# BioScience Trends

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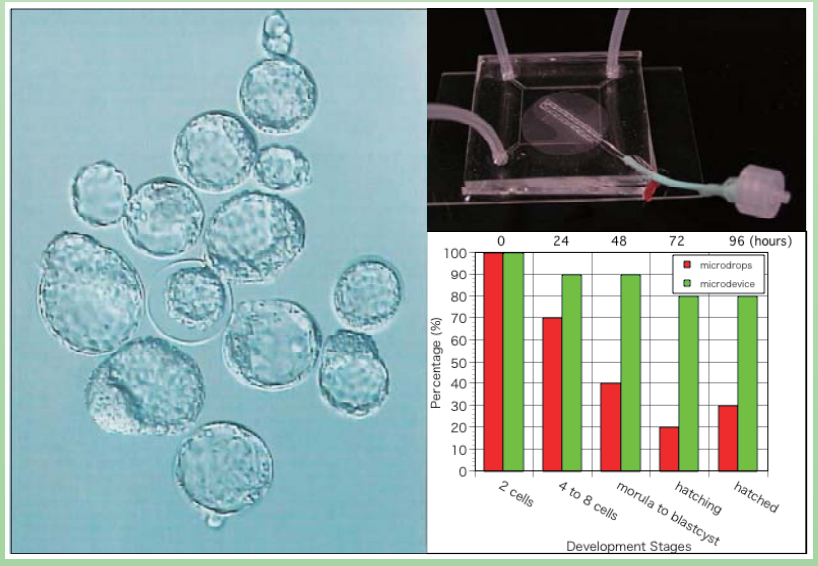


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# BioScience Trends




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**BioScience Trends** publishes original research articles that are judged to make a novel and important contribution to the understanding of any fields of life science, clinical research, public health, medical care system, and social science. In addition to Original Articles, BioScience Trends also publishes Brief Reports, Case Reports, Reviews, Policy Forum, News, and Commentary to encourage cooperation and networking among researchers, doctors, and students.

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# BioScience Trends

## Editorial Board

# CONTENTS

*Volume 1, Number 3, 2007*

## News



## Commentary



## Review



## Original Article

## CONTENTS (*Continued*)

# A more womb-like chip for IVF was born in Japan

**Huanli Xu, Yoshinori Inagaki**

***Key Words:*** *In vitro* fertilization (IVF), womb, embryo, chip

Since the birth of Louise Brown, the first test-tube baby in 1978, *in vitro* fertilization (IVF) has produced approximately three million infants worldwide. Although success rates continue to improve, the technology is still far from reliable enough for infertile couples, many of whom attempt IVF multiple times. Many researchers are focusing on developing technologies that try to mimic the womb in order to improve IVF.

According to Popular Science, Teruo Fujii at the University of Tokyo's Institute of Industrial Science and his colleagues (Yasuyuki Sakai at the University of Tokyo's Center for Disease Biology and Integrative Medicine and the Inui Maternity Clinic) have invented a plastic chip-like incubator that nurtures early embryos like a real womb does. Their research was presented at a meeting of the European Society for Human Reproduction and Embryology in Lyon, France, August 2007. (*October 30, 2007, Sharon Guynup Popular Science. http://www.popsci.com/popsci/science/250216b8ff0f5110vgnvcm1000004eecb ccdrcrd.html.*).

Fujii's team has created a novel “lab on a chip” that is 2 millimeters across and 0.5 millimeters high in which up to 20 eggs can be fertilized. Currently, IVF eggs mature while resting on the top floor of a two-tiered silicone microchip, a very womb-like environment *(see Figure 1 and the cover of this issue*). “We wanted to culture embryos in an environment that is closer to what happens inside the body,” Fujii said.

To test the device, Fujii and his team carried out several experiments on mice, comparing resulting embryos with those produced using conventional IVF. First, 10 mouse eggs were carefully placed individually inside a “cage” on the top floor of a two-tiered silicone microchip. Next, sperm cells were added to fertilize the eggs. Over the next 48 to 72 hours, a pulsing micro-pump washed the early embryos with rhythmic waves of a culture fluid that helped them grow in an attempt to simulate what happens in the womb. Then, the embryos were removed, and the healthy ones were implanted into the actual wombs of mother mice (*26 July, 2007, Linda Geddes, New Scientist. http://www.newscientist.com/channel/sex/mg19526146.200-wombonachip-may-boost-ivf-successes.html*).

Currently, test-tube human embryos are kept in “microdroplets” -- a mixture of mineral oil and culture fluid -- to keep them from drying out. However, the artificially fertilized embryos tend to grow considerably slower in microdroplets because during IVF eggs or embryos were often moved or washed with culture fluid, causing changes in temperature and pH.

In another advanced experiment in mice, Fujii's team suggested the chip was more successful than traditional microdroplets in improving the success rate of IVF. Fertilized eggs grew much faster on the chip than in traditional microdroplets. After 2 days, the chips contained around 77-119 cells, compared to 58-94 cells in microdrops. The faster-growing embryos are believed to stand a better chance of survival after being reinserted back into the mother's womb. At the time for implantation into a real womb, 80% of embryos held inside the chips were ready for implantation, while only 20% in microdroplets grew to that stage in the same amount of time.

“It's a large difference between the conventional method and our device,” Fujii said. He and his colleagues believe that their “womb-on-a-chip” is superior to the conventional system.

First, endometrial cells, which line real wombs, are also grown on the chip. These cells release chemicals tailored to the changing needs of a growing embryo, delivering the amino acids, proteins, and growth

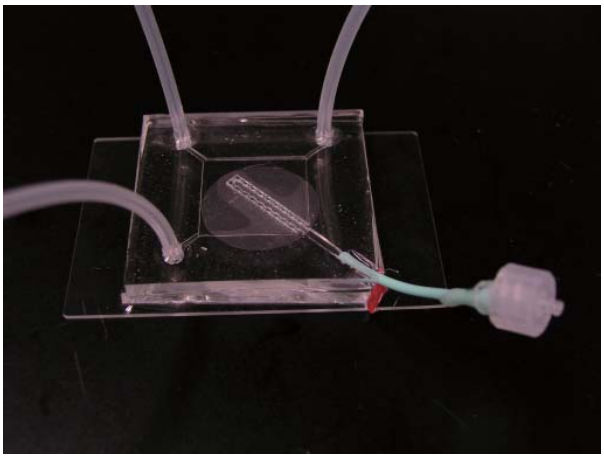

**Figure 1.** The newly developed IVF device (photo by Prof. Fujii).

factors that help the embryo develop. Second, the chip functions a bit like a gentle car wash, using pumps to periodically bathe the cells in the fluid needed to keep them alive. It can be programmed to infuse the inside of the device with as little as one trillionth of a liter of liquid and employs tiny chambers to contain the eggs and prevent nutrients from becoming too diluted. Another benefit is reduced stress to an embryo since it is not sucked into a pipette, which can cause physical damage or fatal changes in pH or temperature.

Following these successful experiments in mice, Fuji's team was granted permission to begin trials on human embryos. There is, however, always a level of uncertainty when research moves from animals to humans, which is not limited to reproductive technologies.

Until recently, IVF was only for those with fertility problems. The strong ethical stance against assisted reproduction, especially that involving human embryos, often puzzles scientists. Some people are worried by increasingly artificial means of reproduction. One concern is that some people are resorting to IVF-pre-implantation genetic diagnosis (PGD) so they can choose their child's most basic characteristic: sex. Another concern is that "assisted reproduction" will help and encourage women to have children ever-later in life (*October 22, 2007, Sharon Guynup, Boston Globe. http://www.boston.com/yourlife/health/articles/2007/10/22/scientists_try_to_build_a_better_womb_for_ivf/.*), which is worthy of concern given the increased risks for mother and infant when an older mother gives birth.

Such concerns have not deterred Fujii and his team, who are now "working towards translating this technology for humans." He is, however, aware that fertility treatment is still an invasive, psychologically challenging procedure and that it could take at least five years for the technology to reach clinics.

(*the University of Tokyo, Tokyo 113-8655, Japan.* .)

# Dams causing algae-induced ill health and poverty? Stories from the Mekong

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***Key Words:*** Dams, poverty reduction, algae, the Mekong

The Se San River is a trans-boundary river originating in Vietnam and running through Northern Cambodia where it culminates in the Mekong River. It is recognized as one of the top three rivers in Vietnam in terms of its hydropower potential. Development of the Hydropower Project started in 1993, and the project was completed in 2002. Today three more projects are under construction on the Vietnamese side of the Se San River. At the same time, several reports (*1*,*2*), as well as information garnered from people living along the Cambodian part of the Se San River have revealed health-related problems and deterioration of the surrounding ecosystem.

In June of 2007, environmental groups obtained the "Final report on environmental assessment on the Cambodian part of the Se San River due to Hydropower Development in Vietnam" published in December 2006. The report has drawn great attention because it highlights for the first time that the possible connection between health problems of people living downstream of the Se San River - including itchiness, skin lesions, stomach aches, headaches, and respiratory problems - and toxic blue-green algae in the river following dam construction on the Vietnam side (*3*). For the local inhabitants, vital daily activities such as cooking, bathing, and washing clothes depend on the water from the river, so their health could be directly affected if the quality of the water deteriorates. The report also indicated that the 10 to 30% reduction in fish numbers and species in the last ten years was due to the dam, and warned of an expected future risk of malnourishment and associated deterioration of health status, especially for growing children, unless viable alternative protein sources to fish can be introduced and the lacking riverbank vegetables can be replaced by other species with equivalent nutritional content (*4*).

In July, the Economist warned of a plan of 240 MW hydro-electric dam in the Mekong River in the Siphandon district (4,000 islands) of southern Laos bordering Cambodia, a region home to picturesque waterfalls, tranquil waterways and a colony of endangered Irrawaddy dolphins. If the dam goes ahead as scheduled, it may block a channel used by over 200 species of migratory fish, such as the giant catfish, to bypass waterfalls between Laos and Cambodia. This would disrupt their bleeding cycles and might destroy the livelihood of the riparian population (*5*). It is notable that this world-renowned economic journal criticized plans for construction of a dam, aiming to bring economically gains to one of the poorest countries in Asia by selling electricity generated by the dam. Laos has an abundance of mountains and is traversed by the surging rivers of the Mekong, which runs its full length. Seven dams are already working and 11 more are under preparation, including the massive, and controversial, 1,088 MW Nam Thuen 2 project. The government sells the electricity to Cambodia, Thailand and Vietnam. However, as reported in the environment assessment

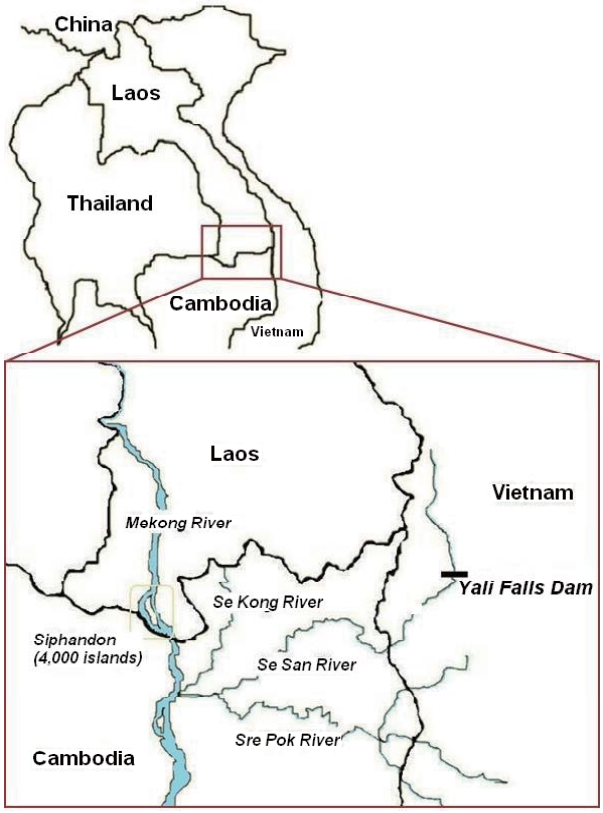


**Figure 1.** Mekong Map.

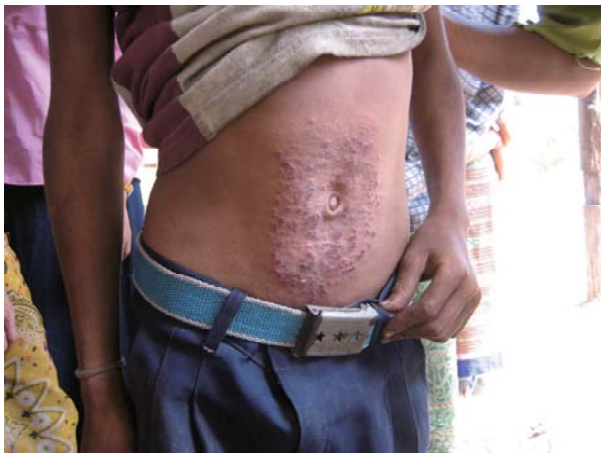

**Figure 2.** Skin lesion of a child in Pau village along the Se San River.

**Figure 3.** Abandoned riverbank vegetable field due to change of water level.

on the Cambodia side, if started, this new project would bring about deterioration in ill health status, as well as a reduction of the fish population so crucial to the livelihood of the local people as a source of income and of dietary protein.

Construction of the dam is generally supported by donor partners and the World Bank granted a loan to the Nam Thuen 2 project because expected revenues from dam-produced electricity are expected to be used for poverty reduction in accordance with the Bank's mission (*6*). However, the issue of support and compensation for local people who have to be relocated elsewhere due to the construction remains unclear (*7,8*). Environmental groups are trying to mitigate these problems for the people living with the enriched Mekong River who are weak and vulnerable, and to preserve the precious ecosystem, which the current world economic system seems intentionally to be ignored.

Japan has started preparation for the G8 summit in Hokkaido next year, and the main item on the agenda is the Environment (*9*). Although Japan is a main contributor to the World Bank, we should take the initiative to address the threatened ecosystem and the interests of the people living along the Mekong. In the past we have overcome serious and shameful environmental pollutions and related human rights abuses, such as Minamata disease and Itai Itai disease (*10,11*). The economy is always deemed more important than the environment and the victims are always vulnerable people like fishermen and farmers.

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# Measuring the economic and social consequences of CVDs and diabetes in India and Pakistan

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**SUMMARY** **In India and Pakistan, CVD and diabetes has assumed alarming levels. However, governments in these countries are ill-prepared for coping with this epidemic. This paper reviews the literature for those studies which have addressed the current and foreseen economic and social consequences of CVDs and diabetes in India and Pakistan. This review adopts a societal perspective by incorporating the impact on the individual, the household, and the health and economic sectors. The review finds that in both countries there has been a paucity of systematic efforts to measure the economic and social impact of CVDs and diabetes. Moreover, the review has found an absence of assessments of direct and indirect costs in the same study, inattention to the social consequences of these diseases and methodological inconsistencies which make comparative analyses restrictive.**

**It is critically important that a research base of studies investigating the impact of the diseases in India and Pakistan be undertaken. Gathering these data is critical since both countries have many competing health priorities reflected in the intransigency of key health indicators and the data emerging from these countries suggests that the social gradient is reversing. Therefore, in the absence of hard evidence to these diseases are likely to remain outside of mainstream public health planning. With these data in hand, the choice for health planners with regard to important decisions may become clearer. Similarly, the implications for productivity and revenue earnings make a powerful argument in order to focus the attention of private sector employers on these issues.**

***Key Words:*** Cardiovascular diseases, diabetes, socio-economic impact, India, Pakistan

## 1. Introduction

Countries in South Asia, especially India and Pakistan in spite of impressive economic and political changes and notable gains in two important health indicators - life expectancy and infant mortality - continue to face severe challenges of social underdevelopment and ever-widening disparities between rich and poor. Although one out of every four people in the world live in South Asia, their annual contribution to global production is only 2% and almost 50% of them live below the poverty line with poor access to healthcare and other essential basic services (*1*).

Although infectious diseases remain a formidable enemy, chronic diseases, especially cardiovascular diseases (CVDs) and diabetes are increasing the health challenges facing India and Pakistan. In 2002, almost 75% of the 45 million adult deaths reported worldwide were attributable to non-communicable diseases (NCDs) (*2*). Of these, CVDs and diabetes, which fall under the rubric of NCDs, accounted for approximately 30% and 2%, respectively of all deaths.

In India, CVD-related deaths accounted for approximately 32% of all deaths in the year 1998 whereas in Pakistan estimates for the year 2001 indicate that they account for 25% of the total deaths within the country (*3*). In India, mortality arising from coronary heart disease (CHD) is expected to increase to 2.03

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million in 2010 (*4*). Diabetes mellitus, an important risk factor for CVDs, blindness, kidney failure, and lower-extremity amputations, has assumed alarming levels in South Asia. India, the "diabetic capital of the world" is estimated to have approximately 30 million people living with the disease (*5*). The picture in Pakistan is equally gloomy: 12% of the population, over the age of 25 suffers from diabetes and 10% have impaired glucose tolerance (IGT) (*6*). This figure is expected to escalate to 14.5 million people affected by the year 2025, only to be exceeded by India with approximately 57.2 million affected (*7*).

As stated by WHO (*2, p85*) "CVDs have not only emerged in all but the very poorest countries, but are already well advanced; this growing burden has real potential to hinder social and economic development". Despite the projections and the urgency in planning for the epidemic, many governments, particularly in the middle- and low-income countries are ill-prepared for coping with this epidemic.

Data that document and quantify the magnitude of the NCD problem in terms of prevalence and incidence of diseases and their underlying risk factors and determinants remains very inadequate, particularly in developing countries. However, in recent years measures have been taken to improve NCD surveillance systems and registries; in particular, progress has been made in developing and subsequently establishing within countries, a risk factor surveillance system, which is suited for application in low resource settings - the WHO STEPwise approach to surveillance (STEPS). However, on the other hand, inadequate attention has been paid to gathering evidence relating to the economic and social impact of NCDs. The WHO Commission on Macroeconomics and Health (CMH) addressed the impact of ill-health in terms of increasing health care costs and productivity losses, however omitted NCDs in the discussion.

This paper reviews the literature for published and unpublished studies, which have addressed the current and foreseen economic and social consequences faced by India and Pakistan in view of the significant CVD and diabetes burden. We also describe here a simple but logical approach for undertaking economic and social analysis of CVD and Diabetes in low resources settings.

## 2. Methods

### 2.1 *Review of literature*

The primary searches were conducted on the electronic databases Medline and Science Direct and the search was limited to English language articles published between 1985 and 2005. Specific key words included "diabetes mellitus", "cardiovascular diseases", "India", and "Pakistan" were used in combination with more general terms including "costs", "cost-of-illness studies", "economic impact", "social impact", "developing countries" and "South Asia". References from selected articles were also reviewed. Since research and published literature on CVDs and diabetes in India and Pakistan - especially that evaluating the economic and social impact - continues to lag behind that of other public health concerns (for instance infectious disease), it was considered necessary to consult the 'grey' literature for regional and country specific articles and reports. This was carried out in two ways: 1) through web searches (*e.g.* google scholar) which carry both published and unpublished resources and 2) contacting leading researchers working in the area of CVDs and diabetes in India and Pakistan.

The electronic search yielded 524 references and the inclusion criteria were relatively broadly specified to include 1) articles which have analyzed the economic and social consequences of diseases and conditions; and 2) articles which have addressed the economic and social impact of CVD and diabetes in India and Pakistan. On this basis, a total of 37 articles were retrieved and included in this review. Of these, the economic cost of CVD and diabetes in India and Pakistan is the primary focus which only 10 articles address.

### 2.2 *Framework for review and analysis*

For this review and analysis, we used a framework, which we found simple and appropriate, which we are describing here for the benefit of other researchers in this field. The assessment of the social and economic impact of CVD and diabetes is important on two accounts; firstly, because the age group at greatest risk includes adults in their most productive years and secondly, because management of these diseases often involves expensive health care. The economic and social impact will be experienced first by the affected individuals and their families, with the effects thereafter filtering to the health and social welfare system and other public and private sectors. The framework adopts a societal perspective by incorporating the impact on the individual, the household, the health and economic sectors.

Step1. The first step was to identify the factors influencing health seeking behaviour: socio-economic variables, such as income, educational level and place of residence; costs and types of services available including physical access and perceived quality of care; and type and severity of illness were considered (see Table 1 for steps 1, 2 and 3).

Step 2. We then went onto explore the coping strategies of households in the advent of illness: Here we looked at financial costs (using cash and mobilizing savings, deferring expenditures such as education, sales of assets, and borrowing loans); time costs (intra

**Table 1.** Social and economic impact on household

| Variable of analysis | Factors |
|---|---|
| **Social consequences** | |
| Factors influencing health seeking behaviour | • *Socio-economic variables* (income, educational level, place of residence *etc*.)<br>• *Costs and types of services available* (costs of services, physical access, perceived quality of care *etc*.)<br>• *Type and severity of illness* |
| Coping strategies | • *Financial costs* (using cash and mobilizing savings, deferring expenditure (*e.g.* education), sales of assets, loans *etc*.)<br>• *Time costs* (intra-household labour substitution, changing capital-labour mix of production, hiring labour, free community labour *etc*.)<br>• *Precautionary Measures* (adapting diet and lifestyle to prevent CVD and diabetes) |
| **Economic consequences** | |
| Direct Costs of care | • Hospital, transport, drug costs *etc*. |
| Indirect Costs of care | • Lost earnings associated with morbidity, mortality and disability<br>• Lost earnings on the part of care-givers |

**Table 2.** Sector wide impact

| | |
|---|---|
| **Health sector impact** | |
| Health care costs (direct costs) | • Costs of inpatient care<br>• Costs of outpatient care (*e.g.* general practitioner, district hospital, pharmacy *etc*.)<br>• Costs of long-term care in the case of disabilities |
| **Economic sector impact** | |
| Productivity losses (indirect costs) | • Costs due to absenteeism<br>• Costs due to permanent disability<br>• Costs due to mortality |

household labour substitution, changing capital labour mix of production, hiring labour, free community labour *etc*.); and precautionary measures (adapting diet and lifestyle to prevent CVD and diabetes.

Step 3. This included an examination of the economic consequences of CVDs and diabetes and was limited to direct costs of care (hospital, transport, drug costs *etc*.) and indirect costs of care (lost earning associated with morbidity, mortality and disability and lost earnings on the part of care-givers.

Step 4. The final was to explore the 'sector wide impact' (see Table 2). This entailed a review of health sector impact (costs of inpatient care, costs of outpatient care and costs of long-term care in the case of disabilities) and impact on other economic sector impact (costs due to absenteeism, permanent disability and mortality).

## 3. Results

The review of 524 articles and reports found less than 20 articles studies which fulfilled the study objective. In the following sections we present the results of our review.

### 3.1 *Individual and household impact*

In developing countries, diabetes exhibits higher prevalence amongst the higher socio-economic groups (SES) than the lower SES (*8-10*). This pattern is evident in India where the more affluent had twice as high prevalence compared to the lower SES (*10*).

Ramachandran and colleagues (*11*) in a study of the impact of poverty on the prevalence of diabetes found that diabetic subjects from a lower SES have a higher prevalence of cardiac disease, neuropathy and cataract but a lower prevalence of retinopathy compared to those from higher SES. Moreover, risk factors including hyperglycaemia, dyslipidemia, hypertension, smoking and alcohol consumption were higher in the low SES group. Mohan *et al*. (*5, p31*) observe "Disparities in health by SES among people with diabetes could reflect the direct effects of deprivation on health or could result indirectly from the effects of unfavourable health behaviours linked to lower SES. Another potential reason could be the 'inverse care law' whereby access to and use of services is reduced, and the quality of care provided is substandard, for patients with the greatest need".

#### 3.1.1 *Health seeking behaviour and coping strategies*

In the Urban District Diabetes study carried out in Bangalore, a large Indian city, it was found that there was a four-year delay in diagnosis of diabetes between the highest and lowest SES (*12*). Education was also found to be an important factor in explaining the time of diagnosis (*13*). The Cost of Diabetes in India (CODI) study undertaken by Kapur *et al*. (*13, p20*) found that delays in diagnosis were directly related to the level of education: "College-educated people were on average diagnosed 7 years before people with no literacy". Kapur further remarks that those with a college education despite having diabetes for a longer period of time, had lower rates of complications (55%) compared to those with little or no education (80%).

The National Diabetes Survey of Pakistan, conducted in the 1990's found that despite a high prevalence of diabetes and IGT in Pakistan, 36.3% were unaware of their condition (*14-16*). Although this data was not analyzed further by socio-economic status, education and other variables, looking at the trends in neighbouring India it is reasonable to assume that both the control and awareness rates would be even lower in the lower socio-economic groups (*16*).

The conclusion that can be drawn from this is obvious: the most socio-economically deprived groups of society are highest at risk for developing diabetes-related complications because of delays in diagnosis. As it has been well documented with respect to other

diseases and conditions, the costs of health care often carries dire consequences for the individual and the household.

What of the social consequences? As described earlier, the death of a household head on the household is quite profound, more especially in the poorer sections of society and carries with it significant inter-generational consequences. Leeder *et al*. (*17*) estimated that in India CVD deaths among those in the 35-64 age categories affect almost 5 million household members[1]. The significance of this is even more profound when one considers that almost 75% of the elderly in India and more especially 86% of urban elderly women are economically dependent on their children. This is likely to increase in the coming years as the population ages.

The gender dimensions of CVD and diabetes have not been sufficiently explored. As observed by Leeder *et al*. (*17, p34*) "The impact of CVD on women is both direct, when they experience the illness themselves, and indirect, when their educational and economic circumstances are affected by death or disability due to CVD of family members". MacKay and Mensah (*18, p42*) remarked that women are "…less likely to be referred to a heart specialist…. More likely to enter the health system with a diagnosis of a second heart attack … after a first stroke, women are more kept in hospital longer, and remain more disabled than men receiving similar care".

3.1.2 *Household costs*

*Diabetes* The CODI study based on a large community-based survey was designed to illicit the direct and indirect costs associated with diabetes (*19*). Table 3 indicates the average annual costs arising from diabetes in India. Indirect costs constitute 64% of cost followed by the annualized costs of hospitalization (12.2%). Generalizing these findings a crude estimate suggests that the economic cost of diabetes to India is about USD 444 million.

Kapur *et al*. (*13*) identify several factors as contributing to the costs of care. Late diagnosis of diabetes often results in as many as 50% of people developing complications (*e.g*. retinopathy, nephropathy *etc*.). These complications often require expensive therapies and prolonged hospitalizations, thereby contributing to increasing direct costs and indirect costs in terms of productivity loss and absenteeism. With 3 or more complications, the costs of care were almost 48% higher.

Of critical importance to this paper, is the question of how costs of care impact different socio-economic groups. A study in India revealed that those with high income spent 12% of their total income on treatment as compared to 59% by the low income group (*20*).

*Cardiovascular diseases* A study in Karachi, Pakistan showed an incidence of 1.66 per 1,000 per year for stroke (*21*). Khealani *et al*. evaluated the cost of acute stroke care and its determinants at the Aga Khan University Hospital (AKUH), a tertiary care hospital in Karachi through a retrospective review of medical and billing records of 443 patients with acute stroke between 1998 and 2001.

Table 4 below presents the total average costs and a breakdown by laboratory, pharmaceutical and radiology. Average total cost of the care was PKR 70,714 (USD 1,179), and more than a third (39%) was incurred by hospital bed/room charges, with pharmacy, radiological investigations and laboratory investigations accounting for 19%, 18% and 12% respectively. The average total cost was directly related to length of hospital stay and was largely driven by laboratory and pharmacy costs. It was also found that the cost was also related to the type of ward the patient was admitted to; the intensive care unit (PKR 155,010 ≈ USD 2,584) was 2.5 times more expensive than the general ward (PKR 60,574 ≈ USD 1,010). The significance of these costs which are borne entirely by the patient is important when considered against the fact that gross national income per capita is USD 690 (*22*). Similar data for inpatient care was not available for India.

We were able to locate only one article which examined the social and economic impact of NCDs as

**Table 3.** Direct and indirect annual patient costs from diabetes in India

| Item | Costs (INR) | Percentage of Total Cost |
|---|---|---|
| Doctor visit | 853 | 4.28 |
| Monitoring and lab | 1,609 | 8.08 |
| Treatment | 2,262 | 11.36 |
| Hospitalization (annualized) | 2,434 | 12.22 |
| Mean total direct cost | 7,158 | 35.94 |
| Mean total indirect cost | 12,756 | 64.06 |
| Total estimated annual cost | 19,914 | 100 |

*Source: Kapur et al. 2004. Extracted from Table 1, p19.*

**Table 4.** Direct patient cost of acute stroke care, Akuh, Pakistan (Cost in PKR, 2003, USD in Parenthesis)

| Length of Stay (days) | Average laboratory cost | Average pharmacy cost | Average radiology cost | Average total cost |
|---|---|---|---|---|
| 1 | 3,272 (USD 55) | 1,743 (USD 29) | 9,148 (USD 152) | 19,597 (USD 326) |
| 2 - 3 | 3,446 (USD 57) | 2,134 (USD 36) | 10,968 (USD 182) | 25,568 (USD 426) |
| 4 - 7 | 6,504 (USD 108) | 11,732 (USD 196) | 12,151 (USD 203) | 49,705 (USD 828) |
| 8 - 30 | 17,404 (USD 290) | 32,258 (USD 538) | 15,074 (USD 251) | 153,586 (USD 2,559) |
| > 30 | 59,298 (USD 988) | 160,291 (USD 2,672) | 35,510 (USD 592) | 588,239 (USD 9,804) |

*Source: Khealani et al. 2003. Extracted from Table 2, p553.*

[1] The authors assumed an urban household size of 5.8 and a rural household size of 5.5.

a whole in comparison to communicable diseases in India and Pakistan. This study arose from a recently reported population-based cross-sectional survey conducted in Pakistan (*23*). The results showed that 37.4% of the households spend an average of PKR 405 (USD 6.77) on the treatment of communicable diseases whereas 45.2% of the households spend an average of PKR 3,935 (USD 65.80) on the treatment of NCDs. These data show that a significantly higher percentage of households spend more on treatment of non-communicable diseases compared with communicable diseases.

3.2 *Sector wide impact*

3.2.1 *Health system*

For this section we could find only one study where estimates were made for India and China for the economic costs of all NCDs. In this study, Popkin *et al*. (*24*) estimated that annual health care system costs in India arising from NCDs were USD 1.1 billion in 1995, of which 10% were state expenditures. Beyond these much aggregated estimates, there has been little inquiry to establish the direct costs (inpatient, outpatient and long term care) of CVDs and diabetes in India and Pakistan.

3.2.2 *Productive economic sectors*

Leeder *et al*. (*17*) estimated that India will experience a dramatic increase of 35% in CVD-related morality for those in the 35-64 age group between 2000-2030 based on WHO mortality rates (*25*) and World Bank population projections (*26*). They also estimated that the number of productive years of life lost to CVD would increase by 95% from 9,221,165 in 2000 to 17,937,070 in 2030. The most dramatic increase will be in the 45-54 age group, where there will almost be a doubling of the years of productive life lost.

Popkin *et al*. (*24*) estimated[2] for India the productivity costs arising from premature deaths to be USD 2.25 billion which was approximately 0.71% of GDP in 1995. They point out that these figures are an underestimate of the costs because they exclude productivity losses arising from morbidity and absenteeism and early retirement on account of disability.

**4. Discussion and Conclusion**

There is a considerable body of evidence supporting the findings that, in the event of catastrophic and chronic illness, poorer households who are often without private insurance, access care at considerable costs, often depleting savings, selling off assets, incurring debt and reallocating waged labour responsibilities within the household (*27-30*).

Ill-health, death or disability carry both direct and indirect costs. Death, especially of a parent often means a permanent loss of income and often displaces other consumption and investment activities of the household. For example, in order to supplement household income and reduce spending on other activities (*e.g*. educational expenses) children are often removed from school and engaged in productive labour.

At the macro-level, the impact of CVD and diabetes like other diseases and conditions will be felt on both the public and private sector. CVD and diabetes episodes of illness associated with these diseases, disability and death often imply increasing costs and productivity losses. The broader impact on economic growth will depend on the extent of the epidemic on savings and investment decisions and the impact on different socio-economic groups. In addition to health, other social sectors are also likely to be affected, the most obvious and immediate being education and social security.

Within the developing countries, as the burden from these two diseases grows, health expenditure will also rise. Evidence from developed countries point to the mounting costs of managing and treating CVD and diabetes, and their treatment is also likely to consume considerable resources in the developing countries. Inpatient costs tend to be largest single contributor to direct health care costs. Inpatient care of CVD and diabetes, in comparison to acute care, often entails lengthier stays and requires more expensive procedures and drugs.

The impact of these two diseases on productive sectors and of the economy at large depends on a range of factors. These include their prevalence, the groups at risk; the structure of the economy and the contributions of its key sectors (*e.g*. agriculture, industry *etc*.); and the size, structure and the skills profile of the labour force. Even at a cursory level, it can be said that CVD and diabetes have important consequences for productivity. Premature death, morbidity, and disability contribute to lower levels of productivity. Lost time due to illness often entails lost earnings, recruitment and training costs to replace workers, all of which contribute to revenues losses. In addition, considerations of impact of morbidity and mortality and on employee benefits[3] also needs to be taken into account.

In the developing world where the epidemic increasingly targets those in their most productive years, the worst-case scenario is that replacement of older and more experienced workers with less-experienced labour will entail reductions in labour productivity and has implications for competitiveness within the industry

[2] The costs of premature mortality from NCDs were estimated on the basis of the following assumptions: 1) average loss of 19 years of working life per death, 2) 60% labour force participation, 3) 3% real wage growth rate per annum and a discount rate of 12% per annum.

[3] Employee benefits include provision of medical services, health insurance, sick leave provision *etc*.

both domestically and internationally.

In both India and Pakistan, there has been a paucity of systematic efforts to measure the economic and social impact of CVDs and diabetes, despite there being a growing consensus and concern over the magnitude of the challenge that both diseases pose. Gathering these data is critical in both countries because of a number of reasons.

Firstly, both countries, which collectively house more than a fifth of the world's population, have many competing health priorities reflected in the intransigency of key health indicators; their meagre health allocations - less than 1% of GNP spent on health in both the countries - are challenged with many competing priorities. Therefore, in the absence of hard evidence to show the magnitude of economic and social impact that these diseases have, they are likely to remain outside of mainstream public health planning.

Secondly, it is widely perceived that CVDs in particular and NCDs in general, affect the affluent (*31*). This is wildly incorrect; both diseases manifest preferentially among the poor, both in the poorest nations and the poor in wealthy nations (*32*,*33*). This appears to be true for India and Pakistan as well.

However in the absence of documented evidence and/or gaps in translating evidence into effective communication and advocacy, these fail to receive due attention. In Pakistan, CVDs and diabetes are now part of the National Program for the Prevention and Control of Non-Communicable Diseases and Health Promotion; the program has been launched as the 8th public health programme and has admirably received budgetary support from public sector development budgets - a result of successful lobbying by the NGO Heartfile, which also has a lead role in the public-private partnership configuration of this programme (*34*). Notwithstanding, the programme is not part of the Poverty Reduction Strategy Framework of Pakistan, which currently guides priority public sector spending within the country. This example shows that even if these diseases are mainstreamed into public health planning, they may not appear as priority areas unless there is enough evidence to show that they have implications for the poor.

Poverty eradication has also assumed a centre stage position in the global development scenario. The current organization of aid and resource allocations from the developed to the less developed countries is being channelled with a greater-than-ever focus on poverty reduction. Poverty eradication is also central to the manner in which bilateral and multilateral international donor aid is being organized for the developing countries. It is therefore no wonder that cardiovascular diseases and diabetes receive negligible support from the donor and development community. Hence highlighting their magnitude of the impact on poverty - in terms of cost of care, lost productivity and the potential to perpetuate the chain of poverty and precipitate an acute poverty crisis - will also have implications for the manner in which donor resources flow to these countries.

Thirdly, it is important to generate locally relevant evidence - from applied, health systems and policy research perspectives - which shows that investments in cost-effective interventions can mitigate the risk of CVDs and diabetes and hence be contributory to saving costs, which incur in treating these ailments, if and when established. This is particularly relevant as the share of public contributions to health financing is dismally low in both the countries and patients, especially the poor, often have to shoulder the burden of health care. In the case of the aforementioned diseases, the prolonged costs of care can be prohibitive and pose access to care issues. In India, the number of the poor who did not seek treatment because of financial reasons increased from 15% to 24% in rural areas and doubled from 10% to 21% in urban areas between 1986 and 1996 (*37*). An analysis by the World Bank (*38*) concludes that "the hospitalized Indian spends more than half of his total annual expenditures on buying healthcare; more than 40% of hospitalized people borrow money or sell assets to cover expenses and 35% fall below the poverty line." The picture is likely to be the same for Pakistan.

Given these considerations, it is of critical importance that a research base of studies investigating the economic and social impact of CVDs and diabetes in India and Pakistan be undertaken as a first step in order to demonstrate the gravity of the epidemic to all stake-holders. With these data in hand, the choice for health planners with regard to important decisions such as including the provision of prevention and early detection services vis-à-vis care of established cases of CVDs and diabetes may become clearer. Similarly, the implications for productivity and revenue earnings make a powerful argument in order to focus the attention of private sector employers on these issues.

It is also important that the envisaged research base should pay close and careful attention to a number of parameters, where gaps have been identified based on the assessment of existing studies reviewed in this paper. These include the absence of assessments of direct and indirect costs in the same study; inattention to the social consequences of these diseases and methodological inconsistencies which make comparative analyses restrictive. In this regard, geographic, cultural and ethnic similarities between India and Pakistan make a strong case for collaborative efforts and capitalizing on sharing of experiences (*37*). It must be clearly recognized that the successful launching of such efforts at a policy and public health level hinges on the availability of appropriate evidence - we must commit ourselves to making that available and effectively communicated.

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# An overview of currently available anti-insulin-like growth factor I receptor antibodies

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**SUMMARY** **A number of studies during the last two decades revealed that the insulin-like growth factor I receptor (IGFIR) is an attractive target for cancer molecular therapy. Different molecular strategies have been developed and evaluated in experimental systems, and one such strategy involves anti-IGFIR antibodies, which have been rigorously tested for their therapeutic potential over the last 5-6 years. This mini-review thus introduces currently available IGFIR antibodies with a particular emphasis on epitope mapping and anti-IGFIR antibody-induced cancer growth inhibition.**

***Key Words:*** Antibody, cancer therapy, epitope specificity, hormone, insulin-like growth factor I receptor

## Antibody engineering for use in cancer therapy

The ground-breaking establishment of monoclonal antibody (mAb)-production technology (*1*) was followed by the use of recombinant DNA technology in antibody engineering (*2*), which laid the groundwork for major advances in producing a variety of antibodies as therapeutics to treat patients with various diseases including cancer. Production of therapeutic antibodies, however, requires humanization of murine antibodies in order to reduce their immunogenicity in humans. Chimeric antibodies with mouse variable regions and human constant regions were constructed (*3*,*4*), but were found to still be immunogenic. Further improvements in producing therapeutic antibodies include complementarity-determining region (CDR) grafting of a murine antibody onto a human variable-domain framework (*5*), screening of recombinant antibody libraries (*6*), and human antibody production from transgenic animals having human immunoglobulin gene loci (*7*).

Antibody-based therapeutics has emerged as an important component of therapies for an increasing number of human malignancies. Rituximab (anti-CD20) was the first FDA-approved agent for treatment of cancer, specifically non-Hodgkins lymphoma, in 1997. Herceptin (Trastuzumab; anti-HER2/neu), which was approved for clinical use in 1998, has successfully been used to treat metastatic breast cancer. These earlier studies encouraged screening of new and more effective target molecules expressed on various malignant cells by a number of laboratories and companies (*8*,*9*). Since accumulating evidence suggests that IGFIR is involved in mitogenic and anti-apoptotic effects of a variety of cancer cell lines, IGFIR is a potentially worthwhile molecular target (*10*-*13*).

## IGFIR axis

The ligands for IGFIR are IGF- I and II, which consist of 70 and 67 amino acids, respectively. They share 62% identity and also show structural homology to proinsulin (*14*). IGF-I is synthesized in the liver under the regulation of growth hormone and secreted into the bloodstream (endocrine action). IGFs also act in an autocrine/paracrine manner in peripheral tissues (*15*). Both ligands bind to IGFIR with equally high affinity, which leads to growth promotion and inhibition of apoptosis. IGFIR is a transmembrane glycoprotein consisting of two α subunits and two β subunits that are linked by disulfide bonds. The α subunit is completely extracellular and responsible for ligand-binding while the β subunit is a transmembrane protein whose cytoplasmic domain carries tyrosine protein kinase activity (*16*). The cytoplasmic domain

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of the β subunit contains tyrosine residues, which are auto-phosphorylated after ligand stimulation, that act as docking sites for several substrates including insulin receptor substrates (IRSs) and Shc (*17*). Following these events, down-stream signal molecules including mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt (*18*) are activated, leading to cell proliferation and attenuation of apoptosis. Furthermore, the actions of IGFs are regulated by the presence of IGF-binding proteins (IGF-BPs) 1-6 that are found in the circulation and extracellular fluids (*17*).

**Structural and functional relationships with respect to the insulin receptor**

Molecular cloning of human IGFIR cDNA (*19*) revealed sequence homology with the insulin receptor (IR). Although both IR and IGFIR signaling pathways overlap, IR and IGFIR mainly play distinct roles in metabolic and mitogenic pathways, respectively. IGFIR is overexpressed in a variety of cancers in which IGFIR signaling plays an important role in proliferation, anti-apoptosis, and tumorigenesis (*20*). IGFIR is also a key mediator of hormone-independent progression in prostate cancer cell lines (*21*). In addition, IGFIR can dimerize with IR, resulting in an IR/IGFIR hybrid receptor. This IR/IGFIR hybrid receptor also acts as a growth receptor through stimulation by IGF-I or IGF-II (*22,23*).

**Production of monoclonal antibodies against human IGFIR**

The first anti-IGFIR mAb, αIR-3, was obtained from mice immunized by IR purified from human placentae that had to have contained IGFIR as a contaminant (*24*). αIR-3 was thus a kind of by-product that has since proven extremely useful. Later, more anti-IGFIR mAbs were produced using a variety of antigens including purified human placental IGFIR (*25,26*), purified ecto-IGFIR (*27*), and IGF-IR-overexpressing cells (*28*).

**IGF-I binding domains and epitope mapping for anti-IGFIR mAbs**

Over the last two decades, studies on binding sites for ligands and mAbs took advantage of two structurally-related receptors, in that receptor chimeras in which IGFIR and IR domains were shuffled within the framework of IR or IGFIR were recombinantly expressed in order to test the reactivity of the grafted domain. A good example for this is the study by Gustafson and Rutter (*29*), which identified the cysteine-rich domains of IR (230-285) and IGFIR (223-274) as primary determinants of hormone binding specificity. Consequent works by Mynarcik *et al.* (*30*), Whittaker *et al*. (*31*), and Keyhanfar *et al*. (*32*) more precisely mapped the IGF-I binding site to the cysteine-rich domain on IGFIR using IR/IGFIR chimeras and point mutational analysis, but suggested other residues, especially $Phe^{701}$, also play critical roles in ligand binding. Interestingly, the IGFIR ectodomain (L1-cysteine rich-L2 domain), produced and structurally determined by X-ray crystallography, was unable to bind to the ligand (*33*). This may indicate that although the cysteine-rich domain contains the IGF-I binding site, the entire α subunit connected to the extracellular domain of the β subunit may be necessary to exhibit ligand-binding activity.

In addition to the aforementioned approach, strategies commonly used to categorize various mAbs obtained include screening the effects of mAbs on IGF-I or -II binding to IGFIR and on cell growth as usually determined by DNA synthesis. Table 1 summarizes various anti-IGFIR mAbs whose epitopes and ligand binding effects have been reported. Siddle and his colleagues developed several mAbs and characterized their epitopes using domain-shuffled chimera receptors (*28*) and reported further analysis of epitope mapping as well as their effects on ligand binding (*34*). For example, 16-13 and 26-3, which bind to respective

**Table 1.** Summary of each anti-IGFIR mAb-epitope on the α subunit of IGFIR and effect on ligand binding

| Number | Name | Epitope | Effect on ligand (IGF-I)-binding | References |
|---|---|---|---|---|
| 1 | 1H7 | 440-514 | inhibition | Li *et al*. (*25*)<br>Kusada *et al*. (*35*) |
| 2 | 3B7 | 62-184 | stimulation | Xiong *et al*. (*26*)<br>Kusada *et al*. (*35*) |
| 3 | αIR-3 | 223-274 | inhibition | Kull *et al*. (*24*)<br>Gustafson *et al*. (*29*) |
| 4 | 24-31 | 283-440 | no effect | Schumacher *et al*. (*34*) |
| 5 | 17-69 | 514-586 | inhibition | Schumacher *et al*. (*34*) |
| 6 | 24-55 | 440-514 | inhibition | Schumacher *et al*. (*34*) |
| 7 | 24-60 | 184-283 | inhibition | Schumacher *et al*. (*34*) |
| 8 | 24-57 | 440-514 | inhibition | Schumacher *et al*. (*34*) |
| 9 | 16-13 | 62-184 | stimulation | Soos *et al*. (*28*) |
| 10 | 26-3 | 283-440 | stimulation | Schumacher *et al*. (*34*) |
| 11 | 7C2 | 131-315 | inhibition | Keyhanfar *et al*. (*32*) |
| 12 | 9E11 | 131-315 | inhibition | Keyhanfar *et al*. (*32*) |

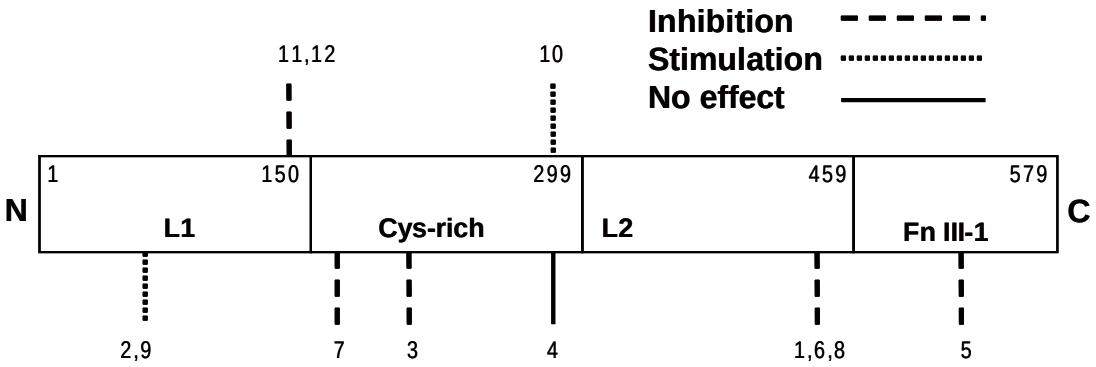


**Figure 1.** Schematic representation of the IGFIR α subunit (1-579) in relation to epitopes of anti-IGFIR mAbs. "N" and "C" indicate the N terminus and C terminus of the IGFIR α subunit (1-579), respectively. "L", "Cys-rich", and "Fn III" are respectively the leucine-rich repeat, cysteine-rich domain and fibronectin III repeat domain. Anti-IGFIR mAbs listed in Table 1 are marked with their corresponding numbers at their epitope sites (amino acids of N-termini in the regions recognized by each mAb). The effects of anti-IGFIR mAbs on ligand binding are shown by bars; the dashed line indicates inhibition, the dotted line indicates stimulation, and the solid line indicates no effect.

**Table 2.** Summary of therapeutic antibodies targeting IGFIR

| Clone name | Generation technology | References |
|---|---|---|
| scFv-Fc | Recombinant chimeric antibody derived from mAb | Li *et al*. (*44*) |
| CP-751,871 | Transgenic mouse producing human antibodies | Cohen *et al*. (*47*) |
| A12 | Phage display screening | Burtrum *et al*. (*48*) |
| 19D12 | Transgenic mouse producing human antibodies | Wang *et al*. (*52*) |
| h7C10 | Recombinant humanized antibody derived from mAb | Goetsch *et al*. (*53*) |

62-184 and 283-440 residues on the IGFIR α subunit, are able to stimulate IGF-I binding whereas 24-60 and 24-57, which recognize respective 184-283 and 440-514 residues of the α subunit, almost completely inhibit IGF-I binding. The ligand-binding inhibition by 24-60 is consistent with the notion that it competitively binds to the IGF-I binding domain. Ligand-binding inhibitory mAbs, whose epitopes are identified to be regions other than the IGF-I binding domain (cysteine-rich domain), most likely either induce conformational changes in IGFIR upon binding or induce steric hindrance, resulting in low ligand-binding ability. In the case of ligand-binding stimulatory mAbs, however, conformational changes in the receptor caused by mAbs must be responsible for the observed higher binding ability of IGFIR. Our laboratory recently determined the epitopes of anti-human placental IGFIR mAbs, 1H7 and 3B7 (*35*), by competitive inhibition assays using mAbs (24-57 and 16-13) produced by Soos *et al*. (*28,34*). 1H7 and 3B7 exhibited opposite effects on ligand binding; that is, 1H7 inhibits ligand binding whereas 3B7 stimulates it (*25,26*). The competitive inhibition study demonstrated that 1H7 recognizes 440-514 regions (since it competes with 24-57) whereas 3B7 binds to 62-184 regions (since it competes with 16-13) on the α subunit (*35*).

As described above, αIR-3, which inhibits IGF-I binding, recognizes the cysteine-rich domain that was determined to be the IGF-I binding site (*29*). 1H7 mAb binds to an epitope other than the IGF-I binding site, indicating that 1H7 induces conformational changes in the receptor or causes steric hindrance. There are conflicting reports regarding the ligand-binding domain (cysteine-rich domain). Delafontaine *et al*. prepared anti-IGFIR polyclonal Abs by immunizing rabbit with peptide fragments of the IGFIR α subunit (*36*). They reported that any Abs recognizing the cysteine rich domain did not interfere with IGF-I binding, but one group of Ab, RAB6, that recognizes the 38-44 residues near the N-terminus of the α subunit, inhibited IGF-I binding. The question of whether or not the cysteine-rich domain is the major binding site for IGF-I is still unresolved. However the antibodies described by Delafontaine *et al*. are polyclonal and showed weak affinity for native receptor. Therefore it is inappropriate to compare these antibodies with other mAbs. The mAbs described above are summarized in Table 1 and in Figure 1, where each epitope of mAbs and its effect on ligand binding are shown with respect to the structure of the IGFIR α subunit.

**Recombinant IGFIR antibodies for cancer therapy**

Since the well-studied anti-IGFIR mAb, αIR-3, was shown to inhibit the growth of human cancer cells *in vitro* and *in vivo* (*37-39*), several other groups have reported on the potential for using anti-IGFIR mAbs to develop cancer therapeutics (*40-43*). With the advancement of recombinant antibody technologies, more therapeutic anti-IGFIR mAbs have been developed (Table 2). Li *et al*. first produced a chimeric IGFIR antibody consisting of a single chain variable

fragment (scFv) derived from mAb 1H7 and the human $IgG_1$ Fc region (*44*). This recombinant antibody, named IGFIR scFv-Fc, was shown to inhibit growth of the human breast cancer cell line MCF7 *in vitro* and *in vivo*. Sachdev *et al*. revealed that scFv-Fc has an agonistic effect on MCF-7 cells but that the long-time treatment of MCF-7 cells with scFv-Fc down-regulated IGFIR, resulting in the cancer cells becoming refractory to ligand stimulation (*45*). Breast cancer tumor growth *in vivo* was inhibited by scFv-Fc in two different systems, MCF-7 (*45*) and T61 (*46*). In combination with Tamoxifen, αIGFIR scFv-Fc treatment suppressed the growth of T61 tumors *in vivo* more significantly than scFv-Fc treatment alone (*46*). Cohen *et al*. produced an anti-IGFIR mAb called CP-751,871 from transgenic mice and demonstrated that this mAb inhibits tumor growth alone or in combination with chemotherapy *in vivo* (*47*). A fully human anti-IGFIR mAb, A12, that was prepared by screening of a phage displayed human Fab library exhibited tumor growth inhibition on breast, colon, pancreatic, and prostate cancer cell lines *in vivo* (*48,49*). A12 was also tested for its efficacy when used in combination with chemotherapy or radiotherapy (*50,51*). Another fully human antibody 19D12, which was produced from transgenic mice by Wang *et al*. (*52*), was found to significantly inhibit tumor growth *in vivo* as a single agent. Goetsch *et al*. produced a recombinant humanized anti-IGFIR antibody, h7C10 (*53*). This antibody showed *in vivo* antitumor efficacy as a single agent against established breast (MCF-7) and non-small cell lung cancer (A549) xenografts when administered intraperitoneally (*53*). Ligand-independent down-regulation of both IGFIR and hybrid receptors (IR-A or IR-B/IGFIR) was demonstrated upon long-term incubation of cells expressing IR-A/IGFIR or IR-B/IGFIR with h7C10 (*54*), indicating that this mAb is a potent inhibitor of both IGFIR and hybrid receptors.

## A major mechanism for anti-cancer growth by IGFIR antibodies

Although several therapeutic strategies for targeting IGFIR, including antisense RNA and tyrosine kinase inhibitors, have been developed (*55-57*), monoclonal antibody therapy has emerged as the most promising approach for anti-cancer applications. What follows is a brief summary of how therapeutic anti-IGFIR antibodies work, as is illustrated in Figure 2. Most anti-IGFIR antibodies developed for cancer therapy thus far seem to down-regulate (internalize and degrade) IGFIR, thereby making cancer cells insensitive to ligand stimulation (*45-49,52,53*). Anti-IGFIR antibodies induce receptor degradation mainly *via* endosomal- and lysosomal-pathways (*45*). The receptor degradation not only causes the loss of cell-sensitivity to IGFs resulting in growth inhibition but also induces apoptosis resulting in cell death. This receptor degradation effect is attributable

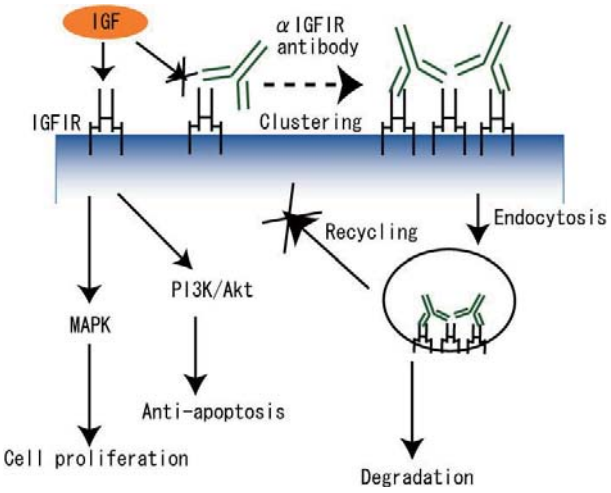


**Figure 2.** Schematic illustration for IGF signaling and IGFI receptor degradation in IGFIR-expressing cells. When ligands, IGF-I or IGF-II, bind to the receptor, down-stream signaling molecules are activated, leading to cell proliferation and counteracting apoptosis through MAPK and PI3K/Akt pathways. Anti-IGFIR antibody treatment not only prevents ligand-binding but also causes receptor-clustering followed by degradation through endosomal/lysosomal pathways.

to the multivalency of antibodies such as IgG or scFv-Fc, since monomeric anti-IGFIR Fab fragments were not able to trigger receptor degradation (*52*). Receptor down-regulation is very effective in cancer cells overexpressing IGFIRs. Anti-IGFIR antibodies are not believed to down-regulate IGFIR in normal cells since anti-IGFIR antibodies that cross-react to mouse IGFIR did not cause any significant side effects in mice. This observation is consistent with the notion that anti-IGFIR antibody-induced down-regulation occurs only in cancer cells overexpressing IGFIRs but not in normal cells that express lower levels of IGFIRs (*49*).

Anti-IGFIR antibodies also down-regulated IR (*58*). IR can form a heterodimer with IGFIR, resulting in IR/IGFIR hybrid receptors. IR exists in two isoforms of IR-A and IR-B (*17*). Since IR-A is expressed predominantly in cancer cell lines and cancerous tissues, IR-A/IGFIR hybrid receptor may exist as a major type in cancer cells. Both IR-A holo-receptor and IR-A/IGFIR hybrid receptor have high affinity for IGF-II, thus having more of a growth effect than a metabolic effect (*23,59*). Zhang *et al.* recently reported that down-regulation of IGFIR by small interfering RNA increases sensitivity of breast cancer cells to insulin (*60*). Because IR also activates signaling pathways similar to IGFIR in cancer cells, agents targeting both receptors may be necessary to disrupt the malignant phenotype regulated by this growth factor system. Thus, IR-A targeted antibodies will be the next generation of antibodies to be developed.

## Concluding remarks

Over the last decade, significant progress has been made in the development of anti-IGFIR antibodies for

therapeutic use. Several are now undergoing clinical trials. As these trials move forward, they should elucidate whether disruption of IGFIR signaling results in relevant clinical outcomes.

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# The growth of *Vibrio vulnificus* and the habitat of infected patients in Kumamoto

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**SUMMARY** **In Japan, *Vibrio vulnificus* (*V. vulnificus*) infection is very rare, and most infections have occurred in Kumamoto Prefecture (*1*), and especially around the Ariake and Yatsushiro seas. To investigate the relationship between the occurrence of *V. vulnificus* infection and environmental factors, including the salinity of seawater and the amount of rain in the Ariake and Yatsushiro seas, we measured the most probable number (MPN) of *V. vulnificus* in seawater and sea mud. In the Ariake Sea, we also observed the temperature and salinity of seawater at one site located on an estuary where the salinity is easily affected by river water and another site located offshore where seawater is little affected by river water. Furthermore, we investigated the MPN of *V. vulnificus* and observed the temperature and the salinity of seawater in 25 sites in the Ariake and Yatsushiro seas from July to August 2003 and 2004. In addition, we collected data on patients with *V. vulnificus* infections in Kumamoto from 1990 to 2006. The MPN of *V. vulnificus* differed by sampling site. More *V. vulnificus* were detected around the inland sea than the open sea, and the increase in *V. vulnificus* levels was affected by rainfall around inland sea areas with many rivers. *V. vulnificus* increases significantly in brackish water areas, and the salinity of seawater was as important as the seawater temperature. In other words, an area's topography and amount of rain are believed to be important factors for the occurrence of *V. vulnificus* infection. *V. vulnificus* infection has been regarded as an infection of hot districts. However, the salinity of seawater may be more important than temperature for the growth of *V. vulnificus*. Therefore, investigating these geographical and meteorological factors can help predict areas with a higher number of *V. vulnificus* infection outbreaks.**

***Key Words:*** *Vibrio vulnificus*, tidelands, salinity of seawater, amount of rain, geographical factors, meteorological factors

## Introduction

In Japan, there have been over 200 cases of *V. vulnificus* infection reported since 1978 (*2*). There are still many questions about the occurrence of *V. vulnificus* infections.

The current authors treated 43 *V. vulnificus* patients between 1990 and 2006 in Kumamoto Prefecture (Figure 1). In addition, there are several reports on *V. vulnificus* infections in Nagasaki (*3*) and Saga (*4*) prefectures but there are none on Kagoshima or Miyazaki prefectures, even though these prefectures neighbor Kumamoto and have higher average temperatures. This may be attributed to the fact that in these prefectures there are few areas with brackish water where *V. vulnificus* can grow.

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## Materials and Methods

Samples were prepared by using subsurface seawater

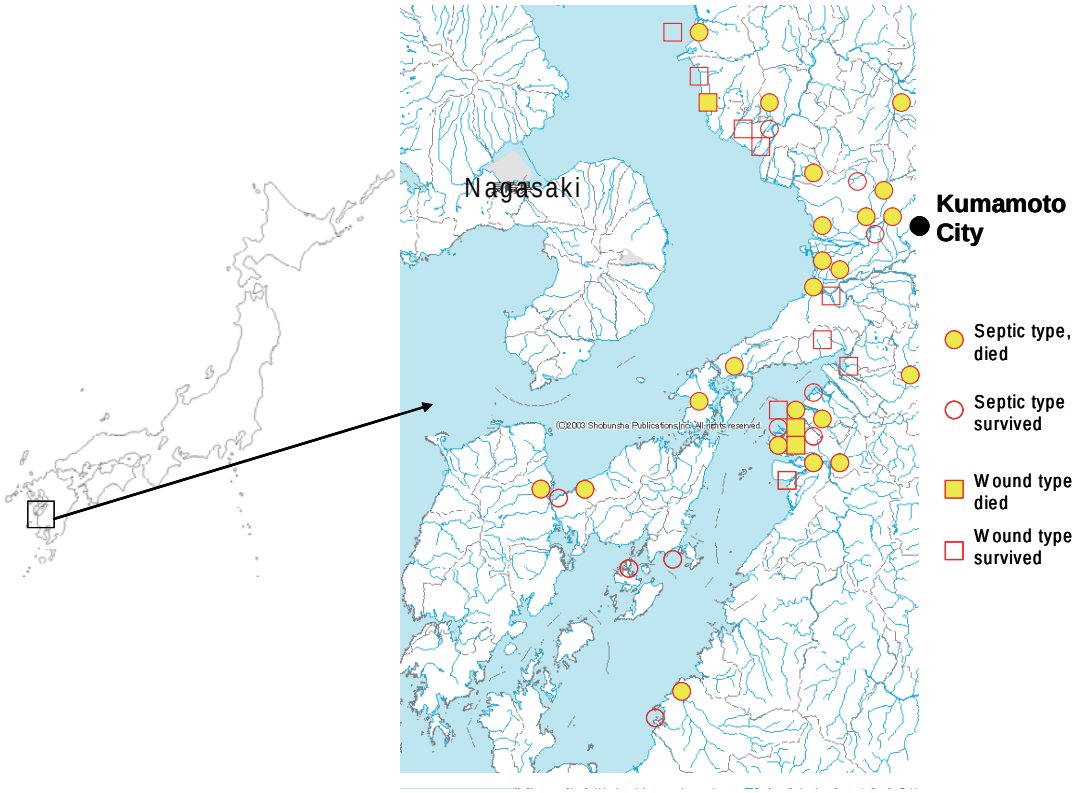


**Figure 1.** Patients with *V. vulnificus* infection in Kumamoto 1990-2006 ($n$ = 43).

collected from Ariake Sea at a depth of between 1 m and 1.5 m. All samples were transported to the laboratory in insulated coolers and examined within 24-48 h of collection.

Seawater and mud samples were collected in sterilized glass bottles from sites A and B. Site A was located on the estuary of the Kikuchi River, where salinity is easily affected by river water, and site B, which was located offshore of the Ariake Sea 1.5 kilometers from Ooyano Island and where the salinity is little affected by river water. Samples were collected once a month from May to December, 2004.

Water samples were also collected in sterilized glass bottles from 25 different sites along the coast in Kumamoto (Figure 2) from July to August of 2003 and 2004.

The numbers of *V. vulnificus* in the seawater samples were estimated by the three-tube most-probable-number (MPN) method. Volumes of 10 mL and 1 mL of seawater were added to 10 mL and 1 mL of double-strength alkaline peptone water (APW), respectively. One mL of dilutions ($10^{-1}$-$10^{-4}$) in PBS was added to 10 mL APW. In addition, 500 mL of each seawater sample were filtered with a filter (pore size: 0.45 μm) and 40 mL APW were added to a tube containing the filter. After incubation at 35ºC for 18 h, 10 μL of the culture were streaked onto CHROM agar Vibrio and incubated at 35ºC for 18 h. Colonies

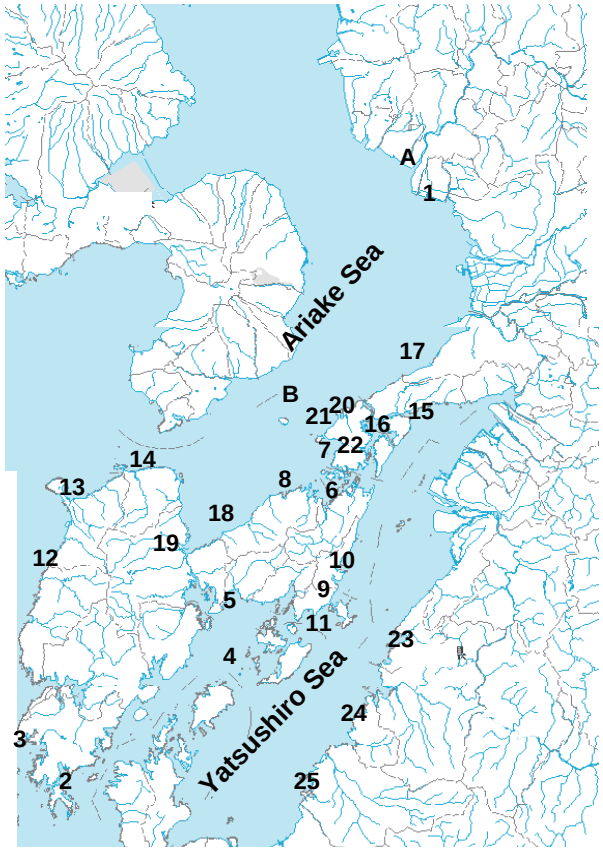


**Figure 2.** Water sampling sites along the coast in Kumamoto.

suspected of being *V. vulnificus* were confirmed to be *V. vulnificus* by the oxidase test, culture in triple sugar iron agar medium (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan) containing 2% NaCl, culture in lysine indol motility medium (Nissui Pharmaceutical Co. Ltd) containing 2% NaCl, culture in VP semi-solid medium (Nissui Pharmaceutical Co. Ltd) containing 2% NaCl, and growth in nutrient broth (Oxoid, Hampshire, UK) containing 0, 3, 8, and 10% NaCl. Furthermore, the suspected colonies were tested for the presence of the cytotoxin-haemolysin gene of *V. vulnificus* by PCR with the primer set used by Hill *et al*. (5).

To serve as a collection stomacher, 500 g of sea mud were placed in a pouch that as then sealed and immediately transported to the lab. Sea mud in the sample poucher was mixed by hand and every bag was handled uniformly. Twenty-five-gram samples were thinly scattered in dishes, dried for 2 h at 110ºC, and weighed after the sample was left in a desiccator for 40 min; then, the quantity of interstitial water was calculated. An additional 25 g of sea mud were diluted 10 × with PBS. This solution was added to 10 mL APW and diluted 10 × with PBS. *V. vulnificus* was identified and the MPN of the interstitial water per 100 mL was calculated by the method described above.

The seawater samples were collected in sterilized plastic bottles. Salinity was measured with a salt analyser (SAT-210, Toa Electronics, Tokyo, Japan). The temperature of seawater was measured with a thermometer. Seawater salinity (19-31‰) was not constant and may have been affected by weather or other factors rather than seasonal factors, although the temperature seemed to be affected by seasonal factors.

## Results

The MPN of *V. vulnificus* in seawater and sea mud from May to December in 2004 is shown in Table 1. *V. vulnificus* numbers started to increase in seawater in May when the seawater temperature exceeded 20ºC

**Table 1.** *V. vulnificus* levels at Site A and Site B

| Month | Site A | | | | Site B | | | |
|---|---|---|---|---|---|---|---|---|
| | MPN (/100mL) | | Sea water Temperature (ºC) | Salinity of seawater (‰) | MPN (/100mL) | | Sea water Temperature (ºC) | Salinity of seawater (‰) |
| | Seawater | Sea mud* | | | Seawater | Sea mud* | | |
| May | 7 | 230,000 | 19 | 18.4 | < 3 | < 3 | 19.5 | 33.5 |
| June | < 3 | 700 | 24.3 | 34.2 | < 3 | < 3 | 29 | 34.1 |
| July | 2,300 | 43,000 | 30.5 | 21.1 | < 3 | < 3 | 28 | 35.2 |
| August | 2,300 | 1,500 | 31 | 34.1 | 3 | 30 | 29.5 | 35.9 |
| September | 1,500 | 230,000 | 29.5 | 30.8 | < 3 | < 3 | 27 | 33.3 |
| October | 75 | 21,000 | 24 | 32.9 | < 3 | 40 | 22.4 | 32.3 |
| November | 3 | 290 | 21 | 30 | < 3 | < 3 | 19 | 32.7 |
| December | < 3 | 150 | 17.5 | 30.7 | < 3 | < 3 | 17 | 29 |

*MPN of interstitial water (/100mL)

**Table 2.** *Vibrio vulnificus* at 25 sites around Kumamoto (from July to August in 2003 and 2004 )

| Site | 2003 | | | 2004 | | |
|---|---|---|---|---|---|---|
| | MPN (/100mL) | Amount of rain (mm)* | Salinity of seawater (‰) | MPN (/100mL) | Amount of rain (mm)* | Salinity of seawater (‰) |
| 1 | 23,000 | 180 | 19.6 | 2300 | 0 | 34.1 |
| 2 | < 3 | 30 | 33.7 | < 3 | 0 | 37.3 |
| 3 | < 3 | 30 | 33.5 | < 3 | 0 | 37.8 |
| 4 | < 3 | 35 | 31.8 | < 3 | 0 | 36.5 |
| 5 | 7 | 174 | 30 | < 3 | 0 | 37.2 |
| 6 | < 3 | 208 | 26.1 | < 3 | 24 | 34.8 |
| 7 | 4 | 208 | 26.1 | 3 | 24 | 34.7 |
| 8 | 4 | 208 | 25.3 | 3 | 24 | 34.4 |
| 9 | 4 | 205 | 21.4 | < 3 | 4 | 34.1 |
| 10 | 3,800 | 205 | 22.1 | < 3 | 4 | 34.4 |
| 11 | < 3 | 205 | 24 | < 3 | 4 | 35 |
| 12 | < 3 | 129 | 33 | < 3 | 6 | 36 |
| 13 | < 3 | 30 | 32.7 | < 3 | 6 | 36.2 |
| 14 | < 3 | 174 | 30.8 | < 3 | 6 | 35.5 |
| 15 | 93 | 128 | 25.6 | < 3 | 6 | 35.3 |
| 16 | 430 | 128 | 24.1 | < 3 | 6 | 36.5 |
| 17 | 230 | 128 | 26.8 | 3 | 6 | 35.9 |
| 18 | < 3 | 278 | 32.7 | 3 | 34 | 35.9 |
| 19 | < 3 | 174 | 31.3 | < 3 | 4 | 37 |
| 20 | 36 | 44 | 31.9 | < 3 | 70 | 35.8 |
| 21 | 6 | 44 | 31.3 | < 3 | 70 | 36.4 |
| 22 | < 3 | 44 | 31.7 | < 3 | 70 | 36.4 |
| 23 | 21 | 251 | 30.3 | < 3 | 30 | 35.6 |
| 24 | 9 | 251 | 31.6 | < 3 | 0 | 35.4 |
| 25 | 23 | 204 | 31.6 | < 3 | 2 | 35 |

* Amount of rain during the seven days before each seawater sampling

at site A, and the peak number of *V. vulnificus* was from July to October when the seawater temperature exceeded 30ºC. *V. vulnificus* inhabits sea mud more so than seawater, *V. vulnificus* was only isolated from seawater once in August at site B. In addition, the MPN was low, as shown in Table 1. *V. vulnificus* was isolated from sea mud in August and October, and the numbers were higher than from seawater. In addition, the salinity was stable. The increase in *V. vulnificus* was probably affected by the salinity, and *V. vulnificus* may have increased in number in sea mud more so than in seawater.

The MPN of *V. vulnificus* at twenty-five sites in Kumamoto in July and August in 2003 and 2004 is shown in Figure 1 and Table 2. The amount of rain in Table 2 shows the total rain during the seven days before each seawater sampling. There was more rainfall in 2003 than in 2004. In 2003, *V. vulnificus* was isolated from 14 of 25 sites but from only five sites in 2004. The salinity of seawater was higher in 2004 than in 2003 at all of the sites investigated. The difference in seawater salinity could have been caused by a difference in rainfall.

Kumamoto is one area where numerous *V. vulnificus* infections occur. Since 1990, 30 patients with *V. vulnificus* infection were seen at Kumamoto University Hospital and 13 patients were seen at 7 other hospitals in Kumamoto Prefecture (Table 3). However, there were no patients from islands facing the open sea.

## Discussion

*V. vulnificus* infection is very rare, but its prognosis is poor once it occurs. *V. vulnificus* infection is divided into several clinical types: septic, wound, digestive, and other types. In Asian countries, including Japan, many

**Table 3.** *V. vulnificus* infection patients in Kumamoto from 1990 to 2006

| No. | Year | Female/Male | Years | Type | Day of onset | Died/Suvived |
|---|---|---|---|---|---|---|
| 1 | 1990 | F | 58 | Septic type | 1990.10.30 | D |
| 2 | 1991 | M | 56 | Septic type | 1991.7.8 | S |
| 3 | 1992 | M | 48 | Septic type | 1992.8.6 | D |
| 4 | 1993 | F | 64 | Septic type | 1993.8.15 | D |
| 5 | 1995 | M | 44 | Wound type | 1995.7.12 | D |
| 6 | | M | 57 | Septic type | 1995.7.13 | D |
| 7 | 1996 | M | 55 | Septic type | 1996.7.22 | D |
| 8 | | M | 56 | Ingestive type | 1996.7.31 | S |
| 9 | | M | 67 | Septic type | 1996.9.18 | D |
| 10 | 1997 | M | 35 | Wound type | 1997.6.13 | D |
| 11 | | M | 53 | Septic type | 1997.7.21 | D |
| 12 | 1999 | F | 66 | Septic type | 1999.6.6 | D |
| 13 | | F | 74 | Wound type | 1999.7.8 | S |
| 14 | | M | 38 | Septic type | 1999.8 | D |
| 15 | | M | 67 | Septic type | 1999.9.21 | D |
| 16 | 2001 | M | 61 | Septic type | 2001.6.29 | D |
| 17 | | M | 72 | Septic type | 2001.7.10 | S |
| 18 | | M | 61 | Septic type | 2001.7.10 | S |
| 19 | | M | 77 | Septic type | 2001.7.4 | S |
| 20 | | M | 62 | Wound type | 2001.7.7 | S |
| 21 | | M | 56 | Septic type | 2001.7.12 | D |
| 22 | | M | 43 | Septic type | 2001.7.17 | D |
| 23 | | M | 74 | Wound type | 2001.7.18 | S |
| 24 | | M | 77 | Wound type | 2001.8. | S |
| 25 | | M | 68 | Septic type | 2001.8.14 | D |
| 26 | 2002 | M | 67 | Septic type | 2002.8.23 | D |
| 27 | | M | 70 | Septic type | 2002.9.19 | S |
| 28 | | M | 63 | Wound type | 2002.10.6 | S |
| 29 | 2003 | M | 77 | Wound type | 2003.7.24 | D |
| 30 | | M | 77 | Wound type | 2003.8.15 | S |
| 31 | | F | 70 | Septic type | 2003.9.5 | S |
| 32 | | M | 32 | Wound type | 2003.9.7 | S |
| 33 | | M | 70 | Septic type | 2003.10.8 | D |
| 34 | 2004 | M | 58 | Septic type | 2004.6.17 | D |
| 35 | | M | 40 | Septic type | 2004.6.21 | S |
| 36 | | M | 66 | Septic type | 2004.9.21 | S |
| 37 | 2005 | M | 59 | Septic type | 2005.7.15 | D |
| 38 | | M | 61 | Septic type | 2005.7.12 | D |
| 39 | | M | 59 | Wound type | 2005.7.28 | S |
| 40 | | M | 55 | Septic type | 2005.7.30 | D |
| 41 | | M | 70 | Wound type | 2005.9.25 | S |
| 42 | 2006 | M | 69 | Septic type | 2006.7.19 | D |
| 43 | | M | 70 | Septic type | 2006.8.3 | D |

patients have the septic type, which is assumed to be a result of eating fresh marine products. *V. vulnificus* infections have been reported all over the world (*6-15*) and especially in warm areas in the summer. The water temperature suitable for growth of *V. vulnificus* is more than 20ºC (*16*), and adequate salinity is 15-25‰ (*17*), but its growth is suppressed when salinity exceeds 25‰. However, there are few studies of *V. vulnificus* in relation to environmental factors and therefore, many questions remain regarding the association of *V. vulnificus* and environmental factors.

There are few reports on *V. vulnificus* in sea mud. The current investigation found a higher concentration of *V. vulnificus* in sea mud than in seawater. For this reason, wound-type *V. vulnificus* infection may occur from sea mud without the patient even being in seawater. Furthermore, there was no clear difference between sites A and B in terms of the seawater temperature; however, the salinity changed greatly at site A in comparison to site B, and a much higher concentration of *V. vulnificus* was found at site A than site B. The salinity of seawater may be as important for the growth of *V. vulnificus* as seawater temperature.

Hoi *et al*. (*18*) isolated *V. vulnificus* from seawater in Denmark, and *V. vulnificus* grew rapidly with a higher seawater temperature. However, there were no changes in the salinity of seawater in Denmark. Hervio-Heath *et al*. (*19*) performed an environmental study in France and clearly confirmed the presence of *V. vulnificus* in Girond littoral in the Bordeaux district; salinities there were 10-20‰, which were lower than in other areas. However, the rate of detection of *V. vulnificus* is assumed to be low, and the salinity of Mediterranean seawater is more than 35‰, which is higher than in other seas. This could be the reason why there are few patients with *V. vulnificus* in areas around the Mediterranean Sea (*20*). *V. vulnificus* was detected in Chesapeake Bay (*21*) even at a time when the seawater temperature was less than 10ºC. However, in North Carolina (*22*), it was detected only when seawater temperature was more than 20ºC. The current study detected high levels of *V. vulnificus* in an inland sea even when seawater temperature was under 15ºC (data not shown). Motes *et al*. (*23*) reported that the MPN of *V. vulnificus* is very high in the Arabian Gulf Coast region. the above reports suggest that the salinity of seawater is important for the growth of *V. vulnificus*.

The current authors reported the first outbreak of *V. vulnificus* infection in Japan in 2001 (*24*) and focused on rainfall and related factors at the time of the outbreak. Since 1990, forty-three patients have been confirmed to have the infection (Table 3) in Kumamoto. Patients were concentrated in a coastal area where there is a large river.

At sites 15, 16, and 17 in the peninsula area, the amount of rainfall in July to August 2003 was 128 mm and that in 2004 was 6 mm. The salinity in 2003 was between 24.1‰ and 26.8‰ and that in 2004 was between 35.3‰ and 36.5‰. The MPN of *V. vulnificus* in 2003 and 2004 was from 93 to 430 and < 3, respectively. At sites 2, 3, 12, 13, and 14 in island areas, in contrast, the salinity of seawater was stable though there was much more rainfall in 2003 than in 2004, and *V. vulnificus* was not detected either in 2003 or 2004. Based on these results, *V. vulnificus* is unlikely to grow in island areas facing the open sea but instead proliferate in island seas following rain. In actuality, no patients with *V. vulnificus* were found around sites 2, 3, 12, 13, and 14 in island areas even though the inhabitants eat a great deal of raw fish.

Thus, the amount of rain and the salinity of seawater are believed to be associated with the occurrence of a *V. vulnificus* infection as much as seawater temperature. Geographical and meteorological factors are believed to greatly influence the occurrence of *V. vulnificus* infection.

The onset of this illness is abrupt, rapidly progressing to septic shock with a high mortality rate. Clinicians managing patients with chronic liver disease need to educate their patients about the risk associated with the consumption of raw shellfish.

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# Prevalence and determinants of obesity and dietary habits among adults in rural area, Chile

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**SUMMARY** **This study was undertaken to examine the prevalence of obesity and its determinants among adults in a rural area of Chile. A community-based cross-sectional study was conducted in April-June 2004 in San Carlos (8th region). Height, weight, and waist and hip circumferences were measured for 603 adults (female 447, male 156) aged 20-64 years, and data on socio-economic factors, dietary intake, and dietary habits were obtained by questionnaire interviews. The prevalence of obese individuals was 45.2% among females and 30.1% among males, whereas that of overweight individuals was higher among males than females. Obesity was associated with socio-economic factors for females but not for males. With regard to diet, different patterns between females and males were observed in terms of frequency of food intake, as well as in dietary habits. Our findings of a high prevalence of obese/overweight individuals, together with the characteristics of their diets including changes in the cooking process, suggest that nutrition transition is underway in rural regions as well. Appropriate interventions, therefore, should be introduced to control obesity among women and to enhance health awareness among men throughout the country.**

***Key Words:*** Obesity, overweight, socio-economic, nutrition transition, Chile

## Introduction

Obesity is known to be the most significant nutritional disorder in developed countries. There are estimated to be 1 billion overweight adults, of which at least 300 million are obese, worldwide (*1*). Being obese or overweight is regarded as a major risk for serious lifestyle-related diseases, including Diabetes Mellitus (DM), hypertension, and cardiovascular diseases. Moreover, additional burdens of obesity on the limited national health budget cannot be ignored (*e.g.* 5.5-7.0% in the US) (*2*,*3*). The problem of obesity is now emerging in developing countries as well, where malnutrition and infectious diseases used to be the most serious problems. As a country achieves economic development, the diet and physical activity patterns of its citizens change greatly. This phenomenon, observed in countries with economies in transition, is known as "nutrition transition" (*4*).

Like other countries in Latin America, Chile is undergoing a nutrition transition (*5*). A nutrition transition is defined as a change in diet and lifestyles, leading to a significant impact on the nutritional status of the population. Dietary changes include the increased consumption of fat, sugar, and animal food products and decreased cereal and fiber intake (*6-8*). Likewise, changes in diet, from a traditional to a "Western" one, and in physical activity patterns have resulted in the increased prevalence of obesity and lifestyle-related diseases in Chile (*9*,*10*). In light of these circumstances, the Ministry of Health started to invest in the control of obesity for children and pregnant women in 1998 (*11*)

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and later for adults in 1999 (*12*). And yet the country' s obesity rates remain high. According to the latest National Health Survey, the prevalence of overweight individuals was 33% for females and 43% for males, and that of obese individuals was 27% and 19%, respectively (*13*). So far, studies on the obesity of adults in this country have tended to concentrate on urban areas like the metropolitan Santiago area and data on other provinces including rural areas are scarce.

This study therefore, aims to examine the prevalence of obesity and investigate its determinants and dietary habits among adults in rural Chile.

## Materials and methods

A community-based cross-sectional study was undertaken from April to June 2004 in San Carlos in the Ñuble Province of the 8th region (of a total 13 regions) in Chile. The 8th region is located 376.2 km south from Santiago (population: 6,061,185), and San Carlos is in the northern part of the region (population: 50,139) (*14,15*). About 44% of the population is engaged in agriculture, mainly farming and cultivating wheat (*15*). Out of a total of 78 districts, the central district and 13 suburb districts were chosen for this study to represent the diverse geographical characteristics of San Carlos. All households in each district were visited individually by interviewers who fully explained the study objective and procedures, and then one person aged 20-64 years per household, excluding pregnant and lactating women, was chosen, upon receipt of consent, to participate. A total of 603 adults (447 females and 156 males) were recruited.

During the household visits, anthropometric measurements were taken for all 603 adults. Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca 214, Seca, Germany). Weight was measured using a digital bathroom scale (Seca 880, Seca, Germany) with capacity of 200 kg × 100 g. All of the sampled adults were weighed barefoot wearing light clothing. In order to assess fatness of each study subject, Body Mass Index (BMI) was calculated as weight (kg) divided by height squared ($m^2$), and subjects were categorized into four groups: underweight (< 18.5 kg/$m^2$), normal (18.5-24.9 kg/$m^2$), overweight (25.0-29.9 kg/$m^2$), and obese (≥ 30 kg/$m^2$) (*16*). Using a plastic tape measure, waist and hip circumferences were also measured as an independent indicator of visceral obesity. An individual is considered at risk of obesity when women have a waist circumference greater than 88 cm and men have one greater than 102 cm (*16*). The waist to hip ratio was calculated by dividing the waist circumference by the hip circumference, and study subjects with a ratio greater than 1.0 were considered at risk. Pregnant and lactating women were excluded from the sampling.

Socio-economic and behavioral (*e.g.* age, residence, marital status, occupation, education, income) were obtained by the interviews using the structured questionnaires. Residence was categorized as suburb ("population < 2,000" or "population of 1001-2000 with less than 50% of those working in industries/service") or central ("population ≥ 2,000" or "population of 1001-2000 with more than 50% of those working in industries/service"). The minimum monthly wage in Chile (120,000 pesos) was used to classify income levels. This study used the "frequency of exercise" as a variable to estimate an individual's physical activity level. A food frequency questionnaire (FFQ) was used to obtain dietary data. The frequency of major food items consumed during the past year was included in order to investigate the characteristics and habits of participants' diets. Dietary habits were asked to investigate the factors affecting their food intake. A focus group discussion was held with 12 housewives as participants in order to obtain information on dietary changes.

All of the data were entered and analyzed with SPSS version 14.0. Software called "*Minuta*" was used to calculate nutrition composition, which was derived from the Chilean food composition table of the Institute of Nutrition and Food Technology (INTA) (*17*). Univariate analyses were performed to examine the association between BMI and socio-economic and behavioral characteristics and food intake. In a *t*-test, $\chi^2$ test, and F-test a cut-off of 0.05 was used as the level of statistical significance.

All subjects gave informed consent, and the study protocol was approved by the ethical committee of the University of Tokyo and the University of Chile and the Chilean Ministry of Health's health council for the 8th region.

## Results

Table 1 shows socio-economic characteristics of study subjects. Distributions of age, residence, and marital status among females and males were almost the same. Types of occupation differed, however, with more males than females being employed. Other occupations include vendor, student, and unemployed. "Income" means the total monthly income of the family, which was higher among the families of male respondents than of female respondents (Median: 130,000 pesos *vs.* 115,000 pesos; $p = 0.002$). The proportion of study subjects who were illiterate or had a primary level (1-8 years) was higher among females (46.1%) than males (35.9%).

The prevalence of overweight or obese individuals was 38.5% and 45.2% for females, and 51.3% and 30.1% for males, respectively (Table 2). There was a significant difference between females and males ($p = 0.009$) in the distribution of BMI. The proportion of overweight individuals was higher among males

**Table 1.** Characteristics of study subjects

| | Female ($n$ = 447) | Male ($n$ = 156) |
|---|---|---|
| | $n$ (%) | $n$ (%) |
| Age, years | | |
| 20 - 29 | 77 (17.2) | 33 (21.2) |
| 30 - 39 | 106 (23.7) | 39 (25.0) |
| 40 - 49 | 141 (31.6) | 40 (25.6) |
| ≥ 50 | 123 (27.5) | 44 (28.2) |
| Residence | | |
| Suburb | 170 (38.0) | 54 (34.6) |
| Central | 277 (62.0) | 102 (65.4) |
| Marital status | | |
| Married | 260 (58.2) | 96 (61.5) |
| Single | 91 (20.4) | 36 (23.1) |
| Widowed | 26 (5.8) | 1 (0.7) |
| Separated | 31 (6.9) | 8 (5.1) |
| Living together | 39 (8.7) | 13 (8.3) |
| No answer | 0 (0.0) | 2 (1.3) |
| Occupation | | |
| Farmer | 1 (0.2) | 26 (16.7) |
| Housewife | 264 (59.1) | 0 (0.0) |
| Employee | 78 (17.4) | 68 (43.6) |
| Other | 104 (23.3) | 62 (39.7) |
| Education | | |
| Illiterate | 14 (3.1) | 4 (2.6) |
| Primary | 192 (43.0) | 52 (33.3) |
| Secondary | 173 (38.7) | 60 (38.5) |
| High schoolor university | 68 (15.2) | 24 (15.4) |
| No answer | 0 (0.0) | 16 (10.2) |
| | Median (Inter-quartile range) | Median (Inter-quartile range) |
| Income, pesos[a] | 115.000 (110.000) | 130.000 (110.000)[b] |

[a] US$1 = 608 pesos (April 2004); [b] Mann-Whitney test, $p < 0.01$.

**Table 2.** Anthropometric characteristics of study subjects

| | Female ($n$ = 447) | Male ($n$ = 156) |
|---|---|---|
| | $n$ (%) | $n$ (%) |
| BMI[a] | | |
| Underweight | 1 (0.2) | 0 (0.0)** |
| Normal | 72 (16.1) | 29 (18.6) |
| Overweight | 172 (38.5) | 80 (51.3) |
| Obese | 202 (45.2) | 47 (30.1) |
| Waist circumference | | |
| Normal | 156 (34.9) | 121 (77.6)*** |
| At risk[b] | 291 (65.1) | 35 (22.4) |
| Waist to hip ratio | | |
| Normal | 412 (92.2) | 131 (84.0)** |
| At risk[c] | 35 (7.8) | 25 (16.0) |
| | Mean (SD) | Mean (SD) |
| Height, cm | 154.3 (6.4) | 166.9 (7.1)*** |
| Weight, kg | 71.2 (12.8) | 78.6 (12.2)*** |
| Waist circumference, cm | 93.2 (12.8) | 94.1 (10.3) |

[a] Only one female was underweight and she was not included in the $\chi^2$ test; [b] ≥ 88 cm for females and ≥ 102 cm for males; [c] ≥ 1.0 for females and males. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

(51.3%) than females (38.5%), though obese individuals were more prevalent among females (45.2%) than males (30.1%). The proportion of females with a waist circumference at risk was higher than males (65.1%, 22.4%; $p < 0.001$), though the reverse trend was observed in terms of waist-to-hip ratio (7.8%, 16.0%; $p = 0.003$).

Tables 3 and 4 show the prevalence of obesity among females and males, respectively, according to socio-economic and behavioral characteristics. Only one female was underweight (Table 2), hence her data were excluded from the analysis in Table 3. Age, residence, marital status, education, income, smoking, and parity were associated with obesity for females ($p < 0.05$ for each), whereas only age ($p < 0.001$) and smoking ($p < 0.05$) were significant factors for males. Regardless of gender, older persons were more likely to be obese than younger ones. Obese individuals were more prevalent in suburb (54.7%) than central (39.5%) areas among females, though no geographical difference was observed among males in this rural region. Subjects who were illiterate or had a primary school education were more likely to be obese than those with higher education (≥ 9 years) (female: 54.9% *vs*. 37.1%; $p < 0.001$, male: 39.3% *vs*. 22.6%; $p = 0.088$). Among the families of female subjects, those with low incomes (< 120,000 pesos) had a higher prevalence of obesity (49.3%) than those with high incomes (≥ 120,000 pesos) (39.8%) ($p = 0.007$). Parity was also a significant factor, and women who had delivered three or more times had a higher proportion of obesity (54.0%) in comparison to those who delivered fewer than three times (once or twice 40.9%, never 24.0%; $p < 0.001$). Next, the association between obesity and behavioral factors was examined. The proportion of obese individuals was the highest for those who had never smoked or had stopped smoking among females (50.3%). With regard to physical activity, a rather small proportion of study subjects in all BMI categories exercised for 30 min more than three times per week. There was no statistically significant association between physical activity level and obesity.

The mean frequency of intake per week for selected food items is listed in Tables 5 and 6 by BMI and gender. There was no significant difference in food frequency between BMIs. Intake of bread, potatoes, vegetables, and fruit was more frequent, followed by rice, beef, chicken, margarine, juice, and carbonated drinks for both genders. The intake of fish, seafood, mushrooms, and alcohol was low. The mean frequency of intake of beef and chicken was more than once a week, but the mean frequency of intake of fish was less than once a week regardless of gender. For males, intake of juice and carbonated drinks was two or three times a week, but intake of milk was two times a week. Although data are not shown in the tables, comparison of females and males indicated that intake of bread, potatoes, cheese, pork, mutton, processed meat, eggs, carbonated drinks, and alcohol was more frequent for males than females ($p < 0.05$ for each), while females consumed yogurt, seaweed, fruit, and jam more frequently than males ($p < 0.05$ for each).

Dietary habits were also asked in this study (Table 7 and 8). More than 70% of the subjects took breakfast everyday, and more than 80% of the normal females and overweight males did so. Regardless of gender, there

**Table 3.** Prevalence of obesity according to socio-economic and behavioral characteristics (females)

| | | Normal ($n$ = 72) | Overweight ($n$ = 172) | Obese ($n$ = 202) |
|---|---|---|---|---|
| | | $n$ (%) | $n$ (%) | $n$ (%) |
| Age, years | < 43 | 51 (23.1) | 92 (41.6) | 78 (35.3)*** |
| | ≥ 43 | 21 (9.3) | 80 (35.6) | 124 (55.1) |
| Residence | Suburb | 17 (10.0) | 60 (35.3) | 93 (54.7)** |
| | Central | 55 (19.9) | 112 (40.6) | 109 (39.5) |
| Marital status | Married | 36 (13.8) | 101 (38.9) | 123 (47.3)* |
| | Single | 25 (27.8) | 30 (33.3) | 35 (38.9) |
| | Widowed | 4 (15.4) | 14 (53.8) | 8 (30.8) |
| | Separated | 1 (3.2) | 14 (45.2) | 16 (51.6) |
| | Living together | 6 (15.4) | 13 (33.3) | 20 (51.3) |
| Occupation | Farmer | 0 (0.0) | 0 (0.0) | 1 (100.0) |
| | Housewife | 35 (13.2) | 105 (39.8) | 124 ( 47.0) |
| | Employee | 20 (25.6) | 31 (39.8) | 27 (34.6) |
| | Other | 17 (16.5) | 36 (35.0) | 50 (48.5) |
| Education | ≤ Primary | 18 (8.7) | 75 (36.4) | 113 (54.9)*** |
| | ≥ Secondary | 54 (22.5) | 97 (40.4) | 89 (37.1) |
| Income, pesos[a] | < 120.000 | 32 (11.8) | 105 (38.9) | 133 (49.3)** |
| | ≥ 120.000 | 38 (22.9) | 62 (37.3) | 66 (39.8) |
| Smoking | Everyday | 19 (22.6) | 36 (42.9) | 29 (34.5)** |
| | Sometimes | 13 (25.0) | 22 (42.3) | 17 (32.7) |
| | Don't smoke, quit | 40 (12.9) | 114 (36.8) | 156 (50.3) |
| Exercise[b] | Yes | 7 (16.7) | 19 (45.2) | 16 (38.1) |
| | No | 65 (16.1) | 153 (38.0) | 185 (45.9) |
| Parity | 0 | 20 (40.0) | 18 (36.0) | 12 (24.0)*** |
| | 1 - 2 | 28 (15.5) | 79 (43.6) | 74 (40.9) |
| | ≥ 3 | 24 (11.2) | 75 (34.9) | 116 (53.9) |

[a] US$1 = 608 pesos (April 2004); [b] More than 3 times a week, more than 30 min; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

**Table 4.** Prevalence of obesity according to socio-economic and behavioral characteristics (males)

| | | Normal ($n$ = 29) | Overweight ($n$ = 80) | Obese ($n$ = 47) |
|---|---|---|---|---|
| | | $n$ (%) | $n$ (%) | $n$ (%) |
| Age, years | < 43 | 21 (26.6) | 42 (53.2) | 16 (20.2)** |
| | ≥ 43 | 8 (10.4) | 38 (49.3) | 31 (40.3) |
| Residence | Suburb | 10 (18.5) | 28 (51.9) | 16 (29.6) |
| | Central | 19 (18.6) | 52 (51.0) | 31 (30.4) |
| Marital status | Married | 14 (14.6) | 49 (51.0) | 33 (34.4) |
| | Single | 12 (33.3) | 17 (47.2) | 7 (19.5) |
| | Widowed | 0 (0.0) | 0 (0.0) | 1 (100.0) |
| | Separated | 1 (12.5) | 7 (87.5) | 0 (0.0) |
| | Living together | 2 (15.4) | 6 (46.1) | 5 (38.5) |
| Occupation | Farmer | 6 (23.1) | 12 (46.1) | 8 (30.8) |
| | Housewife | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | Employee | 10 (12.8) | 35 (55.3) | 23 (31.9) |
| | Other | 13 (30.6) | 33 (44.4) | 16 (25.0) |
| Education | ≤ Primary | 8 (14.3) | 26 (46.4) | 22 (39.3) |
| | ≥ Secondary | 19 (22.6) | 46 (54.8) | 19 (22.6) |
| Income, pesos[a] | < 120.000 | 17 (23.3) | 35 (47.9) | 21 (28.8) |
| | ≥ 120.000 | 12 (15.4) | 44 (56.4) | 22 (28.2) |
| Smoking | Everyday | 6 (21.4) | 18 (64.3) | 4 (14.3)* |
| | Sometimes | 9 (31.0) | 9 (31.0) | 11 (38.0) |
| | Don't smoke, quit | 14 (14.6) | 52 (54.2) | 30 (31.2) |
| Exercise[b] | Yes | 2 (12.5) | 8 (50.0) | 6 (37.5) |
| | No | 27 (19.4) | 72 (51.8) | 40 (28.8) |

[a] US$1 = 608 pesos (April 2004); [b] More than 3 times a week, more than 30 min; * $p < 0.05$; ** $p < 0.01$.

was no statistically significant association between eating out for lunch/dinner or having a snack and BMI. The percentage of individuals who ate out for lunch and dinner was higher among males than females but there was an opposite trend regarding having snacks. Dietary habits were closely associated with factors affecting food purchases: regardless of gender, the most important factor for the present purchase was found to be "price,"

**Table 5.** Mean (SD) of food frequency per week according to BMI (females)

| | Normal ($n$ = 72) | Overweight ($n$ = 171) | Obese ($n$ = 202) |
|---|---|---|---|
| | n (%) | n (%) | n (%) |
| Cereals | | | |
| Bread | 6.6 (1.3) | 6.8 (1.1) | 6.9 (0.7) |
| Noodles | 1.8 (1.2) | 1.8 (1.1) | 1.9 (1.3) |
| Rice | 2.1 (1.0) | 2.2 (1.0) | 2.4 (1.3) |
| Potatoes | 3.6 (2.1)[a] | 4.0 (2.1) | 4.4 (2.1)[a]* |
| Legumes | 1.3 (0.9) | 1.5 (0.8) | 1.4 (0.8) |
| Dairy products | | | |
| Milk | 2.8 (2.8) | 2.4 (2.7) | 2.4 (2.8) |
| Yogurt | 1.7 (2.0) | 1.6 (1.9) | 1.3 (1.8) |
| Unripe cheese | 1.2 (1.5) | 1.3 (1.6) | 1.1 (1.5) |
| Cheese | 2.0 (1.8) | 1.6 (1.6) | 1.4 (1.4) |
| Meat | | | |
| Beef | 2.1 (1.5) | 2.1 (1.5) | 2.1 (1.6) |
| Pork | 0.9 (0.8) | 1.2 (0.9) | 1.2 (0.9) |
| Mutton | 0.2 (0.4) | 0.1 (0.3) | 0.1 (0.4) |
| Chicken | 1.8 (0.9) | 2.0 (1.2) | 2.0 (1.3) |
| Other (rabbit, wild bird) | 0.2 (0.4) | 0.2 (0.5) | 0.3 (0.6) |
| Viscera | 0.3 (0.4)[ab] | 0.5 (0.6)[b]* | 0.5 (0.7)[a]* |
| Processed meat | 1.5 (1.3) | 1.4 (1.2) | 1.4 (1.3) |
| Eggs | 2.1 (1.5) | 1.8 (1.3) | 1.9 (1.7) |
| Fish and seafood | | | |
| Fish | 0.7 (0.5) | 0.8 (0.6) | 0.8 (0.7) |
| Canned fish | 1.1 (0.7) | 1.2 (0.8) | 1.2 (0.9) |
| Seafood | 0.2 (0.5) | 0.4 (0.6) | 0.3 (0.5) |
| Seaweed | 0.4 (0.9) | 0.4 (0.7) | 0.4 (0.6) |
| Vegetables and fruits | | | |
| Vegetables | 5.6 (2.3) | 6.0 (1.9) | 5.8 (2.1) |
| Mushrooms | 0.1 (0.3) | 0.1 (0.4) | 0.1 (0.3) |
| Fruit | 4.9 (2.5) | 5.4 (2.2) | 5.5 (2.2) |
| Fats and oils | | | |
| Butter | 1.2 (2.3) | 1.2 (2.3) | 1.2 (2.4) |
| Margarine | 2.7 (2.9) | 2.6 (2.9) | 2.2 (2.6) |
| Mayonnaise | 1.3 (1.6) | 1.2 (1.2) | 1.0 (1.2) |
| Sugar and sweets | | | |
| Jam | 2.4 (2.2) | 2.3 (2.1) | 1.8 (2.0) |
| Sweets | 2.8 (2.6)[ab] | 2.0 (2.2)[b]* | 1.7 (2.3)[a]** |
| Beverages | | | |
| Juice | 2.6 (2.9) | 2.1 (2.7) | 1.9 (2.6) |
| Carbonated drinks | 2.5 (2.3) | 2.2 (2.0) | 2.4 (2.4) |
| Alcohol | 0.5 (0.8) | 0.5 (0.8) | 0.3 (0.7) |

[a] Significant between normal and obese; [b] Significant between normal and overweight; Bonferroni correction following ANOVA
* $p < 0.05$; ** $p < 0.01$.

followed by freshness (though not significantly different, obese female subjects tended to choose price more than subjects with other BMIs). With regard to factors affecting future purchases, a relatively high proportion of participants answered "nutrition." Since price was quoted as the most significant factor for food purchases, average food prices were surveyed at three markets in San Carlos (Table 9).

During the focus group discussion, the participants discussed the differences in past and present diets. They stated that they had consumed milk, soup, legumes, and fruits during their childhood while they mentioned that their latest children consumed yogurt, sweets, french fries, and hot dogs. Overall, the cooking process was also found to have changed, with "fried" now being favored over boiling/cooking (*e.g.* french fries *vs.* boiled potatoes). A question about when and how dietary patterns have changed received the following responses:

"Our diet changed as technology advanced, and then ready-made and processed food began to appear on the market."

"Since TV sets have increased, we had more chances to eat the foods advertised in commercials. Sometimes my children prefer to have the foods shown on TV rather than the meals I cook."

## Discussion

Although there are many studies on obesity in Chile, little is known about rural areas, especially with respect to the influences of socio-economic and dietary factors. This study confirmed the high prevalence of obesity in a rural province of Chile (female: 45.2%, male: 30.1%), finding it to be much higher than national average (female 27%, male 19%). Females had a higher proportion of obesity than males, especially in suburb areas. Similar results were found in several Latin American countries like Brazil and Peru and two previous studies of the Chilean cities of Santiago and Valparaiso (*18*). One of the possible explanations for females being more likely to be obese is biological differences (*19*). Humans carry a number of genes

**Table 6.** Mean (SD) of food frequency per week according to BMI (males)

| | Normal ($n$ = 29) | Overweight ($n$ = 80) | Obese ($n$ = 47) |
|---|---|---|---|
| | $n$ (%) | $n$ (%) | $n$ (%) |
| Cereals | | | |
| Bread | 7.0 (0.0) | 6.9 (0.6) | 6.8 (0.8) |
| Noodles | 1.9 (1.1) | 2.0 (1.1) | 1.7 (1.2) |
| Rice | 2.2 (1.1) | 2.6 (1.3) [c] | 1.9 (1.1)[c]** |
| Potatoes | 4.3 (2.2) | 4.8 (2.2) | 4.5 (2.4) |
| Legumes | 1.2 (0.7) | 1.5 (0.9) | 1.3 (0.8) |
| Dairy products | | | |
| Milk | 2.3 (2.6) | 2.2 (2.5) | 2.0 (2.5) |
| Yogurt | 1.1 (1.2) | 1.0 (1.4) | 1.0 (1.6) |
| Unripe cheese | 0.8 (1.0) | 1.2 (1.5) | 1.1 (1.4) |
| Cheese | 2.5 (2.2) | 1.8 (1.9) | 1.9 (1.6) |
| Meat | | | |
| Beef | 1.4 (1.0)[b] | 2.4 (1.7)[b]** | 2.3 (1.8) |
| Pork | 1.0 (0.8) | 1.5 (1.3) | 1.5 (0.9) |
| Mutton | 0.3 (0.5) | 0.2 (0.4) | 0.4 (0.8) |
| Chicken | 1.9 (1.2) | 2.0 (1.3) | 1.6 (0.7) |
| Other (rabbit, wild bird) | 0.3 (0.5) | 0.5 (1.0) | 0.3 (0.8) |
| Viscera | 0.4 (0.5) | 0.5 (0.6) | 0.4 (0.5) |
| Processed meat | 2.0 (1.4) | 1.8 (1.6) | 1.7 (1.5) |
| Eggs | 1.8 (1.2) | 2.4 (1.5) | 2.4 (1.9) |
| Fish and seafood | | | |
| Fish | 0.9 (0.4) | 0.9 (0.5) | 0.7 (0.5) |
| Canned fish | 1.4 (1.2) | 1.2 (0.9) | 1.2 (0.9) |
| Seafood | 0.2 (0.4) | 0.4 (0.5) | 0.3 (0.5) |
| Seaweed | 0.3 (0.5) | 0.4 (0.6) | 0.2 (0.4) |
| Vegetables and fruits | | | |
| Vegetables | 5.2 (2.4) | 5.9 (2.0) | 5.8 (2.2) |
| Mushrooms | 0.0 (0.2) | 0.2 (0.4) | 0.2 (0.6) |
| Fruit | 4.5 (2.6) | 4.9 (2.3) | 5.0 (2.5) |
| Fats and oils | | | |
| Butter | 0.9 (1.6) | 1.6 (1.7) | 1.5 (2.4) |
| Margarine | 2.7 (2.7) | 2.4 (2.8) | 2.5 (2.8) |
| Mayonnaise | 1.6 (1.8) | 1.2 (1.6) | 1.0 (1.3) |
| Sugar and sweets | | | |
| Jam | 1.3 (1.1) | 1.8 (1.9) | 1.7 (2.0) |
| Sweets | 2.1 (2.6) | 1.5 (2.2) | 1.3 (2.0) |
| Beverages | | | |
| Juice | 3.4 (2.8) | 2.3 (2.6) | 2.5 (2.9) |
| Carbonated drinks | 3.4 (2.4) | 3.6 (2.7) | 3.0 (2.5) |
| Alcohol | 0.8 (0.6) | 1.0 (1.4) | 0.9 (1.1) |

[b] Significant between normal and overweight; [c] Significant between overweight and obese; Bonferroni correction following ANOVA ** $p < 0.01$.

**Table 7.** Prevalence of obesity according to dietary habits (females)

| | | Normal ($n$ = 72) | Overweight ($n$ = 172) | Obese ($n$ = 202) |
|---|---|---|---|---|
| | | $n$ (%) | $n$ (%) | $n$ (%) |
| Breakfast | Don't eat | 6 (8.3) | 6 (3.5) | 8 (4.0) |
| | Sometimes | 7 (9.7) | 35 (20.6) | 45 (22.4) |
| | Everyday | 59 (82.0) | 129 (75.9) | 148 (73.6) |
| Eat out for lunch, no. of times[a] | 0 | 38 (52.8) | 89 (52.7) | 113 (55.9) |
| | < 3 | 27 (37.5) | 62 (36.7) | 67 (33.2) |
| | ≥ 4 | 7 (9.7) | 18 (10.6) | 22 (10.9) |
| Eat out for dinner, no. of times[b] | 0 | 40 (55.6) | 123 (71.6) | 153 (76.1)** |
| | < 3 | 26 (36.1) | 46 (26.7) | 43 (21.4) |
| | ≥ 4 | 6 (8.3) | 3 (1.7) | 5 (2.5) |
| Have a snack, no. of times[c] | 0 | 37 (51.4) | 82 (47.7) | 114 (56.4) |
| | ≥ 1 | 35 (48.6) | 90 (52.3) | 88 (43.6) |
| Have you heard of dietary fiber? | Yes | 58 (80.6) | 131 (76.2) | 151 (74.8) |
| | No | 14 (19.4) | 41 (23.8) | 51 (25.2) |
| What do you think is most important when buy food? | Price | 34 (16.8) | 59 (29.2) | 109 (63.7) |
| | Freshness | 12 (11.0) | 49 (45.0) | 48 (28.1) |
| | Nutrition | 1 (16.7) | 1 (16.7) | 4 (2.3) |
| | Other | 2 (6.4) | 19 (61.3) | 10 (5.9) |
| In the future, what will be most important for you when buy food? | Price | 19 (38.8) | 38 (29.7) | 70 (40.2) |
| | Freshness | 14 (28.6) | 43 (33.6) | 46 (26.4) |
| | Nutrition | 11 (22.4) | 28 (21.9) | 42 (24.2) |
| | Other | 5 (10.2) | 19 (14.8) | 16 (9.2) |

[a] Frequency of eating out for lunch per week; [b] Frequency of eating out for dinner per week; [c] Frequency of having snacks per day; ** $p < 0.01$.

**Table 8.** Prevalence of obesity according to dietary habits (males)

| | | Normal ($n$ = 29) | Overweight ($n$ = 80) | Obese ($n$ = 47) |
|---|---|---|---|---|
| | | $n$ (%) | $n$ (%) | $n$ (%) |
| Breakfast | Don't eat | 3 (10.7) | 3 (3.8) | 4 (8.7) |
| | Sometimes | 4 (14.3) | 11 (13.9) | 6 (13.0) |
| | Everyday | 21 (75.0) | 65 (82.3) | 36 (78.3) |
| Eat out for lunch, no. of times[a] | 0 | 6 (20.7) | 40 (51.9) | 23 (50.0)* |
| | < 3 | 20 (69.0) | 25 (32.5) | 17 (37.0) |
| | ≥ 4 | 3 (10.3) | 12 (15.6) | 6 (13.0) |
| Eat out for dinner, no. of times[b] | 0 | 8 (27.6) | 56 (71.8) | 32 (71.1) |
| | < 3 | 17 (58.6) | 16 (20.5) | 9 (20.0) |
| | ≥ 4 | 4 (13.8) | 6 (7.7) | 4 (8.9) |
| Have a snack, no. of times[c] | 0 | 17 (58.6) | 52 (65.0) | 32 (68.1) |
| | ≥ 1 | 12 (41.4) | 28 (35.0) | 15 (31.9) |
| Have you heard of dietary fiber? | Yes | 23 (79.3) | 49 (62.0) | 28 (60.9) |
| | No | 6 (20.7) | 30 (38.0) | 18 (39.1) |
| What do you think is most important when buy food? | Price | 9 (56.3) | 28 (59.6) | 13 (56.6) |
| | Freshness | 6 (37.5) | 14 (29.8) | 5 (21.7) |
| | Nutrition | 0 (0.0) | 1 (2.1) | 0 (0.0) |
| | Other | 1 (6.2) | 4 (8.5) | 5 (21.7) |
| In the future, what will be most important for you when buy food? | Price | 7 (43.8) | 19 (40.4) | 9 (37.5) |
| | Freshness | 5 (31.3) | 15 (31.9) | 6 (25.0) |
| | Nutrition | 1 (6.2) | 8 (17.0) | 5 (20.8) |
| | Other | 3 (18.7) | 5 (10.7) | 4 (16.7) |

[a] Frequency of eating out for lunch per week; [b] Frequency of eating out for dinner per week; [c] Frequency of having snacks per day; * $p < 0.05$.

**Table 9.** List of market food prices

| Food | | Unit | Price (pesos)[a] |
|---|---|---|---|
| Cereals | Bread | 1 kg | 580 |
| | Noodles | 1 kg | 598 |
| | Rice | 1 kg | 687 |
| Potatoes | | 1 kg | 100 |
| Beans | | 1 kg | 980 |
| Dairy products | Milk | 1 L | 490 |
| | Powdered Milk | 130 g + water (L) = 1 L | 375 |
| | Low fat powdered milk | 130 g + water (L) = 1 L | 408 |
| | Yogurt | 165 g | 117 |
| | Low fat yogurt | 165 g | 190 |
| | Unripe cheese | 250 g | 695 |
| | Cheese | 250 g | 555 |
| Meat and eggs | Beef | 1 kg | 2406 |
| | Pork | 1 kg | 1352 |
| | Chicken | 1 kg | 1015 |
| | Sausage | 1 dozen | 804 |
| | Eggs | 1 dozen | 680 |
| Fish and seafood | Fish | 1 kg | 2390 |
| | Canned fish | 425 g | 463 |
| | Seafood (with shell) | 1 kg | 650 |
| | Canned seafood | 110 g | 805 |
| Vegetables | | 1 kg | 175 - 450 |
| Fruits | | 1 kg | 200 - 500 |
| Fats and oils | Butter | 250 g | 662 |
| | Margarine | 250 g | 479 |
| | Low fat margarine | 250 g | 500 |
| | Mayonnaise | 250 g | 308 |
| | Low fat mayonnaise | 250 g | 411 |
| Sweets | Jam | 250 g | 374 |
| | Low sugar jam | 250 g | 376 |
| Beverages | Powdered juice | 45 g + water (L) = 1 L | 116 |
| | Low sugar juice | 45 g + water (L) = 1 L | 116 |
| | Carbonated drinks | 1 L | 330 |
| | Low calorie carbonated drinks | 1 L | 338 |

Visits were made to three supermarkets in San Carlos; The price is the average of the three supermarkets; [a] US$1 = 608 pesos (April 2004).

related to body size, and environmental factors would also affect the phenotypic expression of these genes (*20*). Another important determinant of obesity of females was parity. This finding was compatible to a study by Bastian *et al*. (*21*), which showed that the risk of being obese in later life would increase according to the number of children one had.

One of the interesting findings of the current study

is that there was a significant association between BMI and some socio-economic factors for females (Table 3) but not for males (Table 4). A similar trend was observed in a previous study (*22*). In the current study, males had fewer restrictions on access to food, with more chances to eat out for dinner; they were probably influenced less by socio-economic factors than females. Peña *et al*. suggested that the association between obesity and socio-economic characteristics may be influenced by cultural and social background, though in most cases this is not readily apparent (*7*). In a patriarchal society, the intra-household food distribution may be in favor of males.

Contrary to general understanding, subjects with a lower level of physical activity were not necessarily obese in this study. The proportion of those who exercised for more than 30 min three times or more per week was 9.7% (58/600), and thus it was quite difficult to make the statistical comparison. Moreover, this study did not measure actual energy expenditure, so caution is needed when interpreting the results. A National Health Survey noted that the proportion of persons with a low level of physical activity was quite high (*13*). Enhancing awareness of the importance of increasing one's physical activity is therefore crucial.

With regard to diet, no association between frequency of food intake and BMI was found. The possibility of under-reporting of dietary intake of obese subjects was noted in an earlier study (*23*), which might have contributed to the obese subjects having a lower frequency of intake in sugar-rich foods such as sweets and juice than those with a normal BMI.

Another important finding in this study was the possible association between the factors affecting food purchases and frequency of food intake. Comparison of the factors affecting present and future food purchases indicated that the highest proportion answered "price" for both present and future purchases, whereas those who answered "nutrition" increased for future purchases. This shows awareness of the importance of nutrition among the study population, though in actuality individuals would place priority on price. Obese female subjects tended to attach importance to price for their present purchases. Characteristics of obese female subjects like having a family and income constraints might have contributed this priority as well as decision of cooking process. These subjects are thus unable to place a priority on purchase aspects besides price.

Results for food frequency suggested the impact of price. Table 9 shows the list of market food prices. Of course, the frequency of food intake is not simply due to price. The current dietary guideline in Chile recommends the intake of dairy products and fish (*24*). Comparing fish and meat indicates people eat more pork and chicken (1352 pesos, 1015 pesos per 1 kg respectively) which is relatively cheaper than fish (2390 pesos per 1 kg), though beef is an exception (2406 pesos per 1 kg). Another food recommended in the dietary guideline is dairy products including milk. For males, the frequency of intake of milk was about two times a week and intake of juice and carbonated drinks was two or three times a week. In terms of price, milk is more expensive than juice and carbonated drinks, and low fat milk is even more expensive. Similar trends were observed in a previous study where persons with low socio-economic status would consume only what they could afford (*25*,*26*). Ironically, most of the foods affordable to poor populations tend to be energy-dense and high-fat (*27*) and the current study also found that food items recommended by dietary guidelines were rather expensive. An essential aspect to promoting healthy food choices is that recommended foods do not increase the costs for the population. Combined with nutrition education, price controls as have been reported in Mauritius and Finland may play an important role, (*28*,*29*). The current study also suggests that in addition to price the availability at the shop negatively affects access to recommended foods (*e.g*. there were only three fish shops, while meat was available at many shops).

Since Chile is a country about 4000 km long from north to south, the crops cultivated and livestock raised, as well as the availability of markets for other foods, may differ greatly by region. Establishing area-specific strategies, including the dietary guidelines, to control obesity and related diseases is therefore essential. A good model for this may be the Japanese Ministry of Health, Labor, and Welfare's (former the Ministry of Health and Welfare) "Health Japan 21 (Kenkou Nippon 21)," which set out Japanese public health targets for the year 2010, as it employs different aims/strategies by region (*30*).

In conclusion, the current findings of a high prevalence of obese/overweight individuals, together with the characteristics of their diets including changes in the cooking process, suggest that a nutrition transition is underway in rural areas as well. Although assessing actual changes in BMI during the course of a nutrition transition is difficult to do with a cross-sectional study, the findings of this study illustrate the significance of obesity in the area studied. Latin Americans are known to be more likely to have greater body fat for the same BMI than whites in the US and Europe and therefore to have a higher likelihood of experiencing related diseases at lower BMI levels (*31*). Although a lower prevalence of obesity was observed for males, the high frequency of eating out must be curbed and low awareness of dietary fiber must be remedied to prevent an increase in overweight individuals and to enhance health awareness among males. In addition, future nutrition policy should take regional difference into consideration and should be established in collaboration with relevant sectors (*e.g*. health, education, agriculture,

economic) as well as with the mass media.

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# Factors associated with skills of health visitors in maternal-infant mental health in Japan

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**SUMMARY** **This study is a formative evaluation of a training seminar for health visitors, who visit mothers to provide them with support in terms of postpartum mental health, and was performed to examine factors that relate to the skills of these health visitors. Subjects were all health visitors ($n$ = 232) from around Japan who participated in a 2-day training seminar. One-hundred and thirty-three valid responses (57.3%) were received and written consent to participate in the research was obtained. Results of statistical analyses indicated that a health visitor's skill at supporting a mother in terms of postpartum mental health had two domains, such as interpersonal health care skills and skill at formulating measures. In addition to the length of experience ($p < 0.001$), the level of expertise ($p < 0.001$) and the total score on the Generalized Self-Efficacy Scale ($p < 0.1$ for interpersonal health care skills) was related to a higher level of the health visitor's skill at supporting mothers in terms of their postpartum mental health. In contrast, having a university degree ($p < 0.1$) was related to a lower level of the health visitor's interpersonal health care skills. Therefore, a training seminar aimed at promoting the skills of health visitors must provide them with the latest expertise and encourage their self-efficacy by helping them successfully envision supporting mothers in terms of their postpartum mental health. In addition, careful instruction of health visitors with less experience and a university degree is crucial.**

***Key Words:*** Community mental health, home visits, maternal-child health, nursing skills, public health nurse

## Introduction

Among the tasks related to assessing the postpartum mental health of mothers, early detection of postpartum depression is vital. Postpartum depression is a depressive disorder found in women after childbirth and develops within one year after childbirth at a high rate, reaching 10-15% in various countries (*1-3*). Although the period when it most frequently develops has been described as 4-6 weeks after childbirth, recent work has found that the disease most often occurs even earlier, *i.e.* a couple of weeks after childbirth (*4,5*). As well as having a negative impact on the child-rearing ability of family members and the infant's development (*6*), postpartum depression is considered to be a risk factor for infant abuse (*7*). The Japanese Ministry of Health, Labor, and Welfare has designated a decrease in the incidence of postpartum depression and mortality due to child abuse as a major objective of its project named "Sukoyaka Oyako 21 (Sukoyaka Family 21: Sukoyaka means 'sound' or 'well-being')." There is an urgent need to establish measurements, including early screening of postpartum depression, to prevent inappropriate child care or infant abuse.

As part of the Japanese maternal and child health system, a program of visits to new mothers by health visitors, mainly from health and welfare centers in the community, may have contributed to a decrease in mothers' anxieties about child rearing (*8,9*). Improving

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the skills of health visitors, who visit mothers to provide support in terms of their postpartum mental health should prove to be a practical and effective way of early screening for postpartum depression and preventing infant abuse.

The Edinburgh Postnatal Depression Scale (EPDS) is a self-administered questionnaire developed by Cox *et al*. (*10*) for population-based screening of postpartum depression. Research has shown that use of this questionnaire can increase the population-based rate of detection of postpartum depression.

Yoshida confirmed the usefulness of having health visitors who perform postnatal visits utilize the EPDS (*10,11*) and the Bonding Questionnaire (Marks, unpublished) (*12*) in combination with a short self-constructed questionnaire in order to determine high risks for postpartum depression and infant abuse, including socioeconomical items (*3,12*). In 2004, a child-rearing support manual based on these three types of questionnaires was developed through Grants for Health Science (Research on Children and Families) (*13*), and the manual was freely distributed to 127 main branches of maternal and child health organizations in Japan (in 47 prefectures, 13 government-designated cities, 9 public health center-designated cities and 23 wards of Tokyo). In addition, a training seminar using this child-rearing support manual was initiated in 2005 for health visitors who make postnatal visits.

To ensure the validity of the training seminar, factors related to the skills of health visitors who provide mental assessments and provide care support for postnatal mothers and their families in the community must be determined. Thus, the purpose of this study was to explore factors related to the skills of such health visitors.

## Methods

### *Participants and procedures*

Subjects were 232 health visitors who participated in a 2-day training seminar held in August and September 2005 in Tokyo and Fukuoka, respectively (*14*). Subjects came from Tokyo, Fukuoka, and 37 other prefectures. An oral explanation of the objective of the research was provided on the day of the seminar. In addition, the fact that the signed questionnaires would be strictly managed and published without identifying individuals and the institutions where they worked was explained. Subjects were sent self-administered questionnaires by mail in December 2005. Written consent to participate in the research was obtained.

### *Measures*

Skill at supporting mothers in terms of their postpartum mental health is assumed to be related to the characteristics of individual health visitors, such as the length of experience and the characteristics of the institutions where they work, such as the establishment of postnatal visits using the EPDS as a business operation (Figure 1).

Such skills were assumed to consist of a series of processes through which health visitors support mothers after childbirth and the process of planning measures. Specifically, a process was designed in which

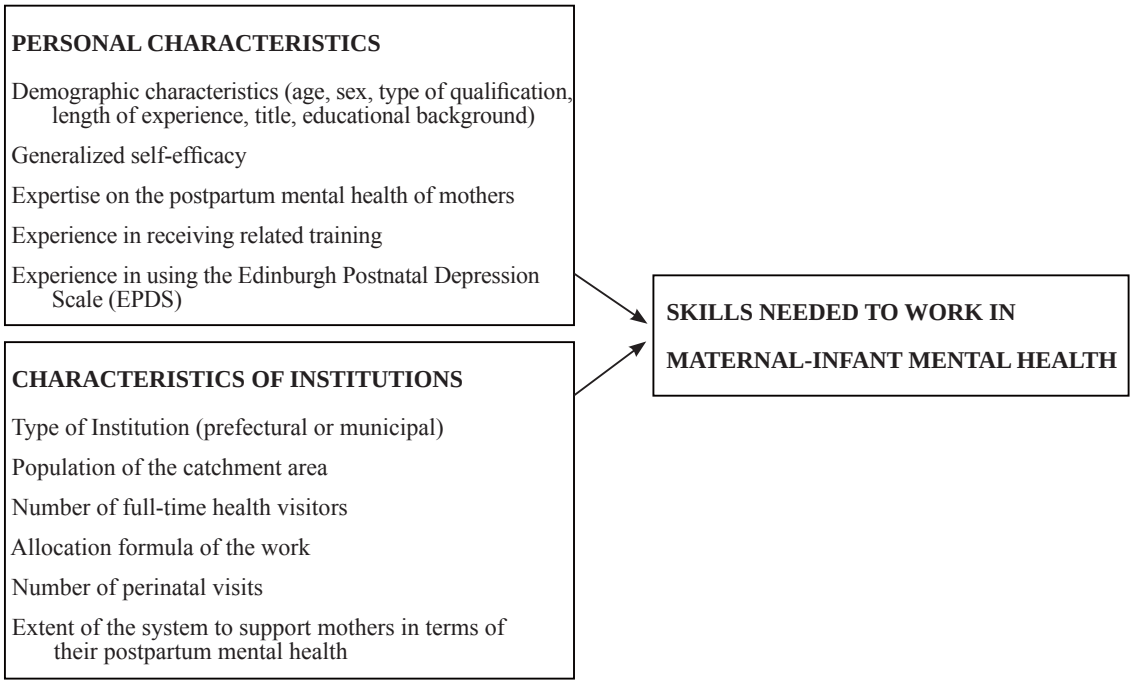


**Figure 1.** Model of factors potentially associated with skills of health visitors working in maternal-infant mental health.

health visitors conducted perinatal visits, interviewed the mothers using the three types of self-administered questionnaires, performed assessment and provided support, cooperated with other institutions as needed, collected data, and developed measures (*15*). Referring to the questionnaire developed by Elliott *et al*. (*16*) and Saeki *et al*. (*17,18*), a self-reported questionnaire was also developed using 11 questions with a 4 point Likert scale, with points ranging from 1 (insufficient) to 4 (sufficient) to evaluate the skills specific to support for mothers in terms of their postpartum mental health (hereinafter referred to as the skill scale).

Questions regarding personal characteristics were administered to identify demographic characteristics (age, sex, type of qualification, length of experience, title, and educational background), experience in receiving related training, experience in using the EPDS, and the time at which use of the EPDS commenced, as well as generalized self-efficacy and self-evaluation of expertise.

Self-evaluation of expertise was done with a self-reported questionnaire with 15 questions prepared with reference to the questionnaire developed by Elliott *et al*. (*16*) and the child-rearing support manual (*13*). The questionnaire had a 4 point Likert scale, with points ranging from 1 (not know at all) to 4 (well know) (hereinafter referred to as the expertise scale).

Self-efficacy is one of the core concepts of Bandura's social cognitive theory (*19*). It shows a cognitive tendency to consider that one's own judgment and effort contribute to success. This concept has been used not only to clinically evaluate the self-management behavior of patients with chronic diseases but also to improve the skills of specialists. Although many case-specific self-efficacy scales have been developed, the current study used the Generalized Self-Efficacy Scale, which was developed by Sherer (*20*) and translated into Japanese by Narita *et al*. (*21*), to measure generalized self-efficacy as a characteristic, cognitive tendency. The scale included a questionnaire with 23 items using a 5-point Likert scale.

Questions regarding the characteristics of the institutions where the health visitors work were administered using six items, such as the type of institution (municipal or prefectural), population in the catchment area, number of full-time health visitors, formula for allocation of work, level of activity (number of mothers and children to visit) and evaluation of the system of operations (hereinafter referred to as the scale of operations).

*Design*

Exploratory factor analysis was performed after calculating descriptive statistics in order to examine the validity of the skill scale prepared for this study. Multiple linear regression analysis was subsequently performed to investigate the factors related to the skills of health visitors, with the points on the skill scale serving as dependent variables. For the points on the skill scale, ANOVA and the test of significance for Spearman's correlation coefficient were performed regarding individual factors, in which individual factors were selected as independent variables when the individual $F$ value was $p < 0.2$. Taking multicollinearity into account, selected valuables were used for multiple linear regression analysis, excluding outliers. Using the step-down procedure, a model with maximum explanatory power (adjusted $R^2$) was determined. Statistical analyses were conducted using SPSS 12.0 J for Windows.

**Results**

*Characteristics of participants* (Table 1)

There were 133 valid responses (valid response rate: 57.3%). The personal characteristics of the participants were as follows: all of them were female, average age was 38.5 ± 9.0 years, they included 123 public health nurses (92.5%) with various lengths of experience, 27 participants (20.3%) had titles such as manager or assistant manager, and 26 (19.5%) had an educational background that included university/graduate school. The average and standard deviation of the Generalized Self-Efficacy Scale were 79.1 ± 9.83, and Cronbach's α in this study was 0.92. The average and standard deviation of the total scores on the expertise scale were 45.3 ± 6.54, and Cronbach's α was 0.94. Thirty-two participants (24.1%) answered "yes" regarding experience in attending related training seminars. In addition, 64 participants (48.1%) had experience using the EPDS.

The characteristics of the institutions where the participants worked were as follows: they included 40 prefectural health institutions (30.1%), and the median of population of the catchment area of the individual institution was 123,587 (a prefectural institution generally draws from 10 to 30 times the population drawn from by a municipal institution). The median number of full-time health visitors at each institution was 4.3. The level of a health visitor's activity was a median of 105 postnatal visits during the first half of FY2005. The average and standard deviation of the total scores for scale of operations was 8.92 ± 1.82, and Cronbach's α was 0.92.

*Factor structure of the prepared skill scale and the new skill scale*

As a result of factor analysis regarding the prepared skill scale, 2 factors were found to have converged (Table 2). Since the factor loading was greater than 0.4 for both factors regarding an item on the skill scale,

**Table 1.** Demographic data ($n$ = 133)

| | | *n* (%) | mean ± SD/median (range) |
|---|---|---|---|
| ***Personal characteristics*** | | | |
| Age | | | mean: 38.5 ± 9.0 (23-57) |
| Type of qualification | Public health nurse | 123 (92.5) | |
| | Other | 6 (4.5) | |
| Years of experience | 10 years or less | 45 (33.8) | |
| | 11-20 | 50 (37.6) | |
| | 21-30 | 29 (21.8) | |
| | 31-35 | 4 (3.0) | |
| Management position | With title | 27 (20.3) | |
| | Without title | 100 (75.2) | |
| Education | Training school | 67 (50.4) | |
| | Junior college | 26 (19.5) | |
| | University/graduate school | 26 (19.5) | |
| | Other | 8 (6.0) | |
| Generalized self efficacy scale | | | mean: 79.1 ± 9.83 (40-107) |
| Expertise scale | | | mean: 45.3 ± 6.54 (28-60) |
| Experience of related training | Yes | 32 (24.1) | |
| | No | 99 (74.4) | |
| Experience of using EPDS | Yes | 64 (48.1) | |
| | *Used before March 31, 2005* | *23 (35.9)* | |
| | *Used since April 1, 2005* | *40 (62.5)* | |
| | No | 62 (46.6) | |
| ***Characteristics of Institutions*** | | | |
| Type of Institutions | Prefectural health institutions | 40 (30.1) | |
| | Municipal health institutions | 89 (66.9) | |
| Population of catchment area | | | median: 123,587 (1,578-3,790,000) |
| Number of full-time health visitors | | | median: 4.3 (1-166) |
| Allocation formula of the work | Allocation by assigned district | 28 (21.1) | |
| | Allocation by health problem characteristics | 45 (33.8) | |
| | Allocation with features of above-mentioned two formulae | 51 (38.3) | |
| Number of perinatal visits during the first half of FY2005 | | | median: 105 (0-2,160) |
| Operation scale | | | mean: 8.92 ± 1.82 (4-12) |

"skill at presenting an assessment and support plan for colleagues or persons in other jobs," this item was excluded; the remaining 10 items were regarded as the new set of items for the skill scale (hereinafter referred to as the new skill scale). The average and standard deviation of the total score on the new skill scale were 23.4 ± 4.89. The first (6 items) and second (4 items) factors were interpersonal health care skills and skill at formulating measures, respectively. Cronbach's α for the total, first, and second factors was 0.92, 0.90, and 0.89, respectively.

*Factors related to the skills*

Multiple linear regression analysis was performed with the whole sum of scores of the new skill scale, the total score of interpersonal health care skills, and the total score for skill at formulating measures as dependent variables, respectively. Independent variables were selected based on the aforementioned standard model, along with 4 variables, including the type of institution where a health visitor worked, size of the catchment

**Table 2.** The new skill scale

| | Factor 1 | Factor 2 |
|---|---|---|
| Skill at interviewing to assess risk of postpartum depression | **0.823** | -0.102 |
| Skill at interviewing to assess risk of infant abuse | **0.859** | -0.051 |
| Skill at making a care plan based on assessments of postpartum mental health of mothers | **0.588** | 0.272 |
| Skill at caring for postpartum mothers and their families | **0.852** | -0.081 |
| Skill at cooperating with other institutions as needed for care | **0.467** | 0.325 |
| Skill at assessing outcomes of care | **0.568** | 0.290 |
| Skill at sharing learned expertise and skills at an institution | 0.060 | **0.771** |
| Skill at modifying and improving ways of care conducted in an institution | 0.020 | **0.876** |
| Skill at accumulating data and analyzing a database | -0.077 | **0.788** |
| Skill at creating a project proposal and developing measures | -0.053 | **0.859** |

Factor analysis by least squares without weight, and promax rotation.
Factor 1: interpersonal health care skills
Factor 2: skill at formulating measures

area, number of full-time health visitors, and the total score for the scale of operations as part of the characteristics of institutions, in addition to 7 variables, such as length of experience, title, educational background, the total score on the Generalized Self-Efficacy Scale, the total score on the expertise scale, experience in receiving related training, and experience in using the EPDS as part of the characteristics of individual health visitors, where the dependent variable was the whole sum of scores on the new skill scale. In the same manner as for the whole sum of scores on the new skill scale, when the dependent variable was the total score for interpersonal health care skills, the independent variables were the 4 variables for the characteristics of institutions and the 7 variables for the characteristics of individual health visitors. Lastly, when the dependent variable was the total score for skill at formulating measures, the independent variables were only 2 variables, such as the type of institution where a health visitor worked and the total score for the scale of operations as part of the characteristics of institutions, and the aforementioned 7 variables as part of the characteristics of individual health visitors. For educational background, graduation from universities/graduate schools served as an independent variable, taking into consideration future trends for the organization of mother-infant support systems and the training of public health nurses.

The independent variables that were commonly found to be significant with respect to the three dependent variables were length of experience and the total score on the expertise scale ($p < 0.001$) as part of the characteristics of individual health visitors (Table 3). In addition, the population of the catchment area ($p < 0.05$) as part of the characteristics of institutions explained the total score on the new skill scale, while the number of full-time health visitors ($p < 0.01$) as part of the characteristics of institutions and educational background ($p < 0.1$) and the total score on the Generalized Self-Efficacy Scale ($p < 0.1$) as part of the characteristics of individual health visitors explained the interpersonal health care skills.

## Discussion

### *Measurement scale for skills*

Elliott *et al*. (*16*), who developed an intervention program for health visitors, prepared a self-evaluated scale using 11 items regarding expertise and 9 items regarding skills to evaluate the improvement of the skills of health visitors. However, they did not evaluate the validity and reliability of their scale. One large difference between the current scale and the scale of Elliott *et al*. (*16*) in terms of evaluating the skills of health visitors is the fact that only interpersonal health care skills were evaluated in the scale of Elliott *et al*., whereas the current scale went as far as evaluating skill at formulating measures. The sub-scale for formulating measures includes the skill to encourage maternal and child mental health care activities and establish measures to improve the mental health of mothers and children in individual communities.

This dissimilarity was a consequence of differences between Japan and the UK in terms of the community health system, and especially in the role of health visitors. In the UK, the responsibility of health visitors as specialists has been clearly established so that health visitors are responsible for providing interpersonal care to local residents and the primary care trust (PCT) and the National Health Service (NHS) trust are responsible for developing health care measures. In Japan, health visitors like the current participants are responsible for both providing individual care and establishing health care measures (*15*). That is, in Japan the individual health visitor, or public health nurse, performs a dual role of identifying issues regarding health measures by providing interpersonal care in communities and of establishing health care measures.

In the US, the Quad Council of Public Health Nursing Organizations (*22*) stipulated the level of required proficiency (awareness, knowledge, or proficiency) for generalists/staff public health nurses (PHN) or managers/clinical nurse specialists (CNS)/program specialists/executives with regard to

**Table 3.** Linear regressions (beta) for skill total, interpersonal health care skills, and skill at formulating measures

| Independent factors | Dependent factors | | |
|---|---|---|---|
| | Skill total | Interpersonal health care skills | Skills at formulating measures |
| Personal characteristics | | | |
| Years of experience | 0.511*** | 0.431*** | 0.488*** |
| University degree | - | -0.157† | - |
| Experience using EPDS | 0.082 | - | - |
| Experience attending related training | - | - | 0.095 |
| Total score on expertise scale | 0.315*** | 0.295*** | 0.300*** |
| Total score on generalized self efficacy scale | 0.106 | 0.131† | - |
| Characteristics of institutions | | | |
| Size of catchment area | 0.158* | - | - |
| Number of full-time health visitors | - | 0.231** | - |
| Total score for scale of operations | - | - | 0.088 |
| Adjusted $R^2$ | 0.462 | 0.460 | 0.377 |

*** Statistically significant differences ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$), † ($p < 0.1$) .

the individual domains of individuals/families and populations/systems, respectively. These domains included 8 domains, such as communication skills, analytic assessment skills, financial planning and management skills. Cross *et al*. (*23*) devised an instrument to measure changes in public health nursing competency, and its validity has long been established. Several investigations have been performed to clarify the competencies expected of public health nurses in Japan (*24,25*). Moreover, a scale to evaluate these competencies was developed by Saeki *et al*. (*17,18*). However, no investigations have been performed to clarify the skills required for postnatal visits and other maternal and child mental health activities. Although Nakaita *et al*. (*26*) conducted a study regarding self-evaluation by public health nurses in activities to prevent child abuse, they did not confirm the validity of the self-evaluation items used in the study.

Factor analysis of the prepared skill scale revealed that factors included interpersonal health care skills and skill at formulating measures, as previously assumed. For the item "skill at presenting an assessment and support plan," the factor loading of both factors was higher than 0.4. This may have been because the skill for this item serves as a bridge between interpersonal health care and formulating measures. Therefore, the item was considered to be necessary in evaluating the skills of health visitors but was excluded from the scale, and two factors were set as subscales. As a result, a new scale with 6 items for interpersonal health care skills and 4 items for skill at formulating measures was used for analysis. Factor analysis was performed again and Cronbach's α was calculated to confirm the construct validity and the internal reliability, respectively.

*Factors related to skills*

Qualitative and quantitative investigations have suggested that length of experience (*18,24,25*) and the institution where a health visitor works (prefectural health institution, municipal health center, *etc*.) (*24,26*) are factors related to the skills and competencies of health visitors. In the current study, the variable with the greatest explanatory power in two subscales and total score on the new skill scale was the length of experience among personal characteristics of health visitors. In addition, the total score on the expertise scale was the variable with secondary greatest explanatory power in two subscales and the total score on the new skill scale. This shows that health visitors with more expertise have greater skills even in comparison to those with the same length of experience.

In addition, a large population of the catchment area, part of the characteristics of institutions, indicated a high level of general skills. This may be related to the opportunities that health visitors have to accumulate experience while working with difficult cases or abundant social resources in such areas.

Health visitors who worked for institutions with a larger number of full-time health visitors had greater interpersonal health care skills. Their skills were believed to have improved through experience in which colleagues acted as a model to learn from, an advisor, or a support resource. In addition, interpersonal health care skills tended to be higher in health visitors with highly generalized self-efficacy. Those who believed in their potential were highly motivated, contributed to successes, continued to make an effort, and were flexible with respect to change (*27*). Such health visitors are believed to have greater skill in support activities provided to various types of households. Health visitors with a university degree tended to have lower interpersonal health care skills. This may be related to the recent difficultly university students have had in sufficiently undergoing practical training because the number of students seeking a license in public health nursing has exceeded the capacity of institutions for practical training in Japan.

A training seminar aimed at promoting the skills of health visitors who conduct home visits to support mothers in terms of their postpartum mental health must provide them with the latest expertise, based on reliable evidence, and encourage their self-efficacy by helping them successfully envision supporting mothers in terms of their postpartum mental health. In addition, this study revealed the importance of carefully instructing health visitors with less experience, a university degree, and who work in institutions with a small population in the catchment area and with a smaller number of full-time health visitors.

**Conclusions**

Results of exploring the factors related to the skills of health visitors who conducted home visits to support mothers in terms of their postpartum mental health were as follows:

1. The skills of health visitors who support mothers in terms of their postpartum mental health were found to have 2 domains, such as interpersonal health care skills and skill at formulating measures.

2. The most explanatory variable for the levels of interpersonal health care skills, skill at formulating measures, and the total skill in the 2 domains was the length of experience, followed by the level of expertise.

3. In addition, the population of the catchment area explained the total score for total skills, while the number of full-time health visitors, educational background, and total score on the Generalized Self-Efficacy Scale explained interpersonal health care skills.

Therefore, a training seminar aimed at promoting the skills of health visitors must provide them with the latest expertise and encourage their self-efficacy

by helping them successfully envision supporting mothers in terms of their postpartum mental health. In addition, careful instruction of health visitors with less experience and a university degree is crucial.

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# Activation of the extracellular signal-regulated kinases signaling pathway in squamous cell carcinoma of the skin

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**SUMMARY** **Activation of the extracellular signal-regulated kinase (ERK) pathway is involved in many human tumors. Little is known about the role of activated ERK1/2 in squamous cell carcinoma (SCC) of the skin. In this study, the expression and distribution of phosphorylated ERK (p-ERK) in normal human skin and SCC with different degrees of differentiation was examined by immunohistochemical analysis using formalin-fixed paraffin embedded sections. PD98059, a specific ERK pathway inhibitor, was used to evaluate the effect a blockade of ERK activation has on the proliferation of a cutaneous SCC cell line (DJM-1) in culture. In this study, p-ERK 1/2 positive staining was observed in all cases of SCC examined but rarely in the control specimens of normal skin. Moreover, the expression of p-ERK1/2 was significantly higher in poorly differentiated SCC in comparison to well-differentiated ones. Expression levels were positively associated with the degree of malignancy and proliferative activity of SCC. In contrast, inhibition of ERK pathway signaling markedly suppressed tumor cell proliferation. These results suggest that ERK1/2 signal pathways play an important role in the proliferation of SCC and that the inhibition of this signal pathway may be effective in the treatment of cutaneous SCC.**

***Key Words:*** Extracellular signal-regulated kinases (ERK), squamous cell carcinoma (SCC), PD98059, proliferative activity, immunohistochemistry

## Introduction

The mitogen-activated protein kinases (MAPKs) are a group of protein serine/threonine kinases that are activated in response to a variety of extracellular stimuli and mediate signal transduction from the cell surface to the nucleus. In mammalian cells, there are three well-characterized subfamilies of MAPKs: the extracellular signal-regulated kinases (ERK), the c-Jun *N*-terminal kinases (JNK), and the p38 MAPK kinases (*1-3*). The ERKs are activated by most growth factors and have been shown to be a key regulator of both proliferation and differentiation in different cell types (*4*), while JNKs and p38 MAP kinase are activated by various forms of cellular stress and have predominantly been implicated in responses to cellular stress, inflammation, and/or apoptosis (*5*). There are three major MAP kinase pathways in human tissues, but that involving ERKs is most relevant to human cancer. A high level of p-ERK protein has frequently been observed in many human tumors (*6-10*). Furthermore, recent studies revealed that activation of ERKs plays a critical role in the proliferation of cancer cells (*11-14*). In contrast, the role of ERKs in cutaneous SCC was less clear. Therefore, the present study examined the expression of p-ERKs protein in normal skin and cutaneous SCC. PD98059 (a MEK/ERK inhibitor) was used as a tool to evaluate the effect of blockade of ERK activation on the proliferation of cutaneous SCC cell lines (DJM-1).

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## Materials and Methods

### *Tissue samples*

Surgically resected specimens used for this study included 5 portions of normal human skin obtained

from healthy patients undergoing plastic surgery; 10 had well-differentiated SCC and 10 had poorly-differentiated SCC. All tissue specimens were selected from the files of the Department of Dermatology & Plastic and Reconstructive Surgery, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University. Informed consent and institutional review board agreement were obtained. For each formalin-fixed and paraffin-embedded tissue block, several 4 μm sections were cut. One section was stained with H&E for histological examination, and the others were used for immunohistochemical staining.

*Antibodies and reagents*

Phospho-p44/42 MAP Kinase (Thr202/Tyr204) rabbit polyclonal antibody was purchased from Cell Signaling Technology (Beverly, MA, USA). PD98059 (a MEK/ERK inhibitor) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The secondary antibody and conjugate were included in the VECTASTAIN Elite universal ABC kit PK-6200 (Vector Laboratories, Burlingame, CA, USA).

*Immunohistochemistry*

Immunohistochemical staining was performed using the standard streptavidin-biotin-peroxidase complex method. Briefly, formalin-fixed paraffin sections 4 μm thick were deparaffinized and subjected to antigen retrieval by microwaving in 10 mM of citrate buffer (sodium citrate, pH 6.0) for 15 min. The sections were then treated with 0.3% hydrogen peroxide in methanol for 20 min at room temperature to block endogenous peroxidase. After they were washed in phosphate-buffered saline (PBS), unspecific binding sites were blocked with 5% normal horse serum at room temperature for 1 h. Excess serum was deleted from the sections. The tissues were then incubated with the primary antibody at 1:100 dilutions at 4°C overnight. Following washing with PBS, the sections were incubated with biotinylated horse-anti rabbit IgG at a dilution of 1:200 for 30 min at room temperature. The slides were rinsed and incubated with the avidin/biotin complex at room temperature for 60 min. Visualization of the peroxidase reaction was achieved with diaminobenzidine (DAB), followed by counterstaining with Giemsa.

A negative control slide for each tissue was incubated with non-immunized horse serum to replace the primary antibody.

*Evaluation of immunohistochemical staining*

Only nuclear staining was considered positive for p-ERK. The extent of immunoreactivity was evaluated in a semiquantitative manner using the following scale: Grade 1: < 5% of cells p-ERK positive; Grade 2: 5-25% of cells p-ERK positive; Grade 3: 26-50% of cells p-ERK positive; Grade 4: > 50% of cells p-ERK positive. Sections were examined in a double-blind manner to reduce bias and ensure consistency of examination.

*Cell line and culture conditions*

The human cutaneous squamous carcinoma cell line DJM-1 (*15*) was used in this study. The cells were routinely cultured in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS; Sigma, Deisenhofen, Germany) and antibiotics (penicillin, 100 U/mL and streptomycin, 100 mg/mL) at 37°C in a 5% $CO_2$ incubator. Cells were trypsinized and subcultured when they were approaching confluency.

*Effect of PD98059 on cell proliferation*

Confluent cells were harvested with an EDTA trypsin solution and re-suspended to appropriate concentrations in MEM medium containing 10% fetal bovine serum. After $1 \times 10^4$ cells/1mL growth medium was seeded in each well of a 24-well culture plate, cells were incubated 24 h to allow for attachment. Prior to addition of inhibitors, cells were cultured in serum-free MEM for 24 h to induce a quiescent state. Cells were then incubated for 24, 48 and 72 h in serum-free MEM containing either 5 μM, 10 μM, 20 μM, or 30 μM PD98059 (dissolved in dimethylsulfoxide (DMSO); final concentration in medium < 0.1%). In addition, cells incubated with serum-free MEM with 0.1% DMSO as a control. All experiments were performed in triplicate. The attached cell numbers were determined using a Coulter counter (Beckman Coulter, Fullerton, CA, USA).

*Statistical analysis*

Data are expressed as mean ± standard deviation. All experiments were performed in triplicate. Significant differences among the groups were determined using the Mann-Whitney *U*-test. A value of $p < 0.05$ was considered significant.

**Results**

*Expression of p-ERK protein in normal control skin and normal skin adjacent to a tumor*

In the control specimens of normal skin, no positive staining was seen in the epidermis and hair follicles; p-ERK immunoreactivity was observed in luminal surface of the acrosyringium and in the luminal surface and the nuclei of luminal cells in intra-dermal portions

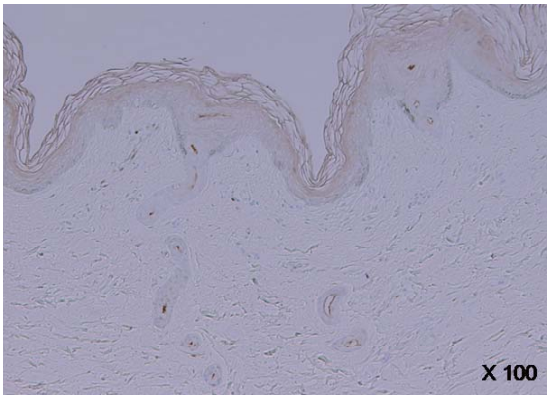


**Figure 1.** Expression of p-ERK protein in normal control skin. Expression was seen in the luminal surface of the acrosyringium and in the luminal surface and the nuclei of luminal cells in intradermal portions of the eccrine sweat ducts. A few vascular endothelial cells with weak nuclear staining were also seen. No signal was seen in the epidermis.

**Table 1.** Summary of p-ERK expression in 20 cases of SCC

| Histological typing | Total | p-ERK expression | | | |
|---|---|---|---|---|---|
| | | Grade 1 (< 5 %)* | Grade 2 (5-25%)* | Grade 3 (26-50%)* | Grade 4 (> 50%)* |
| Well-differentiated | 10 | 2 | 6 | 2 | |
| Poorly differentiated | 10 | | | 3 | 7 |

P-ERK, phosphorylated extracellular signal-related kinase; SCC, squamous cell carcinoma. *Percentage of p-ERK positive cells of tumor cells in the specimen.

of the eccrine sweat ducts. Weak nuclear staining was occasionally found in some vascular endothelial cells (Figure 1). A similar expression pattern was obviously strengthened in normal skin adjacent to a tumor.

*Expression of p-ERK protein in cutaneous squamous cell carcinoma*

Results are summarized in Table 1 and illustrated in Figure 2. Nuclear staining of p-ERK protein was detected in all SCC specimens investigated; there was an obvious difference in the expression levels between poorly differentiated SCCs and well-differentiated SCCs. Immunohistochemical analysis showed that expression of p-ERK significantly increased in poorly differentiated SCCs in comparison to well-differentiated SCCs. In terms of the percentage of positive tumor cells, two cases of well-differentiated SCCs were grade 1, six were grade 2 and two were grade-3. However, three cases of poorly differentiated SCCs were grade 3 and seven were grade 4. Findings demonstrated that p-ERK expression was closely correlated with the degree of tumor cell differentiation. Even in well-differentiated SCCs, only the peripheral cells of the tumor nests were p-ERK-positive, but the central keratin pearls showed a negative immunoreaction.

*Effect of PD98059 on cell proliferation*

Results are shown in Figure 3. Human cutaneous squamous carcinoma cells, DJM-1 cells, were

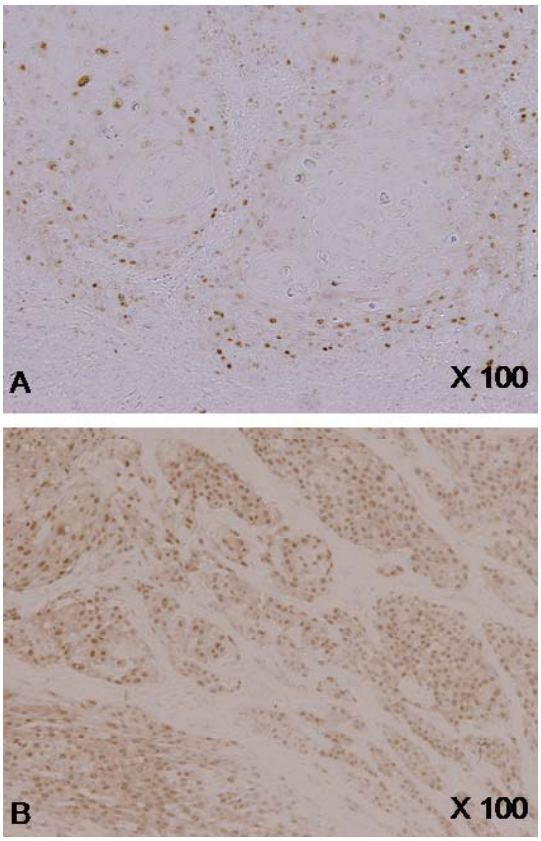


**Figure 2.** Expression of p-ERK protein in cutaneous squamous cell carcinoma. (A) In well-differentiated SCC, nuclear positive staining was noted in the less differentiated cells in the periphery of tumor cell nests. (B) In poorly-differentiated SCC, strong nuclear staining was present in the majority of tumor cells.

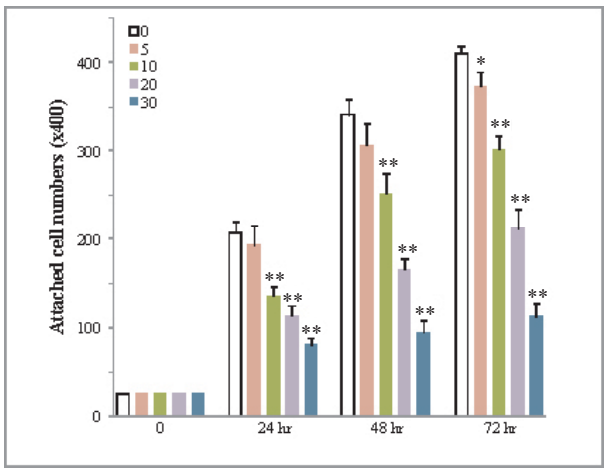


**Figure 3.** Effect of PB98059 on DJM-1 cell proliferation. Cells were plated in 24-well culture plates at a density of $1 \times 10^4$ cells/well in serum-supplemented medium. After a 24 h-attachment period, the cells were grown under serum-free conditions for 24 h. Cells were then incubated in serum-free MEM with different concentrations of PD98059 (0, 5, 10, 20, and 30 μM). The numbers of attached cells were counted at 24, 48, and 72 h after PD98059 treatment using a Coulter counter. Data are expressed as the mean ± standard deviation of three independent experiments. $*P < 0.05$; $**P < 0.01$ when compared to 0 (DMSO also).

incubated with PD98059 for different periods of time at concentrations ranging from 0-30 μM and the cell number was determined with a Coulter counter (Beckman-Coulter). PD98059 was shown to inhibit the proliferation of DJM-1 cells in a dose- and time-

dependent manner. Results showed that SCC cells were extremely sensitive to the growth-inhibiting effects of PD98059, which at a concentration of 30 μM almost completely suppressed DJM-1 cell proliferation.

## Discussion

In the present study, nuclear staining of p-ERK protein was found in all SCC samples investigated. p-ERK expression was significantly higher in poorly-differentiated SCC than in well-differentiated SCC. Even in well-differentiated SCCs, expression was also limited to the less-differentiated area of the tumor, and staining was rarely detected in large keratinized cells at the centre of cell nests or horny pearls. These results revealed that the expression levels of p-ERK increased in accordance with decreasing grades of histological differentiation, suggesting that up-regulation of p-ERK expression reflects a high degree of malignancy and proliferative activity of SCCs.

Among MAPK pathways, the ERK pathway, known to be responsible for unregulated cell proliferation, is thus far one of the best characterized and is closely related to human cancer. High levels of phosphorylated ERKs have been reported in various types of human carcinoma cells (*6-10*). The elevated expression of p-ERK observed in SCCs in this study is consistent with the results of previous studies indicating that increased expression of p-ERK was correlated with more aggressive tumor behavior and higher proliferative activity (*8,11,13,14,16*).

In order to further confirm the functional role of activated ERK in the proliferation in SCCs, experiments were performed *in vitro* using MEK/ERK inhibitor PD98059 to treat DJM-1 in culture. DJM-1 was almost completely suppressed by PD98059 at a concentration of 30 μM. These results corroborate previous experimental studies that suggest p-ERK plays a critical role in the proliferation of malignant tumors (*11-14*).

Multiple factors are associated with the constitutive activation of the ERK pathway, including MEK-dependent and independent mechanisms (*17,18*). In cutaneous SCC cells, the MEK/ERK pathway inhibitor PD98059 completely inhibited cell proliferation, strongly suggesting that MEK-dependent mechanisms are involved. These data suggest that the MEK/ERK pathway is important for cutaneous SCC cell proliferation.

In summary, increased p-ERK is expressed in human cutaneous SCC and is related to proliferative activity. Inhibition of the MEK/ERK signal pathway with PD98059 completely eliminated SCC cell proliferation *in vitro*. Taken together, these results indicate that the MEK/ERK signal pathway may be an important potential therapeutic target in cutaneous SCC.

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# Effect of vibration on skin blood flow in an *in vivo* microcirculatory model

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**SUMMARY** **The effect of vibration on skin microcirculation was studied to investigate the possibility of clinical use of vibration to prevent and treat pressure ulcers. Vibrations at a vibrational intensity of 600, 800, or 1,000 mVpp with a fixed frequency of 47 Hz were applied horizontally to the ear of male hairless mice ($n$ = 6 for each group) under inhalation anesthesia. The control group ($n$ = 6) received no vibrations. Venular blood flow was measured by an intravital videomicroscope at the baseline and at 0, 5, and 15 min after the application of vibrations. A significant increase was observed in the 600 mVpp group 5 and 15 min after vibration in comparison to the control group ($P$ = 0.002 and $P$ = 0.046, respectively). We also detected increased blood flow in the 800 mVpp group ($P$ = 0.028) and the 1,000 mVpp group ($P$ = 0.012) 5 min after vibration; however, these increases attenuated after 15 min. These results indicate that direct skin vibration at a frequency of 47 Hz improves skin blood flow. The present study gives further support to the role of vibration on a short-term increase in skin blood flow.**

***Key Words:*** Microcirculation, chronic wound, vasodilation, intravital videomicroscope

## Introduction

Prevention and treatment of pressure ulcers caused by tissue ischemia is a priority in an aged society (*1*). Many efforts have been made to reduce the incidence and increase the healing rate of these wounds, but some remain intractable due to poor circulation (*2*). This type of wound may not be healed by the use of pressure redistribution devices to reduce the tissue ischemia caused by compression (*3*). The crucial strategy in preventing or treating such pressure ulcers is to actively increase the circulation in the affected regions. Microcirculation of the skin has been widely investigated, and various methods to promote blood flow have been developed, including the use of vasoactive drugs such as prostaglandin (*4*), foot-baths (*5*), and various methods of promoting angiogenesis (*6*). However, these methods are sometimes invasive and time-consuming and can cause side effects, causing difficulties when applying them to vulnerable skin predisposed to ischemic wounds or wounded areas. Thus, the current study focused on vibration, which has been reported to affect circulation of skin in a non-invasive manner (*7*). Although previous studies have demonstrated the effect of vibration on blood flow, they evaluated the blood flow indirectly using near-infrared spectroscopy (*8*), plethysmography (*9,10*), thermography (*11*), or histological examination (*12*). The methods of blood flow evaluation employed in those studies are based on indirect quantification such as using the change in the hemoglobin concentration *via* near-infrared spectroscopy, or the change in the strain derived from the limb volume change caused by vibration. Additionally, the effect of vibration on

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the blood flow cannot be estimated using histological examination. These methods cannot determine to what extent vibration has an impact on microcirculation, so the current authors thus employed a microcirculatory model using the ear of the hairless mouse (*13*). This model uses an intravital microscope to quantitatively visualize the skin microcirculation (*14*). The current study employed a vibrational frequency of 47 Hz since the most effective frequency had been determined in a previous experimental study (*15*). The purpose of the study was to explore the effect of vibrations of varied intensity on skin microcirculation in order to investigate the possibility of clinical use of vibration to prevent and treat pressure ulcers.

## Materials and Methods

### *Experimental animals*

Twelve male homozygous (hr/hr) hairless mice (Saitama Experimental Animals Supply Co. Ltd, Japan) were used. The animals were given a standard diet ad libitum. The experimental procedures conformed to the Guidelines for Animal Studies at Saitama Medical University.

### *Vibration profiles and experimental set-up*

To apply vibrations, a vibrator consisting of a box-shaped vibration source including an electric motor and a virgulate vibration exciter 5 mm in diameter was developed (Figure 1). With this vibrator, changes in the intensity of vibration were achieved by changing the voltage of the power supply. The vibrator was mounted on a triaxially mobile manipulator.

### *Experimental procedures*

Before vibrations were applied, anesthesia was induced and maintained by continuous inhalation (isoflurane; 1.5-2.0%, air; 200 mL/min). The ears of hairless mice were prepared as described in previous reports (*13,14*). Two distal regions of the auricular margin were sewn with nylon thread to spread and fix the ear on the light source in order to measure blood flow. A thermistor probe was sandwiched between the ear and the light source to monitor the local temperature (Figure 2).

In the experimental group ($n$ = 6), the virgulate vibration exciter was placed in contact with the proximal base of the ear, vertical to the auricular axis, to vibrate the whole ear for 10 min. Intensities of 600 mVpp, 800 mVpp, and 1,000 mVpp were used with a fixed frequency of 47 Hz based on a preliminary study (*15*). Control animals ($n$ = 6) were submitted to the same exact conditions, except for energizing the vibrator. The exciter was placed in contact with the part of the ear as in the experimental group. Use of the same mouse for experiments was avoided for 1 week. All experiments were conducted in a thermoneutral laboratory with a room temperature of 24ºC.

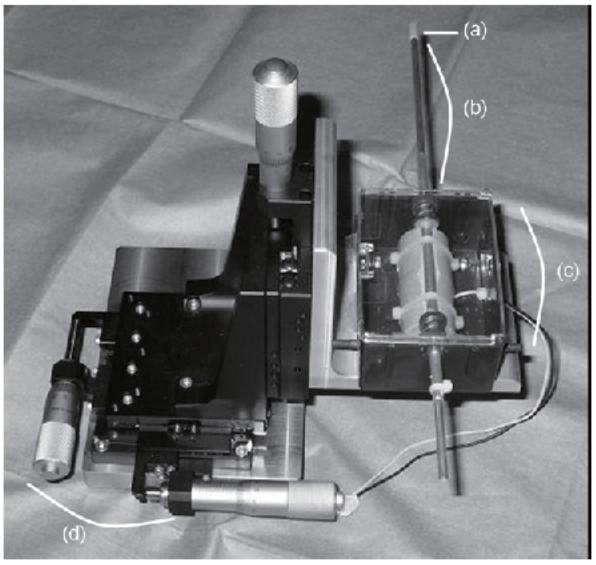


**Figure 1.** External view of the vibrator mounted on a triaxially mobile manipulator. (a) vibration applicator in contact with the ear. (b) virgulate vibration exciter. (c) vibration source including electric motor. (d) triaxially mobile manipulator.

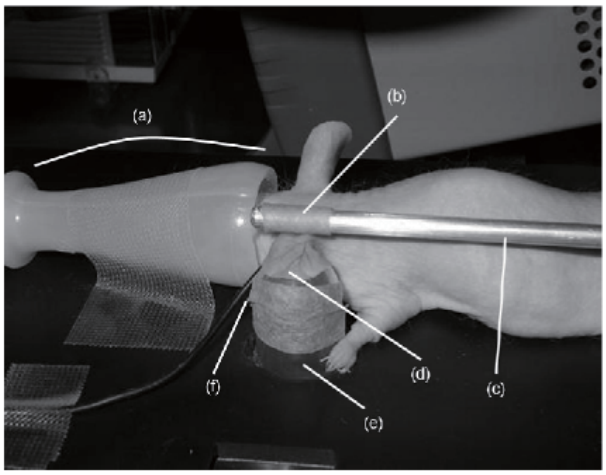


**Figure 2.** Experimental set-up for application of vibrations to the ear. (a) inhalation anesthesia. (b) vibration applicator in contact with the ear. (c) virgulate vibration exciter. (d) ear. (e) halogen lamp. (f) thermistor thermometry.

### *Observation and recording of the microcirculation*

The experimental apparatus used for blood flow monitoring has been described elsewhere (*16*). Briefly, it included a function generator (33220A 20 MHz; Agilent Technologies International Japan, Ltd., Japan), a mounted vibrator, a halogen lamp (PCS-UMX250; Mejiro Precision Inc., Japan), and a charged-coupled device camera (DXC-107a; Sony Corporation, Japan). Images of microcirculation were recorded on a hard disk video recorder (Rec-On; I-O data, Japan) together with time and frame counts (VTG-33; FOR.A, Japan) for later analysis.

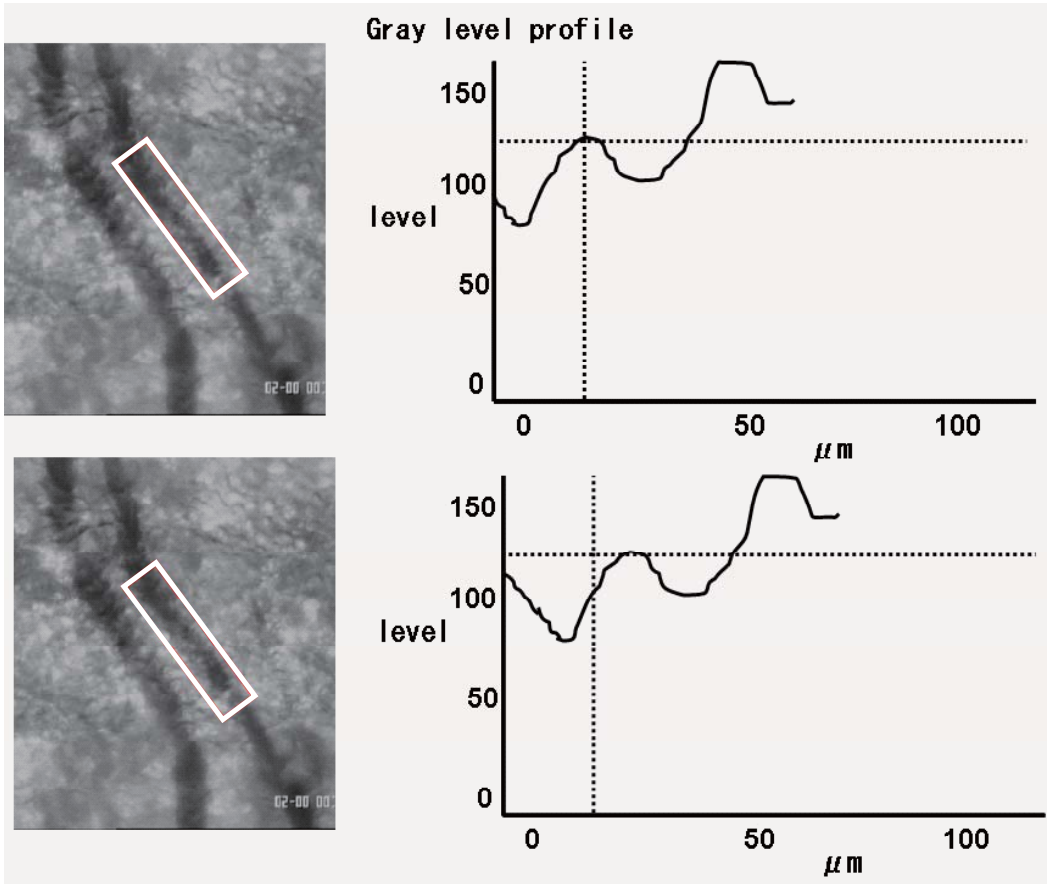


**Figure 3.** Blood flow velocity measurement using the CapiScope. (Above) The gray-level pattern along each line is measured. (Below) The pattern is compared with that from the next field (or several fields later for very low velocities). The velocity can be calculated based on the distance that the correlated pattern has traveled between the two gray-level profile measurements.

*Measurement of blood flow*

Blood flow was measured at the baseline and at 0, 5, and 15 min after the application of vibration. To determine the blood flow in the vessels, the blood velocity (μm/sec) and diameter of individual venules (μm) were measured. Venules with a diameter of 30-60 μm were selected for measurement.

The blood velocity through individual vessels was determined by a spatial correlation technique using recently developed image acquisition and analysis software (CapiScope II; KK Technology, UK) (*17*). This software detects the gray-level profile along a given vessel for each field of recorded images and compares this pattern with that of the next field (or several fields later for very low velocities). The comparison is performed by calculating the correlation coefficient for every possible shift in the previous gray-level profile relative to the new profile. Since the time lapse between the two gray-level profiles is known (*i.e.* 1/60th second for NTSC-based systems), the velocity can be calculated based on the distance that the correlated pattern has traveled between the two gray-level profile measurements (Figure 3).

Venule diameters were measured on acquired images using the calibrated ruler function of image-analyzing software (Beta 4.0.3 of Scion Image; Scion Corporation, MD, USA). Using the velocity (v) and the radius (r) of each venule, the blood flow (F) was calculated as:

$$F = \pi r^2 v$$

*Statistical analysis*

Relative blood flow (%) 0, 5, and 15 min after vibration was calculated using the baseline values as a reference for each intensity of vibration with the following formula: relative blood flow (%) = value at each time point/baseline value × 100. All data are represented as means with standard deviation. To detect differences between the experimental and control groups, analysis of covariance was employed using the baseline data as covariates. All statistical analyses were performed using Statistical Analysis System ver. 9.1.3 (SAS Institute Inc, Cary, NC). The level of significance was set at $P = 0.05$.

**Results**

The representative microscopic appearance of a venule in the 600 mVpp group is shown in Figure 4.

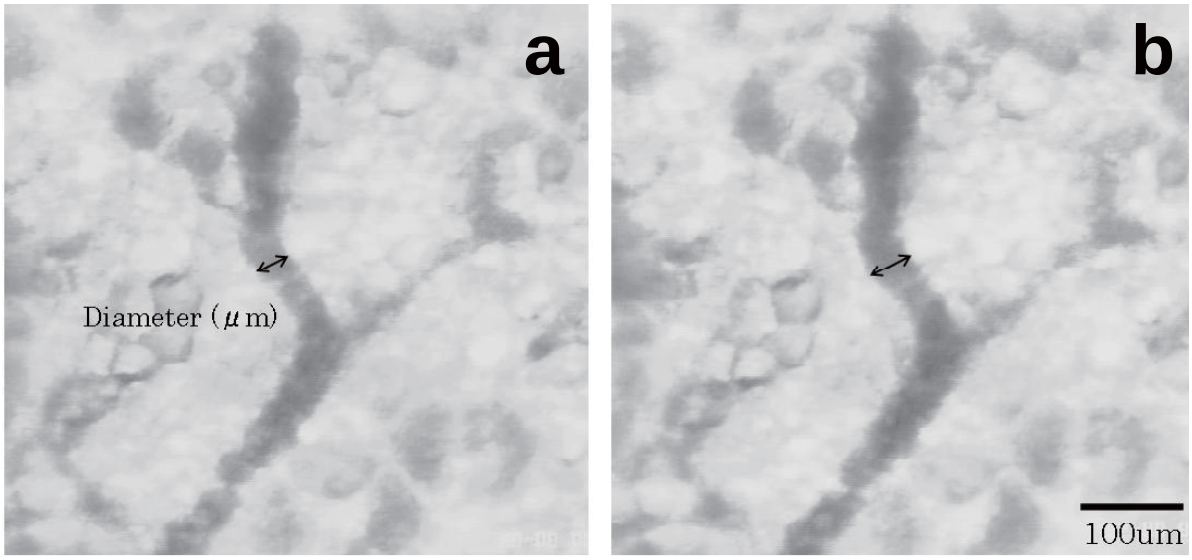


**Figure 4.** Visualization of venules with an intravital videomicroscope. (a) before the application of vibration. (b) 5 min after vibration. Compared to venules prior to vibration, increases were observed in the density of red blood cells and vessel diameter.

**Table 1.** Summary of relative blood flow velocity and vessel diameter.

| | pre | 0 min | 5 min | 15 min |
|---|---|---|---|---|
| Relative blood flow velocity (%) | | | | |
| 600 mVpp (*n* = 6) | 100 | 99.8 ± 7.2 | 103.1 ± 8.3 | 102.8 ± 13.4 |
| 800 mVpp (*n* = 6) | 100 | 103.3 ± 11.6 | 104.9 ± 11.8 | 102.8 ± 16.0 |
| 1,000 mVpp (*n* = 6) | 100 | 104.4 ± 9.4 | 108.9 ± 11.6 | 107.2 ± 15.5 |
| Control (*n* = 6) | 100 | 104.3 ± 6.9 | 103.3 ± 7.4 | 108.6 ± 9.8 |
| Relative vessel diameter (%) | | | | |
| 600 mVpp (*n* = 6) | 100 | 104.7 ± 4.3 | 113.2 ± 6.1 | 112.4 ± 5.4 |
| 800 mVpp (*n* = 6) | 100 | 103.0 ± 5.3 | 106.2 ± 5.0 | 107.5 ± 5.1 |
| 1,000 mVpp (*n* = 6) | 100 | 102.4 ± 6.3 | 103.7 ± 4.6 | 100.1 ± 4.7 |
| Control (*n* = 6) | 100 | 100.3 ± 6.0 | 97.3 ± 6.2 | 97.7 ± 3.0 |

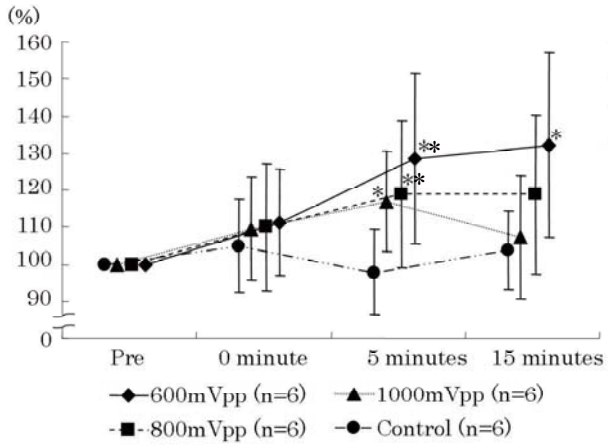


**Figure 5.** Time course of blood flow change. Means with SD are presented. The most significant increase was observed in the 600 mVpp group in comparison to the control. **P* <0.05, ***P* <0.01 compared with the control group.

Compared to venules prior to vibration, increases were seen in the density of red blood cells and vessel diameter. Blood flow velocity and vessel diameter are summarized in Table 1 and blood flow is shown in Figure 5. In the 600 mVpp group, the relative blood flow 5 and 15 min after vibration was 128.6 ± 22.9% and 132.2 ± 24.9%, respectively. These values were significantly higher than those in the control group at the same point in time ($P = 0.0017$ and $P = 0.046$, respectively). In the 800 and 1,000 mVpp groups, the values 5 min after vibration significantly increased to 119.0 ± 19.9% ($P = 0.028$) and 117.0 ± 13.6% ($P = 0.0012$), respectively, in comparison to the control group. However, the increases in the 800 and 1,000 mVpp groups attenuated 15 min after vibration. There were no changes in skin temperatures during the experiments.

## Discussion

The present study first demonstrated the short-term effect of vibration on skin blood flow. These results, based on a newly developed experimental method, can aid further studies in elucidating the mechanism of vasodilation of venules by vibration.

A significant increase in blood flow was observed in the 600 mVpp group 5 and 15 min after vibration in comparison to the control group. Increased blood flow in the 800 and 1,000 mVpp groups was also detected 5 min after vibration. These results indicate

that direct vibration of the skin at a frequency of 47 Hz increases skin blood flow. A study by Kerschan-Schindl using a vibration frequency of 26 Hz demonstrated an increase in muscle blood volume (*18*). Bovenzi reported a decrease with time after stopping vibrations at a frequency of 31.5 Hz (*9*). Skoglund showed that low-amplitude, high- frequency vibration induced vasodilation in human skin (*11*). These facts may point to a relationship between the intensity of vibration and blood flow rather than frequency.

Out of several pathways for vasodilation demonstrated in previous investigations, two main mechanisms were considered to be responsible for the increase in blood flow with vibration. Vibrations in the applicator at 47 Hz may be transmitted to the tissue (*12*), leading to the production of mechanical stresses including shear stress, compression, and stretching of endothelial cells (*19*,*20*). These induce vasodilation of venules via mechanotransduction, which is mainly regulated by nitric oxide (NO). Many studies have reported a relationship between NO production or NO synthase (NOS) expression and mechanical stress created by flow stress (*21*) or exercise (*22*); however, the present study shows that vibration may induce vasodilation by mechanical stress. Experiments using an NOS inhibitor such as $N^G$-nitro-L-arginine are required to elucidate the mechanism of vasodilation through NO production (*23*).

The second mechanism of vasodilation observed in the present study was nerve axon reflex-related microvascular vasodilation (*24*). Vibration may induce impulses *via* polymodal receptors widely distributed on the skin surface, resulting in the release of neuropeptides such as substance P and calcitonin gene-related peptide that dilate the blood vessels (*25*). The 800 and 1,000 mVpp groups showed attenuation of the blood flow increase 15 min after vibration. These results indicate negative feedback, suggesting that caution should be exercised in selecting the intensity of vibrations for clinical use. The attenuation observed may be explained by the habituation of polymodal receptors sensing the vibrations (*26*).

Although there have been reports that type IIa fibers in muscle tissues have typical contraction rates in the range 20-60 Hz (*27*), the ear does not consist of muscle tissue, and hence muscle pump activity cannot be involved in the blood flow increase in the present study (*28*). Since there were no changes in skin temperature during vibration, vasodilation was also not a heat-induced phenomenon (*29*).

The vasodilation achieved by skin vibration may assist the healing of wounds caused by tissue ischemia such as pressure ulcers. The use of vibration to prevent or treat wounds may require that appropriate settings for the intensity of vibration be determined.

The present study does not reveal the detailed mechanism for vasodilation by vibration. In addition, the long-term effect of vibration on skin microcirculation was not determined. Further experiments are needed to address these concerns using an NOS inhibitor to reduce the effect of NO or local anesthesia to block the axon reflex for longer experimental periods.

In conclusion, this study demonstrated that vibration accelerates blood flow *via* venule vasodilation. Accelerated blood flow may be beneficial not only for prevention but also for treatment of cutaneous wounds caused by ischemic complications. Further studies are needed to elucidate the mechanism of vasodilation by vibration.

**Acknowledgment**

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# Inflammatory myofibroblastic tumor of the pancreas – a case report

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**SUMMARY** **Inflammatory myofibroblastic tumor (IMT) of the pancreas is an uncommon tumor with occasional recurrences and rare malignant transformation. We experienced a case of IMT in the body of the pancreas. The patient had no particular symptoms. Dynamic computed tomography revealed a 19-mm lesion in the body of the pancreas. Results: The patient underwent distal pancreatectomy, and has remained in good condition without recurrence for 3 years. Preoperative diagnosis of IMT is difficult due to its rarity and the lack of specific findings. Although the prognosis is better than that for pancreatic carcinoma, long-term follow-up is mandatory.**

***Key Words:*** Pancreas, cancer, resection

## Introduction

Inflammatory myofibroblastic tumor (IMT), proposed by Pettinato *et al*. (*1*), is a rare pathologic entity. IMT is a mass lesion consisting of myofibroblastic spindle cells and plasma cells with inflammatory proliferation (*2*). IMT recurs locally, manifests systemic symptoms, and rarely undergoes malignant transformation (*3*). An inflammatory cell response to infection (*4,5*), trauma, or surgery and immunologic response (*6-8*) are proposed to cause IMT, but the pathogenesis is not clear. IMT most commonly occurs in the lungs followed by lymph node, spleen, liver, heart, orbit, gastrointestinal tract, soft tissue, and bladder (*3,6,9,10*). We report a case of pancreatic IMT.

## Case Report

A 65-year-old man was diagnosed with bladder cancer and underwent transurethral resection of the bladder tumor in 1992. In 2002, a hypoechoic mass in the body of the pancreas was detected in a routine follow-up by ultrasonography examination. He had no particular symptoms. A complete laboratory profile, including the tumor markers carcinoembryonic antigen, cancer antigen 19-9, sialylated carbohydrate antigen (DUPAN-2), and human pancreatic cancer-associated antigen (Span-1), was normal and physical examination was unremarkable.

Abdominal ultrasonography and endoscopic ultrasonography showed a poorly demarcated 2 × 2 cm hypoechoic lesion in the body of the pancreas close to the main pancreatic duct containing several hyperechoic spots. The main pancreatic duct was not dilated. Dynamic computed tomography (CT) showed a 19-mm lesion in the body of the pancreas. Before contrast agent administration, the mass was observed as an iso- dense area. In early - phase contrast CT, the tumor was not enhanced and had a hypo-dense pattern. In late-phase contrast CT, the tumor appeared as an iso- dense mass. Magnetic resonance imaging revealed a low intensity mass in T1 and T2 weighted images, and in dynamic study, the mass appeared as a low intensity mass in the early phase and a high intensity mass in the late phase. Endoscopic retrograde cholangiopancreaticography and celiac angiography revealed no abnormal findings.

Distal pancreatectomy with splenectomy was performed without systemic lymph node dissection. Macroscopically, the tumor was a 2.0 × 1.8 × 1.5 cm mass, poorly demarcated from the normal pancreatic parenchyma. The pancreatic duct was intact and the tumor was as an elastic, hard, solid mass with a yellowish and whitish cut surface (Figure 1). Microscopically, the tumor was fibroblastic and slightly myxoid with moderate infiltration of plasma cells,

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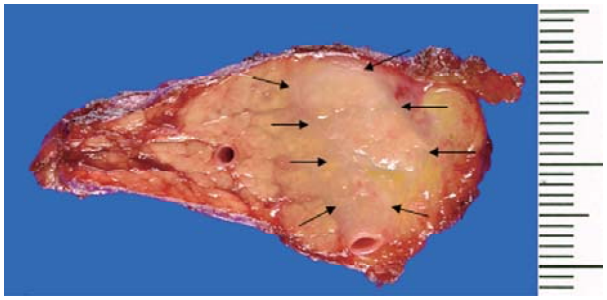

**Figure 1.** Cut surface of the specimen. Arrows indicate the outline of the tumor. The pathologic finding of pancreas without tumor was free from inflammatory changes.

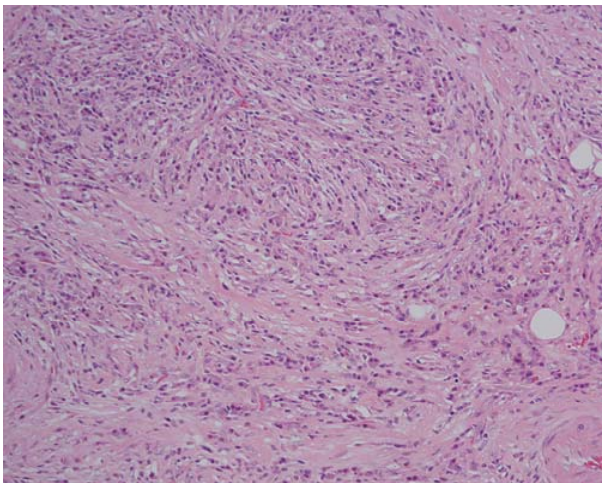

**Figure 2.** Microscopic view of the lesion. Note the increased number of fibromyoblastic cells and moderate infiltration of small round cells.

which are lymphocytes without atypical features. Some of the exocrine and endocrine system remained (Figure 2). Spindle cells were immunohistochemically positive for vimentin and smooth muscle actin (1A-4) and negative for desmin and CD34. The lesion contained small round cells and some were positive for kappa chain and others were positive for lambda chain. Monoclonality was not observed. The estimated MIB1 index of the small round cells was 5%. The patient remains in good condition without recurrence 3 years after the operation.

## Discussion

Although in the presented case a mass lesion was noticed incidentally during routine examination, most patients with pancreatic IMT present with variable signs and symptoms, including abdominal pain (45%), jaundice (45%), weight loss (24%), and abdominal mass (21%) (*11*,*12*). Laboratory findings are variable and non-specific or normal, such as in the present case.

The most typical finding of IMT in diagnostic imaging is a mass lesion without specific findings. Therefore IMT is easily diagnosed as pancreatic cancer. A possible explanation for these misdiagnoses is the low incidence of IMT in the pancreas, the low index of clinical suspicion, the similarity to malignant neoplasm on radiologic examination, and the variability in the histologic appearance of IMT. Fine needle aspiration biopsy or frozen section is of little help for diagnosis (*11*) due to the overwhelming inflammatory infiltration of the lesion.

No previous reports have mentioned positive lymph nodes on pathologic examination. Based on the generally benign behavior of the tumor and the lack of positive swollen lymph nodes in any of the reports, including our experience, additional lymph node dissection is unnecessary. Although IMT has the potential for local recurrence, there are only rare reports of locally aggressive or malignant lesions with distant metastases (*9*,*13*). Those were likely related to factors precluding complete resection, such as adherence to vital strictures and multifocality (*9*), or were connected with some histologic similarities of certain other more aggressive neoplasms (*i.e.* inflammatory fibrosarcoma) even in patients whom received complete resection (*3*,*11*).

In summary, we report a case of pancreatic IMT. Preoperative diagnosis of IMT was difficult due to its rarity and the lack of specific findings. The first choice of treatment should be surgical excision of the tumor as well as excision of detected nodules in the area of the main tumor. Although the prognosis is better than for pancreatic carcinoma, long-term follow-up is mandatory.

## Acknowledgements

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