

# **BioScience Trends**

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## Cover Photo of this issue

### **Physiology and Pharmacology Building, Chinese Academy of Medical Sciences & Peking Union Medical College**

Peking Union Medical College (PUMC) is considered to be the most prestigious medical school in China and the Chinese Academy of Medical Sciences (CAMS) is a unique medical academy. PUMC was founded with the support of the Rockefeller Foundation in 1917 and has been affiliated with CAMS since the latter's establishment in 1956. Fourteen buildings have been rebuilt at the current location, formerly the site of Prince Yu's Residence (豫亲王府). The outsides of the buildings are in typical Chinese Alhambresque style but the insides are designed for medical research, leading many to label CAMS and PUMC a "Western medical school in a Chinese Palace." CAMS and PUMC have produced generations of Chinese leaders in modern medical research and related areas. (by *Li Zhang*)





## China makes impressive achievements in COPD therapy

Xun Li\*

**Keywords:** COPD, Mucolytic agent, Carbocisteine, Therapy

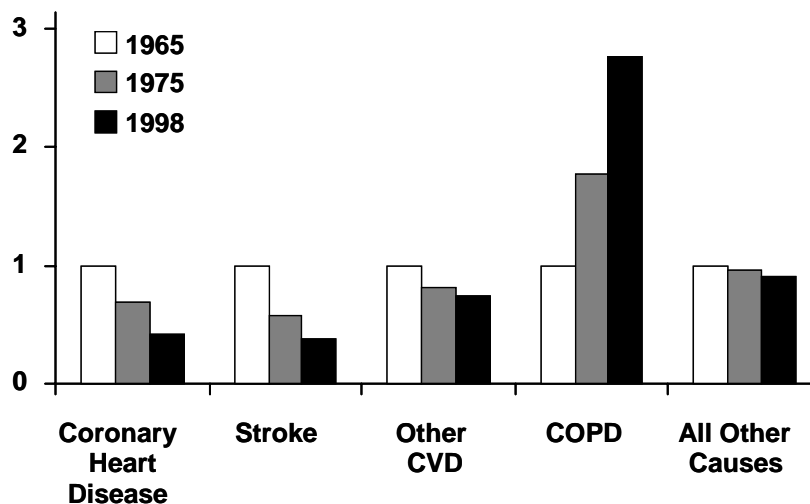
Chronic obstructive pulmonary disease (COPD), a common and frequently occurring lung ailment characterized by obstruction of airflow that interferes with normal breathing, is an overall term for life threatening lung diseases that includes the two conditions of chronic bronchitis and emphysema. The most common symptoms of COPD are breathlessness, or a "need for air," excessive sputum production, and a chronic cough. However, COPD is not just simply a "frequent cough" but often involves lung damage that worsens over time, possibly accelerating deterioration caused by the disease and leading to death.

According to the latest WHO estimates (2007), 210 million people currently suffer from COPD, 3 million died of COPD in 2005, and additional millions are disabled. COPD has been a growing cause of morbidity and mortality worldwide. In the US, for example, the mortality rates for coronary heart disease (CHD) and stroke decreased by 59 and 64 percent, respectively while that for COPD has increased dramatically by 163 percent from 1965-1998, as shown in Figure 1. As a result, the WHO predicts that COPD will become the third leading cause of death worldwide by 2030, particularly in low- and middle-income countries (Data available from <http://www.who.int/respiratory/copden/>).

To date, the pathogenesis of COPD remains somewhat unclear; as a result, the disease is still not curable despite great efforts in seeking effective strategies for COPD treatment and control. However, some potential risk factors have been verified, such as a serious deficiency of  $\alpha_1$ -antitrypsin ( $\alpha_1$ -AT) in the human body, frequent respiratory infections, smoking that includes second-hand or passive exposure, air pollution, occupational dust, chemicals, and poisonous vapors and fumes that irritate or are toxic to bronchial mucosa.

A recent COPD-associated study from China provides encouraging information. According to *China Broadcasting News* from Guangzhou, Zhong Nanshan and his fellow researchers from more than 20 research centers, including the Guangzhou Institute of Respiratory Disease and China Medical University, have achieved exciting results using the mucolytic agent carbocisteine to treat COPD patients ([http://www.cnr.cn/gundong/200806/t20080616\\_504831447.html](http://www.cnr.cn/gundong/200806/t20080616_504831447.html), available as of June 16, 2008).

Zhong is a famous respiratory disease expert and academician of the Chinese Academy of Engineering and president of the Chinese Medical Association (CMA) who has also garnered praise for his significant contribution in fighting Severe Acute Respiratory



**Figure 1.** Percent change in COPD death rates in the US, 1965-1998 (from <http://www.med.monash.edu.au/misc/docs/respiratory-poster-final.pdf>).

Syndrome (SARS) in China. Zhong said less-expensive mucolytics such as carbocisteine could reduce the frequency of self-reported worsening lung damage in approximately 24.5 percent of COPD patients. Furthermore, it significantly improved the quality of life for COPD patients with no obvious adverse effects or toxicity.

Research has demonstrated that pathogenic oxide (e.g. oxygen free radicals), an important pathogenic factor leading to COPD, can affect somatic cells, producing an oxidative stress reaction that results in injury. Therefore, development of effective drugs to counter oxidative stress has become a top priority and an attractive area for drug discovery. Results from Zhong's group also indicated that drugs containing sulfhydryl, usually used as expectorants, counter oxidative stress. However, many difficulties regarding the urgency for prevention and treatment of COPD need to be resolved. These include how to design novel drugs to counter anti-oxidative stress, which drugs containing sulfhydryl should be selected, and the best dosage to administer for them to be effective.

Carbocisteine, a sulfhydryl-containing expectorant with anti-oxidative and anti-inflammatory properties, was selected in order to evaluate its long-term (one year) efficacy and security in terms of reducing acute COPD and improving quality of life; two doses (500 mg) were given three times a day. This study was approved by the local medical ethics committee, and all patients consented to this study and provided informed consent in writing.

Results indicated that mucolytics had clear superiority over conventional therapies such as inhaled corticosteroids, long-acting beta-2 agonists,

and anticholinergics. However, the most significant merit of such drugs is the lower cost of treatment, which was dramatically reduced by up to 3,670 RMB, which was about 85 percent less than that with inhaled corticosteroids. Therefore, "less-expensive mucolytics such as carbocisteine should be recognized as a worthwhile treatment for the long-term management of COPD in the near future," researchers on Zhong's team said, "and this will be exciting news for the majority of COPD patients, and especially for those in low-income countries and regions."

Results of the research, the "Effect of carbocisteine on acute exacerbation of chronic obstructive pulmonary disease (PEACE Study): a randomised placebo-controlled study," were published in the June 14th issue of the respected international medical journal *The Lancet* (Zheng JP, Kang J, Huang SG, et al. *The Lancet* 2008; 371:2013-2018) and have garnered worldwide attention and praise from medical specialists worldwide. Two, Paul Albert and Peter Calverley, commented in an accompanying editorial that this study is particularly prominent in two aspects: (i) it reconsiders the role of existing drugs through rigorous clinical trials, thus offering new insights into COPD care, and (ii) it can greatly reduce the cost of treatment for COPD patients, so that they can benefit from systemic treatment in a standardized way, which is especially suited to low- and middle-income areas in developing countries. Moreover, the journal also held a news conference on the date of publication, providing a special introduction to this exceptional research.

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## Upon completing the 7th Sino-Japanese Symposium on Hepato-Pancreato-Biliary Disease

Mitsugi Shimoda\*

**Keywords:** Liver disease, Biliary disease, Pancreatic disease

The 7th Sino-Japanese Symposium on Hepato-Pancreato-Biliary Disease was held in Beijing from April 18 to 19, 2008 and welcomed four professors as Symposium Presidents: Profs. Ke-Ming Ja, Zhiqiang Huang, and Jia-Hong Dong from China and Prof. Keiichi Kubota from Japan. Top professors from Japan and China gathered together, and results from 75 of the latest studies on themes ranging from hepatocarcinoma, hepatectomy, liver transplantation, treatment of hepatic inflammation, to autoimmune pancreatitis were presented. The symposium attracted many participants, as evidenced by the fact that two conference rooms were packed during the length of the symposium, and featured active question-and-answer sessions. For those not well-versed on medical conditions in China, the symposium offered ample opportunity to learn more through a number of interesting presentations. The assembled research and information on aggressive treatment approaches using surgery on the part of the Chinese participants was impressive and may assist the Japanese participants in their future medical and research practices as well. In addition, the symposium provided a meaningful opportunity to talk with top professors and young doctors from China, with the hopes that such exchanges continue in the future. Many young doctors from Japan also participated in the symposium. They gave a number of interesting presentations, and the symposium offered them the chance to present their results in an international setting. Much as China's drive and determination to host the upcoming Beijing Olympics were apparent, China's influence in the medical field appears to be on the rise as well.

The symposium went well for the two days it was scheduled, and thanks are expressed to everyone who aided with and participated in the symposium. The future looks bright for the upcoming Eighth symposium.



### Main Sessions

- Comprehensive Management of Viral Hepatitis
- Update on Liver Resection Techniques in an Age of Liver Transplantation
- Medical Treatment of Biliary Disease
- Living Donor Liver Transplantation
- Comprehensive Diagnosis and Management of Liver Tumors
- Evaluation and Management of Pancreatic Disease
- Surgical Management of Biliary Disease
- Evaluation and Management of Liver Failure
- Management of Liver Disease
- Current Management of HCC
- Surgical Management of Pancreatic Disease
- Management of Portal Hypertension and Upper Gastrointestinal Hemorrhage
- Systematic Diagnosis and Management of HCC

(\*Second Department of Surgery, Dokkyo University School of Medicine; Secretary general for the Japanese delegation, The 7th Sino-Japanese Symposium on Hepato-Pancreato-Biliary Disease.)

## Brief Report

# Prevalence of HIV infection and HIV-related sex risk behaviors in men who have sex with men in Shandong Province, China

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### Summary

Recently, men who have sex with men (MSM) have become a population at high risk of HIV infection in China, and more investigations of and intervention programs targeting this hidden population are urgently needed. The objective of this study was to assess the prevalence of HIV-positive individuals, HIV-related knowledge, and sex risk behaviors in MSM in large and middle-sized cities. Subjects were 1,617 interviewees who were selected by snowball sampling in 8 cities of Shandong Province and who were interviewed using a structured questionnaire. Their serological specimens were tested in a laboratory to confirm infection with HIV. Results indicated a prevalence of HIV infection of 1.3%, which is low when compared to that in metropolitan areas worldwide, although the prevalence of unsafe sexual behaviors was relevantly high and there was a lack of accessibility to HIV/STD testing and peer education. This study revealed the urgent need to make intervention programs universal, effective, and much more accessible to MSM in typical large and middle-sized cities in China.

**Keywords:** MSM, HIV infection, Risk sexual behaviors, Intervention, China

### 1. Introduction

Since the early 1980s, men who have sex with men (MSM) have fallen victim to an epidemic of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) worldwide. In China, urbanization accelerated by economic changes since the 1980s has complicated the task of HIV/AIDS prevention. HIV/AIDS cases have been increasing over the last several years. At the end of October 2007, the Chinese Ministry of Health, the Joint United Nations Program on HIV/AIDS (UNAIDS), and the World Health Organization (WHO) jointly estimated that China had 700,000 HIV-positive individuals and a prevalence of the infection of 0.05% in the total population (1). Sexual transmission is now the predominant route of transmission. MSM were one of main contributors to the 50,000 cases of emerging infection in 2007, suggesting that they are a high-risk population. According to the joint assessment,

last year HIV-positive individuals among MSM were estimated to have increased by about 30,000 since their level in 2005. The UNAIDS report (2006) indicated growing evidence of HIV outbreaks among Chinese MSM (2).

Unlike in Western countries, MSM remain a "hidden" population in China due to political, socio-cultural, and customary restrictions, and HIV/AIDS prevention and intervention programs targeting MSM in the country have been lacking (3). Academic approaches to such a population that is vulnerable to HIV/AIDS have been limited. Several previous studies focused on MSM and related populations in metropolitan areas such as Shanghai, Nanjing, Guangzhou, and Beijing (4-11) as well as in Kunming, in which the prevalence of HIV is one of China's highest (12). Homosexual transmission was predominant in large and middle-sized cities and areas with large concentrations of migrants. In addition to those metropolitan areas, other major urban areas and other areas experiencing diverse socioeconomic development and urbanization must therefore also be investigated. The objective of this study was to assess the prevalence of HIV-positive individuals, HIV-

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related knowledge, and sex risk behaviors in MSM in Shandong Province, which has China's second highest growth rate and second largest population, as well as geographically diversified socioeconomic conditions in different cities and areas that parallel conditions in the country as a whole. Results should provide evidence of HIV/AIDS prevention and intervention programs for policymakers at both the national and the local level.

## 2. Methods

This is a cross-sectional study conducted in 8 large and middle-sized cities and areas in Shandong Province, including Jinan (the provincial capital of Shandong), Qingdao, Yantai, Jining, Weihai, Dezhou, Liaocheng, and Taian.

In this study, MSM are defined as men who have had sexual activity (anal or oral intercourse) with men in the past year, including male homosexuals, male bisexuals, and male heterosexuals who had sex with men, in the process of receiving commercial sexual services in restrooms, parks, and entertainment venues such as bars, night-clubs, and public baths. This special population was approached *via* a community-based network called the "Rainbow Group" that was voluntarily established among MSM from almost all cities and areas of Shandong Province with the support of experts from the Shandong Province Center for Disease Control and Prevention (Shandong Province CDC). Participants were recruited by snowball sampling due to the difficulty in approaching a hidden population and because of ethical considerations. To minimize the bias from sampling, initial interviewees selected represented diverse demographic, socioeconomic, and behavioral characteristics. Moreover, the interviewees were recruited from different communities and different places in order to improve the representativeness and the generalization of the sampling. As part of ethical considerations, all participants were informed about the study procedures and the investigation was conducted after their informed consent was obtained. The sample size was 1,617 persons in total.

Anonymous participants were personally interviewed using a semi-structured questionnaire that included demographic characteristics, sexual orientation, risk behaviors, and accessibility of related health services. Additionally, a serological test was performed to identify the HIV infection. Venous blood specimens collected from each participant were sent to the laboratory at the Shandong Province CDC. There, sera were separated from the specimens and a rapid HIV test was performed. Frequencies of variables were descriptively calculated using SPSS 11.5 (data analysis is currently underway).

## 3. Results and Discussion

### 3.1. Demographic characteristics

As shown in Table 1, nearly 70% of the surveyed MSM were in their twenties and the average participant age was 23 years. A majority of interviewees had a higher level of education (58.6%). As many as 74% were married or cohabiting with a member of the opposite sex. Interviewees self-identified as homosexual, heterosexual, and bisexual totaled 59.6%, 2.1%, and 31.2%, respectively. This suggests that the range of people exposed to a high risk of HIV and sexually transmitted diseases is widely expanded to include wives, sex partners, and consequently even other related populations (4). A previous review suggested that, as a result of their sexual activity, MSM served as an essential bridge for HIV transmission in sex workers (both male and female) and urban populations (13).

### 3.2. HIV infection and HIV-related risk behaviors

Table 2 summarizes the prevalence of HIV infection and HIV-related risk behaviors. According to the serological test, 18 persons were confirmed as being HIV-positive. The prevalence of HIV infection was 1.3% in the large and middle-sized cities of Shandong Province that were surveyed. Previous studies indicated the prevalence of HIV-positive individuals in related populations was 1.7% among "money boys"

**Table 1.** Demographic characteristics of MSM (*n* = 1,617)

	Number	%
<b>Age</b>		
< 20	19	5.9
≥ 20 - < 30	1,124	69.5
≥ 30 - < 40	340	19.8
≥ 40	77	4.8
<b>Marital status</b>		
Single	398	24.6
Married/cohabiting	1,196	74.0
Divorced/widowed	23	1.4
<b>Education</b>		
Middle school and below	233	14.4
High school	436	27.0
College/junior college and above	948	58.6
<b>Occupation</b>		
Student	409	25.3
Services	245	15.2
Ventures	98	6.1
Liberal occupation/unemployed	865	53.5
<b>Sexual orientation</b>		
Homosexual	946	59.6
Heterosexual	33	2.1
Bisexual	495	31.2
Not sure	113	7.1

**Table 2.** HIV infection and HIV-related risk behaviors (*n* = 1,617)

	Number	%
<b>Serological test</b>		
Positive	18	1.3
Negative	1,599	98.7
<b>Drug user</b>		
Yes	8	0.5
No	1,609	99.5
<b>Consistent condom use in the past 6 months</b>		
Anal intercourse with male		
Yes	791	48.9
No	826	51.1
Patronizing commercial sex		
Yes	741	45.8
No	876	54.2
Sex activities with women		
Yes	621	38.4
No	996	61.6

**Table 3.** Accessibility to HIV-related health services and information (*n* = 1,617)

	Number	%
<b>HIV-related health services (multiple options)</b>		
Free condom	1,095	67.7
Free lubrication	948	58.6
Peer education	446	27.6
Test and treatment of HIV/AIDS	488	30.2
Test and treatment of STD	307	19.0
HIV and STD counseling	952	58.9
Pamphlet	1,137	70.3
<b>Preferring healthcare facilities for STD treatment (multiple options)</b>		
STD-special hospital	726	44.9
General hospital	380	23.5
Private clinic	249	15.4
Self medicine	283	17.5
No treatment	125	7.7
Others	6	0.4

(young male prostitutes) in Shanghai, China (10), 12.5% among gay and bisexual men in Los Angeles and New York City (14), 0.7% among MSM in Zurich (15), 11.0% and 6.9% among gay men in London and Glasgow, respectively (16), 19.1% among MSM in California (17), and 12% among young drug-injecting MSM in San Francisco (18). The assessment of HIV-positive individuals in those studies differed: some implemented a serological test while others relied on self-reported data. In the case of the latter, data on HIV-positive individuals may have been under-reported due to uncertainty as well as social stigma and discrimination. In general, the current study found a low prevalence when compared to that in metropolitan areas worldwide. This suggests that the size of the city in which MSM live may be a factor affecting HIV infection.

With regard to drug abuse, 8 of the total interviewees were drug users (0.5%). Of these, 3 had exchanged needles with others, which is a high risk

behavior for HIV infection.

Interviewees were asked about consistent condom use in the past 6 months. The prevalence of unprotected anal intercourse with men, commercial sex, and sex with women was 51.1%, 54.2%, and 61.6%, respectively; the data were much higher than those in a study carried out in Beijing and Qingdao (19).

### 3.3. Accessibility of HIV prevention health services and information

Since 2005, the Chinese government has enhanced its intervention efforts targeting MSM, and various programs were conducted on condom promotion, testing and counseling, peer education, and follow-up outreach and care services for persons with HIV (1). Usage of HIV prevention health services including free condoms, free lubrication, and HIV & STD counseling and pamphlets was higher than 50% but usage of HIV & STD testing and treatment and peer education was relevantly low (Table 3). Reasons for this were probably the lack of perceived risk, lack of privacy and anonymity, uninteresting program content, and distrust and questionable credibility of providers, as indicated by an ethnographic study in China (20). Moreover, many interviewees did not even know that HIV & STD testing was provided for free.

The healthcare facility most favored for STD treatment by the MSM surveyed was a hospital specializing in STDs (44.9%). The main sources of information related to HIV prevention were television, freely distributed pamphlets, health education notices on the street, books, and magazines.

This study investigated the current state of HIV infection and the related behaviors of Chinese MSM in cities and areas of various sizes. Although HIV prevalence was low when compared to that in metropolitan areas worldwide, the prevalence of unsafe sexual behaviors was relevantly high and there was a lack of accessibility to HIV & STD testing and peer education. This study revealed the urgent need to make intervention programs universal, effective, and much more accessible to MSM in typical large and middle-sized cities in China.

### Acknowledgements

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**Brief Report****Binding of pradimicin A derivative BMY-28864 to neoglycolipids bearing mannose residues at the non-reducing ends**Huanli Xu<sup>1</sup>, Fengshan Wang<sup>1</sup>, Tsuguo Mizuochi<sup>2</sup>, Munehiro Nakata<sup>2,3,\*</sup><sup>1</sup> Institute of Biochemical and Biotechnological Drugs, School of Pharmacy, Shandong University, Jinan, Shandong, China;<sup>2</sup> Department of Applied Biochemistry, Tokai University, Hiratsuka, Kanagawa, Japan;<sup>3</sup> Shandong University China-Japan Cooperation Center for Drug Discovery & Screen, Jinan, Shandong, China.**Summary**

BMY-28864 is a derivative of carbohydrate-binding antibiotic pradimicin A. This study aimed to examine the carbohydrate-binding specificity of BMY-28864 by a direct binding assay using neoglycolipids synthesized by conjugation of various oligosaccharides with dipalmitoylphosphatidylethanolamine (DPPE). Neoglycolipids were chromatographed on a thin layer chromatography plate and then subjected to BMY-28864 binding analysis. Binding of BMY-28864 to neoglycolipids such as Man $\alpha$ 1-3Man-DPPE, Man $\alpha$ 1-6Man-DPPE, Man3-DPPE, and Man5-DPPE and those bearing oligosaccharides derived from ribonuclease B, all of which bear mannose residues at the non-reducing ends, was detected. This study showed that pradimicin A derivative BMY-28864 selectively bound to mannose residues at the non-reducing ends and that neoglycolipids bearing various carbohydrate structures will be helpful as carbohydrate probes to detect carbohydrate-binding low molecular weight compounds.

**Keywords:** Carbohydrate, Neoglycolipid, Lectin, Antibiotic

**1. Introduction**

Pradimicin A is an antifungal antibiotic that Oki *et al.* isolated from the culture filtrate of *Actinomadura hibisca* (I). Pradimicin A and its derivative BMY-28864, which has improved water solubility, have a broad antifungal spectrum against a wide variety of pathogenic fungi such as *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* *in vitro* and in mice (2-4). Notably, several studies have shown that the compounds cause precipitation and alteration of the absorbance spectrum in the presence of mannose in a calcium ion-dependent manner (3-5). Therefore, these low molecular weight compounds are suggested to have a lectin-like carbohydrate-binding ability. The carbohydrate-binding specificity of an increasing number of lectins has been characterized by means of a direct binding assay using neoglycolipids prepared by lipidation of carbohydrates as carbohydrate probes (6,7). The present study used the direct binding assay using neoglycolipids to examine the

interaction of BMY-28864 with carbohydrates.

**2. Materials and Methods****2.1. Materials**

BMY-28864 was kindly provided by Dr. Toshikazu Oki, Toyama Prefectural University, Toyama, Japan, and dissolved in water at 2 mg/mL as a stock solution. The following commercially available carbohydrates were purchased from Funakoshi, Tokyo, Japan: Man $\alpha$ 1-3Man, Man $\alpha$ 1-6Man, mannotriose (Man3) with a structure of Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man, mannopentaose (Man5) with a structure of Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man, core-pentaose (GlcNAc2Man3) with a structure of GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man, maltooligomer with a structure of Glc $\alpha$ 1-(-4Glc $\alpha$ 1-)<sub>0,3</sub>-4Glc, isomaltooligomer with a structure of Glc $\alpha$ 1-(-6Glc $\alpha$ 1-)<sub>0,3</sub>-6Glc, cellooligomer with a structure of Glc $\beta$ 1-(-4Glc $\beta$ 1-)<sub>1 or 3</sub>-4Glc, laminarioligomer with a structure of Glc $\beta$ 1-(-3Glc $\beta$ 1-)<sub>1 or 3</sub>-3Glc, chitooligomer with a structure of GlcNAc $\beta$ 1-(-4GlcNAc $\beta$ 1-)<sub>0,3</sub>-4GlcNAc, and sialyllactose with a structure of NeuAc $\alpha$ 2-3Gal $\beta$ 1-4Glc. Glycoproteins such as bovine pancreas ribonuclease B (RNase B), human

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IgG, and human fibrinogen were purchased from Sigma-Aldrich Japan, Tokyo, Japan. *N*-linked oligosaccharides of these glycoproteins were prepared by hydrazinolysis and re-*N*-acetylation followed by cellulose column chromatography (8,9). Oligosaccharides obtained from IgG and fibrinogen were then asialylated by sialidase treatment as described previously (8).

## 2.2. Synthesis of neoglycolipids

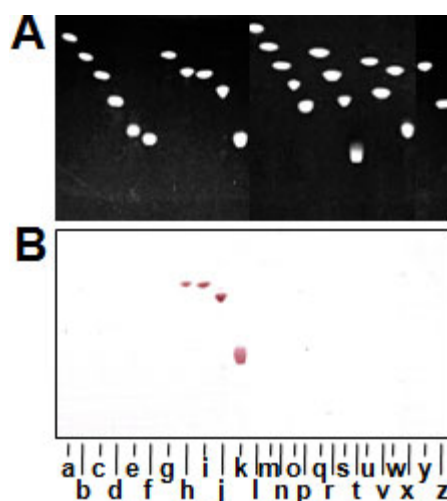
Neoglycolipids were synthesized by conjugation of oligosaccharides with dipalmitoylphosphatidylethanolamine (DPPE, Sigma-Aldrich Japan) by reductive amination (10,11). Briefly, oligosaccharides (0.1-10 mg) were dissolved in water and mixed with 15.7 vol. of DPPE solution (10 mg/mL in chloroform/methanol, 1:1, v/v) and 3.3 vol. of sodium cyanogenborohydrate solution (20 mg/mL in methanol). The reaction mixture was incubated at 80°C for 5 h with occasional sonication. Synthesis of neoglycolipids was confirmed by a thin layer chromatography (TLC) with detection of carbohydrate moiety by orcinol staining and lipid moiety by primulin staining (12). Neoglycolipids derived from commercially available oligosaccharides were then purified by HPLC with a silica gel column (Shim-Pack PREP SIL, 2 × 25 cm, Shimadzu, Kyoto, Japan) and subsequently subjected to chromatography with a Bond Elut C18-cartridge column (Varian, Palo Alto, CA, USA) (13).

## 2.3. Assay of BMY-28864 binding to neoglycolipids

Neoglycolipids were spotted on a TLC plate (Merck, Darmstadt, Germany) and developed using chloroform/methanol/water (60:35:8, v/v) or chloroform/methanol/water (105:100:28, v/v) as a solvent system (12). After drying, the plate was plasticized by soaking it in 0.1% polyisobutyl methacrylate in *n*-hexane for 30 s and then it was blocked by 1% (w/v) gelatin for 2 h at room temperature. The plate was overlaid with 50 µg/mL of BMY-28864 in 10 mM Tris-HCl, pH 7.3, containing 0.15 M NaCl and 1 mM CaCl<sub>2</sub> (Ca-TBS) and incubated for 1 h at room temperature with gentle shaking. After incubation, the plate was rinsed with Ca-TBS and air-dried.

## 3. Results and Discussion

First, purified neoglycolipids derived from commercially available carbohydrates (1 µg each per lane) were chromatographed on a TLC plate and then incubated with 50 µg/mL of BMY-28864 in Ca-TBS for 1 h at room temperature (Figure 1). As shown in Figure 1B, binding of BMY-28864, which was observed as red-colored spots, to neoglycolipids such as Man $\alpha$ 1-3Man-DPPE (lane h), Man $\alpha$ 1-6Man-DPPE (lane i), Man3-DPPE (lane j), and Man5-DPPE (lane k), all of

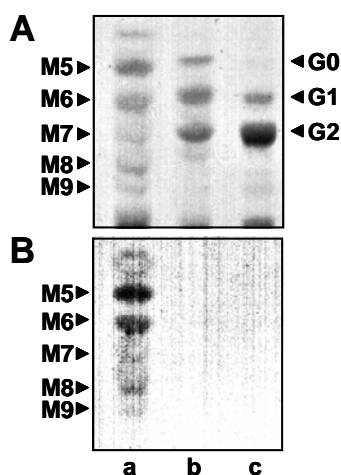


**Figure 1.** A direct binding assay of BMY-38864 with purified neoglycolipids prepared from commercially available carbohydrates. Neoglycolipids were developed on a TLC plate using chloroform/methanol/water (60:35:8, v/v) as a solvent system. (A) The neoglycolipids on the plate were visualized by primulin staining. (B) 50 µg/mL of BMY-28864 in Ca-TBS was incubated on the plate for 1 h at room temperature. Lane a, GlcNAc-DPPE; lanes b-e, chitoooligomer-DPPE bearing 2-5 GlcNAc residues, respectively; lane f, GlcNAc2Man3-DPPE, lane g, Man-DPPE, lane h, Man $\alpha$ 1-3Man-DPPE; lane i, Man $\alpha$ 1-6Man-DPPE; lane j, Man3-DPPE; lane k, Man5-DPPE; lane l, Glc-DPPE; lanes m-p, maltooligomer-DPPE bearing 2-5 Glc residues, respectively; lanes q-t, isomaltooligomer-DPPE bearing 2-5 Glc residues, respectively; lanes u and v, cellooligomer-DPPE bearing 3 and 5 Glc residues, respectively; lanes w and x, laminarioligomer-DPPE bearing 3 and 5 Glc residues, respectively; lane y, lactose-DPPE; lane z, sialyllactose-DPPE.

which bear mannose residue(s) at the non-reducing end(s), was detected. In contrast, binding to Man-DPPE (lane g), in which mannose moiety does not form a ring, GlcNAc2Man3 (lane f), in which mannose residues are not at the non-reducing ends, and neoglycolipids that did not contain mannose residue was not detected. The binding of BMY-28864 to neoglycolipids bearing mannose residue(s) at non-reducing end(s) was completely inhibited in the presence of 100 mM mannose or 2 mM EGTA, while binding was not inhibited by 100 mM of glucose and galactose (data not shown).

Next, neoglycolipids derived from *N*-linked oligosaccharides of glycoproteins (5 µg of carbohydrate equivalent per lane) were developed on a TLC plate and then BMY-28864 binding was analyzed (Figure 2). As shown in Figure 2B, BMY-28864 apparently bound to neoglycolipids bearing high mannose type oligosaccharides derived from RNase B (14) but not to those bearing complex type oligosaccharides derived from IgG (15) and fibrinogen (16).

The present direct binding assay using neoglycolipids showed that pradimicin A derivative BMY-28864 selectively bound to neoglycolipids bearing mannose residues at the non-reducing ends (Figure 1B and 2B). This binding was inhibited by mannose but not by glucose and galactose. Therefore, BMY-28864, a low molecular weight organic compound, can be considered to fall under the new category of "carbohydrate



**Figure 2.** A direct binding assay of BMY-28864 with neoglycolipids prepared from *N*-linked oligosaccharides of various glycoproteins. Neoglycolipids were prepared from high mannose type oligosaccharides of bovine RNase B (lane a) and asialylated complex type oligosaccharides of human IgG (lane b) and human fibrinogen (lane c) and then developed on a TLC plate using chloroform/methanol/water (105:100:28, v/v) as a solvent system. (A) The neoglycolipids on the plate were visualized by orcinol staining. (B) 50  $\mu$ g/mL of BMY-28864 in Ca-TBS was incubated on the plate for 1 h at room temperature. M5-M9 denote neoglycolipids bearing high mannose type oligosaccharides containing 5-9 mannose residues, respectively. G0-G2 denote neoglycolipids bearing complex type oligosaccharides containing 0-2 galactose residues, respectively.

binding compounds" that do not fall under any of the conventional categories.

Mannose-binding compounds will prove useful not only in detecting glycoconjugates bearing mannose residues but also in preventing infection by pathogens with surfaces covered in mannose residues. In fact, the antifungal ability of pradimicin A is thought to be due to its binding to mannan on the fungal surface. Furthermore, research has suggested that pradimicin may prevent human immunodeficiency virus infection (17,18) possibly because of the binding of the antibiotic to virus envelope glycoprotein gp120, which possesses a number of high mannose type oligosaccharides (19,20). Therefore, carbohydrate-binding compounds such as pradimicin A and BMY-28854 will be effective tools for carbohydrate targeting in biochemical and medical studies (21). In addition, other types of carbohydrate-binding antibiotics or organic compounds may exist in nature. Neoglycolipids bearing various carbohydrate structures will be helpful as carbohydrate probes to screen and discover novel carbohydrate-binding compounds.

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**Original Article**

# Risk of sharps exposure among health science students in northeast China

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**Summary**

Previous studies have demonstrated that sharps-related infectious disease is a global concern. Several papers have also reported that students are at a higher risk than healthcare workers. The prevalence of sharps exposure in China, however, is unknown. This study explored the incidence of sharps exposure and its related risk factors among students in all academic years and majors at a medical university in China. This cross sectional study was conducted at a Chinese medical university in May 2005. Stratified random sampling was used. Students in all five academic years (Y1-Y5) who were majoring in clinical medicine, nursing, dentistry, medical technology, pharmacology, acupuncture/massage, and public affairs management were provided questionnaires. Nine hundred seventy of 1,070 (90.7%) students completed the questionnaire. One hundred twenty-two of 968 (12.6%) students reported a total of 131 sharps exposures during the previous 12 months. Of these exposures, 24.7% occurred in academic year five (Y5) students, followed by 23.4% in academic year four (Y4) students. Dental students had the highest incidence rate at 20.6%, followed by medical students (16.0%), nursing students (12.2%), and acupuncture/massage students (5.0%). Only 45 (34.4%) of sharps exposures were reported to a supervisor, and the students displayed a general lack of knowledge of occupational exposure standards (OES). In conclusion, sharps exposures most frequently occurred among students from 3 majors: dentistry, nursing, and clinical medicine. Sharps exposures were underreported to supervisors. Effective OES educational programs need to be developed and should be implemented early in health science students' education.

**Keywords:** Sharps exposure, Sharps injuries, Occupational exposure, Bloodborne pathogens, Medical education

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## 1. Introduction

Among 35 million healthcare workers worldwide, approximately 3 million experience percutaneous exposure to bloodborne pathogens each year, including 2 million to Hepatitis B Virus (HBV), 0.9 million to Hepatitis C Virus (HCV) and 170,000 to Human Immunodeficiency syndrome (HIV) (1-3). The risk of

infection resulting from a single percutaneous exposure to virus-infected blood varies, with rates ranging from 6-30% for HBV, 0-7% for HCV, and 0.2-0.5% for HIV (2). In developing countries, where more than 90% of occupational exposures occur (3), the danger of bloodborne pathogen transmission to medical workers by needle stick and other injuries is of particular concern. Excessive handling of contaminated needles, high demand for injections, and improperly sterilized injection equipment enhance the risk of occupational exposure to bloodborne pathogens (4).

A German study reported a higher incidence of occupational exposure in medical students (24.2%) versus health care workers (15.8%) (5). Students

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majoring in dentistry, nursing, and clinical medicine face the greatest threat of needle-stick injury and other sharps injuries, with the risk of acquiring bloodborne infections while working in a clinical setting (5-9).

Dental students have been shown to be at high risk of sharps exposures. At a United Kingdom dental school, for example, the students' occupational sharps exposure rate during a 12-month period was 39.8%. Y4 and Y5 students were more likely to experience an exposure than Y3 students, and underreporting was common (10). In a Nigerian dental school, 58% of dental students experienced at least one occupational exposure (11), while 32.8% of students experienced occupational exposure in a dental school in the United States (12). Another study from the United States found that the majority of sharps exposures were needle-stick injuries (13).

Medical students throughout the world show a similarly high rate of sharps exposures. One study in Malaysia found a high incidence (23.5%) of sharps injuries among medical students over one year (14). Another study found that 84% of surveyed medical students suffered at least one occupational sharps exposure during their clinical training (6). Lack of experience and technical expertise is related to risk of needle-stick injuries (15), suggesting that unskilled students may be at a heightened risk during their medical training (16,17).

Similarly, nursing students experienced a high prevalence (61.5%) of sharps exposures in a Taiwan nursing school (18). In mainland China, course work for nursing students includes limited information about accidental sharps-related transmission of bloodborne pathogens, and students receive neither conceptual nor concrete information about occupational safety (19).

Educational programs can produce positive changes in both knowledge of and attitudes toward safety protocols (20). Inclusion of blood and body fluid safety precautions in medical school curricula resulted in a more compliant attitude toward safety procedures that protect against accidental bloodborne pathogen transmission (21). There is still little information, however, on bloodborne pathogen transmission prevention in China (19). To address this gap in the literature, this study explored the incidence of sharps exposures and associated risk factors among all academic years and majors at a medical university in China.

## 2. Materials and Methods

### 2.1. Study methods and material

This cross sectional study was conducted at a Chinese medical university in the northeast part of China in May 2005. Thirty-item questionnaires were anonymously sent to 1,070 out of approximately 4,000 registered

students. Fourteen instructors (two for each major) were recruited to administer the survey. All participants were informed of the survey's objectives and gave oral consent before completing the questionnaire. On average, it took approximately 20 min to complete the questionnaire.

Demographic data collected included age, ethnicity, academic year, medical training experience, and evidence of having taken a course on bloodborne pathogen transmission prevention. The students were also asked to recall the frequency of their sharps-related injuries. Knowledge-based questions included: transmission routes of bloodborne pathogens, procedures for using syringes, post-exposure procedures, adherence to universal precaution policy guidelines, and prevalence of HBV and HIV in China.

### 2.2. Sampling and analysis

Stratified random sampling was used such that students from all five academic years and all seven majors (clinical medicine, nursing, dentistry, medical technology, pharmacology, public affairs management, and acupuncture/massage) were included. Fifteen questions were asked to ascertain the students' knowledge of the risk of bloodborne pathogen transmission following sharps exposure. Correct answers were credited with a score of one and incorrect answers with a score of zero, for a maximum aggregate knowledge score of fifteen points.

The variables, including knowledge score, bloodborne pathogens risk perception, adherence of universal precaution, taken course on Occupational Exposure Standards (OES), and hospital training were not normally distributed. For these factors, medians were considered the cut off point. Data analysis was divided into descriptive and inferential groups. Univariate analysis and logistic regression was performed with SPSS 13.0 for Windows. *P* values < 0.05 were considered to be statistically significant.

## 3. Results

### 3.1. Sample demographics

Nine hundred seventy of 1,070 (90.7%) registered students completed the questionnaire. Of these participants, 138 (14.3%) were minorities (not of Han nationality). Demographic characteristics are given in Table 1. There were a disproportionate number of females (67%) in the study.

### 3.2. Sharps exposures

Table 2 breaks down the incidence of sharps exposure by major and year. One hundred twenty-two of 968 (12.6%) students reported at least one sharps exposure

**Table 1.** Demographic characteristics (N = 970)<sup>1</sup>

	Frequency	Percentage <sup>2</sup>
Gender		
Male	342/966	35%
Female	642/966	67%
Age (years) <sup>3</sup>		
18 - 20	335/970	35%
21 - 22	367/970	38%
23 - 27	268/970	28%
Major		
Clinical Medicine	302/970	31%
Nursing	213/970	22%
Dentistry	126/970	13%
Medical Technology	99/970	10%
Pharmacology	99/970	10%
Public Affairs Management	91/970	9%
Acupuncture and Massage	40/970	4%
Academic Year		
First year	248/968	26%
Second year	247/968	26%
Third year	177/968	18%
Fourth year	154/968	16%
Fifth year	142/968	15%
Medical Training Experience		
No	618/964	64%
Yes	346/964	36%
Taken Course on Occupational Exposure Standards		
No	807/967	84%
Yes	160/967	17%

<sup>1</sup> Missing data were not included in some analyses.

<sup>2</sup> The sum of some percentages is not 100% due to rounding.

<sup>3</sup> The median age was 21 years (interquartile range = 20 - 23 years).

during the previous twelve months. Acupuncture/massage was a new major, so for these students, academic years three to five did not exist in 2005. Nursing students only studied for four academic years. Upon combining data from all majors, the highest incidence was 24.7% in Y5, followed by 23.4% in Y4.

Dental students had the highest incidence of sharps exposure (20.6%), followed by medical students (16%), nursing students (12.2%), and acupuncture/massage students (5%). Medical technology students and pharmacology students both showed an incidence rate of 9.1%. Students majoring in public affairs management ranked lowest in incidence rate among majors (2.2%). In a sampling of 131 exposures, only 45 (34.4%) were reported to the student's supervisor, and thus a majority of students displayed a lack of knowledge of OES.

### 3.3. Knowledge on preventing bloodborne pathogens exposure

Fifteen questions (divided into five categories) were asked to ascertain the students' knowledge regarding the risk of bloodborne pathogen transmission following sharps exposure. The results are presented in Table 3. In the first category of questions, the majority of students knew the bloodborne pathogen transmission routes except for HCV (34%). Overall, the students had a good grasp of how HIV was spread. However, only 58% of students knew that blood transmission was the easiest way to transmit HIV, and 62% of them thought professional blood donation could be a risk factor of HIV transmission.

**Table 2.** Incidence of sharp exposure by major and academic year

Majors	Number of students who reported at least one sharp exposure during the last year (%)					
	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Clinical Medicine	0 N = 61	12 (20) N = 60	3 (5) N = 60	14 (23.3) N = 60	19 (32.2) N = 59	48 (16) N = 300
Nursing	2 (2.3) N = 84	9 (11.4) N = 79	4 (12.9) N = 31	11 (57.9) N = 19	— —	26 (12.2) N = 213
Dentistry	1 (3.4) N = 29	4 (13.8) N = 29	3 (10.3) N = 29	7 (53.9) N = 13	11 (42.3) N = 26	26 (20.6) N = 126
Medical Technology	2 (10) N = 20	4 (20) N = 20	1 (5) N = 20	0 N = 19	2 (10) N = 20	9 (9.1) N = 99
Pharmacology	0 N = 16	0 N = 22	2 (9.5) N = 21	4 (19.1) N = 21	3 (15.8) N = 19	9 (9.1) N = 99
Acupuncture and Massage	1 (5) N = 20	1 (5) N = 20	— —	— —	— —	2 (5) N = 40
Public Affairs Management	1 (5.6) N = 18	0 N = 17	1 (6.3) N = 16	0 N = 22	0 N = 18	2 (2.2) N = 91
Total	7 (2.8) N = 248	30 (12.1) N = 247	14 (7.9) N = 177	36 (23.4) N = 154	35 (24.7) N = 142	122 (12.6) N = 968

Missing data were not included in analyses.



**Table 3.** Knowledge of bloodborne pathogen risk following sharps exposure<sup>1</sup>

Items in the questionnaire <sup>2</sup>	Correct response	Percent correct
<b>Transmission Routes of Bloodborne Pathogens</b>		
Which of these diseases do you think can be transmitted by unsterilized syringes or sharp injections?		
1. Hepatitis A Virus (No)	687/970	71
2. Hepatitis B Virus (Yes)	724/969	75
3. Hepatitis C Virus (Yes)	327/970	34
4. Hepatitis E Virus (No)	754/970	78
5. Human Immunodeficiency Virus (Yes)	860/968	89
By which of the following ways may HIV be transmitted?		
1. Ingestion (No)	947/969	98
2. Air (No)	958/970	99
3. Blood (yes)	956/969	99
4. Unprotected sexual Contact (Yes)	960/960	99
5. During pregnancy or breast-feeding (Yes)	930/969	96
6. Daily contact (Handshake, hug, etc) (No)	959/970	99
7. Mosquitoes or other insects (No)	742/969	77
Which is the easiest way to transmit HIV? (Blood transmission)	560/964	58
Do you think professional blood donors are a dangerous source of HIV? (Yes)	593/964	62
<b>Procedures of dealing with syringes:</b>		
Which of the following procedure is correct?		
1. New disposable syringes should be used on all of patients (Yes)	819/967	85
2. Reuse disposable syringes (No)	926/968	96
3. Reuse syringes and sharps but sterilize every time between uses (Yes)	672/966	70
4. Use new sharps every time but reuse the syringes (No)	806/969	83
5. Reuse the glass syringes and sharps; sterilization is not always required (No)	890/966	92
If used syringes are thrown away with regular trash, they pose a danger for people (Yes)	910/961	95
Do you think recapping the used syringes should be done before you throw them away? (No)	107/963	11
Do you think there is something wrong with reusing needles or other sharp instruments on different people without sterilization? (Yes)	937/970	97
Which is the proper way of sterilizing a glass syringes? (Use high pressure)	530/969	55
<b>Post-exposure procedures:</b>		
If you are injured by sharp instruments, what would you do?		
1. Inject the immunity globulin or hepatitis B vaccine as soon as possible (Yes)	275/970	28
2. Let the wound bleed then wash the wound with water (Yes)	714/968	74
3. Put pressure on the wound then bandage (No)	824/970	85
4. Test the blood (Yes)	502/970	52
5. Report to your superior (Yes)	557/970	58
6. Do nothing (No)	962/970	99
When you are injured by syringes or sharp instruments contaminated with blood of HIV patients, if you get treatment as soon as possible, can you reduce the chance of HIV infection? (Yes)	315/963	33
<b>Universal precaution and policy:</b>		
All human blood and certain human body fluids should be treated as if they are known to be infectious for HIV, HBV and other bloodborne pathogens (Yes)	533/960	56
In our country, many provinces are using glass syringes. Which do you think is the best policy? (using the disposable syringes and reuse the sterilized glass syringes)	720/970	74
<b>Current prevalence of HBV and HIV in China, 2005:</b>		
Approximately, what percentage of the country's population are hepatitis B virus carriers? (10%)	359/967	37
At present, which number is closest to the actual number of people living with HIV in the country? (840,000)	249/967	26

<sup>1</sup> The sum of some percentages is not 100% due to rounding. The median total score was 11 points (interquartile range = 9-12 points).

<sup>2</sup> Correct answers are shown in parentheses.

In the second category of questions, the students understood the procedures of handling syringes, but only 11% of students knew that used syringes should not be recapped before being thrown away, while 55% knew that high pressure sterilization was the proper way of sterilizing glass syringes. In the third category of questions, not all students were knowledgeable about post-exposure procedures. Following a sharps exposure, 28% of students indicated that they would receive immune globulin or HBV vaccination, while 52%

would perform blood testing, and 58% would report the incident to a superior. In the last two categories of questions, 56% students were knowledgeable of universal precaution guidelines, and most students did not know the current prevalence of HBV and HIV in China.

#### 3.4. Risk factors related to sharps exposures

Table 4 shows the crude and adjusted odds ratios (OR)

**Table 4.** Crude and adjusted odds ratios and their 95% confidence intervals for risk factors of sharps exposures among 970 Chinese students

Factor	Total	At least one sharps exposure during the last year		Crude OR			Adjusted OR*		
		(N)	%	OR	95% CI	p value	OR	95% CI	p value
<b>Academic year</b>									
First year	248	(7)	2.8	1					
Second year	247	(30)	12.1	4.8	2 - 11.1	<0.01	4.4	1.9 - 10.5	0.01
Third year	177	(14)	7.9	3.0	1.2 - 7.5	0.02	2.4	0.9 - 6.5	0.08
Fourth year	154	(36)	23.4	10.5	4.5 - 24.3	<0.01	6.5	1.9 - 21.7	<0.01
Fifth year	142	(35)	24.6	11.3	4.8 - 26.1	<0.01	6.8	2.1 - 22.5	<0.01
<b>Major</b>									
Clinical medicine	302	(49)	16.2	8.6	2.1 - 36.1	<0.01	10.8	2.5 - 47.0	<0.01
Dentistry	126	(26)	20.6	11.6	2.7 - 50.1	<0.01	15.2	3.4 - 68.2	<0.01
Medical technology	99	(9)	9.1	4.5	0.9 - 21.1	0.04	5.4	1.1 - 27.0	0.04
Pharmacology	99	(9)	9.1	4.5	0.9 - 21.1	0.04	5.5	1.1 - 26.9	0.04
Nursing	213	(26)	12.2	6.2	1.4 - 26.6	<0.01	13.0	2.9 - 58.9	<0.01
Acupuncture and massage	40	(2)	5.0	2.3	0.3 - 17.2	0.39	7.6	1.0	0.05
Public affairs management	91	(2)	2.2	1					
<b>Knowledge score</b>									
High	463	(65)	14.0	1.3	0.9 - 2.0	0.14	0.9	0.6 - 1.4	0.60
Low	453	(49)	10.8						
<b>Bloodborne pathogens risk perception</b>									
High	486	(80)	16.5	2.0	1.4 - 3.0	<0.01	1.3	0.8 - 2.0	0.30
Low	484	(43)	8.9						
<b>Adherence of Universal Precaution</b>									
Yes	533	(60)	11.3	0.8	0.5 - 1.2	0.23	1.4	0.9 - 2.2	0.15
No	427	(59)	13.8						
<b>Taken course on OES</b>									
Yes	160	(31)	19.4	1.9	1.2 - 3.0	<0.01	0.6	0.4 - 1.0	0.06
No	807	(91)	11.3						
<b>Medical training in hospital</b>									
Yes	346	(76)	22.0	3.5	2.3 - 5.2	<0.01	1.8	0.7 - 4.6	0.19
No	618	(46)	7.4						

CI, confidence interval; OR, odds ratio.

\*Adjusted for all variables significant in the univariate analysis.

between risk factors and incidence of sharps exposure. Compared with Y1 students, Y2 students (OR: 4.8; 95% CI: 2-711.1;  $p < 0.01$ ) and Y3 students (OR: 3.0; 95% CI: 1.2-7.5;  $p = 0.02$ ), Y4 students (OR: 10.5; 95% CI: 4.5-24.3;  $p < 0.01$ ) and Y5 students (OR: 11.3; 95% CI: 4.8-26.1;  $p < 0.01$ ) were more likely to have encountered a sharps exposure.

Compared with public affairs management majors, who displayed the lowest incidence of sharps exposure, dental students (OR: 11.6; 95% CI: 2.7-50.1;  $p < 0.01$ ), medical students (OR: 8.6; 95% CI: 2.1-36.1;  $p < 0.01$ ), nursing students (OR: 6.2; 95% CI: 1.4-26.6;  $p < 0.01$ ), and medical technology students and pharmacological students (OR: 4.5; 95% CI: 0.9-21.1;  $p = 0.02$ ) were more likely to encounter a sharps exposure. In contrast, acupuncture/massage students showed no significant difference from public affairs management majors.

The odds of sharps exposure was significantly increased in students who had higher bloodborne pathogen transmission risk perception (OR: 2; 95% CI: 1.4-3;  $p < 0.01$ ), had taken courses on preventing

bloodborne pathogen transmission (OR: 1.9; 95% CI: 1.2-3;  $p < 0.01$ ), or had medical training experience in a hospital (OR: 3.5; 95% CI: 2.3-5.2;  $p < 0.01$ ).

Odds ratios were subsequently adjusted by binary logistic regression analysis. Among all of the significant risk factors noted above, only academic year and choice of major remained significant following regression analysis. Specifically, students in academic years Y2, Y3, and Y5 were more likely to encounter a sharps exposure. Furthermore, dentistry, clinical medicine, and nursing majors were over ten times as likely and medical technology, pharmacology and acupuncture/massage majors were over five times as likely to encounter sharps exposure than public affairs management majors.

#### 4. Discussion

In our study, sharps exposures occurred frequently among students majoring in dentistry (20.6%), clinical medicine (16%), and nursing (12.2%), consistent with

previous research (22-24). The prevalence of sharps exposure among dental students in their final academic year (42.3%) is similar to other reports, including a study of 204 students in a United States dental school (32.8%) (12), and of 153 dental students in a Nigerian dental school (58.8%) (11). In this study, Chinese nursing students in their final academic year had a higher incidence of sharps exposure (57.9%) compared with 13.9% at an Australian nursing school (24) and 21.6% at a Brazilian nursing school (9).

Among medical students in this study, 32.2% had experienced at least one sharps exposure during their final year of training. This prevalence is similar to that observed at medical universities in Brazil (34.2%) (25) and England (30%) (10), higher than that observed at medical universities in Denmark (22%) (8) and Germany (24.2%) (5), and lower than the United States (48%) (7).

Acupuncture/massage majors displayed a low incidence of sharps exposure, although these students may still be at risk. This major was established 2 years before our study, and thus their Y5 training had not yet begun. It is important to note that students in public affairs management following graduation will be responsible for enacting policies directed at the prevention of occupational exposure to bloodborne pathogens in the future. Even though their current incidence of sharps exposure is low, they still need to be educated in OES.

From a sampling of 131 students who experienced sharps exposure, only 34.4% had reported the incident to supervisors. This underreporting rate (65.6%) is much higher than that observed in a Taiwan nursing school (39%) (26), and three Malaysian medical universities (35.6%) (14). Underreporting rates in this study may also correspond to the relative lack of knowledge of post-exposure procedures and universal precaution guidelines (see Table 3).

The majority of students were knowledgeable of bloodborne pathogen transmission routes, except for HCV (34%). Still, further education is needed, since only 58% of the students knew that blood transfusion confers the highest-likelihood risk of transmitting HIV, and only 62% also think professional blood donors could be a dangerous source of HIV. Furthermore, only 37% and 26% of students know the prevalence of HBV and HIV, respectively, in China, suggesting the need for further epidemiological education.

In August 2004, the National Guidelines for the Detection of HIV/AIDS (27) provided instructions for handling used syringes. Since the present study was conducted in May 2005, the dangers of recapping needles (an action which could increase the prevalence of bloodborne pathogens infections due to needle-stick injury) may not have yet been communicated to the local teaching hospital where we conducted this study. This may account for the fact that only 11% of students

knew that recapping needles should not be performed.

According to crude and adjusted odds ratios, Y4 and Y5 students are at least six times as likely to encounter a sharps exposure than Y1 students. This suggests that OES training should occur in earlier academic years. Students majoring in dentistry, clinical medicine, medical technology, pharmacology, and nursing are at the greatest risk and are thus educational priorities. However, training of public affairs management majors should not be ignored, as they represent future policy makers. Finally, binary logistic regression modeling found that acupuncture/massage majors are at least five times more likely to encounter sharps exposures than public affairs management majors.

Surprisingly, students who have knowledge of bloodborne pathogens, have taken courses on bloodborne pathogen risk prevention, or have experienced hospital medical training, are more likely to have sharps exposure than other students. Multiple experiences of sharps exposure may engender students with a higher risk perception and a greater desire to taking training courses. Indeed, 77% of students want to learn more about occupational exposure risks that lead to bloodborne pathogen transmission. Reducing bloodborne pathogen transmission resulting from sharps exposure requires more effective educational programs.

## 5. Conclusions

In this study, we found that sharps exposure occurred frequently at a Chinese medical university. Sharps exposure was highest among dental, nursing, and medical students. Y4 and Y5 students were more likely to encounter sharps exposure than Y1 students. We discovered a lack of knowledge of syringe handling, post-exposure procedures, and universal precaution guidelines. Sharps exposures were underreported to supervisors. While more effective educational programs may be needed in the future, current students should be trained in OES during the earlier academic years of all majors.

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**Original Article****UXO victims and health status of populations living in Xiengkhuang Province, Lao PDR: A household-based survey****Outavong Phathamavong<sup>1,\*</sup>, Nao Boutta<sup>2</sup>, Panome Xayamoungkhoun<sup>3</sup>, Chushi Kuroiwa<sup>1</sup>**<sup>1</sup> Department of Health Policy and Planning, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan;<sup>2</sup> Ministry of Health, Vientiane, Lao PDR;<sup>3</sup> Mother and Child Health Center, Ministry of Health, Vientiane, Lao PDR.**Summary**

During the Vietnam War from 1964 to 1973, over 2 million tons of bombs were dropped on Laos. Approximately 30% of the bombs did not explode and have posed a continued threat to civilians throughout the country. Approximately 200 casualties per year have been reported nationwide. Therefore, we conducted a household survey to better understand magnitude of UXO victims, accessibility to the MCH services and child healthcare seeking behaviors of the population in the Xiengkhuang province. The household-based survey was carried out in February 2006 among 6 of 541 villages. 283 household representatives were interviewed. The cumulative number of UXO victims identified from 1973 to 2005 was 45 casualties, of which 9 (20.0%) occurred in the year 2005. 37.5% reported knowledge of danger zones for UXO contamination. Among the 91 children under the age of 5 years, households on average reported 1.7 episodes per year for diarrhea, 2.7 for respiratory disease, 2.3 for fever and 1.7 for measles. 69.4% of children under five were completed the routine immunization program, 62.6% of mothers used ANC service for their most recent pregnancy, 58.2% accessed family planning services, and 28.6% delivered their most recent child at a healthcare facility. UXO victims in the targeted villages sharply increased in 2005. Insufficient knowledge about UXO danger and MRE seems to be a central factor in the high rate of UXO-associated accidents. Diarrhea, respiratory disease and measles remained health problems for children under 5 years. MCH services utilization were higher than Laos nationally.

**Keywords:** Rural health, Unexploded ordnance, War, Maternal and child health, Injury prevention, Laos

**1. Introduction**

The Lao People's Democratic Republic (PDR) is a small, landlocked country in South East Asia with a population of 5.6 million people (1) surrounded by China, Myanmar, Thailand, Cambodia and Vietnam. Due in part to its geographical location, during the Vietnam War (Indo-China war) from 1964 to 1973, more than 2 million tons of bombs were dropped on Laos, more than any other country involved in the war (2). This amounted to 660 kg per capita (3),

far exceeding even the 280 kg per capita dropped over Vietnam (4). Of these 2 million tons of bombs, approximately 30% failed to explode and remain a persistent threat affecting over 50% of all agriculture land countrywide. The Xiengkhuang province was reported as one of the most heavily UXO-affected provinces in Laos (5).

Since the war ended, the number of victims has been recorded by UXO Laos nationwide. From 1973 through 1997, 11,928 casualties were recorded; during the 1980s, an average of 200 cases per year occurred. In 1996, 199 casualties were identified and in 1997, 140 casualties. One hundred nine casualties in 2003 were recorded and 117 casualties in 2004, in which 54% were children, 85% males (3). In 2005, more than 150 people were injured or killed due to UXO accidents (6).

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The economic burden due to UXO-related accidents and UXO contamination on agriculture lands exacerbates the poor socioeconomic and health conditions of the people (5).

In 1995, the Lao PDR Trust Fund was established in collaboration with the United Nations Development Programme (UNDP) and UNICEF to provide financial support for UXO clearance activities and mine risk education (MRE) (7). The National UXO Programme was established the following year (5). It is estimated, however, that it will take 100 years to clear all UXO from ground surfaces countrywide.

The people of the Xiengkhuang province, already suffering from poor living conditions, are particularly affected by UXO. Previous reports demonstrated just high rates of UXO-associated casualties in the province; however, they did not explore UXO-related information which would raise awareness of the population on UXO explosion and their health (2). Therefore, we conducted a household survey to better understand magnitude of UXO victims, accessibility to the mother and child health (MCH) services and child healthcare seeking behaviors of the population. Our study will provide essential information for future research focusing on health interventions aimed at improving the dire health status of the people of the Xiengkhuang province.

## 2. Methods

### 2.1. Study site

The northern mountainous Xiengkhuang province, which shares a border with Vietnam, is divided into 7 districts consisting of 541 villages. The total population of this province is 228,882 (1). During the Vietnam War, Xiengkhuang was a part of the Ho Chi Minh trail.

Six villages located along main roads from 2 districts (Pek and Kham) were included in this study. These villages were chosen because of their accessibility and their relative safety from UXO accidents over the previous year (2004).

### 2.2. Study design

We conducted a descriptive household survey in February 2006. Trained research assistants recruited household representatives and conducted interviews at temples, schools or village meeting halls. Verbal informed consent was obtained from all household representatives prior to the interview. The National Ethics Committee for Health Research (NECHR) and the Ministry of Health of Laos approved the study.

### 2.3. Data collection

The research assistants conducted face-to-face interviews with a representative of each household

capable and available to participate in the study. When collecting data on children under five, we interviewed their mothers. Structured questionnaire was developed based on the World Health Organization guidelines for conducting community surveys on injuries and violence (8). The questionnaire was developed to investigate demographic characteristics, socioeconomic status, prevalence of UXO-associated trauma, and overall population health status. The latter had a particular focus on maternal and child health (under 5 years of age).

Three trained research assistants interviewed each household representative at the various meeting locations. Additionally, research assistants actively sought out representatives who were absent from the meeting, so as to cover all households for the study.

## 3. Results

### 3.1. Socio-demographic

Two hundred eighty-three household representatives from 6 villages took part in the study. Of these, 256 (90.5%) were lowland Lao (Lao Lum), and 274 (96.8%) were farmers (Table 1). There was no difference in mean educational level between husbands and wives (4.8 year vs. 4.2). The median of household income was US\$300 dollars per year.

### 3.2. UXO-associated trauma

The cumulative number of UXO victims from 1973 to 2005 was 45 casualties, of which 9 (20.0%) casualties occurred in 2005 (Table 2). Thirty-seven (82.2%) of casualties were males. The average age of UXO victims was 18.5 years old. Of the 45 casualties, 17 (37.8%) died and 25 (55.6%) were disabled. Nineteen

**Table 1.** Socio-demographic of study population and their households ( $n = 283$ )

	No	%
Ethnicity		
Lowland	256	90.5
Midland	16	5.7
Highland	10	3.5
Missing	1	0.3
Age (years): Mean (SD)	40.0 (12.5)	
Occupation		
Farmer	274	96.8
Governor	5	1.7
Other	4	1.4
Education level (year)		
Husband: Mean (SD)	4.8 (2.4)	
Wife: Mean (SD)	4.2 (2.3)	
Family income (US\$)		
Median (interquartile range)	300 (162-500)	
Number of children: Mean (SD)	4.4 (2.4)	
Household member: Mean (SD)	6.4 (2.2)	



**Table 2.** Cumulative number of UXO victims (*n* = 283)

	No	%
Household with UXO victims		
Yes	36	12.7
No	244	86.2
Missing	3	1.1
Number of casualties from 1973 to 2005	45	
Only 2005 ( <i>n</i> = 45)	9	20.0
Age Median (interquartile range)	18.5 (12.0-36.0)	
≤ 18	22	48.9
> 18	20	44.4
Missing	3	6.7
Sex		
Male	37	82.2
Female	8	17.8
Casualties situation		
Died	17	37.8
Disabled	25	55.6
Recovered	3	6.7
Disability ( <i>n</i> = 25)		
Arm	7	28.0
Leg	7	28.0
Eye	7	28.0
Ear	1	4.0
Other	2	8.0
Missing	1	4.0
Place of accident		
Agriculture land	19	42.2
Village compound	16	35.6
Forest	7	15.5
Other	1	2.2
Missing	2	4.4
How did it happen?		
Accident	16	35.6
Someone did it	7	15.5
Myself	8	17.8
Don't know who did	14	31.1

(42.2%) and 16 (35.6%) UXO accidents occurred on agricultural land and village compounds, respectively. Sixteen casualties (35.6%) occurred by accident, when victims did not know; while 15 (33.3%) became victims even though they knew that the object was a UXO and nevertheless were playing with it, firing it, or being thrown it from someone else.

Of the 283 representatives, 37.5% reported knowledge of danger zones for UXO contamination (Table 3). Of these, 27.4% had learned about these zones from UXO staffs, whereas 50.0% reported that they had learned about UXO on their own. Surprisingly, 78.3% of those reporting knowledge of danger zones had nevertheless entered into a zone, typically because it was a part of their agriculture land (62.7%), or because their village or house were located within it (8.4%). Approximately 96.8% of the household representatives received UXO-related information from specific UXO staffs who visit the villages once a year (66.1%).

### 3.3. Maternal and child health

Children under 5 years of age were identified from 91 (32.2%) of 283 households (Table 4). The prevalence and number of episodes of illness among these children in the past 12 months was 23.1% and 1.7 for diarrhea, respectively, 42.9% and 2.7 for respiratory disease,

**Table 3.** Sources of UXO-related information (*n* = 283)

	No	%
Know dangerous area for UXO		
Yes	106	37.5
No	177	62.5
<b>If "Know"</b>		
Know from		
Myself	53	50.0
UXO staffs	29	27.4
Other	23	21.7
Missing	1	0.9
Go into dangerous area		
Yes	83	78.3
No	23	21.7
<b>If "Go", Why?</b>		
Bomb in village	7	8.4
Agriculture	52	62.7
Cutting wood	10	12.0
Hunting	8	9.6
Other	6	7.2
UXO-related information		
UXO staffs	274	96.8
Others	6	2.1
Missing	3	1.1
Number of visits in the past year		
1 time	187	66.1
2 times	63	22.3
3 times	23	8.1
> 3 times	7	2.4
Missing	3	1.1

46.2% and 2.3 for fever, and 7.7% and 1.7 for measles. Twenty-three (82.1%) of those children suffering from diarrhea, 42 (75%) of those suffering from respiratory disease, 48 (82.8%) of those who had fever, and 6 (60%) of those who were infected with measles received treatment at a healthcare facility.

A total of 124 children under the age of 5 years old were identified from 91 mothers, of whom 86 (69.4%) had received the complete immunization schedule (one dose of BCG, 3 doses of DPT, 1 dose of measles and 3 drops of polio myelitis) and 35 (28.2%) received an incomplete course (Table 5). Of 91 mothers with children under 5 years of age, 57 (62.6%) received at least one antenatal visit during their most recent pregnancy, 53 (58.2%) had used family planning services, and 26 (28.6%) gave birth to their most recent child at a healthcare facility. Of 283 households, 274 (96.8%) were using insecticide-treated nets (ITNs) and 274 (96.8%) had their own latrine.

## 4. Discussion

UXO-associated trauma remains a serious problem for the people living in Xiengkhuang province. Although this study cannot be completely representative of the entire province due to the non-random sampling methodology and the small sample size, the large number of casualties identified in 2005 (9 out of 45 casualties) is concerning. These were identified among villages located along the main roads and close to the city. This figure suggests an increasing number

**Table 4.** Episode of illness of children under five and health seeking behaviors (*n* = 283)

	No	%
Have under 5 children		
Yes	91	32.2
No	188	66.4
Missing	4	1.4
Prevalence and episode ( <i>n</i> = 91)		
Prevalence of diarrhea	21	23.1
Episode of diarrhea: Mean (SD)	1.7 (1.0)	
Treatment		
Self treatment	3	10.7
Village health volunteer	1	3.6
Traditional healer	0	0.0
Pharmacy	1	3.6
Health facility	23	82.1
Prevalence of respiratory	39	42.9
Episode of respiratory: Mean (SD)	2.7 (2.0)	
Treatment		
Self treatment	3	5.4
Village health volunteer	0	0.0
Traditional healer	4	7.1
Pharmacy	7	12.5
Health facility	42	75.0
Prevalence of fever	42	46.2
Episode of fever: Mean (SD)	2.3 (1.5)	
Treatment		
Self treatment	1	1.7
Village health volunteer	0	0.0
Traditional healer	1	1.7
Pharmacy	8	13.8
Health facility	48	82.8
Prevalence of measles	7	7.7
Episode of measles: Mean (SD)	1.7 (1.3)	
Treatment		
Self treatment	1	10.0
Traditional healer	1	10.0
Village health volunteer	1	10.0
Pharmacy	1	10.0
Health facility	6	60.0

of UXO victims compared with previous years in the Xiengkhuang province and in Laos as a whole. Insufficient knowledge about UXO dangers and MRE seems to be a central factor in the high rate of UXO-associated trauma among the people living in these villages. Indeed, 37.5% of villagers reported that they were aware of the danger zones for UXO; half of these individuals reported that they had learned of these dangers on their own. Specific UXO-awareness workers only visited these villages once a year, however. Furthermore, UXO contamination in agriculture lands and village compounds were repeatedly reported. The study suggests that effective action, especially MRE and UXO clearance, is urgently needed to reduce the risks associated with UXO.

The underlying hypothesis of this study was that UXO contamination plays a detrimental role in the ability of the population to access healthcare. Essential public health interventions such as antenatal care, hospital delivery, family planning and ITNs are operational in the province and enjoy higher utilization

**Table 5.** Mother and child health services among 124 children, 91 mothers, and 283 households

	No	%
Immunization		
Complete	86	69.4
Incomplete	35	28.2
Missing	3	2.4
≥ one ANC visits		
Yes	57	62.6
No	28	30.8
Missing	6	6.7
Family planning		
Yes	53	58.2
No	32	35.1
Missing	6	6.7
Delivery		
Hospital	26	28.6
Home	59	64.8
Missing	6	6.7
Insecticide Treated Nets		
Yes	274	96.8
No	8	2.8
Missing	1	0.4
Latrine		
Yes	274	96.8
No	8	2.8
Missing	1	0.4

rates than are found nationally: the antenatal care utilization rate in the study population was 62.6% compared with 38.7% nationally; 28.6% of mothers gave birth in a hospital for their most recent child, higher than 16.8% reported nationwide; and 96.8% of ITNs coverage in this study, higher than 54% in national level (9). Likewise, 69.4% of children aged less than five completed the routine immunization program, higher than the 53.8% in the national survey (9). These relatively high rates were likely due to the location of these villages, situated close to the provincial and district hospital.

We also expected that this would result in the poor health status of the people living in Xiengkhuang province, particularly mothers and their children. The results demonstrated that diarrhea, respiratory disease and measles are the main public health problems among children under 5 years of age in these villages. The prevalence of diarrhea (23.1%) and respiratory disease (42.9%) in this study were higher than national figures (13.4% for diarrhea and 5.4% for acute respiratory infection) (9). The number of measles cases reported from these villages was also high (7.7% of prevalence and 1.7 episodes). Fortunately, 60% to 80% of parents sought medical care at health facilities for the treatment of these illnesses.

## 5. Conclusions

This descriptive study demonstrated an increase in UXO victims in 2005 among 6 villages in the Pek

and Kham district. Diarrhea, respiratory disease, and measles were observed to be the main public health problems among children under the age of 5 years. Nevertheless, the study population enjoyed utilizing health intervention programs including antenatal care, family planning, birthing at a healthcare facility and immunization than Laos nationally. This is likely due to the location of these villages, which are situated close to the provincial and district hospital, allowing easy access to care by foot, bicycle, or motorbike. UXO must have been cleaned in this area in the past decades. Additionally, studies are urgently needed that focus on remote, rural villages where people experience significant challenges in accessing high-quality healthcare services. Such studies will help in designing appropriate interventions for people living in areas similar to the Xiengkhuang province.

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**Original Article**

## Effects of low-intensity resistance exercise with vascular occlusion on physical function in healthy elderly people

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**Summary**

Some successful fall prevention programs include resistance or balance training, but less is known about the effects of low-intensity resistance exercise with moderate vascular occlusion (LIO) on physical function in healthy elderly people. In LIO, appropriate pressure is applied to the proximal parts of the upper and lower extremities with a specially designed belt. The reduction of muscle blood flow is considered likely to induce the secretion of growth hormone. The aim of this study was to compare the effects of two training programs, LIO versus dynamic balance exercise (DBE) in elderly people in a community. Fifty-one healthy subjects aged 65 and older were randomly assigned to the LIO program ( $n = 24$ ) or the DBE program ( $n = 27$ ). Performance, balance, muscle strength were measured in both groups before and after the 8-week programs. In addition, blood was sampled from LIO participants ( $n = 11$ ) and analyzed for growth hormone and lactate. Overall improvements, but no group differences, were found in performance and balance after the programs. Muscle strength in the lower extremities was significantly increased in the LIO group, but not in the DBE group. Growth hormone was significantly increased immediately after LIO. The 8-week LIO program improved physical function, especially muscle strength, which may be associated with the exercise-induced secretion of growth hormone. Further studies are needed to determine the contents and duration of an LIO program for elderly people.

**Keywords:** Vascular occlusion, Growth hormone, Elderly

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### 1. Introduction

Muscle strength is one of the predictive factors for functional decline in the aged population. Lower limb muscle strength in the elderly is associated with walking ability (1) and activities of daily living (ADL) (2). Performing exercises such as walking, jogging, or recreational physical activities on a regular basis has been reported to improve muscle strength, flexibility, endurance, and balance in older adults (3). In addition, an intervention study on falls prevention demonstrated

the beneficial effects of training for the improvement of muscle strength and balance (4). Exercise programs including balance training are also often recommended for reducing the risk of falling. Dynamic Balance Exercise (DBE), which consists of slow, rotational and multisegmental movements with sequential weight shifting, has been shown to improve balance responses (5). Balance retraining program including DBE was shown to be practical and useful in fall risk reduction (6).

The effects of resistance training have been evaluated using a number of indicators, such as muscle strength of the muscle group to be trained, lean body mass, bone density, lower back pain, muscle function, and performance, including walking velocity and stair ascension and descension (7). A meta-analysis of 62 randomized comparative studies has demonstrated that lower limb muscle strength and walking velocity are

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useful indicators for evaluation (8).

Exercise at an intensity equal to or higher than 65% of 1 repetition maximum (1-RM) has been shown to improve muscle strength (9). Muscle strength can also be improved by a 20-25% low-intensity resistance exercise combined with moderate vascular occlusion (LIO) in active muscle groups (10). The level of muscle strength required for LIO is almost equivalent to that of daily living activities. In this method, low intensity training is performed while applying appropriate pressure to the proximal parts of the upper and lower limbs with a pair of special elastic belts for a given length of time. In the study by Takarada *et al.* (10), plasma levels of growth hormone, norepinephrine, and lactate were remarkably increased after the exercise with occlusion. Furthermore, LIO for a short-time and low-intensity exercise had a significant effect equal to or greater than high-intensity resistance training on an increase in muscular size and strength (11). Among a number of intervention studies designed to slow functional decline, none has been conducted to closely examine performance, muscle strength, and balance in healthy elderly people by using LIO.

The primary effect of DBE is to improve balance, and muscle strength and performance are also expected to increase through the exercise. However, no study has ever compared DBE and LIO directly. In addition, although a significant increase of growth hormone was observed after LIO in young subjects, there has been no data available for the elderly. Therefore, the purpose of the present study was twofold: 1) to determine whether LIO is equally or more effective than DBE regarding performance and balance, and muscle strength and 2) to measure changes of growth hormone and lactate before and after a single bout with LIO, in community-dwelling healthy elderly people.

## 2. Materials and Methods

### 2.1. Study design and subjects

The current study was designed to compare the effects of two different training programs. In April 2004, we compiled a list of all residents aged 65 years and older living in a village in Nagano Prefecture. Among them, 350 people were excluded due to long-term nursing care, hospitalization, or death. Healthcare volunteers distributed a description of the physical training programs to the remaining 1,864 people. In June 2004, eighty-seven of them underwent medical check-ups. A public health nurse explained the contents and schedule of the LIO and DBE programs.

The exclusion criteria were as follows: 1) unable to participate in training sessions for 8 weeks; 2) joint or muscular problems as a result of daily exercise; 3) uncontrolled hypertension: systolic pressure of over 160 mmHg and/or diastolic pressure of over 100

mmHg (those who had controlled blood pressure under antihypertensive therapy were considered eligible); 4) chronic inflammatory conditions; 5) history of myocardial infarction within the last 6 months; and 6) severe paralysis, joint degeneration, or arthralgia in their extremities. In addition, we excluded subjects who were suspected of being at high risk of developing venous thrombosis or other cardiovascular diseases. Among the 87 people who underwent the check-ups, 19 were excluded due to medical problems. Of the 68 who were screened, 51 agreed to participate in the study and signed an informed consent. They were randomly assigned to an 8-week program of either LIO ( $n = 24$ ) or DBE ( $n = 27$ ) (Figure 1). The Medical Ethical Committee of Shinshu University School of Medicine approved the protocol of the study.

### 2.2. Training protocol

#### 2.2.1. LIO program

Low intensity training was performed while applying appropriate pressure to the proximal parts of the thighs with a pair of special elastic belts (width 45 mm, length 1,250 mm). A physical therapist and a public health nurse served as instructors on the training program twice a week for 8 weeks. Twenty-four subjects were divided into 5 groups and participated in 45-min training sessions (Figure 2). Those with systolic pressure over 140 mmHg before the training were instructed to take a rest until the pressure dropped below that level. In the event that their systolic pressure did not drop, they did exercises without a belt. The belt contained a small pneumatic bag (width 33 mm, length 140 mm) along its inner surface that was connected to an electronic pressure gauge (model M.P.S.-700 developed by Y. Sato and manufactured by VINE Medical Instruments, Tokyo, Japan). A varying level of occlusion pressure was applied through pneumatic inflation. The level of pressure applied during the training was determined according to the age and blood pressure of the subjects. The initial pressure on the thighs was set at 70 mmHg, a pressure level where peripheral blood flow is not impaired. The maximum pressure was set at up to 1.2 times the systolic blood pressure level of individual subjects or at a subjectively tolerable level. Applied pressure from the belt was to be released immediately if there was any complaint of discomfort during the training. Table 1 represents a flow of the 8-week LIO training.

The training program consisted of 6 different movements: lowering the body until the knees are flexed at a 60 degree angle (half squats); stepping forward with one leg and lowering the body to 90 degrees with both knees (forward lunges); raising heels up and down in a standing posture (calf raises); lifting one knee and then the other up to waist level, alternating legs (knee lifts); lying on the floor with knees bent and hands



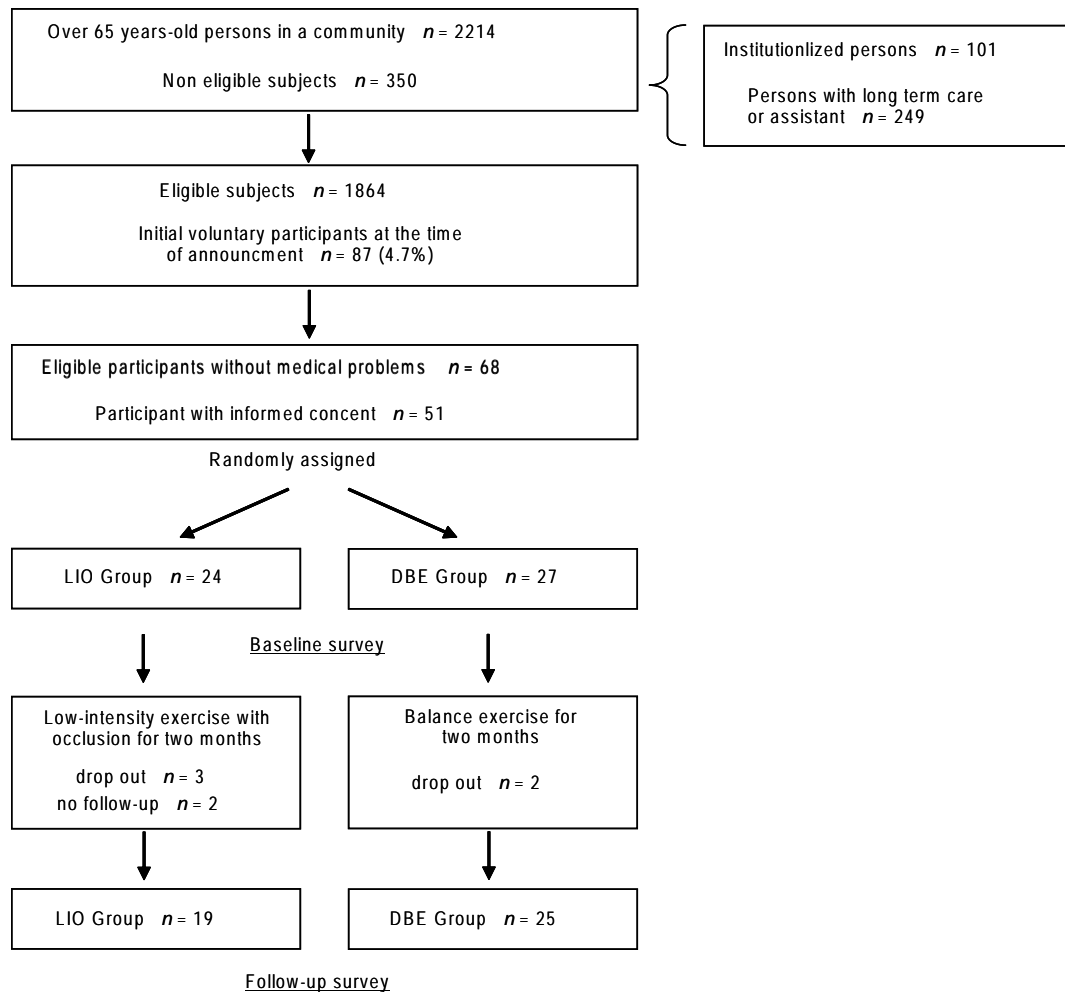


Figure 1. Sampling process.

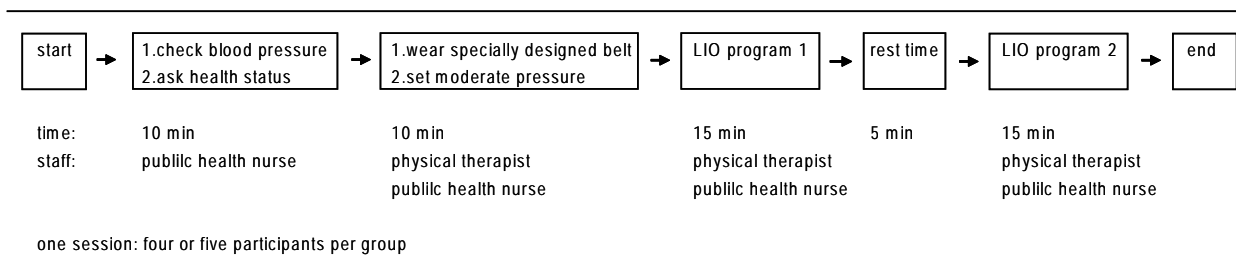


Figure 2. One LIO program session.

behind head, then raising the head and shoulders until the shoulder blades are clear off the floor (crunches); and flexing and extending the knees while sitting on the edge of a chair (knee flexion and extension while seated).

### 2.2.2. DBE program

The aim of the DBE program was to enhance skill in posture position and dynamic stability. The same physical therapist and public health nurse who took part in the LIO also served as instructors on the DBE program once a week for 8 weeks. Twenty-seven subjects were divided into 2 groups, each of which

participated in 90-min training sessions. The following was the content of the DBE instruction: symmetrical and asymmetrical movements; forward and lateral reach; forward and backward steps; standing and walking on a reduced base of support; increasing the complexity of ambulatory tasks; and functional ankle strengthening. Movements were performed on two balance mats (80 mm high × 600 mm wide × 600 mm deep, Toyoda Gosei Co., Ltd., Nagoya, Japan).

### 2.3. Evaluation 1: Physical function tests

In July 2004, we asked subjects concerning their basic



**Table 1.** Eight-week course of low-intensity exercise with occlusion

Period	Max Belt Pressure	Schedule
Week 1	70-80 mmHg	Orientation, 5 sets of 10 repetitions of 6 different movements.
Week 2	90-100 mmHg	3 sets of 10 repetitions of 6 different movements. Rest for 5 min. 2 sets of 10 repetitions of 6 different movements.
Week 3	110-120 mmHg	Same as week 2
Week 4	130-140 mmHg	Same as week 2
Week 5	140-150 mmHg	4 sets of 10 repetitions of 6 different movements.
Week 6	140-150 mmHg	3 sets of 15 repetitions of 6 different movements, adding 20-sec breaks between sets.
Week 7	140-150 mmHg	Same as week 6
Week 8	140-150 mmHg	Same as week 6

characteristics such as sex, age, family structure, current health status, and history of falls. The 8-week intervention was conducted from late July to early September that year. Various physical functions as stated below were evaluated before and after the intervention period.

#### 2.3.1. Performance

The following 4 items were measured. 1) Reaction time: subjects jumped from a standing posture as quickly as possible in response to optical stimulation. Time from stimulation to the onset of performance was measured. The shorter the reaction time, the greater the level of quickness. 2) Timed up and go test (hereinafter referred to as "TUGT"): after standing up at a given signal from a seated position on a chair, the subjects walked up to and around a target object 3 meters away and then returned to sit down on the chair (12). The time taken to complete the process was recorded. 3) Ten-meter walking time: time taken to walk as fast as possible on a 10-meter straight line was measured. 4) Maximum step distance: the length of a step taken as far forward as possible from a standing position was determined.

#### 2.3.2. Balance

Functional reach and the duration of time spent standing on one leg were measured. In functional reach, subjects first stood comfortably upright and then reached forward as far as possible without stepping or losing their balance (13). As for standing on one leg, the duration was measured with eyes open and looking at an object 1.5 meters ahead.

#### 2.3.3. Muscle strength

Grip strength was measured by a digital handgrip dynamometer (Takei Scientific Instruments Co., Ltd., Tokyo, Japan). Isometric knee extension muscle strength was measured in a sitting position at a knee angle of 90 degrees using a dynamometer, GT-30 (OG Giken Co., Ltd., Tokyo, Japan).

#### 2.4. Evaluation 2: Blood tests

In order to evaluate the biochemical parameters, plasma levels of growth hormone and lactate were measured in 11 subjects (3 males and 8 females) who participated in the 8-week LIO program and consented to blood sampling. On the 6th week, LIO with belt pressure of 140 to 150 mmHg was performed for 15 min. Blood was then taken from the 11 subjects, who were instructed to eat more than 2 h before the start of the session. The timing of blood collection was before, after, and 15 min after the session (10).

#### 2.5. Statistical analysis

All analyses were performed with StatView package (ver. 5.0 SAS Institute Inc., Cary, North Carolina, USA) and the significance level was fixed at 5%. The results of physical function tests were analyzed to evaluate differences due to group (LIO versus DBE) and time (baseline versus follow-up) by using repeated measures ANOVA. Regarding characteristics of subjects, age difference between LIO and DBE was compared by *t*-test, and chi-square test was used for the rest of parameters. One-way repeated measures ANOVA was used to compare the variance of growth hormone and lactate before, after, and 15 min after the LIO.

### 3. Results

Participants in the two groups were similar at the baseline for demographics, presence of chronic diseases, self-rated health, self-reports of falls and physical activity (Table 2). The mean age of those who completed the final evaluation was  $70.7 \pm 4.3$  of LIO and  $70.6 \pm 5.0$  of DBE, ranging from 65 to 79 years. Mean participation frequency were  $14.2 \pm 1.5$  times out of a total of 16 times (88.8%) in the LIO and  $6.5 \pm 1.4$  times out of a total of 8 times (81.3%) in the DBE group. No subjects reported serious adverse effects as a result of the programs.

In the LIO group, 19 out of the 24 subjects completed the final evaluation. Three subjects dropped out, of whom one participated 9 times and the others participated only once. Two people failed to undergo follow-up evaluation after the completion of the program. The reasons for not attending the follow-up were poor physical condition and family problems, respectively. In the DBE group, 25 out of the 27 subjects completed the final evaluation. Two people dropped out after attending the program only once (Figure 1).

### 3.1. Evaluation 1: Physical function tests

Table 3 shows the result of physical function tests

before and after the two programs. Both the LIO and DBE programs brought improvements in reaction time, maximum step distance, 10-meter walking time, functional reach test, and standing on one leg (both leg). In repeated measures ANOVA for measuring interactive effects by groups and types of intervention, TUGT and knee extension in the LIO group were items that showed significant improvement (TUGT,  $p < 0.001$ ; left knee,  $p < 0.001$ ; right knee,  $p = 0.007$ ).

### 3.2 Evaluation 2: Blood tests

One-way repeated measures ANOVA showed a significant increase of growth hormone in post hoc test

**Table 2.** Characteristics of subjects<sup>a</sup>

		LIO group (n = 24)	DBE group (n = 27)	Statistical significance
Gender	Female	16 (66.7%)	18 (68.0%)	> 0.99
	Male	8 (33.3%)	9 (32.0%)	
Age		72.3 ± 4.5	71.0 ± 4.1	0.16
Spouse	Yes	18 (75.0%)	20 (74.0%)	> 0.99
	No	6 (25.0%)	7 (26.0%)	
Family composition	Alone	3 (12.5%)	5 (18.6%)	0.45
	Couples	13 (54.2%)	9 (33.3%)	
	With single child	6 (25.0%)	2 (7.4%)	
	With young couples	0	2 (7.4%)	
	With child and grandchildren	2 (8.3%)	8 (29.6%)	
Chronic Diseases	Yes	18 (75.0%)	16 (64.0%)	0.76
	No	6 (25.0%)	11 (36.0%)	
Self rated health	Good	11 (45.8%)	7 (24.0%)	0.14
	Not so good	12 (50.0%)	20 (76.0%)	
	Bad	1 (4.2%)	0	
History of fall	Yes	11 (45.8%)	6 (20.0%)	0.06
	No	13 (54.2%)	21 (80.0%)	
Difficulties from floor sitting position to standing	Yes	10 (41.7%)	11 (44.0%)	> 0.99
	No	14 (58.3%)	16 (56.0%)	

<sup>a</sup> Data including categorical variables are analyzed by chi-square, and continuous data by *t*-test.

**Table 3.** Changes of variables before and after intervention<sup>a,b</sup>

Category of measurements	Variables	LIO group (n = 19)		DBE group (n = 25)		p Value		
		Baseline survey	Follow-up survey	Baseline survey	Follow-up survey	Main effect by group	Main effect by intervention	Interaction
Performance	Reaction time (ms)	509.9 ± 144.6	448.0 ± 66.0	500.3 ± 70.4	475.0 ± 81.2	0.73	0.002	0.18
	TUGT <sup>c</sup> (s)	7.2 ± 1.4	6.1 ± 0.8	6.9 ± 1.4	7.3 ± 1.5	0.22	0.01	< 0.001
	10 m walking time (s)	5.3 ± 0.7	4.8 ± 0.7	5.7 ± 1.2	5.2 ± 0.9	0.09	< 0.001	0.64
	Left maximum step distance (cm)	98.6 ± 25.4	113.9 ± 16.4	99.4 ± 12.6	111.6 ± 13.8	0.19	< 0.001	0.50
	Right maximum step distance (cm)	104.1 ± 14.4	113.2 ± 14.0	101.1 ± 11.3	109.7 ± 12.8	0.30	< 0.001	0.85
Balance	Functional Reach Test	27.8 ± 4.9	30.4 ± 6.0	26.5 ± 6.3	27.6 ± 5.7	0.19	0.01	0.29
	Left leg standing time with open eye (s)	36.0 ± 22.9	38.6 ± 23.1	32.3 ± 19.6	38.6 ± 21.1	0.77	0.03	0.35
	Right leg standing time with open eye (s)	35.8 ± 23.3	23.6 ± 19.6	38.2 ± 23.1	30.8 ± 23.5	0.13	0.09	0.38
Muscle strength	Left knee extension (kg)	21.1 ± 7.4	25.4 ± 8.2	21.4 ± 6.9	20.6 ± 5.8	0.29	0.004	< 0.001
	Right knee extension (kg)	23.1 ± 7.2	24.7 ± 8.1	22.1 ± 6.1	20.9 ± 6.1	0.25	0.70	0.007
	Left grip power (kg)	25.4 ± 6.9	26.7 ± 8.6	25.3 ± 6.4	26.0 ± 7.9	0.85	0.07	0.63
	Right grip power (kg)	26.6 ± 8.1	27.7 ± 9.5	27.0 ± 7.2	27.0 ± 6.8	0.96	0.27	0.26

<sup>a</sup> Values are mean ± SD. Repeated measures ANOVA is used to compare differences between two groups.

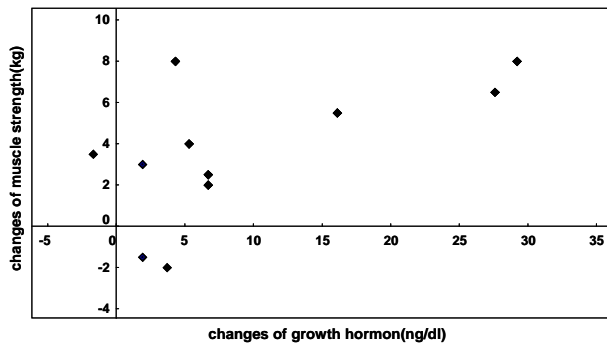
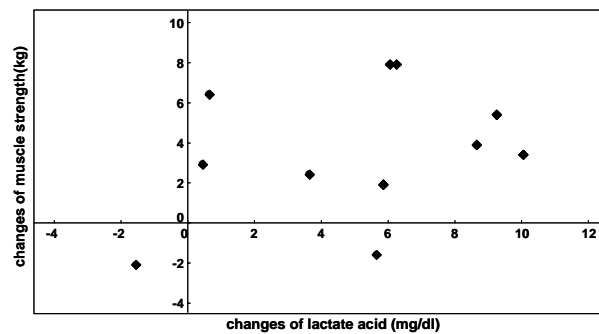
<sup>b</sup> "Main effect by group" means that LIO group (n = 19) and DBE group (n = 25) are compared.

<sup>c</sup> Abbreviations: TUGT, Timed Up and Go Test.

**Table 4.** Biochemical data<sup>a</sup>

	Pre exercise	Post exercise	15 min later	<i>p</i> Value (Post hoc test, pre and post)	<i>p</i> Value (Post hoc test, pre and 15 min later)
Growth hormone ( <i>n</i> = 11)	1.8 ± 2.3	11.1 ± 9.6	7.3 ± 7.7	0.010	0.150
Lactic acid ( <i>n</i> = 11)	10.1 ± 4.3	15.0 ± 4.6	12.2 ± 6.3	0.057	0.053

<sup>a</sup> Values are mean ± SD. One-way repeated measures ANOVA is used.

**Figure 3.** Changes of average knee extensor strength and growth hormone (*n* = 11).**Figure 4.** Changes of average knee extensor strength and lactate (*n* = 11).

( $p = 0.010$ ) (Table 4). In order to explain the relation between muscle strength and biochemical data, we plotted in scatter diagrams the changes of average knee extensor strength before and after the 8-week program with growth hormone (Figure 3), and with lactate (Figure 4). Correlation coefficient were 0.662 ( $p < 0.05$ ) and 0.163 ( $p > 0.05$ ), respectively. In a 75-year-old male, growth hormone was increased approximately 293 times to that before the training (pre-exercise: 0.1 ng/mL; immediately post-exercise: 29.3 ng/mL). After the 8-week intervention program, left and right knee extension of the male was increased by 58.8% and 22.2%, respectively.

#### 4. Discussion

Although the LIO training has been reported to improve muscular function in young males and athletes and in middle-aged women, there has been no research on its effect in healthy elderly subjects. There were even or significant changes in the measured items, including performance, balance, and muscle strength as compared with DBE program without significant adverse events.

In general, performance is thought to be an

integrated ability which is comprised of multiple elements, such as muscle strength, coordination between the central nervous system and muscle, and joint flexibility. There is a report on enhanced performance through resistance training (14). In the present study, 6 different exercises were conducted while wearing a pair of special elastic belts. It is possible that the enhancement of performance can be attributed to the improved nerve-and-muscle coordination, resulting in improved muscle strength of the lower limbs (15).

The results of the functional reach test and standing on one leg were satisfactory after both LIO and DBE programs. It was likely that the subjects relearned to move the center of gravity and maintain a dynamic posture. Namely, when the subjects performed the forward lunges, they moved the center of gravity back and forth without raising their feet from the floor. In the knee lifts, while repetitively and alternately standing on one of the legs, the subjects maintained their center of gravity within the narrow base of support. Similar to a report investigating the effects of Tai Chi (16), such repetitive posture change and the associated improvement in lower-limb strength might have stabilized the range of motion of the center of gravity. Thus, even a short-term LIO could improve balance as effectively as DBE in the elderly.

After the LIO program, left and right knee extension increased by 20.4% and 6.9%, respectively. However, after the DBE program, it decreased slightly by 3.7% and 5.4%, respectively. A difference between the left and right was probably because the participants tended to put an uneven load on both of the legs. Half-squats and forward lunges caused repetitive eccentric and concentric quadriceps contractions. Such local and mechanical stimulation may have been linked with the improvement in muscle strength. Thus, it is certain that even a short-duration and low-intensity LIO training can improve lower-limb muscle strength in the elderly as effectively as resistance machine training in older adults and LIO training in the young athletes (14,17). Prior studies have reported an increase in plasma level of growth hormone in young subjects after the LIO (17,18). In accordance with these studies, we observed similar increased levels of growth hormone in the elderly.

In the study of Takarada *et al.* (10), plasma levels of growth hormone, norepinephrine, and lactate increased remarkably immediately after LIO. Furthermore, LIO had a significant effect equal to or even greater than

high-intensity resistance training on an increase in muscular size and strength (11). The correlation among LIO, lactate, and growth hormone has been discussed as follows: due to moderate inhibition of muscle blood flow at the time of exercise, lactate as a potential cause of fatigue is produced and accumulates in muscles. Accordingly, even in a low intensity exercise with occlusion, additional motor unit recruitment is required. A large amount of growth hormone essential for muscle synthesis is released from the pituitary gland in the brain and carried by blood circulation throughout the body. Growth hormone delivered to all parts of the body via the blood stream is thought to act on muscle tissue, decompose body fat, and produce muscle. In our study, the 8-week LIO program improved physical function, especially muscle strength, which may be associated with the exercise-induced secretion of growth hormone.

The present study had the following limitations: 1) the small samples and short periods did not permit sufficient analysis of the effects on physical function; 2) the results were limited to the case of relatively healthy local elderly residents; and 3) while the results of relatively short-term (8-weeks) trial were obtained, the study could not observe sustained longer-term effects.

This is the first study of LIO designed as a training program targeted at elderly people living in a community. In order to investigate the effects of LIO on physical function, an 8-week LIO program was conducted and compared with an 8-week DBE program. Muscle strength showed a significant increase after the LIO, which may be associated with exercise-induced secretion of growth hormone. This study confirmed that LIO was more effective than DBE in TUGT and muscle strength of knee extension. There were not a large difference of exercise compliance between LIO and DBE, suggesting that LIO could be useful for elderly people. These results suggest that LIO should be seen as one of the most promising physical training programs targeted at healthy elderly people.

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**Original Article**

# Elevation of soluble Fas (APO-1, CD95) ligand in natural aging and Werner syndrome

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**Summary**

The pathophysiological process of natural human aging has not been studied adequately due to the lack of an appropriate human model. Since recent investigations have suggested that inflammation possibly contributes to the pathogenesis of age-related disorders including atherosclerosis, cancer, and diabetes mellitus, the term "inflammaging," a combination of "inflammation" and "aging," has been coined. Werner syndrome (WS), caused by the loss of function of RecQ3 DNA/RNA helicase, is a typical progeroid syndrome mimicking natural aging, although it is extremely rare outside of Japan. We sought to examine WS patients from an immunological/inflammatory perspective. Sera from 14 mutation-proven WS patients (ages: 33-70 years) and 21 healthy Japanese adults ages 15 to 95 years were examined with ELISA for soluble Fas ligand (sFasL) to compare conventional inflammation markers. With natural aging, a statistically significant correlation ( $p < 0.0001$ ) was observed in the serum level of sFasL. The sFasL in WS, a level comparable to that in healthy elderly ages 83 to 95 years, had significantly increased ( $p < 0.05$ ) compared to that in young healthy individuals ages 15 to 70 years. A significant correlation was noted between the serum levels of conventional inflammation markers such as CRP ( $p < 0.025$ ), ESR ( $p < 0.024$ ), and WBC count ( $p < 0.0085$ ). In conclusion, an increased level of serum sFasL in natural aging and WS patients may suggest a common pathophysiological mechanism: inflammation. WS may be a good model for analyzing inflammaging.

**Keywords:** Aging, Apoptosis, Inflammaging, Soluble Fas ligand, Werner syndrome

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## 1. Introduction

Apoptosis, known as programmed cell death, is reported to be essential as a silent, chronic physiological process necessary for part of normal development and organ homeostasis through the interaction of death factors (TNF, FasL, and TRAIL) and cell surface death receptors (Fas, TNFR1, DR3/Wsl-1, and CAR1); thus, it differs from another type of dynamic cell death accompanied by acute inflammation known as necrosis (1-5). FasL is a 40-kd type II transmembrane protein that belongs to the TNF family (5); it induces apoptosis

through its membrane receptor, Fas. FasL is converted to the 26-kd soluble form of the truncated membrane-bound Fas receptor known as the sFas ligand (sFasL) by inflammatory metalloproteinases (6).

Recent investigations have suggested the critical role of apoptosis in the development of a variety of aging-related chronic diseases known as apoptotic syndromes such as Alzheimer's disease, systemic lupus erythematosus, and osteoporosis (7). Because of mounting studies on chronic inflammation, apoptosis is also believed to be the mechanism for the resolution of acute inflammation through the phagocytosis of neutrophils and other cells by macrophages at the site of inflammation (8), clearing up the apoptotic cells at an earlier stage without inciting secondary inflammation due to nitric oxide (9). However, if such a process goes wrong, then apoptosis proceeds to a later stage, following by the possible induction of silent chronic

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inflammation by producing pro-inflammatory cytokines (10).

Aging has been defined as a biologically declining process leading to organismal death at the latter stage of our life in contrast to development, growth, and maturation. Although several researchers have suggested that apoptosis has a role in aging, there has been no concrete evidence indicating its direct role in natural aging (11-13).

Although the pathophysiological functions of sFasL have not been fully clarified, sFasL inhibits Fas-mediated apoptosis by competitively binding with FasL and altering lymphocyte development and proliferation in response to self-antigens (14,15). Elevation of sFasL in the circulation has also been detected in autoimmune rheumatic diseases (14-17), atherosclerosis (18,19), obesity (20), and malignancies (21,22). The relationship between the serum level of sFasL and chronic inflammation has been a matter of considerable discussion especially in the context of the natural aging process (20-24).

Werner syndrome (WS; MIM#27770), a typical progeroid syndrome, has been nominated as the best natural model for analyzing human aging (25-27), as the syndrome manifests a variety of age-related signs and symptoms including skin atrophy, skin pigmentation, sarcopenia, cataracts, diabetes mellitus, hypogonadism, osteoporosis, atherosclerosis, and malignancy at a relatively early stage of life followed by an early death (28,29). Interestingly, a majority of patients with this illness are Japanese in origin, partly because of the relatively high prevalence of consanguineous marriage in rural areas and the extremely high frequency of heterozygotes in Japan (30,31). The current authors previously reported inflammatory conditions observed in WS in a series of publications (32-38). Here, the intent was to study the pathophysiological roles of serum sFasL in the development of aging-related signs and symptoms in Japanese patients with WS and healthy individuals.

## 2. Materials and Methods

### 2.1. Study population

Serum samples were obtained from 14 patients with mutation-proven WS (M = 6, F = 8; ages 33 to 70 years) (28-31,38), 13 healthy young volunteers (M = 4, F = 9; ages 15 to 70 years), and 8 healthy elderly volunteers (M = 1, F = 7; ages 83 to 95 years) who provided informed consent for this study, which was approved by the ethics committee of Tooin University of Yokohama. All samples were stored at -80°C until use. All of the WS patients showed the following manifestations as previously reported: typical body status/face, hoarseness, gray hair/alopecia, skin atrophy/sclerosis/pigmentation, sarcopenia, cataracts, osteoporosis, and subcutaneous calcification. As indicated in Table 1, 11

**Table 1.** Clinical characteristics in Werner syndrome patients

Age	Sex	DM	Hyper-lipidemia	Skin ulcer	Hyper-uricemia	Malignancy pre/post
39	F	+	+	-	-	-/+
39	M	+	+	-	+	-/-
40	F	+	+	+	-	+/-
42	M	-	-	-	+	-/-
45	F	+	-	+	+	-/-
47	M	-	+	-	-	-/+
50	F	+	-	+	+	-/-
50	F	-	+	+	+	-/+
52	F	+	+	+	-	-/-
52	F	-	+	-	+	-/+
54	M	+	+	+	-	-/-
55	M	+	-	-	-	-/-
59	F	-	+	+	+	-/-
70	M	+	+	+	+	-/-

In the malignancy column, -/+ indicates patients did not have a malignancy before sampling but had one after sampling. +/- indicates a patient had a malignancy before sampling but did not have one after sampling.

patients had hyperlipidemia, 9 had diabetes mellitus, 8 had skin ulcers, and 7 had hyperuricemia. No patient had a malignancy at the time of sampling, but one had a malignancy before sampling and 4 had one after sampling (28-30,40).

### 2.2. Determination of sFasL and inflammation markers

sFasL was measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions (MBL, Nagoya, Japan) (41). The ESR level (mm/h) was measured by the Westergren method. CRP (ng/mL) was measured by nephelometry. The number of WBCs was counted by the standard method.

### 2.3. Statistical analysis

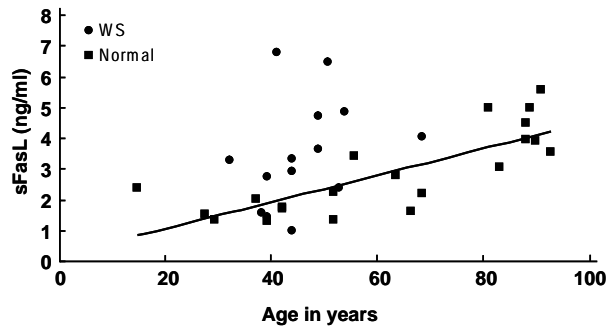
Data are expressed as mean  $\pm$  SE. Significance was tested using the unpaired *t*-test, while correlations were determined using Pearson's formula.

## 3. Results and Discussion

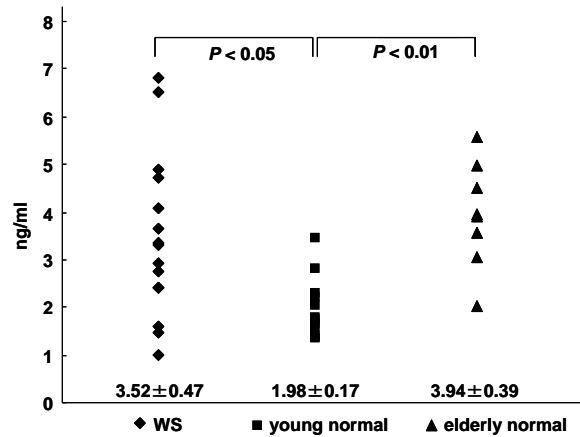
The serum level of sFasL was significantly correlated with natural aging ( $r = 0.8415$ ,  $p < 0.0001$ ) (Figure 1) but there was no gender difference as was previously reported (20,23,42) (Figure 1).

The sFasL level in WS patients was significantly higher ( $3.52 \pm 0.47$  ng/mL;  $p < 0.05$ ) than that in age- and sex-matched young healthy individuals ( $1.98 \pm 0.17$  ng/mL), but was comparable to that in elderly healthy individuals ( $3.94 \pm 0.39$  ng/mL), as shown in Figure 2.

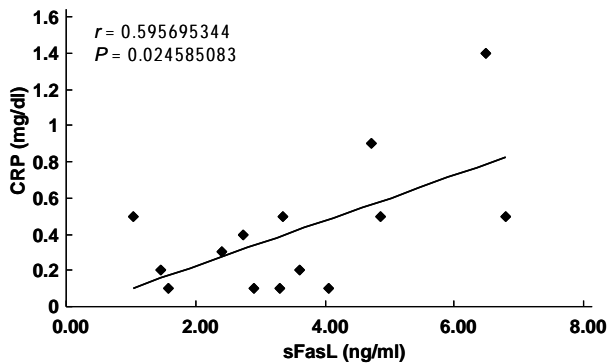
sFasL may be produced as a result of inflammation, and significant correlations were observed between serum levels of sFasL and inflammation markers, *i.e.* CRP ( $r = 0.596$ ;  $p < 0.025$ ) (Figure 3), ESR ( $r = 0.598$ ;  $p < 0.024$ ) (Figure 4), and WBC counts ( $r = 0.67$ ;  $p < 0.0085$ ), in WS patients; these correlations have been described in the general population as well



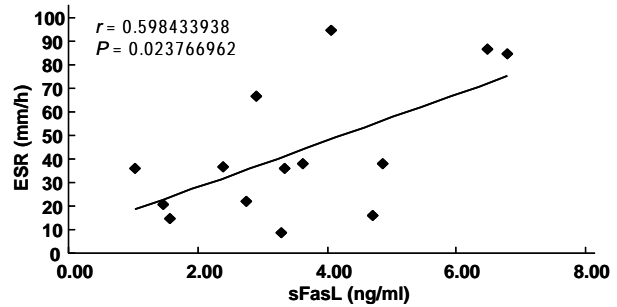
**Figure 1.** sFasL in Werner syndrome and natural aging. Serum levels of sFasL: sFasL were assayed with a commercial sFasL ELISA kit as described in the Materials and Methods section. A statistically significant correlation ( $p < 0.0001$ ) was observed between the serum level of sFasL and natural aging. The sFasL level in most WS patients increased in comparison to that in age-matched healthy individuals.



**Figure 2.** The serum sFasL significantly increased ( $p < 0.05$ ) in comparison to that in age-matched healthy young individuals but was comparable to that in elderly individuals. The mean  $\pm$  SE of the serum sFasL level in each group is indicated in the figure.



**Figure 3.** Correlation between serum levels of sFasL and CRP. The serum level of sFasL was significantly associated with an increasing level of CRP ( $p < 0.025$ ).



**Figure 4.** Correlation between serum levels of sFasL and ESR. The serum level of sFasL was significantly associated with an increasing level of ESR ( $p < 0.024$ ).

(23,41,42). Patients with WS manifest metabolic syndrome consisting of hyperlipidemia, atherosclerosis, diabetes mellitus and hyperuricemia, skin ulcers, and malignancy (28-30,39), all of which have been shown to be closely linked to chronic inflammation (23,37,43,44). Partly because of the limitations of the samples studied, however, statistically significant differences in serum levels of sFas, CRP, ESR, and WBC were not detected between individuals with and without DM, hyperlipidemia, skin ulcers, hyperuricemia, and malignancy.

Although the exact role of serum sFasL in aging has not been clarified, apoptosis in development and maturation may be a prologue to the subsequent chapter of chronic inflammation in senescence, followed by organismal death. Thus, WS may be a good model for studying how low-grade inflammation leads to senescence ('inflammaging') (45).

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## Original Article

# Discriminant analysis: A supportive tool for monogenoidean taxonomy

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### Summary

Data on the measurements of haptoral parts of 47 parasite species of *Bychowskyella* (1), *Cornudiscooides* (2) and *Thaparocleidus* (3), found on Indian freshwater catfish, were gathered from previously reported studies. Based on six morphometric variables concerning haptors, these species were subjected to discriminant analysis in order to more accurately distinguish their generic placement. This paper describes a comparative study of several classification models. Using the original variables in the study, a simple linear discriminant analysis model was constructed and models using principal components (PC) for discrimination have also been explored. The effectiveness of these models is measured in terms of percentage of correct classification. Quadratic discriminant models using original variables and their principal components provided the highest (95%) correct classification for *Bychowskyella* (1). Species of *Thaparocleidus* (3) were correctly classified using dorsal and ventral hard-part measurements (100%) as well as hook measurements. The highest percentage (75%) of correct classification for *Cornudiscooides* (2) was achieved through a quadratic discriminant model using hook measurements.

**Keywords:** Discriminant analysis, Principal component analysis, Monogenoidea, Taxonomy, Hard part

### 1. Introduction

Identification of parasites using only morphological techniques has long been debated by molecular biologists, statisticians, and related researchers. Analysis (characterization) of monogenoideans has often remained an arduous task for the researchers in the field. Their grouping into different taxa has long been questioned. Misidentification and misinterpretation often creates problems with their classification and thus leads to incorrect phylogenies. Many of the Indian species were originally misplaced in incorrect genera due to poor understanding of the morphological boundaries of dactylogyrid genera during early studies in India (4). This confusion can be avoided if assistance is sought from other fields in order to reach the correct classification.

Distinguishing parasites will achieve greater accuracy if appropriate tools are provided by statistics. This will help to clarify the taxonomic status of monogenoidean parasites. In the present work, statistical analysis has been used to supplement the taxonomical work carried out to date regarding the categorization of the species of three genera *Bychowskyella* (1), *Cornudiscooides* (2), and *Thaparocleidus* (3) belonging to the family dactylogyridae in the class monogenoidea. This analysis of morphometric variation is used to assess the congruence of genera and species initially distinguished by morphology, where species are recognized based on morphometric variation of haptoral sclerites. A review of monogenoidean parasites of siluriformes fishes from the Old World (5) indicated that 17 species of *Bychowskyella* (1), 19 species of *Thaparocleidus* (3), and 11 species of *Cornudiscooides* (2) from India are considered valid. The validity of certain species of these genera is still disputed. To overcome this problem, this work uses discriminant analysis, a numerical taxonomy technique that divides data into groups so that subjects within a group are similar to one another and differ from subjects in other

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groups. Relationships among measurement variables with respect to the grouping variable can be expressed by their mean values and variance-covariance matrices. Discriminant analysis has been used in monogenoidean work by previous researchers (6) who examined hook, ventral bar, and anchor features of only four *Gyrodactylus* species: *G. gondae*, *G. flavescens*, *G. arcuatus*, and *G. arcuatoides*. Approximately 8-23 specimens per species were measured. This is a rather low number for proper statistical analyses. Other researchers (7-9) discriminated between only 2 closely related and morphologically similar species of *Gyrodactylus*; *G. salaris* and *G. thymalli*, using the statistical classification methodologies of linear discriminant analysis (LDA) and k-nearest neighbors (KNN). To overcome the shortcomings of earlier research, an attempt has been made here to select as many variables as possible for accurate analysis. The goal of this paper is to investigate the role of discriminant analysis in conjunction with principal component analysis in order to validate the species of three monogenoidean genera: *Cornudiscoides* (2), *Thaparocleidus* (3), and *Bychowskyella* (1).

## 2. Materials and Methods

Data of the hard parts of 47 parasite species belonging to three genera *Cornudiscoides* (2), *Thaparocleidus* (3), and *Bychowskyella* (1) were compiled from published studies and subjected to discriminant analysis. Several published and ongoing studies concerning monogenoidean communities were reviewed. Morphometrical distances of haptor sclerite parts were used. The terminology followed here is that of Gusev (1976) (10). This study provided a set of quantitative observations on several species of parasites. This was done to classify organisms into taxonomic categories based on their quantitative measurements.

Discriminant analysis is a statistical technique in which the quantitative measurement of cases is used to create a model that explains the classification of the cases into different groups. This model can further be used to assign additional observations to the correct group. Such models can be fit in a variety of ways depending on the covariance or correlation structure of the variables. In the simplest case, when the covariance matrices are equal for each group, the linear discriminant model (function) is used. In the most general case, when covariance matrices are unequal for the groups, the discriminant function is quadratic (*i.e.* quadratic discriminant analysis or QDA) (11).

A classical linear discriminant analysis (LDA) of a three-class outcome with two or more feature variables results in two linear discriminants that are linear combinations of the features. A scatter plot of these two variates, with the data points marked by class, shows the effectiveness of the discrimination between classes. Sometimes when the number of variables is

large or there is multicollinearity (12) among the feature variables, the analysis can be simplified by considering a smaller number of linear combinations of the original variables (13). Principal component analysis (PCA) is a technique that finds such linear combinations (called principal components). Usually the first two or three principal components explain most of the variation in the original data. This paper uses a classification method based on PCA and DA. The method consists of two steps: first, the original vector space is projected to a subspace *via* PCA, and then DA is used to obtain a best classifier. The basic idea of combining PCA and DA is to improve the generalization capability of DA when only few samples per class are available. S-PLUS and SPSS software were used for statistical processing in this study.

## 3. Results

### 3.1. Statistical analysis

The variables in this study were (measurements in microns):

1. Dorsal Anchor inner length (DALI)
2. Dorsal Anchor outer length (DAO)
3. Dorsal Bar length (DBL)
4. Ventral Bar length (VBL)
5. Ventral Anchor length inner (VALI)
6. Hook measurements (H1, H2, H3, H4, H5, H6, H7)

The classes (groups) are described by the variable GENUS.

A univariate analysis of variance (Table 1) indicated a significant difference between the three group means with respect to dorsal bar length ( $p = 0.007$ ), ventral bar length ( $p = 0.006$ ), and ventral anchor inner length ( $p < 0.001$ ). Although the differences in group means for DALI were not significant ( $p = 0.301$ ), they might serve as an ancillary variable and provide, along with significant variables, additional information for classification purposes.

The relationships between the variables used for classification should be explored.

The matrix plot in Figure 1 shows the direction of relationships between the original variables. The last row and column indicate the differences between the three genera. As is apparent, the variables for

**Table 1.** Mean and standard deviation (Mean; SD) of variables with respect to each genus

	<i>Bychowskyella</i> (n = 20) Mean; SD	<i>Cornudiscoides</i> (n = 12) Mean; SD	<i>Thaparocleidus</i> (n = 15) Mean; SD	p-value
DALI	70.63; 23	97.82; 174.8	43.8; 13.21	0.301
DBL	50.98; 25.57	31.75; 5.17	34.3; 11.03	0.007
VALI	40.29; 17.33	25.25; 9.55	21.63; 6.35	<0.0001
VBL	63.1; 43.76	36.92; 6.44	31.27; 10.05	0.006



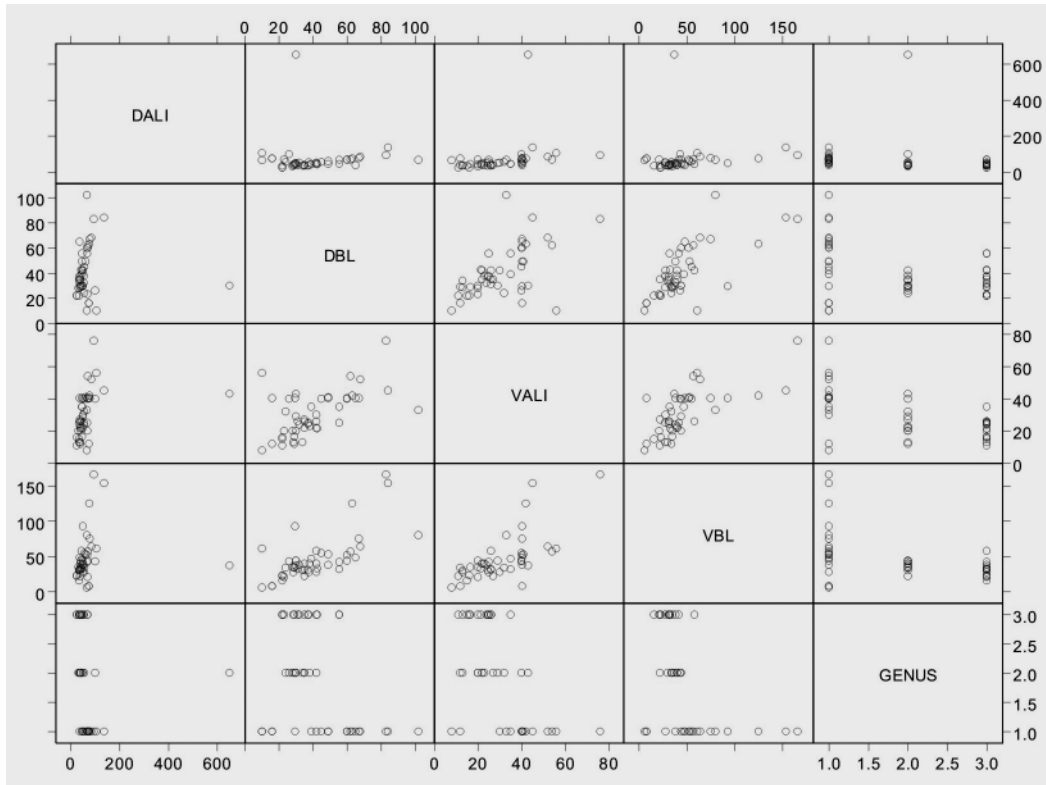


Figure 1. Matrix plot indicating strength and direction of relationship between variables.

*Bychowskyella* (1) have a markedly different location and scatter pattern while *Cornudiscooides* (2) and *Thaparocleidus* (3) have a similar pattern.

An important assumption in linear LDA is that the within groups covariance matrices should be equal for each group. The differences in covariance matrices for each of the three groups were found to be highly significant ( $p < 0.05$ ). Hence, the data suggests a heteroscedastic covariance structure.

### 3.2. Construction of discriminant models

Next, the classification obtained by linear and quadratic classifiers was compared using original variables. The best overall classification is obtained by using a quadratic discriminant function with original variables as independents (Table 2). An overall correct classification of 80.9% is obtained. While the *Bychowskyella* (1) and *Thaparocleidus* (3) species are correctly classified, the classification of *Cornudiscooides* (2) is merely 33.3%.

In order to determine the maximum separation between *Cornudiscooides* (2) and *Thaparocleidus* (3), the two were studied separately. Since dorsal anchor outer length is an additional feature available for the two genera, it was also incorporated in the classification model. The matrix plot in Figure 2 shows the direction of relationships between the previous four variables and the additional variable (dorsal anchor outer length) for the two genera.

Table 2. Comparison of various linear and quadratic discriminant models

Genus	Correct classification (%)	
	LDA	QDA
<i>Bychowskyella</i>	90	95
<i>Cornudiscooides</i>	8.4	33.3
<i>Thaparocleidus</i>	94	100
Overall	70.21	80.9

LDA, linear discriminant analysis; QDA, quadratic discriminant analysis.

The best model for the discrimination of species in the two groups, based on dorsal and ventral hard parts, is the linear discriminant model with the separate principal components of the dorsal and ventral hard parts. The first principal component of the dorsal anchor outer length and dorsal bar length accounts for 80% of variability in the data and the first principal component of the ventral anchor inner length and ventral bar length also accounts for 80% of variability in the data. Discriminant analysis using these two components as new predictors yielded the following classification, as shown in Table 3.

The apparent error rate (APER) for the above classification was 29.6%, which is quite reasonable. The linear discriminant function is shown in Figure 3.

In order to find an even better way to classify the two genera *Cornudiscooides* (2) and *Thaparocleidus* (3), measurements of the seven pairs of hooks in each species were also used. The best classification was

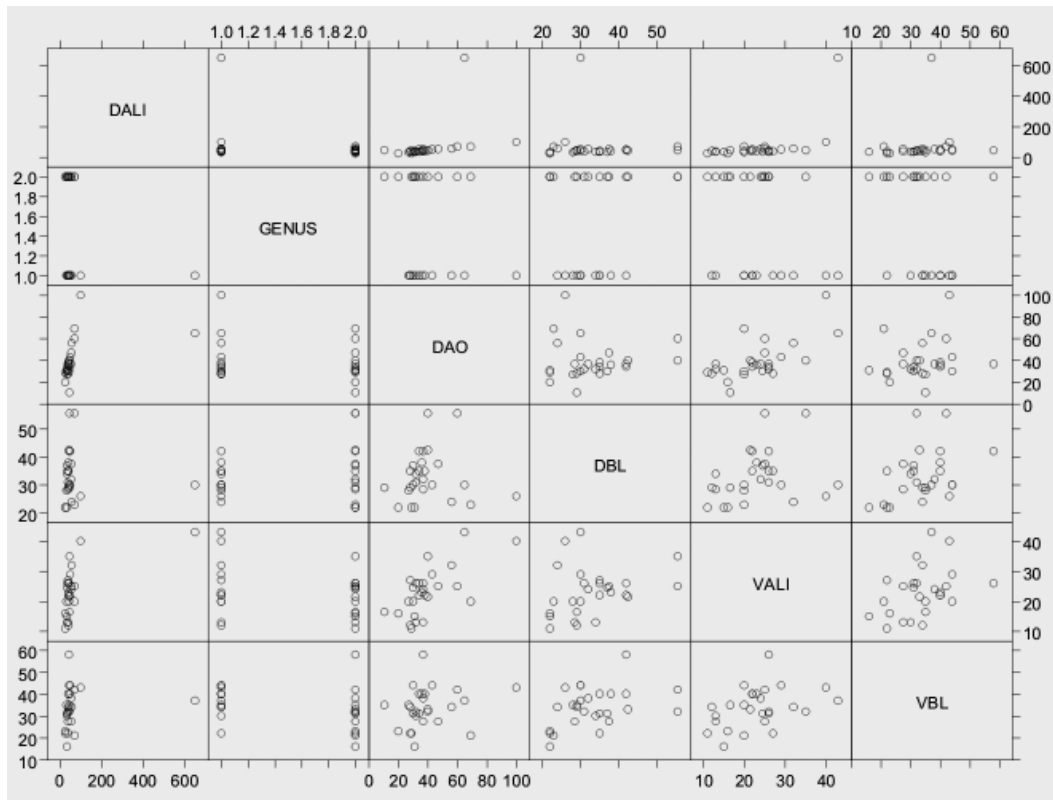


Figure 2. Relationships between variables for genus *Cornudiscoides* (2) and *Thaparocleidus* (3).

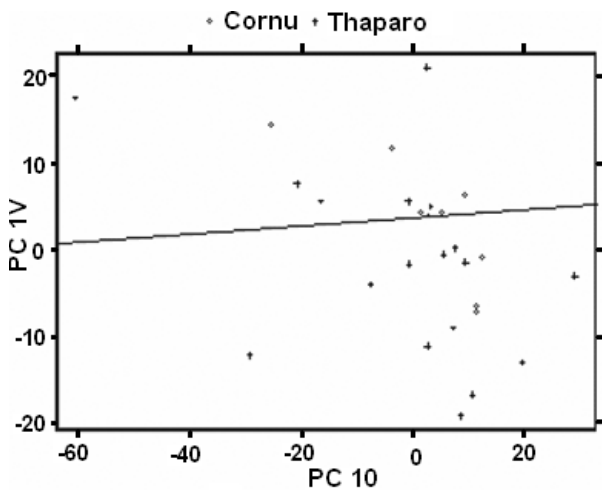


Figure 3. Linear discriminant function using two principal components.

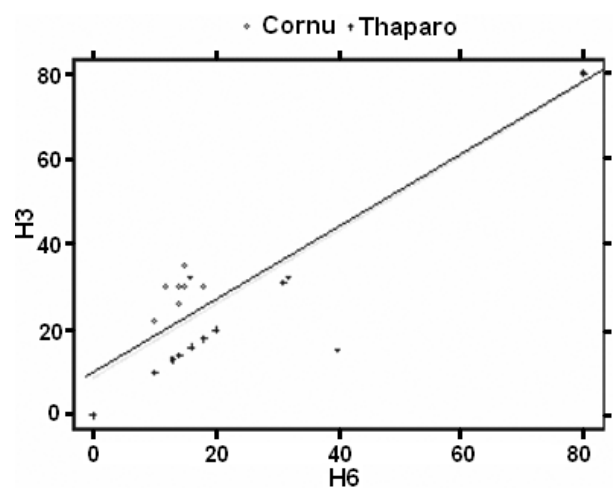


Figure 4. Quadratic discriminant analysis using hooks data. H3 is the third hook and H6 is the sixth hook.

Table 3. Summary of LDA using principal components of dorsal and ventral hard parts

		Predicted genus		Total number of parasites
		<i>Cornudiscoides</i>	<i>Thaparocleidus</i>	
Actual genus	<i>Cornudiscoides</i>	8	4	12
	<i>Thaparocleidus</i>	4	11	15

Table 4. Summary of QDA using hook measurements

		Predicted genus		Total number of parasites
		<i>Cornudiscoides</i>	<i>Thaparocleidus</i>	
Actual genus	<i>Cornudiscoides</i>	9	3	12
	<i>Thaparocleidus</i>	0	15	15

found to be a quadratic discriminant function depending on hook variables (H3 and H6) with overall correct classification of 88.9% (Table 4).

The highest percentage (75%) of correct classification for *Cornudiscooides* (2) and 100% correct classification for *Thaparocleidus* (3) genera were achieved through the QDA model. The quadratic discriminant function is shown in Figure 4.

#### 4. Discussion

The order Siluriformes, or catfishes, includes 13 genera and about 100 species of freshwater fishes and has a wide geographical distribution, being found in Africa, Syria, and southern and western Asia (Philippines to Java) (14). Parasites classified based on statistical technique are found on catfishes that belong to this order. Discrimination among these parasites was required, necessitating their critical reexamination. The current analysis identified significant differences ( $p < 0.05$ ) between species within each genus for six measured haptor sclerite characteristics. Quadratic discriminant models using original variables and their principal components provided the highest (95%) correct classification for the genus *Bychowskyella* (1). The single species that remains incorrectly classified in *Bychowskyella* (1) is *Bychowskyella tripathii* (15). The species of *Thaparocleidus* (3) were correctly classified using dorsal and ventral hard part measurements (100%) as well as hook measurements. The highest percentage (75%) of correct classification for *Cornudiscooides* (2) was achieved through a QDA model using hook measurements. The three species that are incorrectly classified in *Cornudiscooides* (2) are *Cornudiscooides heterotylus* (2), *Cornudiscooides megalorcis* (2), and *Cornudiscooides geminus* (10). These species need to be re-examined. Any method of classification should thus take into account the heteroscedasticity in variables for the different groups. Some hard parts were also observed to serve as a more effective method of classification (e.g. hooks). Therefore, such methods should identify morphometric characteristics that best identify species belonging to the three genera.

Statistical implications of the measurements of these parasites are that taxonomists, who consider *Bychowskyella* (1), *Cornudiscooides* (2), and *Thaparocleidus* (3) to be three different and well-established genera, are correct in their claim. Thus, their correct interpretation offers potential for the correct mapping of parasite phylogeny. The blurred distinctiveness of these taxa was restored by subjecting them to discriminant analysis. The fact that the three discriminated genera were quite different from each other and not statistically similar posed no problem whatsoever.

#### Acknowledgements

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