 0.0001$	1.2	0.2
After school	0.9 (0.5 - 1.6), $p = 0.901$	1.0	0.9
Friday PM	0.7 (0.2 - 2.7), $p = 0.739$	1.0	0.7
Holiday	4.0 (1.8 - 9.0), $p < 0.001$	1.0	2.5

[a] ST = Students; PS = Preschoolers.

both preschoolers and students, the frequency was low between 9 PM and 6 AM. Frequency rose during the day, peaked between 3 PM and 6 PM, then declined after 6 PM for preschoolers and after 9 PM for students.

The risk of injury further depended on the day of week, and whether the child attended school. Table 2 shows the odds ratios for comparisons of students, preschoolers, and the model. "Holiday Time" was a critical period of risk for students compared to preschoolers, with an odds ratio of 4.0 ($p < 0.001$). On the other hand, "School Time" was a period of pronounced safety, with an odds ratio of 0.1 ($p < 0.0001$). The same patterns emerged when students were compared to the model, with odds ratios of 2.5 for "Holiday Time" and 0.2 for "School Time." Comparison of preschoolers with the model showed odds ratios of 1.0-1.2 in four of five time periods. This implies that the model successfully predicted the distribution of preschooler injury.

From the results one can deduce three overall risk factors: (i) lack of adult supervision, as demonstrated by low rates of adult supervision in the case sample and by elevated odds ratios for times when students were outside of school; (ii) crowded homes, as demonstrated by elevated odds ratios for children with relatively more siblings or relatively smaller homes; and (iii) poor maternal education, as demonstrated by higher odds ratios for children whose mothers had little or no schooling. The value of these results is that they provide clues for further research to identify children at risk.

Poor supervision is a recurrent theme. Only 21% of the children in our sample were supervised when the injury occurred. Weekends and holidays were associated with the most risk of injury for students, with an odds ratio of 4.0 ($p < 0.001$) compared to preschoolers. Students were relatively safe at school with an odds ratio of 0.1 ($p < 0.0001$) compared to preschoolers. In contrast, preschoolers did not vary in

injury frequency, with odds ratios of ~1 for most time periods. These results provide multiple convergent lines of evidence for the importance of adult supervision. More specifically, the results show that more vigilance is needed on weekends, holidays, and afternoons, and they imply that the risk of injury can be reduced by improving access to school.

Low maternal education was a risk factor in chi-square analysis ($p = 0.016$). Logistic regression showed that this effect was present even when controlled for income. We postulate that less educated mothers, particularly those who cannot read, are not as empowered to recognize and promote healthy behaviors in their children.

Crowded homes, expressed in our results by the number of rooms and number of children in the home, also differed between cases and controls ($p = 0.023$ and $p = 0.047$, respectively). We postulate that crowded homes: (i) made supervision more difficult and (ii) displaced children to more perilous play spaces such as roadsides.

Worldwide, boys show higher overall injury rates than girls (*2*). We found similar results in the present study, with 67% males and 33% females. For burns, however, the distribution was 50% and 50% (see above). A result we did not anticipate was that the control group also consisted of 67% males and 33% females. These values differ dramatically from the nearly equal numbers of boys and girls found in the Pakistan 1998 census (*11*). The literature has established that some families in Pakistan are more likely to seek medical care for their male children as compared to their female children (*12-14*). While the results of this study are provocative in this respect, the study was not designed to test whether a child's gender influences the parents' health-seeking decisions. Carefully designed household-based studies will be needed to test this hypothesis and understand to what extent such gender biases still exist.

Pakistan is a large and remarkably diverse nation. The present study only represents a small portion of Pakistan's geographic, cultural, and economic diversity. Further work is needed to test whether these results generalize to other regions. Another potential limitation to the results is that we collected data over the months of November and December. While many types of injury occur year-round, we postulate that other types, such as drownings and snake bites, are likely to be less common in months characterized by colder weather.

We limited our study to children admitted to the hospital. Our sample may not generalize to children who die outside of the hospital or those who are treated in the emergency department and released. This consideration limits our ability to generalize to minor injuries, or very serious ones such as high-speed motor vehicle collisions.

As commonly occurs in case-control studies, not all differences seen are likely to be causally related to injury. Cases were older than controls ($p < 0.001$), a difference which is likely to be an artifact of our control group. As mentioned above, the control group included a number of children with infections of infancy (pneumonia and gastroenteritis) as well as congenital conditions. These diagnoses may have skewed the analysis in a number of ways. Furthermore, there are selection biases, both geographic and economic, inherent to samples from any one hospital in Pakistan. The findings of this study should be re-tested in a multi-center context.

Based on the above results, as well as the interviews we conducted, we can briefly enumerate some areas that would benefit from further study and policy considerations. Where possible we have referred to works by authorities in these areas. Falls were the most prominent cause of injury in our sample, accounting for 59% of injury admissions. An Indian study found similar results, with 44% of children injured by a fall (*15*). This is in contrast, however, to studies in Iran (*16*) and Thailand (*17*), where falls were less common than motor vehicle collisions. Compared with other causes of injury, falls were associated with less adult supervision (15%). Most Pakistani roofs are unprotected, and the vast majority of fall victims in our sample fell from unprotected roofs. A special cultural issue is kite flying, which is popular in Pakistan and is the focus of the Basant celebration.

Road collisions (*8,9*) accounted for 16% percent of injuries in our sample, consistent with the result of 26% found in India by Tandon *et al.* (*15*). There is a paucity of sidewalks in many areas of Pakistan. Where the government has provided sidewalks and pedestrian overpasses, pedestrians frequently fail to use them. Many pedestrians walk with their backs to traffic. Many cars lack seatbelts, and education about seatbelt use has been inconsistent.

Fires were the third principal cause of injury, accounting for 13% of the sample. The vast majority of burns were at home, from fires at ground level (*18*). There was a clear association between burns and meal times, particularly supper time. Notably, half of fire victims were girls, compared to falls and road collisions, for which boys were over-represented. This reflects that girls spend more time in the kitchen. Many Pakistani females wear loose clothing, which is more prone to ignite.

With over 60 million children, Pakistan is home to one of the world’s five largest pediatric populations. As in many countries, child injury has become one of Pakistan's most pressing public health problems. The social and financial burdens have important implications for Pakistan's future development. We hope the results of this study will be useful in guiding future injury research and focusing injury prevention efforts on children most at risk.

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Original Article

A social marketing approach to quality improvement in family planning services: a case study from Rawalpindi, Pakistan

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Summary **In the 1990s, social marketing approach was introduced in Pakistan to improve the quality and accessibility of family planning methods involving private practitioners. This study measured six quality elements using a Bruce-Jain framework. Cross-sectional survey data were collected from 29 randomly selected *Green Star* clinics. The study's four components were 1) an inventory of each outlet (infrastructure, equipment, and supplies); 2) an observation guide for interaction between family planning clients and service providers; 3) exit interviews with clients attending the outlet; and 4) interviews with providers at the outlet. Of the 29 clients participating in the exit interviews, 72% were new users of family planning. The clients' mean age was 32 years; all clients were married; 93% had received formal education. Housework was the principal activity of 93% of clients. The mean number of children reported was three. Both hormonal and intrauterine contraceptives (IUCDs) were available in all facilities; 86% of the clients reported being able to obtain their contraceptive of choice. Most facilities had the equipment and supplies needed to deliver services; service personnel were trained and regularly supervised; the service outlets emphasized mechanisms to ensure continuity of use. Notable shortcomings included a shortage of information on alternative methods, contraindications, and side-effect management, as well as a dearth of registration records. In conclusion, this is a good example of public-private partnership involving private practitioners using a social marketing approach. The quality components of a Bruce-Jain framework were achieved, resulting in a satisfied clientele. Involvement of private service outlets increased the accessibility and enhanced the use of services. Social marketing may be expanded to improve quality and access by involving further components of health care.**

Keywords: Social marketing, Public-private partnership, Private clinics, Family planning, Pakistan

Introduction

Of 175 million pregnancies worldwide each year, half are unwanted or ill timed. Around 120 million women do not want another pregnancy within the next two years or at all. Over 350 million women do not use safe and effective contraceptive methods (*1*), because they lack access, information, or support from families and communities.

In Pakistan, national surveys have indicated that, although the majority of the population is aware of at least one family planning (FP) method, the contraceptive prevalence rate is still very low (*2*). Factors hindering bridging of the gap between knowledge and practice of family planning include service delivery inefficiencies and social and cultural barriers. Decisions to adopt family planning methods are also influenced by acquaintance with clinic locations and supply sources, distance from clinic, and the provider's reputation. Quality of care concerns tend to be implicit rather than explicit (*3*).

During the past decade, the international family planning community's focus has shifted towards quality in services. Efforts to define and measure quality are motivated by an interest in identifying areas for improvement in a given family planning program and determining whether the level of quality affects

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outcomes, such as continuation rates (*4*). A program of high quality is one that is client oriented and aims to help individuals achieve their reproductive intentions or goals. Improvement in the quality of care is likely to reduce fertility by generating a more committed clientele of satisfied contraceptive users (*5*). Work over the past decade in this area has been guided by the Bruce/Jain framework that outlines six elements of quality: choice of method, information to the client, technical competence, interpersonal relations, mechanisms to encourage continuity, and constellation of services (*6*).

With this increased focus on quality, there has been a parallel interest in developing means of measuring quality, for several reasons. First, client-provider interactions can be understood as intervening elements in a causal chain through which organized family planning efforts meet or generate demand for fertility regulation. Learning more about these processes with the aim of improving them can have important programmatic payoff (*7*).

The challenge in measuring quality is the complexity of the topic. Although the Bruce/Jain framework outlines six elements of quality; there are literally hundreds of possible "sub-elements" that might be measured. In 1990, a Task Force created to explore the measurement of quality; identified over 200 indicators of quality in family planning services (*8*). In 1997, the MEASURE *Evaluation* project, developed and field-tested a low-cost, practical approach to monitoring quality of care, later named the "*Quick Investigation of Quality*". To this end, staff used a modified Delphi approach to arrive at a short list of 25 indicators of quality of care for family planning programs. Three instruments were developed that draw directly from Situation Analysis: a facility audit, an observation of the client-provider interaction, and an exit interview with clients leaving the facility (*9*). These instruments were field tested in four countries (Ecuador, Turkey, Uganda, and Zimbabwe) between October 1998 and March 1999 to determine the feasibility of data collection and reliability of the data (*10*).

Studies exploring the linkages between the quality of care and continued contraceptive practice have revealed an apparent association between clients receiving inadequate counseling and higher levels of contraceptive discontinuation. A study in India confirms that women who opted for an intrauterine device and who received proper counseling and information on side effects are more likely to continue using it than those who did not (*11*), and similar findings were cited from studies in Gambia and Niger (*12*).

In response to perceived inefficiencies and lack of capacity in public health care delivery systems, many developing countries have contracted out health services to private providers (*13*). An estimated 70 percent (*14*) of Pakistanis seek health care from private sector clinics and pharmacies; private health facilities are disproportionately based in urban areas and focus on providing curative services. Quality, cost containment, and equity issues arise from this situation, as the private sector is unregulated. Relatively few private providers offer reproductive health services because their training is limited and curative services are more lucrative. Collaborations with the private sector such as social marketing non-governmental organizations (NGOs) might help effectively to manage such niches, as recognized by the Ministry of Population Welfare.

Social marketing is the application of traditional marketing principles towards the promotion of health behavior change (*15*). It is based on traditional market exchange theory, which states that consumers will adopt behavior changes when barriers are reduced and benefits highlighted, according to their specific needs (*16*). Social marketing manages behavior change by creating incentives or consequences that invite voluntary exchanges. Specifically, social marketers seek to identify barriers to behavior change and to highlight benefits that are relevant to the audience (*17,18*).

Social Marketing Pakistan is an NGO that works in the health sector in collaboration with the Government of Pakistan and the private sector. The Government of Pakistan considers social marketing intervention as an important strategy to advance the cause of population welfare.

Green Star (*19*) is currently the country's second-largest family planning provider. It has developed a social franchise network of private health care providers to increase access to reproductive health care targeting low-income Pakistanis. More than 18,000 specially-trained private-sector doctors, paramedics, and chemists have received extensive training in counseling and service provision and offer quality FP services.

The training program provides a 10-day course (classroom & clinical) to lady doctors and paramedics. It is mainly focused on intrauterine contraceptive device (IUCD) insertion, counseling, provision of hormonal and barrier contraceptives, management of side effects, infection prevention, and record keeping. Franchise members also receive refresher training and are monitored on a regular basis to ensure adhesion to *Green Star* quality standards.

Green Star uses a variety of communications channels to address key barriers to FP, such as low confidence in the safety or efficacy of available methods, ignorance about where to seek quality services and lack of social support for FP, especially from husbands. Messages highlight the availability of products and services and link clients to delivery points (clinics) displaying the *Green Star* logo.

This report assesses quality provision in services from franchised *Green Star* service outlets. It has attempted to measure the six elements of quality in accordance with the Bruce-Jain framework and to

determine whether the above dimensions of care could be enhanced through public-private partnership. The study's four components were 1) an inventory of each outlet (infrastructure, equipment, and supplies); 2) an observation guide for interaction between family planning clients and service providers; 3) exit interviews with clients attending the outlet; and 4) interviews with providers at the outlet.

The following sections provide details on client and provider perspectives on quality in family planning services, and a description of the range of services they offer.

Methods and Material

Study site

This study was conducted in Rawalpindi city. According to the latest census it has a population of 1,406,214 (Males: 747,923, Females: 658,291) (*20*). Women of reproductive age (15-49 years) comprise 52% of the female population and the contraceptive prevalence rate is 41%. The literacy rate is 76%. The Ministry of Population Welfare provides family planning services through its 20 family welfare centers and four reproductive health centers. A handful of NGOs (*e.g.*, Behbood, Family Planning Association of Pakistan, Key Social Marketing, and Social Marketing Pakistan) also provide family planning services through their outlets.

Study methods and materials

A cross sectional study assessed the quality of care in family planning services in Social Marketing Pakistan "*Green Star*" clinics in Rawalpindi. As mentioned above, we utilized the *Quick Investigation of Quality* methodology; that consisted of: an observation of the client-provider interaction, an exit-interview with the client, and a facility inventory (*9*), in addition to the above the authors also interviewed the service providers. Data were mainly collected using the questionnaire developed by Miller *et al.* (*9,21*). The exit interview questionnaire, client-provider interaction checklist and checklist for facility infrastructure and equipment was adapted and modified according to local conditions. An ethnographic field guide was used to interview service providers. The triangulation used, thus enhanced the study's reliability.

The questionnaires were translated into the local language (Urdu), and back-translated to check the sequence, relevance, and clarity of the questions. After pilot-testing for face validity with a few family planning clients outside the study area, the question order was adjusted to facilitate client comprehension.

For the family planning client-provider interactions, the principal investigator; a physician himself, obtained consent from both the provider and client to be present during individual counseling and clinical examination. He used an observation guide to record yes/no answers to a series of actions reflective of quality of care (questions that the provider should ask, points of information that should be covered, clinical procedures that should be used in administering certain contraceptives, *etc*.) As the client left the facility after her visit, the interviewer approached her to ask if she could interview her about the visit and her satisfaction with the services received. The interviewer explained to the client that he did not work for the clinic; that all responses would remain confidential; and that her answers would in no way affect her getting services in the future. After obtaining the consent, the interviewer then proceeded to ask her a series of questions. The clinical facilities, equipment, and services provided were assessed by direct observation.

Samping and analysis

There are 140 *Green Star* clinics in Rawalpindi city offering a full range of *Green Star* products (58 owned by paramedics and 82 owned by doctors). A sample of 20% (n = 29) of clinics were randomly selected; they included clinics owned by paramedics (n = 12) and owned by doctors (n = 17) (*22*). The inclusion criteria were all *Green Star* clinics with at least 1-2 family planning clients per day where provider and client observation and client exit poll interviews could be performed. Neither the provider nor the researcher knew the day of the visit in advance. In selected health centers, 29 in-depth interviews with health care providers (12 paramedics and 17 doctors) were performed, in addition to 29 observations of client-provider interactions and exit poll interviews. Before the start of the study, approval was obtained from Social Marketing Pakistan and individual informed consent was also obtained before each interview.

Data processing and analysis were carried out using SPSS version 10 (SPPS Inc., Chicago, IL, USA) to produce frequencies and percentages. Separate files were created for entering data from the inventory, observations, and client exit interviews. In-depth interviews were recorded in detail and later transcribed. Domains and sub-domains were identified and the responses were analyzed and on their bases and results were drawn.

Results

Characteristics of client population

In the study sample, all respondents at the *Green Star* clinics were females and their average age was 32 ± 16 years. Five clients refused to take part in exit poll interviews. Seventy-two percent (n = 21) of the clients who took part in the exit poll interviews were

new clients, while 28% of the clients were continuing users. Seven percent of the clients were illiterate, while 31% had primary, 45% had secondary, and 17% had university level education. The mean number of children was 3 ± 1 and the mean age of the youngest child was 1.5 years.

Interpersonal relations

All clients were observed to receive friendly greetings from the provider. The majority (83%) of the providers replied to the client's questions satisfactorily. Overall 73% had provided visual privacy, 62% had provided auditory privacy (contrasting with only 8% in paramedic outlets). The duration of the client-provider interaction exceeded 10 minutes in most observations.

All clients were of the opinion that their provider was friendly, but few clients (10%) had been told to ask questions if something was unclear. The majority (97%) regarded their provider as easy to understand and also felt that their privacy was adequate (76%). All replied that the waiting time in the clinic was reasonable, the consultation time with the provider was about right (93%), and the provider listened to their concerns about family planning (97%). From the provider's perspective one mentioned, "Most of the clients come regularly and are like friends and they even discuss family matters with us" (*40-year-old female health care provider*).

Choice of method

Green Star Social Marketing Pakistan is promoting four types of family planning methods: oral contraceptive pills, progesterone injections, IUCDs, and condoms. No product except condoms has ever been out of stock since joining *Green Star*.

The client's ability to make informed choices is determined by the variety of contraceptive methods discussed with her. It was observed that all clients (specifically new users) were told about more than one method and the majority of clients (86%) received the method they chose.

Sixty-two percent of clients were informed about all four methods. The majority (88%) of doctors, but only a minority (25%) of paramedics informed their clients about all four methods. The majority of clients (86%) received their method of choice, as noted in exit interviews.

Regarding predilections for different methods, one health worker stated, "Clients choose either hormonal or IUCD, no one takes condoms from the clinic" (*40-year-old female health care provider*). While explaining how the clients select the methods, it was stated, "First of all, I leave the choice to clients and I help them in choosing an appropriate method for them, but if they depend on my choice then I advise them" (*36-year-old female health care provider*).

Provider - client information exchange

It was observed that in general 79% of clients were asked by their doctor about their reproductive goals; only half of paramedics asked their clients about this. All clients were asked about breast-feeding and if they had any problem or the desire to change the current method.

Exit poll interviews revealed that 55% of clients do not want more children, and an equal number were also breast-feeding at the time of survey. Almost 86% of clients made their own decision to accept their current method.

Health care providers put special emphasis on information exchange: "In the training they said it is important to listen to clients and then help accordingly. I follow that" (*38-year-old female paramedic*). Another declared, "I spend more time on counseling to satisfy my clients" (*46-year-old female paramedic*).

Information given to clients

In all clinics visited, Information, education & communication (IEC) material for use during counseling about family planning was present in examination rooms, waiting rooms, and inside clinics. During client provider interactions, 86% of providers utilized IEC material to explain contraceptive methods. All were told about method usage and effectiveness; 72% were also told about the side effects. Sixty-nine percent were actually shown various contraceptive samples (see Table 1).

Regarding information sharing, one mentioned "I tell my clients especially about how to use the product and possible side effects, so she does not panic if anything happens" (*36-year-old female health care*

Table 1. Information given to clients about contraceptive methods

Information given	Total (*n* = 29)		Doctors (*n* = 17)		Paramedics (*n* = 12)	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Method of application	100	0	100	0	100	0
Effectiveness	100	0	100	0	100	0
Contraindications	72.4	27.6	82.4	17.6	58.3	41.7
Side effects	72.4	27.6	82.4	17.6	58.3	41.7
Sample contraceptive shown	69	31	64.7	35.3	75	25
IEC material shown	86.2	13.8	76.5	23.5	100	0

Table 2. Technical competence of doctor and paramedic providers in Green Star clinics

Components of examination	Total (*n* = 29)		Doctors (*n* = 17)		Paramedics (*n* = 12)	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Medical history	93.1	6.9	100	0	83.3	16.7
Date of last menses	100	0	100	0	100	0
Abnormal vaginal bleeding	69	31	100	0	25	75
Pelvic pain	62.1	37.9	88.2	11.8	25	75
Weight	44.8	55.2	76.5	23.5	0	100
Breast examination	51.7	48.3	88.2	11.8	0	100
Blood pressure	65.5	34.5	100	0	16.6	83.3

provider). Asked how they help clients make a choice, one said "I use brochures, flip charts, and posters to provide information about family planning methods, and I also show them the actual products" (*46-year-old female paramedic*).

Technical competence

All the providers had an average of two years of working experience since training with the *Green Star* program. All clinics had functioning sterilizers, blood pressure monitoring apparatus, weighing scales, antiseptic solutions, an examination couch, gloves, IUCD insertion and removal kits, and relevant equipment. Sixty-five percent (*n* = 19) of clinics had proper waste boxes for disposal of needles. Most clinics were clean and well furnished with adequate seating. Providers were observed during service delivery and clinical procedures. The details are shown in Table 2.

When health care providers were asked about the indications and contraindications for contraceptive prescription, one stated, "I will not insert an intrauterine contraceptive device in a woman whom I suspect to have a reproductive tract infection" (*42-year-old female health care provider*). One maintained, "I will not give hormonal methods to women who are not having their menses, as they emphasized in my training" (*48-year-old female paramedic provider*).

Another stated, "…I advise breast-feeding mothers to avoid hormonal methods and instruct them about alternatives" (*38-year-old female health care provider*).

Mechanism to ensure continuity

None of the *Green Star* clinics had ever experienced any shortages in the supply of hormonal and IUCD contraceptives. Most providers had no client registration log-books; however, they did have registration cards for family planning clients provided by the *Green Star* program. All clients were told when to return; the 35% of clients who chose hormonal contraceptives were given written reminders. It was observed that 69% were instructed how to manage complications, and all were informed how to replenish their supplies.

The providers highlighted the importance for continuity of proper counseling. One mentioned, "Clients should be told in advance about the side effects of those contraceptives. This decreases their anxiety and increases their continuation rate" (*38-year-old female paramedic*). Another stated, "…sometimes it is important to give a written reminder about the next visit: many remember better that way" (*42-year-old female health care provider*).

Constellation of services and accessibility

The questionnaire also captured topics other than family planning that were discussed during counseling sessions. All the clinics surveyed provide family care; and family planning was one component of the services offered. In exit interviews, many clients also mentioned that besides family planning they received services for child care and also obtained information on HIV or other sexually transmitted diseases (STDs), when asked.

Most of the *Green Star* clinics could be reached by local residents on foot. Only 10% of clients used public transport, 7% a private car, and 14% a motorcycle to come to the clinic. The average duration of the trip to the clinic was seven minutes. Most clients were satisfied with the clinic hours, as services were offered both morning and evening. *Green Star* signboards were properly placed at clinics to indicate the availability of family planning services. One provider noted, "The government facilities are far away, but clinics like mine are easily accessible and clients can visit anytime" (*38-year-old female health care provider*).

Discussion

Quality of care should be defined in terms of the provider's technical standards and the patient's expectations. In 1990, Judith Bruce offered a framework for assessing quality of family planning services from the client's perspective. This research has investigated whether the framework dimensions of care could be enhanced through public-private partnership at the level of program efficiency and impact.

The results suggest not only that all the selected service centers were geographically accessible but also that the infrastructure, equipment, and contraceptive supplies were adequately available. Most providers

demonstrated commitment to the program and they satisfied the various quality component criteria in addressing women's reproductive needs. Client-provider relations were based on mutual trust in these clinics and most of the clients considered providers to be warm and technically competent. The present study reveals that providers were encouraging and helping clients to make informed decisions when selecting family planning methods. Through this they sought to build the client trust that would eventually lead to increased continuation rates and greater client satisfaction.

Social Marketing Pakistan's *Green Star* program offers four methods (two hormonal, one barrier, and one IUCD method); all member clinics therefore offer these services. According to providers, supplies have never been out of stock, because *Green Star* supervisors perform their duties efficiently, visit clinics regularly, and provide technical assistance. The clinics were generally well equipped. The majority of providers took detailed medical histories, checked blood pressure, and inquired about menstrual history; half also weighed the patient before prescribing any method.

Most clients were asked about their reproductive goals and were informed about more than one method. The majority of clients received the method of their choice. Most of the clients were given additional information about side effects and contraindications and, to alleviate anxiety and reduce avoidable visits, were told what measures to take in the event of minor side effects.

During counseling, all providers used IEC materials and contraceptive samples and the majority of clients were given an information brochure to take home. Providers and clients both considered revisits as an opportunity to review and revise their choice of methods.

There was an appropriate constellation of family welfare services (*23*) in *Green Star* clinics, including trained providers, medications, equipment, and supplies; besides they also offered mother and child care services. Our research results demonstrate that clients expressed satisfaction with services they received at *Green Star* clinics, citing their geographical accessibility, adequate facilities, provider's technical competency, reasonable waiting times, and convenient clinic hours. The majority stated that contraceptive services were affordable, reported having satisfactorily received the services they desired, and stated that they would recommend this clinic to their acquaintances.

The study had certain limitations. First, the sample size is small. We believe that the clinics studied are representative of *Green Star* member clinics in Rawalpindi city and provided useful information; however, extrapolation of the findings to the rest of Pakistan requires careful consideration, as clinics and services in other geographic areas may vary due to diverse client characteristics and demographic and other factors. Second, the exit poll interviews were conducted just outside the clinic and client responses may have been biased by proximity.

Certain aspects of services require improvement and should be emphasized more in future training programs and clinic follow-ups. For example, some providers and most paramedics failed to inform their clients about side effects and contraindications. Some also failed to take detailed medical histories, including asking about abnormal bleeding, taking blood pressure, and weighing, and never encouraged clients to ask questions. Clients lacked privacy in some clinics. Client registration records were also noticeably lacking. Many providers also felt the need to have more regular refresher training courses so they can keep up with the latest developments in the field.

This study provides an interesting view of a public-private partnership to improve the quality and accessibility of family planning services by involving private practitioners. Public-private partnerships are joint ventures sharing a set of attributes, the most important of which is a shared objective: in this case, improving quality and access to family planning services.

Given the pressure on government resources, it is likely that the private sector will continue to play an increasing part in the provision of services alongside other institutions. Based on the current private sector experience, it is important for the government to review past procedures and move towards developing systems which can extend longer-term relationships and involve the private sector in planning.

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Original Article

Cell growth of the mouse SDHC mutant cells was suppressed by apoptosis throughout mitochondrial pathway

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Summary **SDHC E69 cells, which overproduce superoxide anions in their mitochondria, were previously established that had a mutation in the SDHC gene of complex II of the respiratory chain. We now demonstrate that tumors formed by NIH 3T3 and SDHC E69 cells showed significant histological differences. Cytoplasmic cytochrome c release from mitochondria was significantly elevated in SDHC E69 cells and was likely caused by superoxide anion overproduction from mitochondria. In addition, the p53 and Ras signal transduction pathways were activated by oxidative stress and may play a key role in the supernumerary apoptosis in SDHC E69 cells. Our results suggest that the development and growth characteristics of hereditary paragangliomas, which are defective in the same complex of electron transport as mouse SDHC E69 cells, may be caused by apoptosis induction by mitochondrial oxidative stress.**

Keywords: Mitochondria, Superoxide anion, Oxidative stress, Apoptosis, Paraganglioma

1. Introduction

Major endogenous reactive oxygen species (ROS) are generated from electron leakage during cellular respiration in mitochondria (*1*). The *mev-1* mutant of the nematode *Caenorhabditis elegans* is mutated in the SDHC subunit of complex II in electron transport system (*2*) and produces excessive superoxide anions (O_2^-) in its mitochondria (*3*). This mutant has proven extremely useful for the study of endogenous oxidative stress and its effects on lifespan, apoptosis and mutagenesis (*4,5*). We have recently constructed a transgenic mouse cell line (SDHC E69 cells) with a mutation that mimics *mev-1* (*6*). As in *C. elegans*, excess O_2^- were generated, which led to supernumerary apoptosis and hypermutability (*6*). Interestingly, the SDHC E69 cells that escaped from apoptosis were frequently transformed and, when the cells were injected under the epithelium of nude mice, they resulted in the production of tumors two weeks after implantation (*6*). Conversely, after wild-type cells (NIH3T3 cells) were injected, they were on the verge of disappearance but were transformed at high frequency by spontaneous mutations during long passage time or culture time. We show in this report that the NIH3T3 wild-type tumors developed with considerable proliferative abilities over the course of further incubation while the tumor mass of SDHC E69 transformed cells did not significantly enlarge.

It has been reported that mutations in the SDHC or SDHD gene of mitochondrial complex II cause some nonchromaffin and hereditary paragangriomas (PGLs) in humans (*7,8*). Therefore, further analysis of SDHC E69 cells may help clarify the molecular mechanism of the tumorigenesis in neoplasms such as PGLs. It is clear that apoptosis and cell-cycle arrest serve as defensive mechanisms to rid organisms from potentially neoplastic cells. However, the molecular mechanisms by which apoptosis is stimulated by ROS overproduction

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from mitochondria are not completely understood. In this report, we explored the mitochondrial and cytosolic responses on apoptosis to ROS overproduction in SDHC E69 mitochondria. Specifically, excess apoptosis in SDHC E69 cells was mediated virtually exclusively through the mitochondrial pathway. In addition, p53 and Ras-MEKK pathways were presumably stimulated in the SDHC E69 cells.

2. Materials and Methods

2.1. *Cell culture*

The cells were cultivated in DMEM medium (Nissui Company, Tokyo) including 2.5% FBS + 2.5% CS in a 5% CO_2 incubator. Cell division and proliferation were examined after synchronous culture in G0 phase into exhaustion of serum medium and at the contact inhibition state in 100-mm tissue culture dishes. For cell growth, 5×10^4 synchronized cells were cultured in a standard medium of 35-mm tissue culture dishes.

In this manuscript, three-month SDHC E69 cells after the establishment and their wild-type cells (NIH3T3 cells) for 3 month cultured cells as progenitors were used. In brief, the three-month SDHC E69 cells showed the loss of contact inhibition and had many apoptotic molecule-like granules during the first month after establishment (*6*). During the period of colony formation, some clefts characteristics of programmed cell death were found in the center of some colonies (*6*). In the SDHC E69 cells, the morphology was changed from the typical solid and elongated fibroblasts to smooth and rounded cells (*6*). Similar changes were evident, although to a lesser degree in the one-month SDHC E69 cells after establishment (*6*). In addition, the three-month SDHC E69 cells formed multiple layers (*6*).

2.2. *Antibodies and chemicals*

Caspase inhibitors *Z*-Leu-Glu(OMe)-Thr-Asp-(OMe)-FMK (an inhibitor of caspase 8) and *Z*-Leu-Glu(OMe)-His-Asp(OMe)-FMK (an inhibitor of caspase 9) were purchased from ICN Pharmaceuticals (Irvine). p53 antibody, phospho-p53 (Ser15) antibody, phospho-p38 MAP kinase (Thr180/Tyr182) antibody, phospho-SAPK/JNK (Thr183/Tyr185) antibody and Bid antibody were purchased from Cell Signaling Technology. p21 (C-19) antibody, Bax (N-20) antibody and MEK kinase-1 (C-22) were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz). Anti-cytochrome c monoclonal antibody was purchased form BD PharMingen (San Jose). Anti-rabbit Ig, horseradish peroxidase linked $F(ab')_2$ fragment (from donkey) and anti-mouse Ig, horseradish peroxidase linked $F(ab')_2$ fragment (from sheep) were purchased from Amersham Pharmacia Biotech. The luciferase reporter gene pp53-TA-Luc and pTA-Luc vector used in the p53 reporter assay were purchased from Clontech.

2.3. *Transfection and luciferase assay*

An AP-1 cis-element dependent transcriptional expression vector (pAP-1-TA-Luc) was constructed using the AP-1 cis-element 5' primer (CTAGCTGAGT CAGTGAGTCACTGACTCACTGACTCATGAGTCA GCTGACTCA) and the AP-1 cis-element 3' primer (G ATCTGAGTCAGCTGACTCATGAGTCAGTGAGTC AGTGACTCACTGACTCAG). These were annealed, and this oligonucleotide was inserted at an *NheI*-*Bgl*II site in the pE2F-TA-Luc plasmid vector without the E2F binding site. A pE2F-TA-Luc plasmid vector without the E2F binding site (p-TA-Luc) was used as the negative control vector. Transient transfection of pp53-TA-Luc (0.5 μg), pTA-Luc (0.5 μg) and pCMV-β-galactosidase (0.05 μg) or pAP-1-TA-Luc (0.5 μg), p-TA-Luc (0.5 μg) and pCMV-β-galactosidase (0.05 μg) into NIH3T3 and SDHC E69 cell lines was performed using LipofectAMINE Plus reagent (Invitrogen Inc.). Proteins were prepared for luciferase and β-galactosidase analysis 48 h after transfection by addition of lysis buffer (0.625 mM Tris-PO_4 (pH 7.8), 15% glycerol, 2% CHAPS, 1% Lecichin (*L*-α-phosphachigilcholine), 1% BSA, 0.1 M EGTA, 1 M $MgCl_2$, 1 M DTT, 0.1 M *p*-APMSF). These protein lysates were measured for luciferase and β-galactosidase activities using Luminescencer-PSN (ATTO) or SPECTRA MAX 250 (Molecular Devices) after addition of luciferese buffer (20 mM Tricine-NaOH, 1 mM $4MgCO_2 \cdot Mg(OH) \cdot 5H_2O$ (pH 2.3), 2.7 mM $MgSO_4 \cdot 7(H_2O)$ (pH 2.3), 0.1 mM EDTA, 33 mM DTT, 0.27 mM CoA-Li, 0.47 mM luciferin, 0.53 mM ATP)) or β-galactosidase buffer（60 mM $Na_2HPO_4 \cdot 12H_2O$, 10 mM KCl, 1 mM $MgCl_2$, 40 mM $NaH_2PO_4 \cdot 2H_2O$, 1.1 mM ONPG, 47.5 mM 2-mercaptoethanol). Samples were then incubated for 1 h at 37°C. Luciferase relative activity was normalized based on β-galactosidase activity levels and luciferase activity levels of p-TA-Luc, pTA-Luc negative control vector.

2.4. *Northern blot analysis*

Mouse cDNA's for Northern blot analysis were obtained by RT-PCR method using oligonucleotides for MDM2 (5'-GCC ACC AGA AGA GAA ACC-3' and 5'-GCC TGA GCT GAG TTT TCC-3'), p21-Ras (5'-TTG GAG CAG GTG GTG TTG-3' and 5'-ACA CAT CAG CAC ACA GGG-3'), M-Ras (5'-AGT AGT GGT GGG AGA TGG-3' and 5'-AGT TTG TGA GTG CCG GTG-3'), Raf-1/C-Raf (5'-CAT GAG CAC TGT AGC ACC-3' and 5'-ATC TCC ATG CCA CTT GCC-3'), 18s rRNA (5'-TAC CTG GTT GAT CCT GCC-3' and 5'-TTT CGT CAC TAC CTC CCC-3'), actin (5'-TGG AGA AGA TCT GGC ACC-3' and 5'-ACC CAA GAA GGA AGG CTG-3'), and G3PDH (5'-CAC GGC AAA TTC AAC

GGC-3' and 5'-CTT GGC AGG TTT CTC CAG-3'). 3 μg of mRNA which was extracted by Oligotex-dT30 Super (Roche) was subjected to Northern Blot analysis. Mouse cDNA's were random-prime-labeled using the High prime kit (Roche) with ^{32}P-dCTP (Amersham Biosciences) and purified using ProbeQuant G-50 Micro Columns (Amersham Biosciences). After hybridization for mouse cDNA's filters were stripped and reprobed for actin and G3PDH to verify that comparable amounts of RNA had been loaded in all lanes.

2.5. *Western blot analysis*

After a particular treatment, cells were washed twice with phosphate buffered saline and incubated on ice in lysis buffer containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 100 mM NaCl, 0.1% NP-40, 1 mM DTT and 0.1 mM *p*-APMSF for 10 min. This was followed by brief sonication for Western blot analysis of p53 or p21. Cell lysates for p21 analysis were then cleared by centrifugation (400 × *g*) for 5 min, and the supernatants were used. In Western blot analysis of Bax or cytochrome c, both the mitochondrial and cytoplasm fractions were employed. These cell lysates (containing 10-100 μg of protein) were solubilized by boiling after the addition of 2 × SDS-PAGE sample buffer (0.125 M Tris-HCl (pH 6.8), 10% 2-mercaptoethanol, 4% SDS, 10% sucrose and 0.004% bromophenol blue). For the analysis of p38, p-JNK, Bid or MEKK1, cells were washed twice with phosphate buffered saline and incubated in SDS-PAGE sample buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 50 mM DTT and 10% glycerol for 10 min on ice followed by brief sonication. Cell lysates were then cleared by centrifugation (400 × *g*) for 5 min and the supernatants were directly subjected to SDS-PAGE. After electrophoresis, the proteins were transferred to PVDF (polyvinyliden difluoride) membrane Clearblot membrane (ATTO) using a Semi-dry blotting machine AE-6677 (ATTO). To block nonspecific protein binding, membranes treated for 8 h at 20-25°C with either 0.1% Tween 20, 5% nonfat dried milk in phosphate buffered saline for analyses of p53, p21, Bax, cytochrome c, Bid or MEKK1 or 5% bovine serum albumin, 0.1% Tween 20 in TBS (0.02 M Trizma base, 0.137 M NaCl (pH 7.6)) for analyses of phospho-p53 (Ser15), phospho-p38 MAP kinase (Thr180/Tyr182) or phospho-SAPK/JNK (Thr183/Tyr185). The membranes were treated with anti- p53, p21, Bax, cytochrome c, or MEKK1 antibody in phosphate buffered saline containing 0.1% Tween 20, 5% nonfat dried milk, or phospho-p53 (Ser15) antibody in TBS containing 0.1% Tween 20, 5% bovine serum albumin at room temperature for 1 h or with Bid antibody in phosphate-buffered saline containing 0.1% Tween20, 5% nonfat dried milk, or phospho-p38 MAP kinase (Thr180/Tyr182) or phospho-SAPK/JNK (Thr183/Tyr185) antibody in TBS containing 0.1% Tween 20, 5% bovine serum albumin at 4°C for 8 h. The membranes were then washed with the antibody dilution buffer for 30 min. They were then treated with the ECL-plus Western blotting detection system (Amersham Biosciences) after treatment with anti-rabbit or anti-mouse antibody (for analysis using Bid). They were then exposed to Hyperfilm™ ECL chemiluminescence film (Amersham Biosciences) at room temperature for 2 min. The chemiluminescent signals were visualized with a CS Analyzer and AE-6962 light capture (ATTO).

3. Results

3.1. *Morphology of the SDHC E69 cells on nude mouse*

It is known that NIH3T3 cells are transformed at high frequency by spontaneous mutations during long passage time or culture time. In fact, the cells, which did not proliferate within two weeks after the injection under the epithelium of nude mice (Figure 1A), grew into huge malignant tumors one month (Figure 1B). Some of the SDHC E69 cells that escaped from apoptosis underwent transformation, as evidenced by the fact that SDHC E69 transformed cells caused tumors within two weeks when injected under the epithelium of nude mice (Figure 1C) (*6*). The size of tumors remained unchanged even after one and a half month (Figure 1D). As expected of actively proliferating neoplasms, the tumors derived from the NIH3T3 cells had evidence of the apocytes and the nuclear divisions with characteristic dense staining of the cytoplasm (Figure 2A). In addition, the margin between the tumor and the blood vessel was distinct (Figure 2B). In contrast, The SDHC E69-derived tumors showed no evidence of nuclear divisions and showed nuclear aggregation (Figure 2C). They had assumed the posture of a fibrous tumor, and the blood vessel and tumor border was not distinct. Moreover, tumor-associated cells were present (Figure 2D). Thus, the thickness of the tumor cells layer was less when derived from SDHC E69 cells (Figure 2F) versus the wild type cells (Figure 2E).

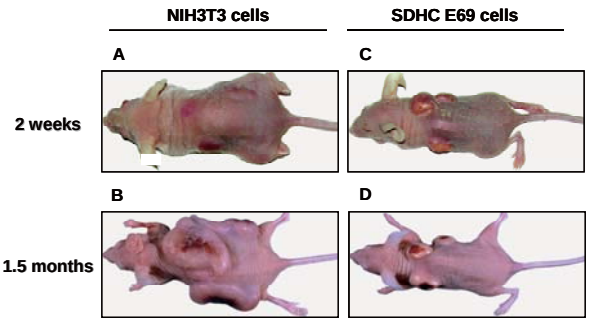

Figure 1. Comparisons of tumorigenesis using NIH3T3 cells and SDHC E69 cells injected into the epithelia of nude mice and cultured for two weeks and one and a half-months. Transplantation of spontaneous transformation NIH3T3 cells (A and B) and SDHC E69 cells (C and D) in epithelia of nude mice.

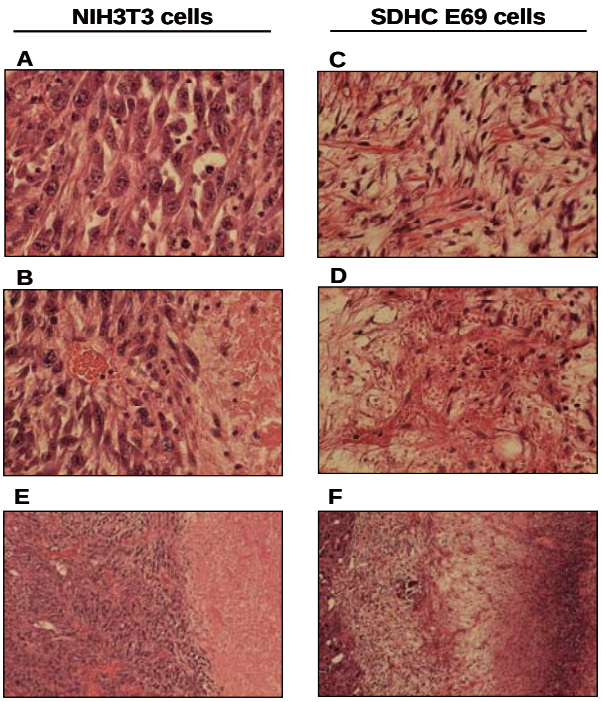

Figure 2. The tissue specimen of the tumor into epithelia of nude mice in spontaneous transformation NIH3T3 cells (A, B, E) and in SDHC E69 cells (C, D, F).

3.2. *The role of mitochondrial function to apoptosis*

Cellular cytochrome c was found in the mitochondria in both the SDHC E69 cells and NIH3T3 cells (Figure 3A). Cytosolic levels in the SDHC E69 cells and NIH3T3 cells were lower than mitochondrial levels. However, cytosolic cytochrome c levels increased significantly in the SDHC E69 cells.

In the mitochondria of the SDHC E69 cells compared to NIH3T3 cells, the Bax levels were significantly higher (Figure 3A). Bid and tBid levels were barely detectable in the SDHC E69 cells (Figure 3A).

In NIH3T3 cells, MDM2 mRNA expression was equally distributed between the 3.0 kbp and 1.7 kbp mRNAs, which are translated into the p90 and p76 MDM2 proteins, respectively (Figure 3B). A dramatically different pattern was observed in the SDHC E69 cells, as only the short-form type was expressed, which is incapable binding to and promoting p53 protein degradation (Figure 3B). p53 protein levels were below the level of detection in NIH3T3 cells (Figure 3C). Conversely, p53 protein existed in copious

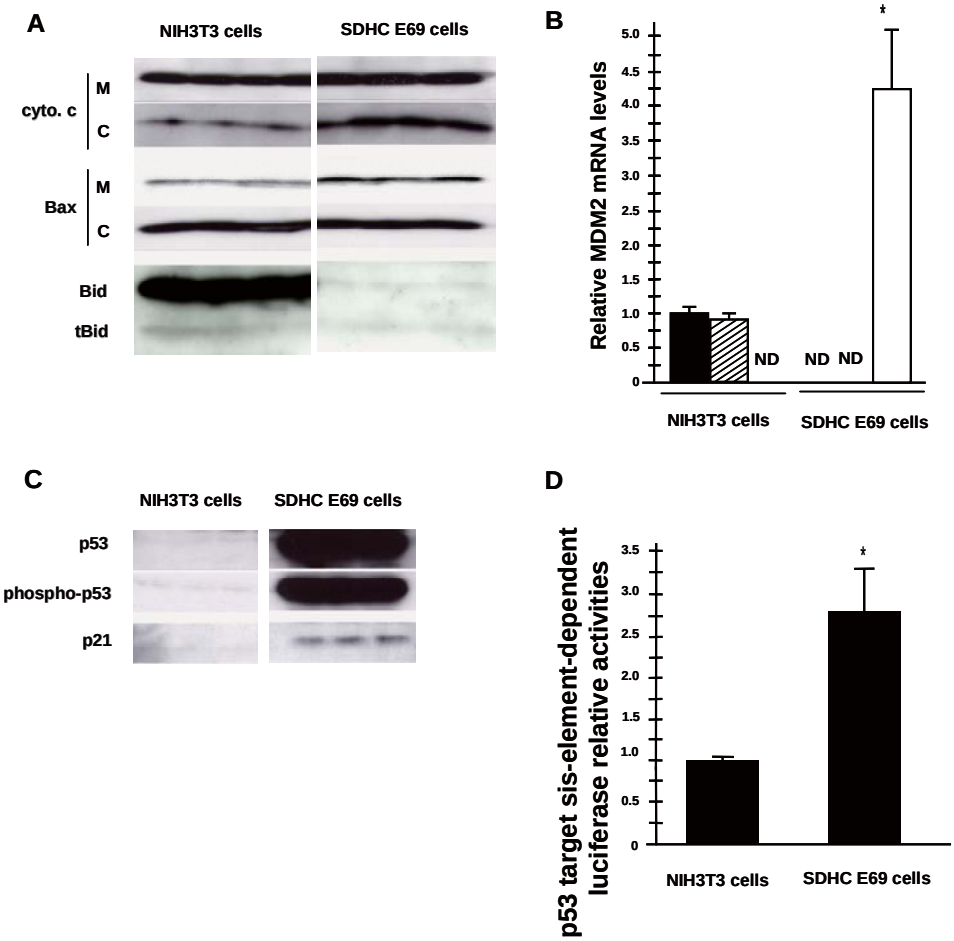

Figure 3. The alteration of cytochrome c release, inducible protein localization and p53 activation in NIH3T3 and SDHC E69 cells. A: Detection of cytochrome c, Bax and Bid proteins levels by Western blot analysis. cyto. c: cytochrome c, M: mitochondrial fraction, C: cytoplasmic fraction. $n = 3$. B: Detection of MDM2 mRNA patterns and expression levels by Northern blot analysis. MDM2 mRNA patterns of 3.0 kbp (p90, full-length MDM2) (closed boxes), 1.7 kbp (p76, not containing p53 binding motif) (hatched boxes) and 1.0 kbp (short form, not containing proteins binding motif) (open boxes) were detected. * $p < 0.01$, $n = 3$. C: Detection of p53, phospho-p53 and p21 protein levels by Western blot analysis. $n = 3$. D: Measurement of p53-dependent transcriptional activation levels by p53 target cis-element-dependent luciferase activity. * $p < 0.01$, $n = 3$.

amounts in the SDHC E69 cells, in which the short-form type MDM2 mRNA was expressed (Figure 3C). Moreover, most of the p53 protein was phosphorylated at serine residue 15 (Figure 3C). In addition, p21 protein was highly expressed in the SDHC E69 cells but not in NIH3T3 cells (Figure 3C). In the each cell lines transiently transfected a luciferase-containing construct with a p53 binding cis-elements, luciferase activity was over 2.8 times higher in the SDHC E69 cells than in the NIH3T3 cells (Figure 3D).

3.3. *The role of caspase 8 and caspase 9 on apoptosis*

As demonstrated previously, caspase 3 levels were higher in the SDHC E69 cells than in NIH3T3 cells (Figure 4A) (*6*). In the NIH3T3 cells, caspase 3 activity was slightly decreased by each caspase antagonist ($p < 0.01$) (Figure 4A). Caspase 3 activity was not further reduced by addition of both caspase 8 and 9 antagonists in the NIH3T3 cells (Figure 4A). In the SDHC E69 cells, both caspase 8 and caspase 9 inhibition had a larger effect on caspase 3 activity (Figure 4A). Moreover, we tested the viability of cells cultured in the presence of each caspase antagonist. The survival rate of the NIH3T3 cells was decreased by treatment with a caspase 9 antagonist (Figure 4B).

In contrast to the results obtained with NIH3T3 cells, the presence of each caspase antagonist resulted in increased the cell growth and proliferation in the SDHC E69 cells (Figure 4C). Moreover, both caspase 8 and 9 antagonists were inadequate to substantially reduce caspase 3 activity in the SDHC E69 cells ($p < 0.01$) (Figure 4A).

3.4. *The role of transduction pathway to apoptosis and tumorigenesis*

First, we analyzed p21Ras (H-, N-, K-Ras) and M-Ras mRNA expression levels by Northern blot analysis. Relative to the actin and G3PDH internal controls, p21Ras and M-Ras mRNA expression levels in the SDHC E69 cells were increased in comparison with the NIH3T3 cells (Figure 5A).

Relative to the actin and G3PDH internal standards, Raf-1/C-Raf mRNA expression, which induces cell growth and proliferation, was significantly increased in the SDHC cells in comparison to the NIH3T3 cells (Figure 5B). 195 kDa full-length MEKK1 protein was present in unchanged amount in the SDHC E69 cells, but the activated p91kDa MEKK1 protein was increased (Figure 5C). In addition, activated 54 kDa and 46 kDa JNK proteins were present in increased amounts in the SDHC E69 cells (Figure 5C). Conversely, the accumulation of activated p38 MAPK protein was not altered (Figure 5C). An AP-1 cis-element-dependent luciferase assay showed that JNK-dependent transcription was activated in the SDHC E69 cells (Figure 5D).

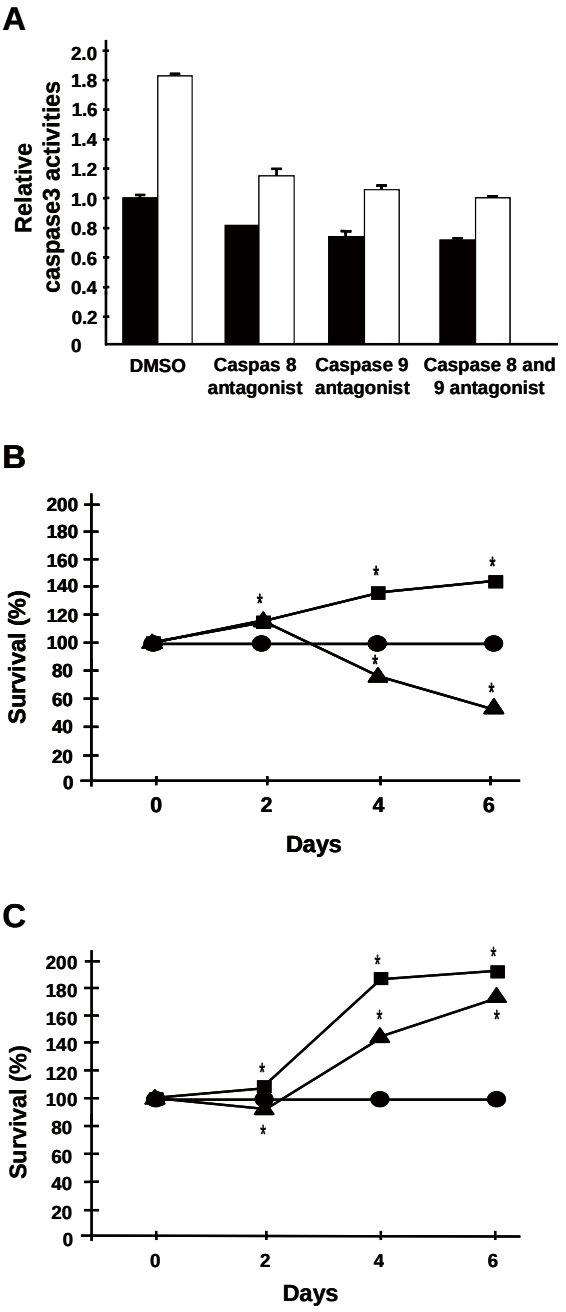

Figure 4. Variation of caspase 3 activity and survival rate by in response to caspase 8 and 9 antagonists. A: Caspase 3 levels were measured in NIH3T3 cells cultured for 3 month (closed boxes) and SDHC E69 cells (open boxes) in the presence of caspase 8 and 9 antogonists. $n = 3$. B and C: Viability of NIH3T3 cells (B), SDHC E69 cells (C) grown in the presence of caspase 8 (■) and caspase 9 (▲) antagonists and DMSO (●) additions. * $p < 0.01$, $n = 3$.

4. Discussion

We have previously established a transgenic SDHC E69 mouse cell line that contains a mutated SDHC subunit in complex II of the electron transport system (*6*). The SDHC E69 cells overproduced superoxide anion from mitochondria had elevated cytoplasmic carbonyl proteins and 8-OHdG in their DNA as well

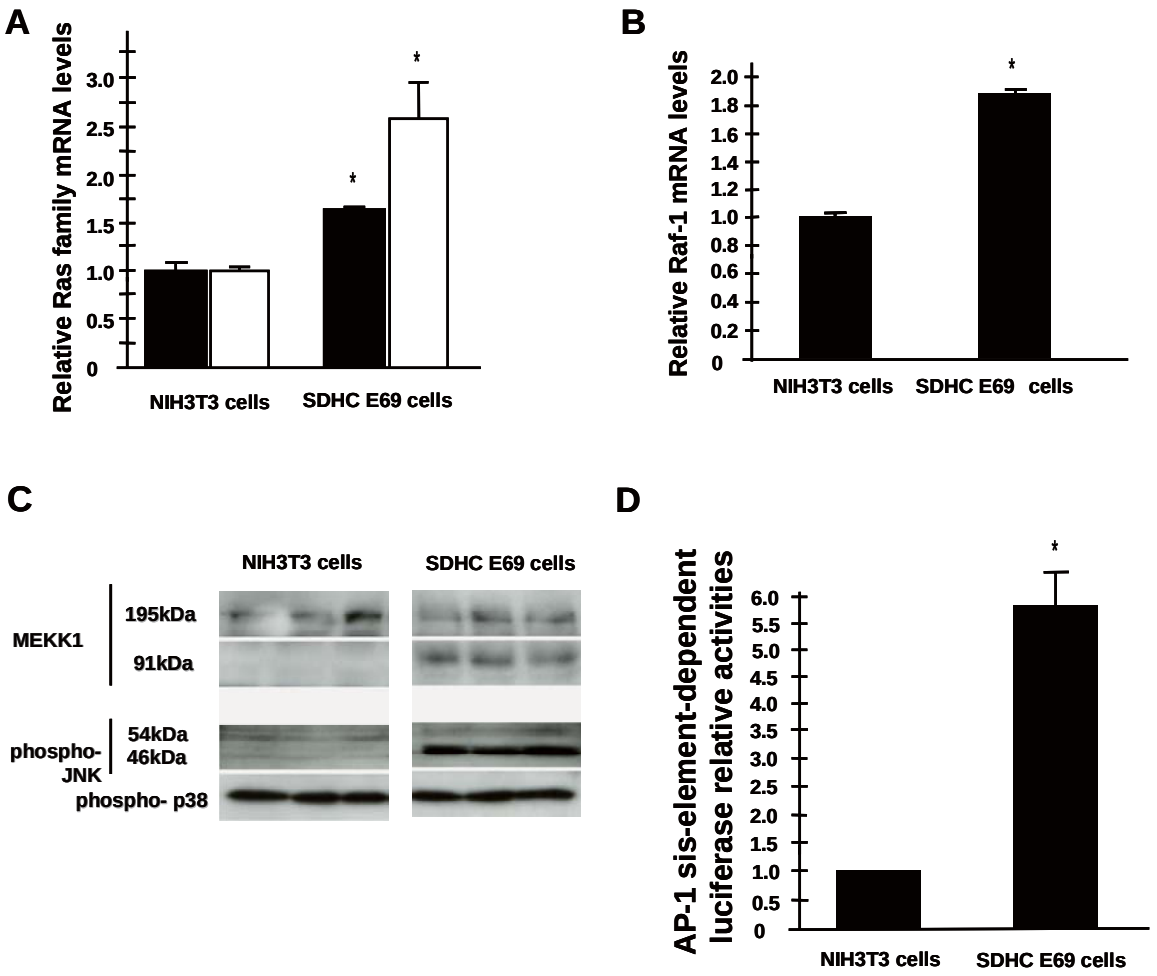

Figure 5. Alteration of stress-induced Ras-Raf and Ras-MEKK signal transduction pathways. A and B: Measurement of p21Ras (H-, N-, K-) (closed boxes), M-Ras (open boxes) (A) and Raf-1 mRNA expression levels (B) by Northern blot analysis. * $p < 0.01$, $n = 3$. C: Detection of MEKK1, phosphorylated JNK and p38 MAPK proteins, which are located and activated in downstream of MEKK1 activation, by Western blot analysis. $n = 3$. D: Measurement of transcriptional activation dependent on JNK activity by AP-1 cis-element-dependent luciferase activity. * $p < 0.01$, $n = 3$.

as significantly higher mutation frequencies than wild type (NIH3T3). There were many apoptotic cells in this cell line, as predicted by the observed increase in caspase 3 activity, decrease in mitochondrial membrane potential and structural changes in their mitochondria (*6*). In addition, some cells that escaped from apoptosis underwent transformation, as evidenced by the fact that SDHC E69 cells caused tumors within two weeks when injected under the epithelium of nude mice (Figure 1C) (*6*). The size of tumors remained unchanged even after one month (Figure 1D). It is known that NIH3T3 cells are transformed at high frequency by spontaneous mutations during long passage time or culture time. In fact, the cells, which did not proliferate within two weeks after the injection under the epithelium of nude mice, grew into huge malignant tumors one month (Figure 1A and B).

The SDHC E69-derived tumors showed no evidence of nuclear divisions and showed nuclear aggregation (Figure 2C). They had assumed the posture of a fibrous tumor, and the blood vessel and tumor border was not distinct. Moreover, tumor-associated cells were present (Figure 2D). In contrast, as expected of actively proliferating neoplasms, the tumors derived from the NIH3T3 cells had evidence of the apocytes and the nuclear divisions with characteristic dense staining of the cytoplasm (Figure 2A). In addition, the margin between the tumor and the blood vessel was distinct (Figure 2B). Thus, the thickness of the tumor cells layer was less when derived from SDHC E69 cells versus the NIH3T3 cells (Figures 2E and F). These histological data are consistent with the notion that the NIH3T3 cells are actively proliferating while the SDHC E69 cells are not. We speculate that there was no increase in overall tumor mass because cell proliferation was counterbalanced by increased levels of apoptosis.

Mitochondria from SDHC E69 cells overproduced O_2^-, leading to wide-spread apoptosis as caspase 3 levels were elevated (*6*). Caspase 3 acts relatively late in the caspase cascade, after cells are inextricably committed to apoptosis. To elucidate the mechanisms by which overproduction of O_2^- leads to supernumerary apoptosis, we examined the activities of various components of the apoptotic machinery as well as the various signaling

pathways that promote apoptosis. We first examined cytosolic and mitochondrial levels of cytochrome c in the SDHC E69 cells after the establishment and their NIH3T3 progenitors. Cytochrome c is known to be released from mitochondria and combines with apoptosis protease activating factor-1 (Apaf-1), procaspase 9, and dATP in the cytosol, triggering the activation of caspase 3 (*9*). As expected given its role in electron transport, cellular cytochrome c was found in the mitochondria in both the SDHC E69 cells and NIH3T3 cells (Figure 3A). Cytosolic levels in the SDHC E69 cells and NIH3T3 cells were lower than mitochondrial levels. However, cytosolic cytochrome c levels increased significantly in the SDHC E69 cells. This is consistent with the increase in caspase 3 that we have documented previously (*9*) and suggests strongly that elevated O_2^- production in the SDHC E69 cells sets into motion events that enable cytochrome c leakage from mitochondria, with caspase 3 activation and apoptosis the downstream consequences. We then examined the status of two proapoptotic members of the Bcl-2 family. First, we looked at Bax, which regulates cytochrome c release from mitochondria by translocating from the cytoplasm to mitochondria and subsequently altering mitochondrial membrane permeability (*10*). Bax primarily localized to the cytosol. In the mitochondria of the SDHC E69 cells compared to NIH3T3 cells, the Bax levels were significantly higher (Figure 3A). These results suggest that the increased cytosolic cytochrome c levels observed in the SDHC E69 cells could be attributed at least in part to Bax translocation. Second, we analyzed the Bid protein, which is activated by caspase 8 cleavage to produce 15 kDa tBid. tBid translocates to mitochondria to facilitate cytochrome c release by interacting with Bax (*11*). Bid and tBid levels were barely detectable in the SDHC E69 cells (Figure 3A). These results suggest that cytochrome c release was mainly caused by Bax localized in mitochondria. We also examined the activation of the tumor suppressor gene p53, as p53 has long been known to act as a transcription factor to promote apoptosis via Bax action (*12*). More recently, p53 has been also been shown to localize to mitochondria and, in a transcription-independent fashion, play an important role in the mitochondrial apoptotic pathway (*13*). We asked whether p53 might be involved in the cellular responses to the oxidative stress inherent to the SDHC E69 cells. Since p53 levels are normally held low via the action of MDM2, we examined MDM2 mRNA expression using Northern blot analyses. MDM2 mRNA exists in three isoforms: 3.0 kbp, 1.7 kbp and several short-forms (of roughly 1.2 kbp) which are generated by alternative splicing. They are translated into several forms, including p90 (which possesses p53 binding capacity), p76 (which lacks p53 binding ability) and short-form types (which also lack p53 activity and are found in transformed cells) (*14*). In NIH3T3 cells, MDM2 mRNA expression was equally distributed between the 3.0 kbp and 1.7 kbp mRNAs, which are translated into the p90 and p76 MDM2 proteins, respectively (Figure 3B). A dramatically different pattern was observed in the SDHC E69 cells, as only the short-form type was expressed, which is incapable binding to and promoting p53 protein degradation (Figure 3B). These results led us to test p53 protein levels by Western blot analysis. p53 levels were below the level of detection in NIH3T3 cells (Figure 3C). Conversely, p53 protein existed in copious amounts in the SDHC E69 cells, in which the short-form type MDM2 mRNA was expressed (Figure 3C). Moreover, most of the p53 protein was phosphorylated at serine residue 15 (Figure 3C), a modification known to activate p53 as a transcription factor, leading to cell-cycle arrest and apoptosis (*15*). These results strongly suggest that p53 exists as an active transcription factor in the SDHC E69 cells. We confirmed this in two ways. First, p21 protein, which is a p53 target gene and promotes the cell-cycle arrest, was highly expressed in the SDHC E69 cells but not in NIH3T3 cells (Figure 3C). Second, a luciferase-containing construct with a p53 binding cis-elements was transiently transfected into the each cell lines. Luciferase activity was over 2.8 times higher in the SDHC E69 cells than in the NIH3T3 cells (Figure 3D). Collectively, these data show that in the SDHC E69 cells, the oxidative stress resulting from mitochondrial overproduction of O_2^- leads to altered mRNA expression of MDM2 which in turn results in p53 accumulation and activation. As a consequence, p21 and presumably other p53 target genes are induced, resulting in cell-cycle delays and apoptosis. It is also possible that p53 is present in the mitochondria of the SDHC E69 cells to promote cytochrome c release and trigger apoptosis.

We next analyzed the relative roles played by caspase 8 and caspase 9 in elevated apoptosis in the SDHC E69 cells. Caspase 8 acts as an initiator caspase in the extrinsic apoptotic pathway while caspase 9 is activated by cytochrome c to initiate the intrinsic (mitochondrial) apoptotic pathway (*9*). When cleaved, both proteolytically activate executioner caspases such as caspase 3. We employed caspase 8 and 9 antagonists for this purpose and measured caspase 3 activity. As demonstrated previously, caspase 3 levels were higher in the SDHC E69 cells than in NIH3T3 cells (Figure 4A). In the NIH3T3 cells, caspase 3 activity was slightly decreased by each caspase antagonist ($p < 0.01$) (Figure 4A). Caspase 3 activity was not further reduced by addition of both caspase 8 and 9 antagonists in the NIH3T3 cells (Figure 4A). Since both caspase 8 and 9 participate in the extrinsic pathway, these results suggest that both caspases are active in NIH3T3 cells, albeit at low levels, in response to the low levels of extrinsic oxidative stress normally present in cultured cells. In the SDHC E69 cells, both

caspase 8 and caspase 9 inhibition had a larger effect on caspase 3 activity (Figure 4A). Since caspase 9 acts downstream of mitochondria, this suggests that the elevated apoptosis in the SDHC E69 transformed cells was the result of increased ROS generation in their mitochondria rather than involving the extrinsic pathway, in which case both caspase antagonists would be expected to have roughly equal inhibitory effects. Moreover, we tested the viability of cells cultured in the presence of each caspase antagonist. The survival rate of the NIH3T3 cells was decreased by treatment with a caspase 9 antagonist (Figure 4B). Some necrotic cell death was observed under these conditions (data not shown). Given that necrotic cell deaths were rarely observed in cells not treated with the caspase antagonists, we speculate that one consequence of the caspase 9-induced apoptosis is to protect tissues from stress-induced necrotic cell death that would otherwise result from mitochondrial oxidative stress.

In contrast to the results obtained with NIH3T3 cells, the presence of each caspase antagonist resulted in increased the cell growth and proliferation in the SDHC E69 cells (Figure 4C). This suggests that the SDHC E69 transformed cells might have developed oxidative stress resistance or even dependent cell growth and proliferation mechanisms. Moreover, both caspase 8 and 9 antagonists were inadequate to substantially reduce caspase 3 activity in the SDHC E69 cells ($p < 0.01$) (Figure 4A). This led us to speculate that a signal transduction pathway might be operative in these cells. Thus, while cytochrome c release from mitochondria was an important intermediary participant, components upstream of the mitochondria and independent of the mitochondria were responsible for initiating the apoptotic process in the SDHC E69 cells.

The Ras-Raf and Ras-MEKK signal transduction pathway has been shown to promote apoptosis in a mitochondria-independent fashion (*16*). For example, MEKK1 can be cleaved by many stimuli to generate a 91 kDa kinase that is a strong inducer of apoptosis (*17,18*). We tested to see if some of the elevated apoptosis in the 3-month SDHC E69 cells might be due to the activation of such signal transduction pathways. First, we analyzed p21Ras (H-, N-, K-Ras) and M-Ras mRNA expression levels by Northern blot analysis. Relative to the actin and G3PDH internal controls, p21Ras and M-Ras mRNA expression levels in the SDHC E69 cells were increased in comparison with the NIH3T3 cells (Figure 5A). Second, we tested Raf-1/C-Raf mRNA expression and MEKK1 protein concentration by Northern and Western blot analyses. Relative to the actin and G3PDH internal standards, Raf-1/C-Raf mRNA expression, which induces cell growth and proliferation, was significantly increased in the SDHC E69 cells in comparison to the NIH3T3 cells (Figure 5B). In additional, 195 kDa full-length MEKK1 protein, which can be cleaved to activate caspase 3, was present in unchanged amount in the SDHC E69 cells, but the activated p91 kDa MEKK1 protein, which induces apoptosis independent of the mitochondrial pathway, was increased (Figure 5C). Thus, it appears that Ras acts to increase MEKK1 expression in response to the oxidative stress and MEKK1 has been cleaved into its active form in the SDHC E69 cells. In addition, we analyzed JNK and p38 MAPK, which are located further downstream in these signal transduction pathways. Activated 54 kDa and 46 kDa JNK proteins were present in increased amounts in the SDHC E69 cells (Figure 5C). Conversely, the accumulation of activated p38 MAPK protein was not altered (Figure 5C). We also performed an AP-1 cis-element-dependent luciferase assay, which serves as a measure of JNK activity. JNK-dependent transcription was activated in the SDHC E69 cells (Figuer 5D).

We speculate that this explains the phenotypes we have previously observed in the SDHC E69 cells; namely, those of increased apoptosis, high levels of transformation into neoplasms and hypermutability. In addition, the growth characteristics of SDHC E69 cells in the epithelium of nude mice appear to mimic the slow growth of PGLs. It has been reported that the hereditary PGLs that are usually characterized by the development of benign, neural-crest-derived, slow-growing tumors of parasympathetic ganglia which are caused by mutations in the SDHC gene. Between 10% and 50% of cases are familial and are transmitted in an autosomal dominant fashion with incomplete and age-dependent penetrance. We speculate that these characteristics are related to the apoptosis induction caused by the mitochondrial.

Acknowledgements

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Original Article

Multilevel modeling of geographically distributed vitamin A deficiency

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Summary **Vitamin A deficiency is a common health problem in developing countries like India. Present study involves data on children aged between 6-36 months from northern part of India collected geographically, to find prevalence and important factors for risk of night-blindness. Both traditional logistic models and multilevel logistic models were applied to achieve our aim. All individual level variables vitamin A diet intake, age, vitamin A capsule intake and awareness about vitamin A were found significant for risk of night-blindness ($p < 0.05$) in individual level analysis. The effect of risk factors for night-blindness was smaller in multilevel modeling as compared to individual level model. The reason is that the previous model takes into account the within-block as well as among-block variations. Multilevel analysis, did not find, individual level variables vitamin A diet intake, awareness of vitamin A and vitamin A capsule intake significant for the outcome variable ($p > 0.10$). There was about 139% change in odd-ratio for vitamin A capsule taken once. Block level variable, average age of subjects in blocks comes out as significant factor ($p = 0.01$) for night-blindness. Thus, this paper demonstrates the usefulness of multilevel modeling in the analysis for epidemiology of disease risk, which is structured in a hierarchiary, with particular reference to geographical analyses of small area data.**

Keywords: Vitamin A deficiency, Multilevel models, Variance components

Introduction

Vitamin A plays an important role in body's defenses against infection. Children are vulnerable to vitamin A deficiency from the time they are born right up to three years of age. During this time, vitamin A deficiency is more lethal as it can cause permanent blindness, even death (*1*). The risks become less in older children, but vitamin A deficiency reduces overall immunity and makes all children susceptible to diseases like measles and diarrhoea. UNICEF estimates that vitamin A deficiency is a public health concern in 72 countries in Asia and Africa including India. The first repeat survey of the National Nutrition Monitoring Bureau (NNMB) in India, conducted during 1988-90 in the same villages that were surveyed earlier during 1975-79 showed that the prevalence of bitot's spot has declined from 1.8 percent to 0.7 percent (*2*). However, the second repeat survey conducted in 1996-97 showed no further improvement (*3*) and the prevalence is still above 0.5 percent, which is the WHO cut off level for a public health problem (*4*). The national averages do not give a full picture because the prevalence rates vary widely, not only between the states but also within a state (*5*). In many areas of public health research including night-blindness prevalence, the data structures are often hierarchical in nature. Generally, two statistical procedures are used to deal with these types of data. The first is to disaggregate all higher order variables to the individual level and carry out the analysis at individual level. Thus, the assumption of the independence of observations that is basis to the classical statistical technique becomes invalid. The other is to aggregate individual's level variables to higher level and does the analysis at higher level. Thus, all the within group variation, which may account for as much as 80% or 90% of the total variation is discarded before the analysis is carried out. Consequently, relations between aggregated variables are often much stronger giving

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distorted interpretation at the individual level (*6-15*). A number of papers in the epidemiological literature contend that ignoring the above issues (unobserved group effects) in data analysis produces downward biases in the standard errors of the estimated parameters which lead to erroneous estimates of the impact of the individual variable. This leads in some instances to faulty conclusions (*8-10*).

In this paper, our aim is two fold: (i) find the factors responsible for night-blindness and (ii) use hierarchical model to take into account group effects. The data was collected in a multistage scheme consisting of villages within blocks. The clusters of individuals within villages and villages within blocks naturally tend to have similar outcomes within clusters than between clusters. When the design of the study includes clusters, the inherent correlational structure can effect significance levels if it is not appropriately incorporated in the analysis (*16*). Therefore, two types of analyses were applied for achieving our aim, an individual level analyses as well as multilevel model approach. The factors that were found significant in individual level analysis, does not remain significant when multilevel models was applied. The present work illustrates how the use of multilevel modeling provides a greater insight than is possible from a single level approach that considers hierarchical structure and makes it possible to incorporate variables from all levels which leads to correct analysis and proper interpretation of data. Unobserved block and village effects were taken into account in multilevel models. It also takes into account individual level variable and block level variable of the subjects under study. Therefore, we define our objectives as: (i) to find block wise and overall prevalence of night-blindness using two types of model, and (ii) to illustrate why multilevel analyses are more appropriate and considered before doing any such analyses.

Methods and Materials

Data

The present study involved data from Hardoi district, a rural part of North India. The district, with an area of 5,986 sq km has a population of 3,397,414 (*17*). This population is distributed in 19 administrative block and 1883 villages. In first stage, the selection of eight blocks was done using the method of simple random sampling. In the second stage, 25 villages were randomly selected from each block. Finally, 16 households were chosen using systematic and purposeful sampling from each village. Only those households that had at least one child in the eligible age range were selected. Within the selected household, one child was randomly chosen from all the eligible children. Thus, 3,200 children aged 0.5-3 years were selected for the study (*18*). Four well trained teams-each comprising a medical officer and a nonmedical research assistant-conducted the survey. The medical officer examined the child for night-blindness and interviewed the parents for night blindness, immunization and actual doses of vitamin taken by the child during the past year. A questionnaire is used for obtaining data from the parents concerning their awareness of night blindness and dietary habits, focusing on the quantitative estimate of intake of vitamin A rich food. The medical officer examined the child for Xeropthalmia which includes conjunctival Xerosis, Bitot's spot, Active corneal Xerosis, corneal ulceration, cornear Scars. The presence of night-blindness in subject was taken as outcome variable, for which prevalence was found. The possible confounders (lowest level variables) for night-blindness considered were (i) weekly vitamin A diet intake by the child, (ii) age of child (iii) vitamin A capsule intake of child (iv) parents' awareness about vitamin A. The block (highest) level variables considered were (i) number of family health centre, and (ii) the average age in blocks. The later inclusion in the model was done to evaluate the group effect on the coefficients of individual level variables and the variance components.

Model description

A hierarchical structure is presented here. Since only one child was selected from each household, the lowest level was child or household. The households are nested within villages and villages are nested within blocks. This data structure implies that a multilevel analysis may be appropriate, and individual, village and blocks will be taken as first, second and third level, respectively of a multilevel model hierarchy. The multilevel logistic model (*12*) used to estimate the individual and block level variable's effect on outcome variable is

$$\log it\left[p_{ijk}\right] = \gamma_o + \sum_{h=1}^{4} \gamma_h x_{hijk} + \sum_{l=1}^{2} \mu_l z_{lk} + u_{jk} + v_k$$

where p_{ijk} is the probability that i^{th} individual in j^{th} village of k^{th} block has night-blindness, x_h (h = 1, 2, 3, 4) represents individual level variables and z_{lk} (l = 1, 2) represents block level variables in the model. Thus a unit difference between the x_h values of two individuals in the same block is associated with a difference of γ_h in their log odds, or equivalently, a ratio of exp (γ_h) in their odds. u_{jk} is the error term for the deviation from average proportion for j^{th} village in k^{th} block which is assumed to have mean zero and variance τ^2. Similarly v_k is the error term for k^{th} block which is assumed to have mean zero and variance φ^2. It doesn't include a level one residual because it is an equation for the probability p_{ijk} of the outcome rather than for the outcome. The individual-level model does not contain the last three terms of above equation.

The purpose of using this model is to control for

the correlation between subjects in a particular block. In this study, the model is estimated using computer software MLwin (version 1.1) for multilevel analysis (*19*). However, simple logistic regression analysis (*20*) was carried out using SPSS (version 11) software.

Results

The prevalence (in %) for night-blindness among different blocks were given in Table 1. The proportion was as low as 0.3% for block Kachauna whereas it was as high as 7.0% for block Tadiyawan. The overall prevalence rate for night blindness was 2.8%. The overall bitot's spot was found in 0.8% subjects, which is about 1.6 times WHO cut-off point, indicating a public health problem (*4*).

Table 2 presents statistical result when three separate analyses were applied on risk of night blindness. Model A is a simple logistic regression model with four individual level explanatory variables as total vitamin A intake, vitamin A capsule intake, age and awareness about vitamin A. Model B in table II is random intercept model with same variables as in Model A, and model C presents the results when block level variables, number of family health centers and average age of subjects in blocks was added to model B. Each model presents, the odds ratios and 95% confidence intervals for the fixed effects. However, models B and C, also shows the village-level and the block-level variance components with their standard errors. The categories in parentheses in Table 2 were reference groups and the odd-ratios for other categories were considered relative to them. All variables included in model A were found to be significant for the outcome variable (with a maximum *p*-value of 0.04). The night-blindness was two times more likely to be present in the individual whose mother was not aware of vitamin A than the one whose mother has knowledge about vitamin A. There was a protective effect of age, *i.e.* as the age increases the child was more likely to suffer from night blindness. All odd-ratios for individual level factor changed with addition of random intercept in model B *i.e.* when the intercept was allowed to vary between blocks as the blocks differ in proportion of outcome variable. The confidence interval of odd-ratio for all variables gets wider with maximum of 240% for vitamin A capsule taken once. There was 24% reduction in the risk of night-blindness for those who were not aware about vitamin A as compared to model A. Although there was only 1% change in the odd-ratio for the weekly vitamin A diet intake, its confidence limits gets wider by 50% making it non-significant ($p > 0.10$) for the outcome variable. The most dramatic change was in the protective effect of those who had taken vitamin A capsule once, as it was reduced by 139%. Except for the last two categories of age-groups all independent level variables vitamin A diet intake, awareness of vitamin A and the vitamin A capsule intake were found non-significant for the night-blindness in model B with a maximum *p* value change of 0.86 for vitamin A capsule taken once. This change

Table 1. Distribution of night blindness with blocks

Block	Prevalence (%)[a]
Ahirori	2.75
Behendar	3.25
Kachauna	0.25
Madhoganj	0.75
Mallawan	5.78
Sandi	2
Sandila	0.5
Tadiyawan	7
Total	2.78

[a] Total number of subjects in each block is 400.

Table 2. Odd ratios and 95% confidence intervals for fixed effects and coefficients and standard errors for random effects

	Model A	Model B	Model C
Fixed part	*ORs* (*95%CI*)	*ORs* (*95%CI*)	*ORs* (*95%CI*)
Weekly vitamin A diet intake	0.953 (0.913-0.994)[a]	0.97 (0.664-1.495)	0.973 (0.932-1.016)
(Aware about vitamin A)	1.00	1.00	1.00
Not Aware about vitamin A	1.962 (1.012-3.806)[a]	1.50 (0.660-3.400)	1.595 (0.715-3.556)
(Never taken Vitamin A capsule)	1.00	1.00	1.00
Once in last six months	0.383 (0.175-0.835)[a]	0.420 (0.146-1.190)	0.382 (0.139-1.050)
Once in life	0.384 (0.178-0.828)[a]	0.918 (0.297-2.840)	0.784 (0.269-2.282)
Age 6-12 months	0.510 (0.271-0.963)[a]	0.530 (0.271-1.010)	0.509 (0.265-0.979)[a]
13-18 months	0.490 (0.253-0.948)[a]	0.600 (0.311-1.160)	0.579 (0.298-1.125)
19-24 months	0.235 (0.097-0.573)[b]	0.260 (0.190-0.640)[b]	0.250 (0.101-0.618)[a]
25-30 months	0.427 (0.213-0.858)[a]	0.470 (0.240-0.940)[a]	0.455 (0.228-0.907)[a]
(31-36 months)	1.00	1.00	1.00
Block level variable			
Number of Family health centre			0.886 (0.704-1.118)
Average age of block			0.367 (0.165-0.819)[a]
Random part		*Variance component (S.E)*	*Variance component (S.E)*
Φ^2 (at block level)		0.656 (0.469)	0.443 (0.363)
τ^2 (at village level)		4.509 (0.701)	3.468 (0.630)

[a] $p < 0.05$, [b] $p < 0.01$.

would attribute to average proportion difference of night-blindness between the blocks and villages. There was seven times less variation between blocks than between villages within blocks. Here the usefulness of multilevel modeling comes out as if the variation at village and block level were not considered, the factors that were not important taken to be significant, thus affecting the outcome variable. All individual level interaction effects were found to be non-significant for the models considered.

Adding block-level variables in model C, the variability at block-level reduces by 33% and at village-level by 23%. It indicates that a definite amount of variability at block-level was explained by the block-level variable number of family health centers and the average age of subjects in blocks, which was found significant for the outcome variable ($p = 0.01$). There was almost no change in odd-ratio of weekly vitamin A intake, but its confidence limits reduced by 32% with addition of the block-level variables. The risk of night blindness increased by 6% for those who were not aware about vitamin A, but the relationship was still not significant ($p = 0.33$). Interactions between individual and block-level were explored but none was detected.

Discussion

There have been numerous individual/district/state level analyses dealing with prevalence percentage of night-blindness (*21,22*), but to our knowledge, in India, no study so far tried to analyze data using the more appropriate procedure of the present study. It is well known that studies involving a large number of important variables categorized suitably combined with the appropriate analytical procedure, will provide more valid and stable results. This study shows the significant factors for outcome variable along with the consequences of not choosing appropriate analysis. Hence, we say that age was significantly affecting the occurrence of night-blindness along with weekly vitamin A diet intake. There is a slight difference in the interpretation of an odds ratio estimated from a multilevel logistic regression model compared with the one obtained from a standard logistic regression model owing to the addition of the random variation at village and block-level. As for former the coefficients are generalized for a wider population of blocks, not restricted to eight blocks only. The effect of risk factors for night-blindness was smaller in multilevel modeling as it takes into account the within-block as well as among-block variation. The effect of the individual level variable, vitamin A capsule intake on the risk of night-blindness was increased with the addition of block effects. However, this variable was non-significant for night-blindness variable in multilevel analysis ($p > 0.10$). The independent variables were considered to have fixed effect. As, no further improvement in model was observed, when random slope was added to the model *i.e.* when the relationship between night-blindness and independent variables varied across blocks In general, while the direction of effects were similar between two methods considered, multilevel modeling led to wider the confidence intervals of fixed effects, specially of weekly vitamin A intake.

Austin *et al*. paper demonstrated that the confidence intervals of group-level variables gets wider when hierarchical structure of data was incorporated in analysis, whereas it was shown in our case that confidence interval of individual-level variables would also get wider when the variability among villages and among blocks was added to the residual variability among individuals. The implication of this will be that, a variable, which were significant in individual-level analysis does not remain significant when multilevel models, are used.

In summary, this study has demonstrated the potential usefulness of multilevel modeling in epidemiology analysis of disease risk measured across a heterogeneous population. Estimating a relationship between risk of night-blindness and individual level variables without taking into account the hierarchical nature of the data was shown to be mistaken. So, before reporting results for analyses where data has hierarchical structure, multilevel models should always be considered.

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Original Article

Validation and determination of the sensing area of the KINOTEX sensor as part of development of a new mattress with an interface pressure-sensing system

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Summary **The purpose of the present study was to examine the validity of the KINOTEX sensor via comparison with an existing sensor and to determine the sensing areas for a new alternating-air mattress that incorporates an interface pressure-sensing system. The study design was an evaluation study to validate and determine the sensing area of the KINOTEX sensor in comparison with another sensor. Study participants were fifty-one healthy volunteers over eighteen years of age, and the two sensors were placed between participants and an alternating-air mattress. We measured the contact area, full weight load, and maximum pressure in the calcaneal region using two sensors and obtained a graphic pressure distribution of > 40 mmHg in the lateral and supine positions. Correlation coefficients between sensors were $r = 0.88$ ($p < 0.001$) for the contact area, $r = 0.89$ ($p < 0.001$) for full weight load, and $r = 0.72$ ($p < 0.001$) at maximum pressure in the calcaneal region. Ninety-one percent of the pressure distribution was recorded in the central 50 cm of the bed, and 94.6% was recorded within an area 160 cm in length need the foot of the bed. We investigated the correlation between the KINOTEX sensor and an existing sensor and determined the necessary sensing area. Results suggested the feasibility of developing a new alternating-air mattress incorporating an interface pressure-sensing system to help prevent pressure ulcers.**

Keywords: Pressure ulcer, Prevention, Mattress

Introduction

Pressure ulcers are a common complication among the elderly and patients with spinal cord injuries or other conditions and have a significant effect on the length of hospital stay and unplanned rehospitalization (*1*,*2*). An ulcer is both difficult and costly to manage once it occurs, and tends to recur easily. Prevention of pressure ulcers is therefore a critical component of care in encouraging patients' rehabilitation and independence (*3*). A large-scale survey of pressure ulcers in Japan revealed a prevalence of only 3.6% in all types of hospitals (*4*). In contrast, the prevalence of pressure ulcers in the United States is reported to be 14.3-15.6% in acute-care settings (*5*) and 27.7% in long-term care facilities (*6*). Although the prevalence of pressure ulcers in Japan is relatively low, the proportion of severe pressure ulcers (stage III or IV) is high: stage III ulcers represent 22.5% of all pressure ulcers and stage IV ulcers represent 12.1% (*7*); the equivalent figures in the United States are 10% and 7%, respectively (*6*). This

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ailment has a great impact on patients' quality of life and medical costs in Japan, and the need to prevent this ailment is clear (*8*,*9*).

Pressure ulcers occur as a result of irreversible ischemic tissue dysfunction caused by prolonged diminishment or cessation of soft tissue perfusion between bone and the skin surface due to external forces applied to the body (*10*). Thus, the intensity and duration of external force are the most important factors in their development (*11*), and these factors are commonly controlled by the use of an alternating-air mattress (*12*). This type of commercially available mattress reduces the intensity of the normal force of the patient's body by adjusting the pressure within internal air cells according to the patient's weight and increasing the contact area with the body. It also limits the duration of that pressure via the inflation or deflation of air cells (*13*,*14*). However, this type of adjustment system presents some difficulties in its use with Japanese patients. Many Japanese patients have extreme bony prominences, which constitute one of the most important risk factors for pressure ulcers (*15*). The extremely high interface pressure from these prominences occurs independent of body weight and can result in severe pressure ulcers. Therefore, a method of preventing pressure ulcers based on the actual interface pressure itself is required.

A Cello (CR-270, Cape Ltd., Kanagawa, Japan) (*16*) pressure-measuring device was used to evaluate the intensity of local interface pressure at a specific point in time; however, the interface pressure changes readily with the slightest motion by the patient, and detecting how pressure is usually applied has not been possible. Inconclusive identification of the regions to which interface pressure is applied leads to situations in which the pressure is not sufficiently reduced, leading to the development, deterioration, and recurrence of pressure ulcers (*17*).

To overcome this problem, the interface pressure must be constantly monitored and regions where the critical pressure is applied for certain duration must be identified. If these steps were part of the alternating-air mattress system, the interface pressure could be successfully reduced according to the patient's position and movement, resulting in optimal efficacy for the alternating-air mattress. Considering the problems noted above, a new alternating-air mattress containing a pressure-sensing system must be developed in order to adequately control the interface pressure for the whole body.

Some currently available mattresses measure air pressure with a sensor pad; the purpose of such a system, however, is the automatic adjustment of air pressure to suit the user's weight, size, and position, rather than measurement of interface pressure intensity (*18*).

Sensors that employ various sensing principles are available: pressure-sensitive and conductive-ink film sensors, Piezo-resistive sensors, and air-pressure appreciation sensors. In pressure-sensitive and conductive-ink film sensors, the applied principle is that the electrical resistance is inversely proportional to the load of ink between the electrodes (*19*,*20*). This sensor is the most sensitive of all sensors but is very expensive and less versatile because of the low durability of the ink. Piezo-resistive sensors that utilize elemental devices convert stress into voltage (*21*). This sensor cannot follow curved surfaces and this causes difficulties when applied to the human body and to air mattresses. Air-pressure appreciation sensors detect air leaks from small air-filled pockets when a load is applied to the top sheet (*22*). This system is difficult to use in clinical practice because the equipment used to detect the air pressure is unwieldy. These examples indicate that the available sensors are inadequate for use in air mattresses.

With these problems in mind, the present study focused on a fiber-optic tactile sensor (KINOTEX sensor, NITTA Corp., Osaka, Japan) incorporating new sensing technology and developed an instrument to measure interface pressure over the entire body. Because the problems inherent in available sensors have been resolved in this sensor, it should enjoy clinical use. Features of the new sensing technology include envelopment, noninvasiveness, and durability. The mechanical properties of the KINOTEX sensor have already been validated in comparison to the BIG-MAT sensor, which is a sensor commonly used for research purposes. When simultaneously applying a particular pressure to the KINOTEX and BIG-MAT sensors, Pearson's product-moment correlation coefficient between the output values of the two sensors was $r = 0.88$ ($p < 0.001$). As correlations above 0.75 indicate a very good to excellent relationship (*23*), this indicates the high degree of sensing validity for the KINOTEX sensor, despite the differences in measurement principles.

The validity of using the KINOTEX sensor with the human body must be demonstrated prior to its clinical use, and placement of the sensing points must be considered when measuring interface pressures over the whole body and in various body positions. The purpose of the present study is to investigate the validity of the KINOTEX sensor when sensing a human body and to determine the sensing areas and placement of the sensing points based on the interface pressure distribution data for the whole body in supine and lateral positions.

Materials and Methods

Participants

Healthy volunteers over the age of eighteen were recruited and informed of the research methods. Only

those volunteers who understood the design, objectives, and risks of the study and who gave informed and written consent participated in the study. They were informed that participation was entirely voluntary and that data obtained from the survey would be analyzed in a depersonalized format.

Materials

KINOTEX is a technology that employs a new sensing principle: a change in optical properties is proportional to the extent of deformation in common polymer foam materials caused by an external influence such as pressure (Figure 1). Optical fibers are implanted in pairs. The light from a light-emitting diode is carried to the integrating cavity of a sensing point by one fiber, the light in the cavity is passed to a photodiode through another fiber, and the external force is calculated by measuring the illumination energy intensity. In the KINOTEX sensor prototype used in this study, the pitch indicating the interval between sensing points was 3.2 × 2.2 cm and sensing area was 50 × 180 cm in the center of 88 × 180 cm polymer foam that included 1,080 sensing points. Saturation sensitivity, which meant the maximum value of normal force which could be read by the sensor, was 4 N; creep, which was gradual deformation of a material under pressure, was 0.9% (120-180 sec); hysteresis, which was a lag occurring between the application and removal of a force in elastic materials, was 30%; and the sampling rate, which indicated the number of samples per second, was 1.0 Hz. The KINOTEX sensor is fabricated from urethane foam as a polymer foam material. The sensor was enclosed within a shade cover because the sensing principle utilizes a change in optical properties.

The KINOTEX sensor was evaluated in comparison to the BIG-MAT sensor. The latter is a pressure-sensitive and conductive-ink film sensor that measures the reduction of resistance for each sensor element due to loading of the element in the normal direction. The sensing principle of this sensor has been described elsewhere (*18*). Briefly, the striped matrix of row and column electrodes consist of an electrically conductive silver ink and are printed directly onto two separate polymer films. The pitch was 1.016 × 1.016 cm, the sensing area was 89.4 × 195.1 cm and included 16,896 sensing points, saturation sensitivity was 171 mmHg, creep was 1.2% (120-180 sec), hysteresis was 6.1%, and the sampling rate was 4.0 Hz.

The placement of the two sensors was considered beforehand. Basically, the BIG-MAT sensor is not suitable for curved surfaces. Therefore, the BIG-MAT was placed on the KINOTEX sensor to prevent the body from sinking too far, and the two were placed on an alternating-air mattress (TriCell, CAPE CO LTD., Kanagawa, Japan); the centers of the sensors and the mattresses were aligned.

The BIG-MAT sensor does not always measure pressure accurately, as it tends to wrinkle easily. This problem was addressed for each position of the participant by measuring the pressure distributions after wrinkles were removed. The two sensors were then equilibrated and calibrated before they were set. For all participants, the mattress was set to static mode and based on initial tests the input value of the user's weight was standardized to 30 kg to meet certain conditions and to prevent bottoming-out or saturation of the sensor.

Protocols

The room temperature was adjusted to 28 degrees Celsius. After the sensors were set as described above, the KINOTEX and BIG-MAT sensors were turned on. Participants were informed of the objectives and procedure of the research and gave their written consent after they entered the room. The participants wore short-sleeved T-shirts and gym shorts to standardize the measuring conditions. After verifying that each sensor was in working order, the participants were asked to lie on the sensors in the lateral position. At this time, wrinkles in the BIG-MAT sensor were removed as much as possible. Since creep could reduce the accuracy of this study, the pressure distribution data from the KINOTEX and BIG-MAT sensors were recorded and saved 2 min after the positions were stabilized; simultaneous digital photographs were taken to capture the position. Participants were then asked to lie in a different lateral position, which was captured by digital

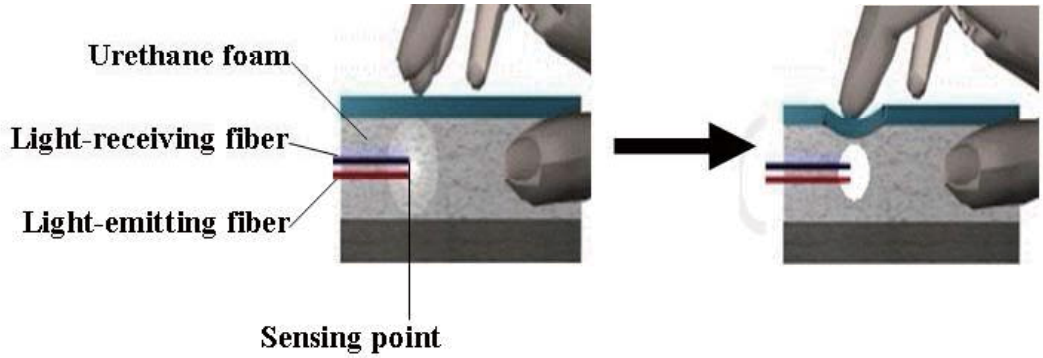

Figure 1. Optical properties of the KINOTEX sensor change in proportion to the extent of deformation in the common polymer foam material.

photography, and the pressure distribution data were similarly recorded. Finally, participants were asked to lie in the supine position with their hands folded on their stomach, this position was captured by digital photography, and the pressure distribution data were similarly recorded. This study was conducted between December 22, 2005 and January 17, 2006.

Data analysis

Pearson's product-moment correlation coefficients were calculated from the output of the sensors using the pressure distribution data from the supine position alone. A linear relationship between the output of the two sensors was sought because of the different measurement principles used by each. The items considered in the data analysis were the contact area for the sensor, full weight load, and maximum pressure at the calcaneal region. The contact area (cm^2) was defined as the superficial measurement of the sensing region of the sensor. Full weight load (kg) was defined as the total load in the sensing region of the sensor. Maximum pressure at the calcaneal region (mmHg) was defined as the highest individual sensor value in the region. Pressure at the calcaneal region was chosen to represent maximum pressure because (i) the calcaneal region is a common site of pressure-ulcer development; (ii) this region has the most pronounced bony prominence, even in healthy subjects; and (iii) measurement in this region is difficult because of its small contact area. As there was a relatively large difference in pitches between the two sensors, comparison of the maximum pressure at the calcaneal region involved the mean pressure for a square area; in relation to a sensing point area for the KINOTEX sensor (7.04 cm^2), this area consisted of nine sensing points with a center point yielded the highest pressure with the BIG-MAT sensor (9.29 cm^2). This method accounted for differences in area between the pitches of the two sensors.

The distribution of data measured by the BIG-MAT sensor in the lateral and supine positions was used to determine sensing areas. Pressure distribution information for the head was considered unnecessary and was excluded from analysis because pillows are placed under the head in clinical practice. The pressure distribution data for all participants and all positions were overlaid, the maximum values at each sensing point were identified, and a graphic representation of data equal to or greater than 40 mmHg (Figure 2) was created. Pressure of 40 mmHg and above is perceived to be the critical pressure for pressure ulcer development (*17*). A rectangular sensor with a longitudinal side of 192 cells, as illustrated in Figure 2, was then visualized within the 88 × 192 cells of the BIG-MAT sensing area d (BIG-MAT cell interval: 1.016 cm). This rectangle was moved by 1 cell, from a to b in Figure 2, and percentages for the number of pressure distribution

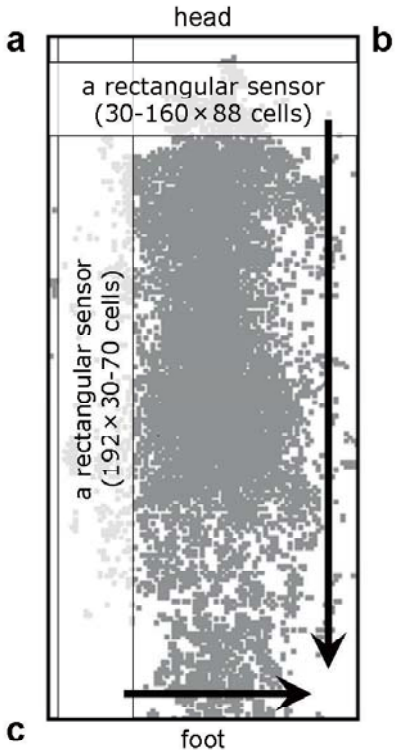

Figure 2. Rectangular sensors are visualized to calculate the percent coverage of the pressure distribution area.

points within the rectangle were plotted for all pressure distribution points (the percent coverage for the pressure distribution area) at each point. This analysis was repeated for 30, 40, 50, 60, and 70 cells from the transverse side, and the maximum percent coverage for the pressure distribution area in each rectangle was plotted; the best transverse length and sensor location were determined according to the result. To determine the longitudinal dimension, rectangular sensors with a transverse side of 88 cells and longitudinal side of 30-160 cells were visualized, and the percent coverage when moving the rectangles from a to c in Figure 2 and the maximum percent coverage for each rectangle were plotted.

All statistical analyses were performed using Statistical Analysis System ver. 9.1 (SAS Institute Inc, Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Results

Participant characteristics

Table 1 shows the demographic data for study participants. Twenty-seven (52.9%) of the 51 participants were female. The mean age ± SD of all participants was 34.4 ± 10.1 years. Body Mass Index (BMI), which indicates relative weight for height, was normal (BMI: 18.5-24.9) in forty-one (80.4%) participants according to the Classification of Overweight and Obesity by BMI (*24*). Three

Table 1. Participant characteristics (n = 51)

Sex: *n* (%)	
Male:	24 (47.1)
Female:	27 (52.9)
Age: mean ± SD (y)	34.4 ± 10.1
Height: mean ± SD (cm)	164.5 ± 8.7
Weight: mean ± SD (kg)	57.4 ± 10.4
BMI: *n* (%)	
≤ 18.4	7 (13.7)
18.5 - 24.9	41 (80.4)
25.0 ≤	3 (5.9)

Abbreviations: SD, standard deviation; BMI, body mass index.

participants classified as overweight (BMI ≥ 25.0) were male and none of the participants were classified as obese (BMI ≥ 30.0).

Validity of KINOTEX sensor

Pearson's product-moment correlation coefficients between the KINOTEX and BIG-MAT sensors were $r = 0.88$ ($p < 0.001$) for the contact area, $r = 0.89$ ($p < 0.001$) for the full weight load, and $r = 0.72$ ($p < 0.001$) for maximum pressure at the calcaneal region (Figures 3, 4, and 5). Correlation coefficients of 0.50-0.75 indicate a good relationship, while those above 0.75 indicate a very good to excellent relationship (*23*); all three of the coefficients obtained in the present study met this criterion.

Determination of the sensing area

Figure 6 presents the pressure distribution for values equal to or greater than 40 mmHg, as obtained from the maximum pressure distribution data for all participants and all positions. Figure 7 shows the maximum percent coverage of the pressure distribution area with respect to changes in the transverse length of the rectangular sensor. These values occurred when the rectangle was positioned in the approximate center of the BIG-MAT sensor for all transverse lengths. The maximum percent coverage of the pressure distribution area with respect to changes in the longitudinal length of the rectangular sensor is shown in Figure 8. Wide variations in pressure distribution were recorded in the foot region in comparison to other areas.

Discussion

The clinical validity of the KINOTEX sensor was assessed by comparing it to an existing sensor and by taking the sensing area into consideration. The results provide a solid foundation for the development of a new air mattress that contains a pressure-sensing system and that contributes to the prevention of pressure ulcers in clinical settings.

Validity test for the KINOTEX sensor

This study compared the output of the KINOTEX

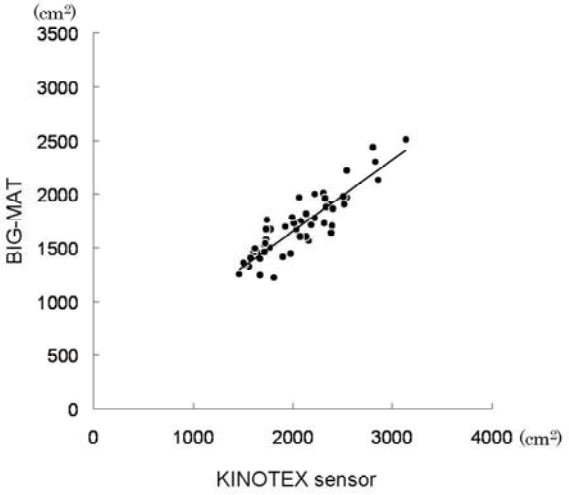

Figure 3. Correlation coefficients for contact areas.

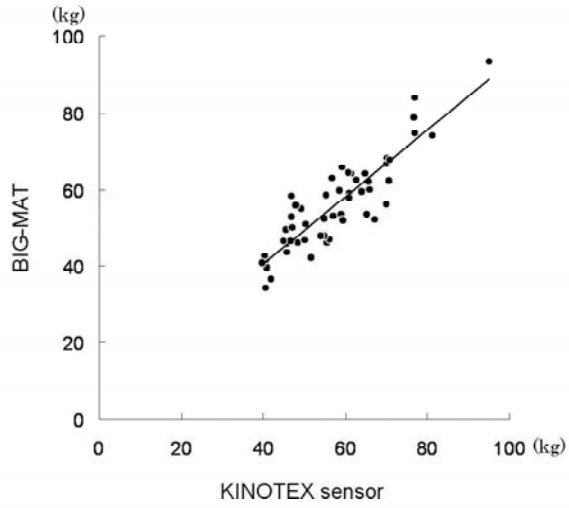

Figure 4. Correlation coefficients for a full weight load.

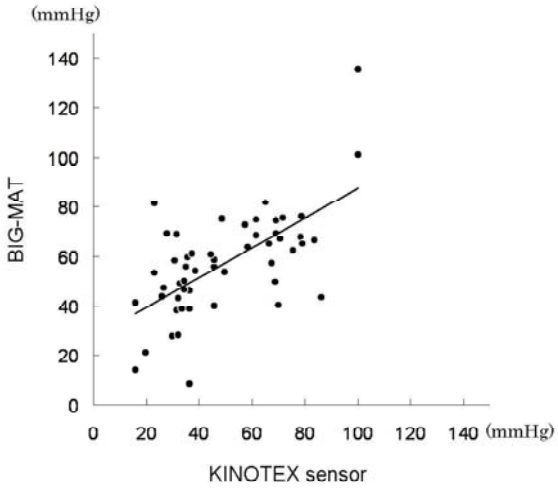

Figure 5. Correlation coefficients for maximum pressure at the calcaneal region.

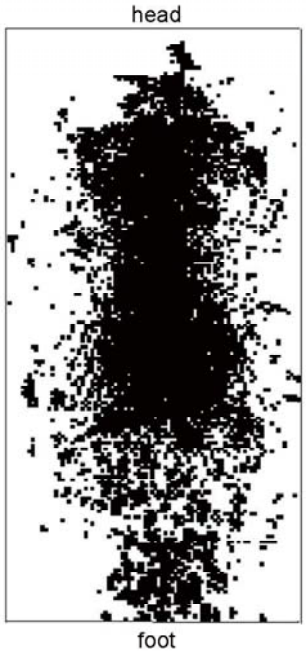

Figure 6. Pressure distribution of over 40 mmHg, obtained from the maximum values of pressure distribution data for all participants and all positions.

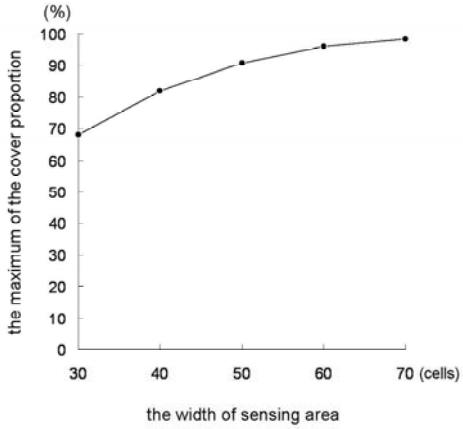

Figure 7. Maximum percent coverage of the pressure distribution area in each rectangle when the transverse side is changed.

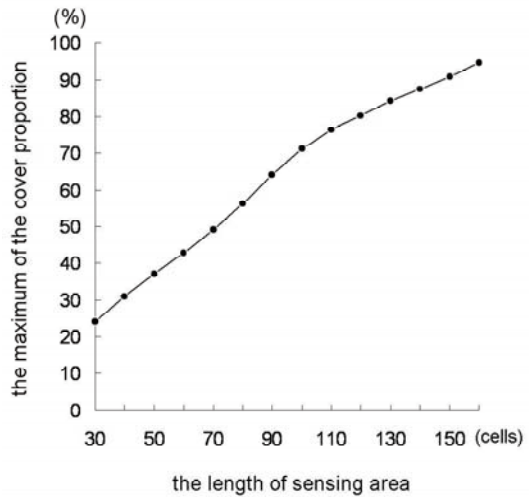

Figure 8. Maximum percent coverage of the pressure distribution area for each rectangle when the longitudinal side is changed.

and BIG-MAT sensors and used Pearson's product-moment correlation coefficients to test the validity of a new instrument that measures interface pressures for human patients. The BIG-MAT sensor is one of the most accurate and reliable devices for pressure-sensing (*25*,*26*). In preliminary analysis, Pearson's product-moment correlation coefficient between the full weight load measured by the BIG-MAT sensor and the participants' weight was $r = 0.88$ ($p < 0.001$, data not shown); the output of this sensor was therefore considered accurate.

The results of this study indicate a good to excellent correlation between the output values for the contact area, full weight load, and maximum pressure at the calcaneal region and similar values obtained with existing high-sensitivity pressure mapping sensors. This demonstrates the validity of the KINOTEX sensor as an instrument for interface-pressure measurement. Although the measurement of pressure at bony prominences is difficult due to the small contact area, which can easily fall between sensing points, good results were obtained for maximum pressure at the calcaneal region; this suggests the clinical usefulness of the KINOTEX sensor. However, other conditions such as a smaller contact area and a higher pressure than saturation sensitivity should be considered in order to accommodate various body sizes and positions.

The correlation coefficient was also used to compare the two sensors with differing measurement principles. While a strong correlation was shown, the slope of the line was relatively low for maximum pressure at the calcaneal region. A conversion factor is therefore needed to map one to the other.

Determination of the sensing area

Ideally, sensing points would be placed throughout an air mattress; however, it is uneconomical to place sensors in areas where interface pressure is applied less intensely. Thus, the sensing area must be limited to enable efficient use of the sensors. The measurement results for the BIG-MAT sensor in the lateral and supine position suggest that a width of 50 cm in the center of a bed is sufficient for the transverse side of the sensing area, taking the whole body and each position into consideration. In this study, 90% of the pressure distribution equal to or greater than 40 mmHg occurred in the 50 BIG-MAT cells (= 50.8 cm), and the percent coverage of the pressure distribution area increased insignificantly, even when the width exceeded 50 cells (Figure 7). The longitudinal side of the sensing area should cover the entire area except the 30-cm head region. Results demonstrated that the percent coverage increased significantly as the length increased (Figure 8) and that the pressure distribution results for the calcaneal region varied widely compared with those for other areas (Figure 6). This variation in the calcaneal

region could reflect the fact that different participants' heels were placed at different locations for each body position. A larger sensing area at the foot of the bed is needed in order to monitor pressure at the calcaneal region, which is one of the most common and important sites of pressure ulcer development. A longitudinal sensing area 160 cm in length is recommended, and no sensors should be located in the head region.

This determination enabled the distribution of sensing points to be restricted to 47.3% of the BIG-MAT sensing area (88 × 192 cm).

Clinical use of the KINOTEX sensor

The current study found the KINOTEX sensor to be valid for monitoring interface pressure over the whole body. This is a new approach that has not previously been attempted in clinical settings. Reflecting the current sensing area results when using the KINOTEX sensor will facilitate more efficient monitoring.

The KINOTEX system enables the simplification of nursing care and improved education for patients and family members regarding pressure-ulcer prevention. This is because pressure values can be presented graphically and the pressure distribution can be displayed in color on-screen.

KINOTEX sensors may be incorporated into alternating-air mattresses in the future. However, reducing the thickness of the urethane foam, 10 mm in the current KINOTEX sensor, should help to increase its efficacy as a clinical tool. Its clinical durability and economic efficiency must also be examined in comparison to the standard protocols for the prevention and management of pressure ulcers.

The present results may be generally applicable for certain individuals like, for example, patients whose physical attributes are close to the standard Japanese attributes and for inpatients in acute care settings. This study did not include physical attributes that are unique to the elderly in Japan or those from other countries. The elderly in Japan are at very high risk of developing pressure-ulcer because of problems concerning weight loss, bony prominences, and joint contracture. An additional point to note is that there are fewer obese patients in Japan. Further research is needed regarding sensing areas and pitch before KINOTEX sensors can be used with every patient.

The present study was conducted to test the static criteria of the KINOTEX sensor; further studies are needed to evaluate dynamic criteria for its use in clinical settings.

Conclusions

The current study investigated the correlation between the KINOTEX sensor and an existing sensor and evaluated the sensing area of the KINOTEX sensor. Results demonstrated that the KINOTEX sensor is a valid an instrument for measuring interface pressure in healthy volunteers. The sensing area of the KINOTEX sensor required a width of 50 cm in the central area and a length of 160 cm, excluding the head region, to measure interface pressures for the whole body. The results of this study suggest that new air mattresses incorporating this pressure-monitoring sensor are feasible. Such mattresses would help to prevent pressure-ulcers.

Acknowledgments

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Original Article

Fever of unknown origin: Revisit of 142 cases in a tertiary Chinese hospital

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Summary **To investigate the causes of fever of unknown origin (FUO), we analyzed the clinical data on 142 patients with FUO admitted to our department from January 2002 to December 2003. After various examinations and specific treatment, a definitive diagnosis was reached in 122 cases. Of them, 51 cases (35.9%) were caused by infections, 46 (32.4%) were due to autoimmune diseases, 18 (12.7%) were due to tumors, 7 (4.9%) were due to other diseases, and in 20 (14.1%) the cause was still unknown after hospitalization. In conclusion, infection is the main cause of FUO. Autoimmune diseases and malignant tumors are both significant causes. Most patients with an FUO were ultimately diagnosed with various examinations and careful analysis.**

Keywords: Fever of unknown origin, Retrospective studies

Introduction

Fever of unknown origin (FUO) is extremely difficult to diagnose. It has been defined as an illness with a rectal temperature exceeding 38.3°C on at least three occasions, lasting at least 3 weeks, and with no diagnosis reached after 1 week of inpatient investigation (*1*). Many prospective studies of patients with FUO have been performed around the world using this definition. In 1991, Durack and Street (*2*) proposed that the requirement of one week of inpatient investigation be modified to either days of inpatient investigation or three visits without discovering the source of fever. China has followed the original criterion of Petersdorf RG because of economic factors. The spectrum of diseases seems to be determined by geographic and economic factors, and it appears to change with time (*3*).

Materials and Methods

A total of 142 patients seen by this department from January 2002 to December 2003 were included in a 2-year study. The inclusion criteria were: (a) fever for at least 3 weeks; (b) fever > 38.3°C at least twice; (c) absence of diagnostic suggestions (diagnostic hypotheses) after history, clinical examination, and a series of screening investigations. Patients with immune deficiency were excluded.

Results

During the two-year period of the study, 142 patients were followed up as FUO. Of the 142 patients, 69 were male and 73 were female. The mean age was 48.7 years, with ages ranging from 14 to 81. Twenty-five patients (17.6%) were older than 65. A diagnosis was reached for 122 (85.9%) patients. No diagnosis was reached for twenty patients (14.1%), three of whom recovered spontaneously. Of those, 14 deteriorated rapidly; these patients were presumed to have had a malignant disease upon discharge and died without necroscopy because of customs prohibiting it. Infections were found in 51 (35.9%) patients, autoimmune diseases in 46 (32.4%), neoplasm in 18 (12.7%), and miscellaneous diseases in 7 (4.9%).

Infections were observed to be the most common aetiology of FUO (Table 1). Infectious causes of FUO are shown in Table 2. The mean age of affected patients was 51.7 years of age. Patients broke down into 26 males with a mean age of 49.8 years and 25 females with a mean age of 53.8 years. Salmonellosis was diagnosed in 8 patients who had atypical and mild

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Table 1. The diagnostic categories of 142 cases

Diagnostic category	No of patients (n = 142)	%
Infection	51	35.9
Autoimmune disease	46	32.4
Neoplasm	18	12.7
Miscellaneous	7	4.9
No diagnosis	20	14.9

Table 2. Infectious causes

Diagnostic category	No of patients (n = 51)
Bacterial infection	36
Salmonellosis	7
Infective endocarditis	7
Tuberculosis	6
Pulmonary tuberculosis	4
Tuberculous peritonitis	1
Intestinal tuberculosis	1
Urinary tract infection	5
Blood stream infection	4
Pneumonia	4
Biliary tract infection	1
Liver abscess	1
Odontogenic fever	1
Viral infection	5
Fungal infection	3
Mycoplasma pneumonia	5
Malaria	2

Table 3. Autoimmune disease

Diagnostic category	No of patients (n = 46)
UCTD	10
Adult Still's disease	8
SLE	8
Dermatomyositis	3
Ankylosing spondylitis	3
Multiple myositis	2
Polymyalgia rheumatica	2
Mixed connective-tissue disease	2
Rheumatoid arthritis	2
Urticarial vasculitis	1
Scleroderma	1
Behcet's disease	1
Autoimmune liver disease	1
Overlap syndrome	2

Table 4. Neoplastic causes

Diagnostic category	No of patients (n = 18)
Lymphoma	8
Myelodysplastic syndrome	2
Cancer of unknown primary site	2
Gastrointestinal tractus Ca	2
Malignant histocytosis	1
Hemophagocytic syndrome	1
Langerhans cell histiocytosis	1
Thyroid adenoma	1

Table 5. Miscellaneous diseases

Diagnostic category	No of patients (n = 7)
Drug fever	2
Necrotic lymphadenitis	2
Digestive tract ulcer	1
Post-infection status	1
Chronic fatigue syndrome	1

symptoms. Infective endocarditis was determined to be the cause of fever in seven patients. Urinary tract infection was diagnosed in 5 patients. Chronic pyelonephritis with obstruction of the ureter was diagnosed in 2 patients by abdominal CT. Chronic recurrent prostatitis was found in two male patients. Atypical pneumonia was found in 4 patients who were elderly or had already received extensive antimicrobials.

Table 3 shows autoimmune causes. The mean age of affected patients was 45.5 years of age, with ages ranging from 14 to 80. Patients broke down into 16 males with a mean age of 49.9 years and 32 females with a mean age of 43.4 years. There were more female than male patients, and the mean age was lower for females than males but the difference was not statistically significant. The most significant autoimmune causes were UCTD (undifferentiated connective tissue disease), SLE, and adult Still's disease.

Table 4 shows neoplastic causes. The mean age of affected patients was 53.7 years of age, with ages ranging from 19 to 71. Patients broke down into 12 males with a mean age of 54.3 years and 6 females with a mean age of 52.5 years. Lymphoma was the most significant cause. All 8 lymphoma cases were diagnosed by clinical and pathological evidence, *i.e.* bone marrow biopsy in 2 patients and lymph node biopsy in 6 patients. There were more male than female patients; the mean age differed but the difference was not statistically significant.

Details on miscellaneous diseases not falling under the preceeding categories are shown in Table 5.

Discussion

In this study a diagnosis could not be reached for 14.1% of the cases. There may be no definitive diagnosis (9-25.6%) in some cases (*1*,*2*,*4-6*). In the current study, the causes of FUO were infections (35.9%), autoimmune diseases (32.4%), neoplasms (12.7%), and miscellaneous diseases (4.9%). Infections were still the most frequent cause of FUO but their frequency was lower than that reported in other studies (*1*,*2*,*4-6*). There were many factors contributing to these causes. One factor may be the ease with which patients receive extensive antimicrobial therapy. In this study, autoimmune diseases were the second most significant cause of FUO and were more frequent than in other studies (*7*). Many doctors who work in non-teaching hospitals had limited awareness of autoimmune diseases, so patients were limited to teaching hospitals.

Neoplasms were the third most significant cause of FUO in this study. The use of CT and MRI imaging allows tumors to be more easily detected (*3*). However, diagnosing hematological malignancies can still be difficult because of the absence of localized symptoms. Perhaps this explains why lymphoma and myelodysplastic syndromes were frequently identified in patients with FUO. Diagnosis of atypical lymphoma is difficult; multiple lymphonode biopsies may help

in diagnosis. Cancer of unknown primary site (CUPS) (*8*) was also a significant cause of neoplasms. In these cases, cancer cells were found in pleural or peritoneal effusions but the primary tumor was not found.

There were more undiagnosed cases at discharge than in other studies (*6*). Only three of those patients recovered spontaneously. Fourteen who deteriorated rapidly were presumed to have had a malignant disease upon discharge; they died but a necroscopy was not performed because of local customs and economic factors. In other studies, most cases of FUO led to a diagnosis and in undiagnosed cases all or most of the patients recovered spontaneously (*4*,*7*,*9*).

Causes of FUO and their relative frequencies in the population should be known because FUO is usually caused by either an uncommon condition or an unusual presentation of a well-known disease. Thus, frequent diseases rather than rare ones should be considered first when diagnosing FUO.

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