EDITORIAL

THE BIRD FLU THREAT – WHY AREN’T WE WORRYING?

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Key words: Bird flu, avian influenza, influenza pandemic

The World Health Organization (WHO) and influenza experts worldwide are concerned that the recent appearance and widespread distribution of an avian influenza virus, influenza A/H5N1, has the potential to ignite the next influenza pandemic. It is estimated that even in the best-case scenarios, the pandemic will cause 2 to 7 million human death and tens of millions would require medical attention. If the pandemic virus is a very virulent strain, deaths could be dramatically higher.

The global spread of influenza pandemic cannot be stopped but preparedness will reduce its impact. Given the current threat, WHO has urged all countries to develop or update their influenza pandemic preparedness plans for responding to the widespread socioeconomic disruptions that would result from having large numbers of people, including health care workers, unwell or dying. Some of the countries have already developed structures and processes to counter this threat but some plans are far from complete and many countries have yet to begin. While it is impossible to accurately forecast the magnitude of the pandemic, it is a fact that much of the world is unprepared for a pandemic of any size. Why is there such complacency in the face of a very urgent and serious threat?

Influenza, to most people, is “just the flu” - an annoying, but tolerable upper respiratory tract infection. Thus, to generate widespread concern about its power as a global threat was as hard as creating interest in the power of asthma, another hidden killer. Why worry about an influenza virus while there are so many more exotic viruses, like Ebola, to fret about? Even the virus that caused the SARS outbreak in 2003 got more front-page coverage than the current outbreaks of bird flu, caused by yet another respiratory virus.

For all its familiarity and apparent harmlessness, complications of influenza had killed 10,000 to 20,000 people in the United States yearly. In the most devastating influenza pandemic that occurred in the winter of 1918-1919 (dubbed the “Spanish flu”), 196,000 Americans died in the month of October 1918 alone. Before the dreadful winter was over, 2 billion people around the world had come down with influenza with worldwide death estimated at 20 to 40 million. The “Spanish flu” had caused more deaths and socioeconomic disruptions in one six-month period then any other comparable period – more than the Black Death of the 14th century, more than the smallpox of the 16th century and even more than AIDS has killed so far. The rapid onset and dissemination of influenza relates to its short incubation period (which averages 2 days) and the high concentrations of the virus in aerosolized respiratory secretions (caused by coughing, sneezing, or speaking).

Modern medicine has given us an influenza vaccine, efficacious anti-influenza drugs and plenty of antibiotics (to treat secondary bacterial infections). All these led many to believe that whatever was killing people so ruthlessly in 1918 must certainly be something we can now treat, if history repeats itself. But during the 1918 debacle, many victims fell ill without prodrome and death occurred within hours of disease onset, making treatment a mockery. Once pandemic caused by a new influenza virus had started, prevention and control by vaccination would not be worthwhile as by the time the vaccine is ready for use (at least six months are needed for a vaccine to be concocted) the pandemic had already peaked and near ending. The current influenza vaccine offers no protection at all against bird flu, because no one can see it coming. Thus a global surveillance system for influenza is a necessity for detection of novel viruses before pandemics begin, as it would dramatically
increased the time to organize a response, including production and distribution of vaccines.

H5N1 is now widely entrenched in Asia and this signals that the world has moved closer to the next pandemic. Poultry husbandry in much of East Asia generally pays scant regard to hygiene practices and the movement of infected poultry, either by design or accident, is thought to have played a part in spreading the disease. Domestic and international trade in wild birds for both food and caged pets, involves millions of individuals annually and the frequent occurrence of mixed markets could allow viruses to pass between species and cross borders. The hiding of fighting cocks to avoid slaughter in Thailand may also have contributed to the spread, or at least hampered eradication. The role of migratory birds in the movement of H5N1 could not be discounted but it is noteworthy that surveillance of wild birds has resulted in very few isolations of H5N1 during either the current or previous Asian outbreaks.

Prior to 1997, avian influenza was confined to animals with no direct spread to humans. However in 1997, 18 human cases of avian influenza with 6 deaths were reported in Hong Kong. Coinciding with outbreaks of highly pathogenic H5N1 in poultry. In 2003, two other avian influenza viruses, A/H7N7 and A/H9N2, had also caused human illnesses in the Netherlands and Hong Kong respectively.

Since time immemorial, influenza A virus had been evolving with the production of totally different subtypes every 10 to 15 years. The evolution is largely due to its unique segmented genome that allows for easy recombination of its genetic materials when two different strains of influenza viruses infect a cell. Environmental factors provide selective pressure in producing mutant viruses and it was postulated that the current A/H5N1 virus could have resulted following widespread vaccination of poultry with H5 vaccine in mainland China, as a preventive response to the 1997 Hong Kong’s avian influenza outbreak. The role of influenza vaccine in the prevention of avian influenza in poultry is controversial, at best. Despite vaccination, sub clinical infections could still be occurring and these would not be detected if post-vaccination surveillance were not put in place. The scenario allows the virus to circulate longer than usual in the avian host population with the risk of viral mutations highly likely to occur. Animals are almost always involved in production of new influenza viruses and in each of the three pandemics – the 1918 “Spanish flu”, the “Asian flu” of 1957, and the “Hong Kong flu” of 1968 – the viruses originated in Asia, most likely somewhere in China.

Since January 2004, the outbreaks of H5N1 avian influenza in Asia had caused 50 deaths in 80 confirmed human cases, with cases reported only from Vietnam, Thailand and Cambodia. Why make a fuss when the mortality is small? This is precisely the right time to make a fuss. The last global influenza pandemic, in 1968, spread around the world killing 45,000 people within five months following detection of a single case. The H5N1 virus had already caused limited human-to-human transmission and it is only a matter of time before the virus adapt more efficient human transmission method. The advent of air travel adds more concern about the magnitude of a future pandemic as the spread of the new epidemic strain may be hastened.

As the scientific community continues to be on alert to the inevitable and possibly imminent massive influenza pandemic, the general public is only now gradually, grudgingly, learning that all our medical sophistication is still relatively helpless in the face of the elusive tactics of our tiniest enemy.

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**References**


CANCER TREATMENT - OBJECTIVES AND QUALITY OF LIFE ISSUES

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The first aim of cancer treatment is to achieve a cure, and when cure is not possible, a good palliation (life prolongation and relief of sufferings) is warranted. This article highlights the aim of cancer treatment and also attempts to assess the issues of quality of life experienced as a result of the disease and its treatment. Palliative therapy should be less intensive than radical treatment and should cause less morbidity than disease itself. It must be effective, completed in a short time and should be tolerable. It is also essential for a physician to give a clear explanation of illness to the patient and realistic advice regarding the likely outcome of therapy and the long and short term morbidities which may occur. The patient may opt for a palliative treatment with a reduced chance of cure but a better quality of life than accepting a radical treatment with a potentially higher degree of morbidity. Quality of life in oncology practice should be seen as a process and as a part of this process it seems sensible to pursue several different lines of questionnaire development rather than constructing one ‘perfect’ quality of life instrument.

Key words: QOL issues, cancer treatment

The most important aim of cancer treatment is to achieve cure and secondly to palliate (life prolongation and relief of sufferings) where cure is not possible due to advanced disease. Nowadays, 30% of all cancers are routinely cured. Treatment should achieve cure whenever possible and that the quality of life is acceptable. The relief of symptoms may follow on from curative treatment, but where cure is not possible the speedy relief of symptoms becomes important.

Treatment undertaken with a curative intent is “radical therapy” while that given solely to relieve symptoms is “palliative”. Palliative therapy should be less intensive than radical treatment and should cause less morbidity than the disease itself. When doctors undertake to treat patients with cancer, they should have a clear idea of the purpose of treatment before therapy is started. If the probability of cure is high and the patient is reasonably fit, considerable short and long term morbidity are acceptable. For example, bowel surgery, necessitating a colostomy causes great inconvenience but may result in long term benefit. However, if the patient is old and frail, even if there is a possibility of cure, careful consideration must be given to the expected side effects, the resulting quality of life and the anticipated life span of the patient. When the patient is suffering from an advanced incurable cancer, the palliative therapy given must cause as little morbidity as possible. It must be effective, completed in a short time and its acute morbidity must be tolerable. It is also essential for the doctor to give a clear explanation of the illness to the patient and realistic advice regarding the likely outcome of therapy and the long and short term morbidities which may occur. The patient may opt for a “palliative” treatment with a reduced chance of cure but a better quality of life than accepting a radical treatment with a potentially higher degree of morbidity. For example, a total laryngopharyngectomy for a pyriform fossa tumour may have a higher chance of cure but the morbidity of the operation, the extensive resection and permanent loss of voice may be too high a price to
pay. Radiotherapy which is non-invasive may have a smaller chance of cure but this modality preserves the anatomy and normal function and may be more acceptable to the patient. In treating terminal cancer, the wise use of adequate doses of analgesics such as morphia coupled with steroids may prove more effective than high technology therapies or chemotherapy. Additional support from the local health facilities may enable the patient to have satisfactory symptom control and in many cases to die in the comfort of home.

Quality of Life

An operational definition of quality of life has been advanced by WHO to capture the three dimensions of health(1). Health is not only the absence of infirmity and disease, but the state of physical, mental and social well being. Only the patient can make a truly valid assessment of quality of life. Early attempts to quantify the general condition of the patient resulted in development of scales of performance status, such as Karnofsky (KPS) and WHO scales, which extended from totally normal activity with no complaints through lesser states involving the presence of symptoms to morbidity (in fact death).

Survival and Life Quality

Favourable prognosis of patients, e.g. with malignant gliomas, has been shown to be mainly related to age, tumour grade, level of function at diagnosis and the completeness of surgical resection (2,3,4,5). Thus young patients who had gross resection of low grade astrocytoma have the best prognosis. How is the duration of survival (prognosis) linked to the quality of life? The KPS has been widely used as a simple and reliable scale of quality of life. Lieberman et al were among the first to examine this problem and evaluated these patients at New York University who lived two or more years after treatment. Of the 57 patients treated with surgical resection, radiation and chemotherapy, 8 patients lived two or more years. Median survival for these patients was 143 weeks and 50% died of their tumour. The conclusion drawn from this study is that a small but gratifying gains have been made in the treatment of patients with malignant astrocytomas with some patients achieving a good quality of life for at least two years.

More recently, there has been an attempt to broadly define quality of life end points in the treatment of patients with cancer (7). While KPS measures external level of function based on factors that can quickly be estimated in a patient encounter, it is not sensitive to a wide range of more intrinsic and psychosocial aspects of the patient. This concept has also been regarded as too abstract and complex to be measured. Various other studies (8,9) suggest that it is possible to devise an indicator of the quality of life that has wide applicability. Aaronson et al (10,11) have recommended that 12 components be evaluated in an assessment in clinical trials: pain and pain relief, fatigue and malaise, psychological distress, nausea and vomiting, psychological functions, symptoms and side effects, body image, sexual functions, social functioning, memory and concentration, economic disruption and global quality of life. Physicians often focus on the disease-related outcomes like tumor response, but patients are often equally concerned with the impact of the disease and therapy on their life and daily function. Such a scale if properly devised and applied may permit a way of translating the medical approach to outcomes that are more meaningful and understandable to patients and their families. More recently many quality of life instruments have been developed like the European Organization for Research and Treatment of Cancer quality of life questionnaire C30 (EORTC QLQ - C30) and Functional Assessment of Cancer Therapy General (FACTG) (12). Both the FACTG and EORTC QLQ-C30 seems to have their specific merits and there may be scope for the development of a new instrument. However, in our opinion, the availability of several widely used assessment instruments for the quality of life of cancer patients has its advantages.

Conclusion

Quality of life issues are at the core of treatment of all malignant neoplasms. As therapy becomes more effective, the quality of survival will emerge as an important consideration. This concern has been regarded by basic scientists and oncologists as a meaningful information. Quality of life research in oncology practice should be seen as a process and as a part of this process it seems sensible to pursue several different lines of questionnaire development rather than constructing one “perfect” quality of life instrument.
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References

ANTITUMOUR-PROMOTING AND CYTOTOXIC CONSTITUENTS OF 
ETLINGERA ELATIOR

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Phytochemical studies on rhizome of Etlingera elatior have resulted in the isolation of 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone (1), demethoxycurcumin (2), 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (3), 16-hydroxylabda-8(17),11,13-trien-16,15-olide (4), stigmaster-4-en-3-one (5), stigmaster-4-ene-3,6-dione (6), stigmaster-4-en-6b-ol-3-one (7), 5β,8α-epidioxystigmast-6,22-dien-3β-ol (8). 1 and 4 were new compounds. Compounds 5 and 7 displayed high antitumour-promoting activity. Ethyl acetate extract showed a very significant cytotoxic activity against CEM-SS and MCF-7 cell lines (4 µg/ml and 6.25 µg/ml respectively). The antitumour-promoting activity was determined by EBV-EA assay and cytotoxic activity was determined by MTT assay.

Key words : Etlingera elatior, antitumour-promoting, EBV-EA, cytotoxic, CEM-SS, MCF-7

Introduction

Etlingera elatior belongs to the Zingiberacea family, and is classified under the genera of Etlingera. This plant is * locally known as kantan. The mature fruits of Etlingera elatior are edible but sour, and are reputed for their antihypertensive activity. In many parts of Southeast Asia, the young inflorescence is used as “ulam” or as ingredients in laksa, curry, and mixed vegetables. A decoction of the fruits may be dropped into the ear to treat earache, and a decoction of leaves may be used to clean wounds (1). The decoction of young shoots is used to reduce body odour after giving birth. Mackeen et al. (1997) reported that the aqueous ethanol extract of the flower shoots of E. elatior possessed antimicrobial activity and was cytotoxic to HeLa cell line (2). Habsah et al. in 2003, reported the antioxidant activity and antitumour promoting activity of the crude dichloromethane and methanol extracts of E. elatior (3). The diarylheptanoids 1-3 from the ethyl acetate extract was reported to have high antioxidant activity (4). The previous screening of its flower shoot extract showed promising antitumour promoting activity (5). No phytochemical study has been done on this species except their essential oil of it young flower shoots (6), thus the objective of this study was to isolate cytotoxic and antitumour promoting compounds from the rhizome of E. elatior.

Materials and Methods

Plant material. One hundred kg of the fresh E. elatior rhizomes were collected in Klang and Banting, Selangor in October 1999. The rhizomes were cleaned, chopped into smaller pieces (3-5 mm thickness) and dried under the shade. A voucher specimen (No. SK 80/01) was deposited at the Herbarium of Laboratory of Phytomedicines (LF),
Extraction and isolation. Sixteen kg of the dried powdered rhizomes (16% w/w of fresh rhizomes) were extracted three times each, first with CHCl₃, then with acetone, and finally with MeOH, to give 120 g, 50 g and 8 g of extracts, respectively. The CHCl₃ extract was triturated with hexane and filtered to give hexane (60 g) and CHCl₃ soluble extracts (60 g). The acetone extract was triturated with ethyl acetate to give 8 g of ethyl acetate soluble extract. Column chromatography (CC) of CHCl₃ extract (40 g) on silica gel (5 x 40 cm) eluted with hexane/diethyl ether, diethyl ether/ethyl acetate, ethyl acetate/MeOH, gave combine fractions A-J respectively. Repeated CC of fraction C (3 g) on silicas gel using diethyl ether in hexane (1:9) gave 7 (20 mg) and 8 (8 mg). Repeated CC of hexane extract (20 g) on silica gel (5 x 40 cm), eluted with hexane/diethyl ether, afforded eight fractions (A-H). Repeated column of fraction F (3 g) afforded four fractions (F1-F4), from which 5 (65 mg) was isolated from fraction F2 (138 mg) after recrystallisation with MeOH. Compound 6 (50 mg) was isolated from fraction F4 (77.3 mg) after preparative TLC (20% diethyl ether in hexane). Compound 4 (11.9 mg), was isolated from fraction H (80 mg) after repeated column chromatography on silica gel eluted with 10% ethyl acetate in CHCl₃, Repeated CC of the ethyl acetate soluble extract (8 g) on sephadex LH 20 (2.5 x 40 cm), eluted with MeOH, afforded 14 fractions (fractions EA-EN) Repeated CC of fraction EK (160 mg) on silica gel, with 10% ethyl acetate in CHCl₃, as the eluent, followed by CC on sephadex LH-20, using MeOH as eluent, gave 2 (5 mg). Compound 1 (4 mg) and 3 (5 mg) were afforded after reversed phase HPLC of fraction EI (50 mg) (Waters PrepPak Cartridge C₁₈, HPLC column (25 x 10 cm), 30% methanol in water as a solvent system, flow rate 5 ml/min, PDA detector, wavelength 254 nm).

1,7-Bis(4-hydroxyphenyl)-2,4,6-heptatrienone (1): Yellow powder; UV (CH₃OH) λₑₑₑ (log ε) 395 (4.51); IR (KBr) νₑₑₑ 3300, 1653, 1578, 1511 cm⁻¹; ¹H NMR (CD₂COCD₂, 500 MHz) δ 7.96 (2H, d, J = 8.8 Hz, H-2’,6’), 6.95 (2H, d, J = 8.8 Hz, H-3’, 5’), 7.41 (2H, d, J = 8.5 Hz, H-2”,6”), 6.85 (2H, d, J = 8.5 Hz, H-3”,5”), 7.20 (1H, d, J = 15.0 Hz, H-2), 7.46 (1H, dd, J = 15.0 Hz, J = 11.0 Hz, H-3), 6.64 (1H, dd, J = 15.0 Hz, J = 11.0 Hz, H-4), 6.94 (1H, dd, J =15.0 Hz, J = 11.0 Hz, H-5), 6.92 (1H, dd, J = 15.0 Hz, J = 11.0 Hz, H-6), 6.80 (1H, δ, J = 15.0 Hz, H-7); ¹³C NMR (CD₂COCD₂, 125 MHz) δ 131.3 (C-1’), 131.5 (C-2’, 6’), 116.1 (C-3’,5’), 162.5 (C-4’), 129.5 (C-1”), 129.3 (C-2”,6”), 116.5 (C-3”,5”), 158.9 (C-4”), 187.9 (C-1), 124.9 (C-2), 144.1 (C-3), 130.8 (C-4), 143.1 (C-5), 126.5 (C-6), 137.6 (C-7); EIMS m/z 292 [M⁺] (94), 171 (38), 121 (100); HREIMS m/z 292.1113 (calcd for C₁₉H₁₆O₃, 292.1099)

16-Hydroxyabada-8(17),11,13-trien-15,16-olide (4): Gummy solid; UV (CH₃OH) λₑₑₑ (log ε) 260
(4.51); IR \nu_{max} (KBr) cm^{-1}: 1750 (\alpha,\beta\text{-unsaturated } \delta\text{-lactone}), 3090, 892 cm^{-1} (exo-methylene). \text{^1}H \text{NMR (CDCl}_3, 500 MHz) \delta 1.04 (1H, ddd, J = 13.2, 13.2, 3.7 Hz, H-1a, ax), 1.38 (1H, m, H-1b), 1.40 (1H, m, H-2a), 1.52 (1H, m, H-2b), 1.18 (1H, br dd, J = 13.2, 13.2 Hz, H-3a, ax), 1.42 (1H, m, H-3b), 1.10 (1H, dd, J = 2.7 Hz, H-7a, eq), 2.47 (1H, ddd, J = 12.9, 2.7, 2.7, 2.7 Hz, H-6b, eq), 2.09 (1H, ddd, J = 13.4, 13.4, 5.6 Hz, H-7a, ax), 2.44 (1H, m, H-7b, eq). 2.47 (1H, d, J = 10.0 Hz, H-9), 6.58 (dd, J = 16.0 Hz, J = 10.0 Hz, H-11a), 6.59 (dd, J = 16.0 Hz, J = 10.0 Hz, H-11b), 6.31 (1H, d, J = 16.0 Hz, H-12), 5.85 (1H, s, H-5), 6.25 (s, H-16a), 6.27 (s, H-16b), 4.38 (d, J = 1.5 Hz, H-17aa), 4.87 (2H, brs, H-17ab, H-17ba), 4.47 (d, J = 1.5 Hz, H-17bb), 0.90 (3H, s, H-18), 0.85 (3H, s, H-19), 0.87 (3H, s, H-20); \text{^{13}C \text{NMR (CDCl}_3, 125 MHz)} \delta 40.9 (C-1a), 39.6 (C-1b), 19.0 (C-2a), 19.0 (C-2a), 42.1 (C-3), 33.5 (C-4), 54.5 (C-5a), 54.5 (C-5b), 23.2 (C-6), 36.6 (C-7), 148.7 (C-8a), 148.9 (C-8b), 62.1 (C-9a), 62.1 (C-9b), 39.5 (C-10a), 39.6 (C-10b), 144.0 (C-11a), 144.1 (C-11b), 122.6 (C-12a), 122.7 (C-12b), 161.0 (C-13a), 161.0 (C-13b), 115.5 (C-14), 171.2 (C-15), 97.5 (C-16a), 97.6 (C-16b), 108.5 (C-17a), 108.9 (C-17b), 21.9 (C-18), 33.6 (C-19), 15.1 (C-20a), 15.2 (C-20b); EIMS m/z 316 [M]+ (13), 180 (30), 162(14), 137(100), 123(25); HREIMS m/z 316.2030 (calcd for C_{20}H_{28}O_{3}, 316.2038)

\textbf{Demethoxycurcumin (2):}

Yellow powder, m.p. 168-170 °C; EIMS m/z 291.9 (M^+, C_{19}H_{18}O_3); \text{^1}H\text{-NMR and } \text{^{13}C\text{-NMR are in agreement with (9).}}

\textbf{Stigmaster-4-en-3-one (5):}

White needles, m.p 80-82 °C; EIMS m/z 426 (M^+, C_{29}H_{46}O_5); \text{^1}H\text{-NMR and } \text{^{13}C\text{-NMR are in agreement with (10).}}

\textbf{Stigmaster-4-en-3,6-dione (6):}

White needles, m.p 75-76 °C; EIMS m/z 437 (M^+, C_{29}H_{46}O_6); \text{^1}H\text{-NMR and } \text{^{13}C\text{-NMR are in agreement with (10).}}

\textbf{Stigmaster-4-en-6\beta\text{-ol-3-one (7):}

White needles, m.p 217-218 °C; EIMS m/z 428 (M^+, C_{29}H_{48}O_6); \text{^1}H\text{-NMR and } \text{^{13}C\text{-NMR are in agreement with (10).}}

\textbf{5\alpha,8\alpha-Epidioxyergosta-6,22-dien-3\beta\text{-ol (8):}

Off-white amorphous solid, m.p 176-178 °C; EIMS m/z 428 (M^+, C_{29}H_{46}O_7); \text{^1}H\text{-NMR and } \text{^{13}C\text{-NMR are in agreement with (11).}}

\textbf{Antitumour Promoting Activity}

\textbf{Stock solution of pure compounds.}

The extract was dissolved in dimethyl sulfoxide (DMSO) as a stock solution, with concentrations and 10 mg/ml for crude extract and 4 mg/ml for pure compound.

\textbf{Cell Lines}

The Raji cells were maintained in medium RPMI 1640 (Flow Lab., UK) supplemented with 10% foetal calf serum (Gibco, UK), 100 IU/ml penicillin/streptomycin, 50 mg/mL Amphostat B and

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Extract} & \textbf{Cell Viability (\%)} & \textbf{Inhibition Rate (\%)} \\
\hline
Hexane & 83.3 ± 1.3 & 18.3 ± 1.3 \\
CHCl\textsubscript{3} & 54.16 ± 1.5 & 92.18 ± 1.5 \\
EtO\textsubscript{Ac} & 19.05 ± 0.9 & - \\
MeOH & 92.5 ± 1.6 & 85.9 ± 1.6 \\
\hline
\end{tabular}
\caption{Table 1: Antitumour promoting activity of crude extracts of Etlingera elatior using EBV-EA assay in Raji cell line.}
\end{table}
120 mg/mL L-glutamine as a static suspension culture at 37°C in a humidified atmosphere of 50 % CO₂ in air.

**Antitumour-promoting Activity in Raji Cells Assay**

The inhibitory activity of Epstein-Barr virus (EBV) activation assay was performed as previously described (4). Raji cells were activated with 20 ng/ml of TPA (Sigma, USA) and 4 mM/ml of sodium-n-butyrate (Nacarai Tesque, Japan) to induce the expression EBV EA. The plant extracts at the concentration of 200 mg/ml were added immediately after the addition of TPA as tumour promoter. The cells were incubated at 37°C for 72 hours, after which they were subjected to indirect immunoflourescence assay using EBV EA positive nasopharyngeal carcinoma serum and FITC-conjugated anti-human IgG (Sigma, USA). The inhibitory rate (IR) of each test sample against the EBV activation was classified into four ranks as follows: ++++, strongly active (IR ≥ 70% ); ++, moderately active (70% > IR ≥ 50%); +, weakly active (50% > IR ≥ 30%); - , inactive (30% > IR) (5). All tests and analyses were run in triplicate and averaged.

**Cytotoxicity Assay**

**Plant Extract**

The extract was dissolved in dimethyl sulfoxide (DMSO) as a stock solution, with concentrations 4 mg/ml for pure compounds and fractions.

**Microculture Cytotoxicity Screening Using Methyl Thiazole Tetrazolium (MTT) Assay**

A 10-fold dilution gradient microtitre cell culture was adopted and modified to 3-fold dilution cell plating. All cells were cultured in sterile RPMI-1640 complete media, supplemented with antibiotic-antimycotic mixture (containing 103 U/mL Penicillin G; 100 mg/mL Streptomycin SO; 2.5 mg/ L Amphoterin B), 2 mM L-Glutamine and 10% FBS (all from Sigma). Exponentially growing cells were pre-determined by trypsinisation (0.25% p.p trypsin) and/or re-suspension, with a 100% confluency and 96% viability (using 0.2% typan blue exclusion cell-count in an Improved Neubauer Haemacytometer). A final cell concentration of 2.5 x 10⁴ cells/well was used as inoculation density for all anchorage

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Cell Viability (%)</th>
<th>Inhibition Rate (%)</th>
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<tbody>
<tr>
<td>A</td>
<td>100.0 ± 0.2</td>
<td>69.7 ± 0.2</td>
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<tr>
<td>B</td>
<td>89.8 ± 0.5</td>
<td>85.6 ± 0.5</td>
</tr>
<tr>
<td>C</td>
<td>80.3 ± 1.2</td>
<td>97.9 ± 1.2</td>
</tr>
<tr>
<td>D</td>
<td>100.0 ± 1.4</td>
<td>69.1 ± 1.4</td>
</tr>
<tr>
<td>E</td>
<td>80.0 ± 1.7</td>
<td>74.4 ± 1.7</td>
</tr>
<tr>
<td>F</td>
<td>87.5 ± 0.8</td>
<td>2.7 ± 0.8</td>
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<td>G</td>
<td>50.0 ± 0.5</td>
<td>17.6 ± 0.5</td>
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<td>H</td>
<td>4.0 ± 0.7</td>
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<tr>
<td>I</td>
<td>94.4 ± 0.8</td>
<td>43.8 ± 0.8</td>
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<tr>
<td>J</td>
<td>100 ± 1.2</td>
<td>73.4 ± 1.2</td>
</tr>
</tbody>
</table>

*50 μg/ml, b200 μg/ml, c25 μg/ml, cytototoxic at 200 μg/ml

Table 2: Antitumour promoting activity of the fractions from crude CHCl₃ extracts of Etingera elatior using EBV-EA assay in Raji cell line.
dependent cell lines, and 5 x 10^4 cells/well for CEM-SS cell suspensions.

Into a sterile and labeled NUNCLON™ 96 well (180 mL volumes) micro-titre plates (Nunc, Denmark), 180 mL volumes were pipetted appropriately for all cell lines. The plated cells were then incubated overnight, under standard culture conditions of 5% CO₂, 95% air and 100% humidity, to allow cell settling and differentiation.

Stock solutions of all samples were prepared as 10 mg/mL in absolute dimethylsulphoxide, DMSO (HPLC-grade, Sigma, USA). Using the same culture media as diluent, a sub-stock solution of 1000 mM was prepared immediately before addition and serially diluted in sterile sample containers, to give 10x working stock solutions for each of the final test-range concentrations, topping from 100 mM down to the lowest of 0.1 mM. Having prepared the above mentioned dilutions, 20 mL quadruplicates of the corresponding 10x working stock samples were all added up to give the required final concentrations, in total volume of 200 mL. Plates were returned to the incubator for a further 4-day culture period. Cultures were regularly observed for any visual interference(s), with morphological changes and or cell killing effects being monitored using CK2 light microscope (Olympus, Japan).

At the end of each culture successfully attained, devoid of any contaminating and other interfering notifications, cells were aseptically subjected to further analysis, using the MTT biochemical assay (11). A 20 mL volume of MTT (Sigma, USA), prepared at 5 mg/ml in phosphate buffered saline (PBS), was added into each 200 mL culture of the 96 well microtitre plate, wrapped with aluminium foil and incubated for a further 4 hours culture under similar conditions as above. This allowed the activation of mitochondrial dehydrogenases of the CEM-SS cells to reduce the yellowed colour MTT into a crystallized blue-violet

### Table 3: Antitumour promoting activity of compounds isolated from Etlingera elatior using EBV-EA assay in Raji cell line.

<table>
<thead>
<tr>
<th>Compounds [20 μg/ml]</th>
<th>Cell Viability (%)</th>
<th>Inhibition Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>97.93 ± 1.5</td>
<td>78.4 ± 1.5</td>
</tr>
<tr>
<td>7</td>
<td>77.5 ± 0.7</td>
<td>80.6 ± 0.7</td>
</tr>
<tr>
<td>8</td>
<td>80.4 ± 0.3</td>
<td>14.1 ± 0.3</td>
</tr>
<tr>
<td>β-Sitosterol and stigmasterol</td>
<td>89.2 ± 0.9</td>
<td>85.1 ± 0.9</td>
</tr>
<tr>
<td>6</td>
<td>91.0 ± 1.4</td>
<td>56.9 ± 1.4</td>
</tr>
<tr>
<td>Tetracosanoic acid</td>
<td>85.9 ± 0.7</td>
<td>72.4 ± 0.7</td>
</tr>
</tbody>
</table>

### Table 4: Cytotoxic activity of Etlingera elatior extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>CEM-SS</th>
<th>MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>42.5 ± 0.1</td>
<td>36.0 ± 0.3</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>14.0 ± 0.5</td>
<td>26.0 ± 0.5</td>
</tr>
<tr>
<td>EtOAc</td>
<td>4.0 ± 0.1</td>
<td>6.25 ± 0.1</td>
</tr>
<tr>
<td>MeOH</td>
<td>46.0 ± 0.4</td>
<td>47.0 ± 0.9</td>
</tr>
</tbody>
</table>

Note: The standard use was tamoxifen with IC₅₀ = 30 μM and IC₅₀ = 15 μM against MCF-7 and CEM-SS cell lines respectively.
colour complex product, formazan. A total volume aspiration followed by 200 mL addition of pure DMSO (Ajax, Australia) was carried out, with gentle mixing and 5 minute incubation at room temperature, to allow for faster and more enhanced formazan solubility. Optical densities (O.D.) of the respective concentrations of samples were then measured using DYNEK MRX ELISA reader (Dynex Instruments, Inc. USA), at a 550 nm test and 630 nm reference wavelengths. Percentage proportions of the control O.D. values were then compacted in a dose-response standard curve to enable a more accurate and standardized determination of the 50% growth inhibitory concentration, IC$_{50}$. All tests and analyses were run in triplicate and averaged.

Results

Ten compounds were isolated from E. elatior after extensive chromatography of the crude extracts. Compounds 1-8 (Figure 1), a mixture of stigmasterol and β-sitosterol, and tetracosanoic acid were identified based on spectral data (UV, MS, IR, $^1$H NMR, $^{13}$C NMR, H-H COSY, HMQC and HMBC) and comparison with literature values (7-11, 13-15). The antitumour promoting activity of the crude extracts, fractions and compounds is shown in Table 1-3, respectively. The cytotoxic activity of the crude extracts is shown in Table 4.

Discussion

The preliminary screening showed both CHCl$_3$ and MeOH extracts of E. elatior possessed high antitumour promoting activity, with 92.18% and 85.9% inhibition rate, respectively. Both hexane and ethyl acetate were cytotoxic against Raji cell at initial concentration (200 mg/ml) (Table 1). Five fractions (fractions A-C, E and J) of CHCl$_3$ extract showed strong antitumour promoting activity. The less polar fractions showed high antitumour promoting activity compared to the more polar fractions (Table 2). Seven compounds (5, 6 and tetracosanoic acid from the hexane extract; 7, 8 and a mixture of stigmasterol and sitosterol from CHCl$_3$ extract) were screened for antitumour promoting activity (Table 3). Among them, 5, 7, a mixture of b-sitosterol and stigmasterol, and tetracosanoic acid showed high antitumour promoting activity, with inhibition rate of 78.4%, 80.6%, 85.1% and 72.4% respectively. Compound 6 only showed moderate activity with inhibition rate of 56.9%. Compound 8 and the mixture of stigmast-4-en-6a-ol-3-one and 8 did not show any significant activity. Our finding suggested that the Δ$^{4(5)}$-3-keto steroids (5-7) displayed high antitumour promoting activity. The activity of the Δ$^{4(5)}$-3-keto steroids increased due to the b-hydroxy group at C-6 as in the case of 7. The activity decreased when this 6-hydroxy group was in its oxidised form as in the case of 6. In a related study, a Δ$^{8(9)}$-11-keto steroid, 5α,14α-dimethyl-ergosta-8,24(28)-dien-11-one from Euphorbia chamaesyce also displayed a potent inhibitory effect on EBV-EA (15). This finding suggested that both Δ$^{4(5)}$-3-keto and Δ$^{8(9)}$-11-keto steroids could act as potent antitumour promoters. Four extracts of Etlingera elatior rhizome were tested for their cytotoxic activity against CEM-SS and MCF-7 cell lines (Table 4). The in vitro cytotoxic assay was based on modification of Monsman’s method (11). The ethyl acetate extract was found to show a significant cytotoxic to both CEM-SS (IC$_{50}$ 4 mg/ml) and MCF-7 (IC$_{50}$ 6.25 mg/ml). From the ethyl acetate extract, we successfully isolated three diarylheptanoids, 1-3, which showed strong antioxidant activity (4). However the cytotoxicity of each diarylheptanoid could not be evaluated because of insufficient amount. It was reported that demethoxycurcumin had cytotoxicity effect against ovarian cancer OVCAR-3 cells (7), displayed DPPH free radical scavenging activity and showed significant hepatoprotective effects on tacrine-induced cytotoxicity in human liver–derived Hep G2 cells (17). The other extracts, including the hexane, CHCl$_3$ and MeOH extracts, also showed significant cytotoxicity against both CEM-7 and CEM-SS cell lines. It was also reported that the ethanol aqueous extract of the young flower shoots was cytotoxic against HeLa cell line with IC$_{50}$ value of 10 mg/ml (2). This implied that besides the young flower shoots, the rhizome is also a potential source for cytotoxic compounds.

Acknowledgements

The authors wishes to thank the Ministry of Science, Technology and the Environment Malaysia for the fund provided under the Intensified Research in Priority Areas Research Grant (No. 09-02-04-0067). HM thanks the University College of Science and Technology Malaysia for granting her study leave as well as N. Nakatani and H. Kikuzaki for their assistance and gratefully acknowledges the Program for Promotion of Basic Research Activities for Innovative Biosciences (BRAIN).
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References


OPEN LABEL CLINICAL TRIAL TO STUDY ADVERSE EFFECTS AND TOLERANCE TO DRY POWDER OF THE AERIAL PART OF ANDROGRAPHIS PANICULATA IN PATIENTS TYPE 2 WITH DIABETES MELLITUS

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Adverse effects and tolerance to dry powder of aerial part of Andrographis paniculata (Burm.f.) Nees were studied in 20 patients with type 2 diabetes mellitus for a period of 12 weeks. Patients were given powdered A. paniculata starting with 600 mg daily, gradually increasing to a maximum of 1.8 gm daily. Parameters monitored included body weight, blood pressure, liver function tests, renal function tests, cardiac enzymes, hemogram, serum electrolytes, fasting blood glucose, HbA1c, blood cholesterol, serum triglycerides and blood hormone levels (triiodothyronine, thyroxine, thyrotropin, insulin, fasting cortisol). None of the above mentioned parameters showed significant change during the study period except for a fall in HbA1c by 5.46% (p<0.01) and fasting S. insulin by 20.93% (p<0.003). In conclusion, A. paniculata powder did not induced significant adverse events based on parameters observed in our study but significantly lowered HbA1c and fasting serum insulin in patients with type 2 diabetes.

Key words: Andrographis paniculata, diabetes mellitus type 2

Introduction

Traditional medicines in South East Asia are sourced from plants, animals and minerals. People often turn to traditional medicine when modern western medicines fail or to treat chronic medical illnesses such as diabetes mellitus. Andrographis paniculata (Acanthaceae) is among many local herbs in South East Asia, which are claimed to have antidiabetic properties. The known active chemical
of this herb is a diterpene lactone, andrographolide (Fig. 1). Other chemicals include 14-deoxyandrographolide, 14-deoxy-11-oxo -andrographolide, 14-deoxy-11, 12-didehydroandrographolide and neoandrographolide.

For centuries leaves of this tropical plant are used by local people either as complementary treatment or the only medication to treat diabetes. Antidiabetic properties of *Andrographis paniculata* are substantiated by a number of animal experiments conducted in Malaysia (1) and elsewhere (2). Moreover it has not been associated with any significant side effects in animal and human studies (3,4). *Toxicity of Andrographis paniculata*, has been studied by a number of researchers in rats (5) and rabbits (6). A few animal studies suggest antifertility (7) action when the herb was given in very high doses (2gm / kg body weight for 6 weeks), but these results are controversial (8). The aim of the current study is to assess the tolerance and adverse effects to dry powder of aerial part of *Andrographis paniculata* in patients with type 2 diabetes mellitus.

### Materials and Method

**Andrographis paniculata dry powder capsules**

The plant *Andrographis paniculata* (AP) was cultivated at Universiti Sains Malaysia herbal garden according to physiological and agronomical guidelines. The species is identified by the Division of Medicinal Plants, Forest Research Institute of Malaysia. Voucher specimen deposited to Forest Research Institute of Malaysia [PPSP/HB/1/(2002)]. Total amount of raw material (the aerial part of the plant) required for this 12-week study was collected in one batch to avoid changes in the concentration of active chemical (batch to batch variation). After thorough cleaning of aerial part of the plant it was subjected to drying at 40°C for 72 hours in electric oven. The dried plant extract was then made into powder. Capsules of this powder were then prepared at Pharmacology Laboratory, School of Medical Sciences, Universiti Sains Malaysia. Each capsule contained 300 mg of dry herb.
**HPLC method validation to measure andrographolide contents**

For validation of HPLC method Waters™ 610 fluid unit coupled with Waters’ 600 controller, Waters’ 486 tunable UV absorbance detector (230 nm). Lichrosorb reverse phase (RP-18) analytical column (250×4.6 mm, ID) and 50 μl loop was used. The column consisted of particles of the size 5 μm, 3390A integrator from Hewlett Packard. The mobile phase was prepared using 30% acetonitril and 70% distilled water. A flow rate of 1.1 ml/min was maintained throughout and injection volume was 50 μl each time. By comparing retention times our HPLC assay was found to be free from possible interference from other diterpene compounds present in the dry leaves of *Andrographis paniculata*. A calibration graph was constructed in the concentration range of 1μg/ml to 60μg/ml using 5 concentrations (n=2 each). The linearity of calibration graph was demonstrated by good determination coefficient (r²=0.9983) obtained for regression line. The precision of the method was evaluated by determining the interday RSD% (5.49-11.58) intraday RSD% (3.24-14.93) of the measured peak areas for different concentrations. The LOQ defined in the present experiment as lowest andrographolide concentration in the calibration curve that can be measured routinely with acceptable precision (RSD<20%) and accuracy (80-120%) was 1μg/ml. Recovery data was determined at three concentration levels as recommended by center for Drug Evaluation and Research (96.20% to 103%). Andrographolide contents of the herb were found to be 5.45% as determined by first extracting the dry powder with methanol and then using above mentioned validated high pressure liquid chromatography method.

**Selection of subjects**

Twenty patients, already diagnosed with diabetes mellitus type 2 and undergoing treatment...
in diabetes clinic, Hospital Universiti Sains Malaysia (USM) were recruited for this study. These included 6 males and 14 females. For subjects to be included into the trial, they were required to fulfill inclusion and exclusion criteria. Subjects of both sexes between the age of 35 and 70 years, body mass index between 25 and 40 kg/m², glycosylated haemoglobin (HbA1c) between 7% and 10% and fasting plasma glucose (FPG) between 7mmol and 15mmol were included in the trial if they fulfilled all the exclusion criteria. Exclusion criteria were, impaired hepatic function (ALT or ALP >2x ULN), impaired renal function (S. creatinine>150 µmol/l), history of cardiac problem (IHD, cardiac failure), uncontrolled blood pressure (SBP>160mmHg DBP >100mmHg), history of treatment with insulin, proteinuria (2+ and above) and pregnant/breast feeding mothers). All subjects were required to give written informed consent.

Clinical evaluation

At first screening visit, subjects were evaluated for eligibility criteria. After physical examination, blood was collected to determine eligibility for the study. On 2nd visit (2 weeks after 1st visit) all subjects came after overnight fasting and blood results were reviewed for eligibility. For eligible subjects, general physical examination was done, blood was collected for baseline investigations, which included FBG, HbA1c, complete haemogram, LFT, RFT, electrolytes, CK, LDH, cholesterol, triglycerides, insulin, T4, TSH and cortisol.

Subjects were then instructed to take 2 capsules of AP (600 mg) daily with breakfast as an add on treatment to their ongoing medication. Subsequently subjects were asked to attend Clinical Trial Unit at 2 weekly intervals after overnight fasting for a total of 7 visits. During visits 3, 4, 5 and 6 general physical examination was repeated and blood was collected for FBG, ALT, ALP, and
creatinine, Urine was also taken for testing of protein and sugar. Those subjects who had FBG above 7 mmol/l, the dose of AP was increased by 1 capsule (300 mg) each time. Maximum doses achieved were 3 capsules (900 mg each) twice a day for 2 weeks. On visit 7 all investigations were repeated as in visit 2. During all visits after starting this herbal treatment subjects were inquired about adverse events experienced since last visit and state of compliance to the medication.

Results

Adverse events

During all visits subjects were asked about occurrence of any adverse event since last visit. During the entire period of trial one patient complained of gastric irritation and nausea after swallowing the capsule. She was prescribed antacid gel and was able to continue with the trial without further experiencing similar symptoms.

Clinical and biochemical

Physical examination conducted during all 7 visits showed no significant changes in mean values of body weight (BMI), systolic blood pressure, diastolic blood pressure and pulse rate (Figure 2 and 3). Mean fasting blood glucose was 10.19 (S.E.± 0.53) mmol/L at screening visit and 9.71 (S.E.± 0.76) mmol/L at visit 7 (Figure 2), thus showing no significant change during treatment. However mean HbA1c fell from 8.61 (S.E. ± 0.25)% at screening visit to 8.13 (S.E. ± 0.29)% at visit 7, a fall by 5.46% (p<0.01). Liver function tests showed no changes as seen in Figure 4a & 4b. In the same way renal functions were well preserved and comparing results of blood urea, creatinine and uric acid (Figure 5) showed no significant change. Cardiac functions were monitored by measuring CK and LDH enzyme levels from visit 2 to 7. Comparison of these enzyme levels in Figure 6 showed no significant rise or fall. Serum electrolyte values measured from visit 2 to 7 were well within normal limits (serum sodium 139.5 ± 0.97 mmol/L to 139.85 ± 0.54 mmol/L, serum potassium 4.15 ± 0.21 to 4.34 ± 0.09 mmol/L, serum calcium 2.23 ± 0.08 mmol/L to 2.39 ± 0.03 mmol/
L, serum phosphorus 1.77 ± 0.64 mmol/L to 1.23 ± 0.03 mmol/L). Mean hemoglobin, total WBC, platelet counts and ESR also showed no changes (Figure 7). Blood lipid levels (cholesterol 5.7 ± 0.26 mmol/L to 5.85 ± 0.26 mmol/L and triglycerides 1.59 ± 0.12 mmol/L to 1.56 ± 0.15 mmol/L) showed no significant change from visits 2 to 7 and similar results were observed for thyroid hormone (FT4 14.03 ± 0.61 nmol/L to 13.6 ± 0.44 nmol/L, TSH 0.98 ± 0.08 mU/L to 1.06 ± 0.10 mU/L) and fasting cortisol levels (319.63 ± 30.42 nmol/L to 342.16 ± 35.46 nmol/L). Fasting insulin levels however showed a significant fall from 11.18 (S.E.± 1.46) IU/L at visit 2 to 8.84 (S.E.± 1.05) IU/L at visit 7, a reduction by 20.93% (p<0.003) (Table 1). Average patient compliance as determined by capsule counting was 90.93%.

**Discussion**

Many researchers have studied the effects of *Andrographis paniculata* in animals and in human. In various animal experiments it has been found to have hepatoprotective (9, 10), antioxidant, antidiabetic (2), antihypertensive (11) and anti HIV (12) effects. In human clinical trial it was found to be effective against common cold (3, 4) and pharyngotonsillitis (13). Various animal experiments have also been done to study toxicity to *Andrographis paniculata* (5, 6). These experiments have shown *Andrographis* to be a safe herb. The results of our study showed no changes in physical and biochemical parameters of toxicity with doses as high as 900mg twice a day for 2 weeks.

These results support the results of previously conducted studies. However as reported by Zhang et al. (2) the increase in body weight and fall in fasting blood glucose of diabetic rats after treatment with ethanolic extract of AP was not observed in our clinical trial. The fall in HbA1c and fasting insulin level in diabetic patients in our study suggests its efficacy as an antidiabetic agent. These results suggest that the mechanism of action of *Andrographis* is by increasing peripheral utilization of glucose, probably by potentiating insulin action and not by a direct insulin releasing action on islet cells in pancreas. However other possible extrapancreatic effects on glucose metabolism (enzymatic and non enzymatic) in liver or carbohydrate absorption in GIT remain unclear.

In conclusion, dry powder of aerial part of *Andrographis paniculata* caused no significant adverse effects in parameters observed in patients with type 2 diabetes mellitus. Physical and biochemical parameters of toxicity were not affected during treatment with *Andrographis paniculata* for a period of 12 weeks and at the same time it showed antidiabetic property. *Andrographis paniculata* therefore was found to be a safe herb, which needs to be further evaluated for its long term safety and efficacy as antidiabetic agent.

**Acknowledgements**

We would like to thank Suhaila binti Che Dir, Roslina binti Mat Zain, Mr. Othman Ismail and Mr. Manaf Jusoh for their dedicated research assistance at Clinical Trial Unit and the Malaysian Ministry of Science and Technology for financial support under IRPA. (Intensified Research in Priority Area) research grant.

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**References**


A PRELIMINARY RESULT OF THE CARDIOVASCULAR RISK FACTORS INTERVENTION STUDY (PIKOM STUDY): DIABETES MELLITUS, HYPERTENSION AND THEIR ASSOCIATED FACTORS

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Cardiovascular disease (CVD) has been the number one cause of death since the last three decades in Malaysia and diabetes mellitus and hypertension are considered as major risk factors. A study to reduce risk factors for cardiovascular diseases in the community (PIKOM) through education and lifestyle changes was undertaken. The study population was from four different areas in Peninsular Malaysia – Kota Bharu and Bachok in Kelantan; Raub in Pahang; Gunung Besout in Perak and Felda Palong in Negri Sembilan. The subjects invited to participate in this study were aged between 30 – 65 years, did not have any debilitating illnesses and no known history of diabetes mellitus, hypertension or cardiovascular disease. Subjects were asked to come to the local clinic in a fasting state and after physical examination, blood was taken for plasma glucose and lipids. Oral glucose tolerance test (OGTT) was then performed. A total of 4,121 subjects participated in the study. The proportion of subjects with diabetes mellitus was highest in Felda Palong area (20.3%) and lowest in Raub area (7.1%). The proportion of subjects with hypertension was also highest in Felda Palong area (38.6%) and lowest in Raub area (29.1%). This could be attributable to the subjects in Felda Palong having the highest mean Body Mass Index (BMI) and Waist-to-Hip Ratio (WHR). There were significant associations between diabetes and hypertension with age and obesity. Subjects with diabetes mellitus and hypertension also had the highest mean age, BMI, WHR and plasma cholesterol.

In conclusion, the proportion of patients with risk factors for CVD was high and intervention studies through education and lifestyle changes were being carried out to see their effectiveness.

Key words: Cardiovascular disease, diabetes mellitus, hypertension, obesity, smoking, cholesterol

Submitted-30.12.2004, Accepted-05.01.2004

Introduction

Cardiovascular disease (CVD) is becoming a major health problem in the developing countries and in Malaysia, it has been the number one cause of death since the last three decades (1). Among the risk factors, diabetes mellitus and hypertension are considered as major risks (2, 3). The National Cholesterol Education Program (NCEP) of the United States, has classified type 2 diabetes mellitus as coronary heart disease equivalent (4). Obesity, smoking and sedentary lifestyle have also been associated with cardiovascular disease (5, 6). Intervention studies have shown that lifestyle modification can decrease the incidence of diabetes mellitus (7, 8) and decrease blood pressure (9, 10). In 1996, the Malaysia 2nd National Health and Morbidity Survey showed that the prevalence of diabetes mellitus was 8.3% of whom 2.5% was previously undiagnosed and the prevalence of
hypertension was 29.9% (11). In 1998, a multi-centre community based cardiovascular risk factors intervention project (PIKOM) was started. This project was approved and sponsored by Ministry of Science, Technology and Environment Malaysia. The objective of the project was to determine the effect of education and lifestyle changes on cardiovascular risk factors. This paper describes the study population with respect to the cardiovascular risk factors and their association.

Materials and Methods

Survey Procedure

This study was conducted in four different areas and centres in Peninsular Malaysia. The areas were Bachok and Kota Bharu, Kelantan (Universiti Sains Malaysia, USM, as centre); Raub, Pahang (Universiti Kebangsaan Malaysia, UKM / Universiti Islam Antarabangsa, UIA as centre); Gunung Besout, Perak (Kementerian Kesihatan Malaysia, KKM as centre); and Felda Palong, Negri Sembilan (Universiti Malaya, UM / Universiti Putra Malaysia UPM, as centre). Ethical approval for the study was obtained from the respective Ethics Committees of the institutions concerned.

Subjects were invited to enroll for the study. All respondents age between 30 – 65 years were included in the study. All those with known diabetes mellitus, hypertension or cardiovascular disease (past history of angina, myocardial infarct or stroke) or debilitating illnesses were excluded from the study. Subjects were asked to come in a fasting state after an overnight fast (at least 8 hours) to the local health clinic for examination by the visiting research team. Informed consent was obtained and history taken. Height and weight were recorded and body mass index (BMI) calculated. BMI classification was based on the World Health Organization criteria (12); underweight < 18.5 kg/m², normal 18.5-24.9 kg/m², overweight 25.0-29.9 kg/m² and obese ≥ 30 kg/m². Waist and hip circumferences were also measured.

Table I : Respondents by age, sex, and centre

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age Group</th>
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<th>UM/UPM</th>
<th>USM</th>
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<td>47</td>
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<td></td>
<td>40 – 49</td>
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<td>Female</td>
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<td>5</td>
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<td>351</td>
<td>1181</td>
<td>1337</td>
<td>4117*</td>
</tr>
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</table>

(*)4 missing for age

Table II : Mean (± s.d.) age, BMI, WHR for the different centres

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>BMI (kg/m²)</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>KKM</td>
<td>46.9 ± 5.9</td>
<td>25.4 ± 4.4</td>
</tr>
<tr>
<td>UKM/UIA</td>
<td>43.3 ± 8.6</td>
<td>24.9 ± 4.3</td>
</tr>
<tr>
<td>UM/UPM</td>
<td>46.1 ± 6.6</td>
<td>25.8 ± 4.5</td>
</tr>
<tr>
<td>USM</td>
<td>46.9 ± 9.5</td>
<td>24.4 ± 4.5</td>
</tr>
</tbody>
</table>

p < 0.001
and waist to hip ratio (WHR) was calculated. Desirable WHR was defined as < 1.00 for men and < 0.85 for women (13). Blood pressure was measured using a mercury sphygmomanometer. If the blood pressure was found to be high, patient was asked to rest for half an hour before another reading was made and the lower of the 2 readings was recorded. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg (14). After a physical examination, blood was taken and oral glucose tolerance test (OGTT) was done. Blood taken was sent for fasting plasma glucose, total cholesterol and 2 hours post-glucose load glucose. Diabetes mellitus was defined as fasting plasma glucose (FPG) ≥ 7.0 mmol/L or 2 hours post-glucose load glucose (2HPG) ≥ 11.1 mmol/L (15).

**Statistical Analysis**

Statistical analysis was performed using SPSS statistical software version 11.0. Descriptive statistics of the study subjects was calculated as means ± SD and compared using ANOVA and t-test for continuous variables and x² test for categorical variables.

**Table III**: Mean (± s.d.) of various parameters for subjects with hypertension, diabetes mellitus, hypertension and diabetes mellitus and without diabetes mellitus or hypertension.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HT (n=415)</th>
<th>DM (n=576)</th>
<th>DM + HT (n=77)</th>
<th>no DM/HT (n=2466)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>48.4 ± 7.8</td>
<td>46.9 ± 6.9</td>
<td>49.2 ± 7.0</td>
<td>45.1 ± 7.6</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 ± 4.7</td>
<td>25.8 ± 4.6</td>
<td>27.4 ± 4.5</td>
<td>24.1 ± 4.0</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.08</td>
<td>0.88 ± 0.09</td>
<td>0.90 ± 0.08</td>
<td>0.90 ± 0.08</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>145.2 ± 14.8</td>
<td>123.3 ± 8.8</td>
<td>147.8 ± 16.6</td>
<td>119.4 ± 9.7</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>91.9 ± 8.1</td>
<td>78.9 ± 6.3</td>
<td>92.8 ± 9.2</td>
<td>76.9 ± 6.8</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>FPG (mmol/L)</td>
<td>5.4 ± 0.7</td>
<td>9.1 ± 4.0</td>
<td>9.4 ± 3.6</td>
<td>5.2 ± 0.7</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>2HPG (mmol/L)</td>
<td>6.9 ± 1.6</td>
<td>13.2 ± 6.6</td>
<td>12.8 ± 5.9</td>
<td>6.5 ± 1.6</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>Chol (mmol/L)</td>
<td>5.9 ± 1.5</td>
<td>6.0 ± 1.2</td>
<td>6.1 ± 1.2</td>
<td>5.6 ± 1.3</td>
<td>P &lt;0.001</td>
</tr>
</tbody>
</table>

**Table IV**: Proportion of diabetes mellitus and hypertension by centre

<table>
<thead>
<tr>
<th>Centre</th>
<th>DM (n=576)</th>
<th>HT (n=415)</th>
<th>Both DM + HT (n=77)</th>
<th>no DM/HT (n=2466)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KKM</td>
<td>154 (28.4%)</td>
<td>415 (33.3%)</td>
<td>77 (6.2%)</td>
<td>755 (60.5%)</td>
</tr>
<tr>
<td>UKM/UIA</td>
<td>25 (7.1%)</td>
<td>102 (28.6%)</td>
<td>10 (2.8%)</td>
<td>237 (67.0%)</td>
</tr>
<tr>
<td>UM/UPM</td>
<td>240 (20.3%)</td>
<td>456 (38.6%)</td>
<td>132 (11.2%)</td>
<td>617 (52.2%)</td>
</tr>
<tr>
<td>USM</td>
<td>157 (11.7%)</td>
<td>385 (28.6%)</td>
<td>60 (4.5%)</td>
<td>857 (64.0%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>576 (14.0%)</td>
<td>1358 (33.0%)</td>
<td>279 (6.8%)</td>
<td>2466 (59.8%)</td>
</tr>
</tbody>
</table>
Results

A total of 4,121 subjects participated in this study. Of these, 1,247 were from KKM centre, 353 from UKM/UIA centre, 1,181 from UM/UPM centre and 1,338 from USM centre (Table I). The mean age, BMI and WHR of the subjects in each centre is shown in Table II. There were significant differences in the mean age, BMI and WHR of the subjects between the centres. The means age, BMI, WHR and cholesterol of the hypertensive, diabetic and hypertensive and non-diabetic/hypertensive subjects is shown in Table III. The mean age, BMI, WHR and cholesterol was highest in the diabetic and hypertensive subjects compared to the other groups. The proportion of subjects with diabetes was highest in Felda Palong (20.3%) and lowest in Raub (7.1%) (Table IV). The proportion of subjects with hypertension was also highest in Felda Palong (38.6%) and lowest in Raub (28.8%) (Table IV). Similarly with hypertension and diabetes, the proportion were highest in Felda Palong (11.2%) and lowest in Raub (2.8%) (Table IV). Subjects in the 50 to 59 years age group had the highest proportion of diabetes (17.5%) compared to the other age groups (Table V) whilst for hypertension, subjects in the 60 years or older age group had the highest proportion (9.6%) (Table V). There was a significant association between BMI and diabetes (Table VI); between BMI and hypertension (Table VI) and between BMI and hypertension with diabetes (Table VI). There was also a significant association between WHR and

<table>
<thead>
<tr>
<th>Age Group (yrs)</th>
<th>DM</th>
<th>HT</th>
<th>Both DM + HT</th>
<th>no DM/HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 – 39</td>
<td>66</td>
<td>172</td>
<td>24</td>
<td>598</td>
</tr>
<tr>
<td></td>
<td>(8.1%)</td>
<td>(21.2%)</td>
<td>(3.0%)</td>
<td>(73.6%)</td>
</tr>
<tr>
<td>40 – 49</td>
<td>275</td>
<td>583</td>
<td>125</td>
<td>1210</td>
</tr>
<tr>
<td></td>
<td>(14.2%)</td>
<td>(30.0%)</td>
<td>(6.4%)</td>
<td>(62.3%)</td>
</tr>
<tr>
<td>50 – 59</td>
<td>197</td>
<td>483</td>
<td>109</td>
<td>557</td>
</tr>
<tr>
<td></td>
<td>(17.5%)</td>
<td>(42.8%)</td>
<td>(9.6%)</td>
<td>(49.4%)</td>
</tr>
<tr>
<td>&gt;,60</td>
<td>38</td>
<td>120</td>
<td>21</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>(16.0%)</td>
<td>(50.4%)</td>
<td>(8.8%)</td>
<td>(42.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>576</td>
<td>1358</td>
<td>279</td>
<td>2466</td>
</tr>
<tr>
<td></td>
<td>(14.0%)</td>
<td>(33.0%)</td>
<td>(6.8%)</td>
<td>(59.8%)</td>
</tr>
</tbody>
</table>

Table VI : Relationship between diabetes mellitus and hypertension with BMI

<table>
<thead>
<tr>
<th>BMI Status (kg/m²)</th>
<th>DM</th>
<th>HT</th>
<th>Both DM + HT</th>
<th>No DM/HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18.5</td>
<td>12</td>
<td>46</td>
<td>1</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>(5.6%)</td>
<td>(21.4%)</td>
<td>(0.5%)</td>
<td>(73.5%)</td>
</tr>
<tr>
<td>18.5 - 24.9</td>
<td>159</td>
<td>465</td>
<td>65</td>
<td>1316</td>
</tr>
<tr>
<td></td>
<td>(8.5%)</td>
<td>(24.8%)</td>
<td>(3.5%)</td>
<td>(70.2%)</td>
</tr>
<tr>
<td>25 - 29.9</td>
<td>263</td>
<td>570</td>
<td>139</td>
<td>779</td>
</tr>
<tr>
<td></td>
<td>(17.8%)</td>
<td>(38.7%)</td>
<td>(9.4%)</td>
<td>(52.9%)</td>
</tr>
<tr>
<td>&gt;,30</td>
<td>142</td>
<td>277</td>
<td>74</td>
<td>213</td>
</tr>
<tr>
<td></td>
<td>(25.5%)</td>
<td>(49.6%)</td>
<td>(13.3%)</td>
<td>(38.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>576</td>
<td>1358</td>
<td>279</td>
<td>2466</td>
</tr>
<tr>
<td></td>
<td>(14.0%)</td>
<td>(33.0%)</td>
<td>(6.8%)</td>
<td>(59.8%)</td>
</tr>
</tbody>
</table>
diabetes (Table VII); between WHR and hypertension (Table VII) and between WHR and hypertension with diabetes (VII).

**Discussion**

As the study was done in subjects with no known history of diabetes or hypertension, the proportion of patients with undiagnosed diabetes or hypertension was high. However, this study might not reflect the true prevalence of undiagnosed diabetes or hypertension in the general population. The nature of this study was to find subjects with risk factors for CVD and it was likely that subjects who thought themselves having risk factors for CVD would attend the screening visit. Thus it was not surprising to find a higher proportion of these subjects having diabetes or hypertension compared to previously reported prevalence rates.

The prevalence of diabetes mellitus and hypertension in the Malaysian 2nd National Health and Morbidity Survey was 8.3% and 29.9% respectively (11). In another study in Kelantan, Malaysia, the prevalence of diabetes was 10.5% (12). Obesity and especially abdominal obesity are significantly associated with diabetes and hypertension (13, 14) and this was also reflected in our study which showed significant association between BMI and WHR with diabetes and hypertension.

The proportion of patients with diabetes or hypertension in this study was highest in Felda Palong area (20.3% and 38.6% respectively). This could be due to this area having subjects with the highest mean BMI (25.8 ± 4.5 kg/m²) and highest mean WHR (0.87 ± 0.08) which implied that subjects here were not only more obese but also had more abdominal fat. Subjects with diabetes and hypertension also had the highest mean age, BMI, WHR and plasma cholesterol. There was also a significant association between age and diabetes and also hypertension. This is in keeping with the current knowledge that the prevalence of diabetes and hypertension increases with age (17,18). Type 2 diabetes mellitus and hypertension are considered part of the Metabolic Syndrome or the Insulin Resistance Syndrome and increasing age and obesity are believed to be contributory factors (19).

In conclusion, the proportion of patients with diabetes or hypertension was high. Diabetes and hypertension were significantly associated with age, body mass index and waist-hip ratio. Intervention studies by education and lifestyle changes had begun to study their effectiveness in reducing cardiovascular risks.

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**References**


15. The sixth report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure. *Arch Intern Med* 1997; **157**: 2413 - 46.


STUDENTS’ PERCEPTIONS OF ‘TECHNOLOGY-BASED’ LECTURE HANDOUTS


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Lecture handouts are widely used instructional tools. Handouts supplement rather than substitute students’ regular reading. It is now a common practice to supply PowerPoint handouts and publish lecture handouts on the web for students’ access. A study was conducted among the first year medical students (n=142) of School of Medical Sciences (SMS), Universiti Sains Malaysia (USM) in order to determine their perceptions and expectations with regards to lecture handouts provided to them. The majority of the students reported that they read the lecture handouts as a reference and found them useful as a guide for future learning. More than half (68%) of the students expressed dissatisfaction with the overall presentation format of the handouts which is mainly technology-related i.e. PowerPoint and photocopying. This study indicated that students’ expectations and experiences were positive towards the use of handouts. They used handouts as a means of supplementing rather than substituting their learning. Much care is needed when educators supply computer-based handouts, as this study shows a number of limitations when students use them. Medical schools should consider publishing web-based handouts with online and other facilities to make it interesting and effective.

Key words: Lecture; Handout; Medical education, Malaysia


Introduction

In spite of its limitations, lecturing is the most commonly used method of teaching in higher education. As a result of advanced technology, cost-containment pressures and a desire to make innovative improvements in medical education, dramatic changes have occurred in planning and delivery of lecture and its handouts(1,2). Apart from the conventional classroom mode, lectures are now delivered through a number of innovative ways such as making it computer-based, web-based/on-line, video-taped or through video-conferencing.

Lecture handouts are widely used instructional tools. Handouts make the lectures “portable and enduring”(3) and lead to improved recall of information and improved test performance. In traditional settings, written handouts are used as a meaningful supplement of the lectures. Teachers use the handout as a discussion aide to accompany the lecture and as a means of disseminating additional information not included in the lecture. It is now a common practice to supply PowerPoint handouts when teachers rely on computer-based lectures. A well-known basic option of PowerPoint is the ability to make custom handouts of a presentation, which offers a huge advantage to the teachers. Handouts may be made with one slide per page or as many as six per page. Many institutions and individuals are now routinely publishing lecture handouts on the web for students to access. This method of distribution of information provides many advantages over conventional paper-based distribution as it provide links to other online materials e.g. image archives and journal articles(2). In addition, handouts are cheaper to distribute on the web compare to paper form as they are easy to update; offer interactivity with questions and ‘click-based’ contents; display images, diagrams, video and animated contents; and allow assessment of students’
performance. Although handouts are widely used instructional tools, little research has been done on conventional paper-based, and also on computer-based and web-based handouts. A study was conducted among the first year medical students of School of Medical Sciences (SMS), Universiti Sains Malaysia (USM) in order to determine their perceptions and expectations with regards to lecture handouts provided to them.

Method

In SMS, most teachers deliver computer-based lectures for the first year students and supply hard copies of PowerPoint slides as handouts. To evaluate these handouts, students were asked to complete a questionnaire at the end of their first year. The questionnaire consisted of statements to which students listed their level of agreement using five-point Likert type scale as well as two open-ended questions on strength and weaknesses of the handouts provided to them over the past one year.

Table 1: Students’ perceptions on lecture handouts

<table>
<thead>
<tr>
<th>Statements</th>
<th>Agree (%) [Strongly Agree + Agree]</th>
<th>Undecided (%)</th>
<th>Disagree (%) [Strongly disagree + disagree]</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading handout as a reference</td>
<td>96.5</td>
<td>2.8</td>
<td>0.7</td>
<td>4.61</td>
</tr>
<tr>
<td>Handouts are useful</td>
<td>90.9</td>
<td>6.3</td>
<td>0.7</td>
<td>4.41</td>
</tr>
<tr>
<td>Learning objectives are used as guide</td>
<td>71.5</td>
<td>15.5</td>
<td>4.9</td>
<td>4.12</td>
</tr>
<tr>
<td>Filling the empty spaces of the handouts, if there are any</td>
<td>49.2</td>
<td>38.8</td>
<td>10.8</td>
<td>3.49</td>
</tr>
<tr>
<td>Reading other resources along with handouts</td>
<td>82.9</td>
<td>13.6</td>
<td>3.6</td>
<td>4.07</td>
</tr>
<tr>
<td>Satisfactory presentation style</td>
<td>45.1</td>
<td>41.2</td>
<td>13.7</td>
<td>3.37</td>
</tr>
<tr>
<td>Good content sequence</td>
<td>62.7</td>
<td>22.9</td>
<td>8.4</td>
<td>3.63</td>
</tr>
<tr>
<td>Appropriate length of text</td>
<td>55</td>
<td>34.5</td>
<td>10.6</td>
<td>3.56</td>
</tr>
<tr>
<td>Text is readable</td>
<td>67.6</td>
<td>22.5</td>
<td>9.2</td>
<td>3.68</td>
</tr>
</tbody>
</table>

Table 2: Students’ comments on lecture handouts

- “Actually, first year students need to be guided by handouts, because they still don’t know how to manage their time and which books need to be referred.”

- “It acts as a guide to my further reading and act as an overall summary to me for my understanding and will encourage me to read more. It helps me to know the important points that we ought to know for that particular chapter.”

- “The pictures and diagrams were not clear when we Photocopy it.”

- “Quite often the PowerPoint background presentation is superimposed on the notes which makes the notes difficult to read.”

- “Lack of picture in the handouts.”

- “…… sometimes, the text was unreadable. The diagrams/pictures not clear. …… not supplied before the class.”

- “Printed with 6 slides in a page, they’re too small to read.”

- “Some of the pictures are not clear.”

- “…… the size of the words especially in PowerPoint handouts are very small.”
Results

One hundred and forty two (79%) students answered the questionnaire, where 69% were females and 31% were males. Almost all students reported that they read the lecture handouts as a reference and found them useful. Eighty percent of the students used the learning objectives of the handouts as a guide for future learning. More than 80% of the students read other reading resources along with handouts. However, a majority (68%) of the students has shown their dissatisfaction with the overall presentation format of the handouts. Sixty-three percent of the students are satisfied with content sequence while 68% found the handouts readable. A substantial number of students (45%) was of the opinion that the length of the text is inappropriate i.e. too little information when PowerPoint slides are provided as handouts. (Table 1)

Among the problems which students perceived regarding the overall format of the handouts included: difficult and ambiguous language, fade diagrams in photostat copies, short and incomplete text, illegible handouts printed with PowerPoint background and small letter size, and contradictions between the contents of handouts and standard text books. (Table 2)

The main strengths of the handout from the students view were that it acts as a guide for learning and revision before the examination. The students expected the handouts to be clear, brief, well structured; in simple language; compilation of summary from standard textbooks; outline clinical applications; contain key references and definitions of new terms; and most importantly, should be online for students ready reference. (Table 2)

Discussion

First year medical students are mostly teacher dependent because of exposure to a new curriculum. They also show greater demand for learning resources, especially lecture handouts(5). It is often necessary to guide them through handouts, as they may not be matured enough to manage their time for learning and to look for the right references. Teachers expect that lecture handouts should supplement rather than substitute students’ regular reading(5). This study indicated that first year medical students’ expectations and experiences were positive towards the use of handouts. However, it was noticed that students’ dissatisfaction about handouts are mainly “technology-based i.e. adequacy of PowerPoint and qualities of photocopies supplied. While technology brings revolution in education, it often needs careful application. Educators should pay much attention when they supply computer-based handouts. Teachers should be trained on producing and presenting lectures using the PowerPoint and other computer programs. Medical schools, especially in Asian, should consider publishing web-based resource materials with online links and up-to-date interactive and click-based contents(6). Other considerations would include graphics, images, video and animated web-based handouts that can be utilized for students’ assessment. These areas need further in-depth research by seeking feedback from the students and teachers to ensure effective use of the handouts.

Acknowledgements

The findings of the research was presented in the “9th National Conference on Medical Sciences” held at Universiti Sains Malaysia, Health Campus, Kota Bharu on 22 – 23 May, 2004.

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References

2. Rous, BA., Rashbass, JL. What can Internet technology add to pathology education? Histopathology 2002; 41 Suppl 2: 216-21
THE MAXILLARY ARCH AND ITS RELATIONSHIP TO CEPHALOMETRIC LANDMARKS OF SELECTED MALAY ETHNIC GROUP

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The objectives of this study were to estimate the maxillary arch measurements, to assess the validity of Pont’s & Korkhaus’ Indices; to determine the relationship between maxillary arch form with head form; and to estimate the cephalic index (CI) of the study population. A cross-sectional study was conducted on 85 mature Malay students, 28 male students (32.98%), 57 females (67.02%) attending Teachers’ Training College. Their mean age was 23.9 yr, and Cephalic Index (CI) 86.4 (95% Confidence Interval 85.5-87.3). Arch and head dimensions were significantly larger in males than in females. CI was not significantly different between males and females. Means of anterior arch width (AAW), posterior-arch-width (PAW) and arch-length (Lu) were 35.57mm, 47.3mm and 18.01mm respectively. They were significantly different from their corresponding Indices. Correlation Coefficient between bizygomatic width and anterior-arch-width was 0.18 and was not significant in both sexes of the present population.

Key words: Dental arch form, Pont’s Index, Korkhaus’ Index, Malay, Cephalic Index

Introduction

A sound knowledge of tooth size and dental arch dimensions of a population is important for several dental treatment procedures. For example in Restorative Dentistry, where teeth is being restored to its original morphology, knowledge of the tooth size will certainly implement in the treatment administered. Differences in dental arch and head dimensions of different populations can be inherited and these inherited differences are useful for the practice of Aesthetic Dentistry and for effective orthodontic treatment(1) It is therefore important to have knowledge of certain cephalometric and dental arch parameters and their relationships for a given population. There are several indices derived from these measurements; indices of Pont(2) Linder(3), and Korkhaus(4), are mostly used in German-speaking countries(5). These indices predicts the ideal values (standard values) of the arch width and length from the sum of upper four incisors (SIu). A certain correlation exist between the arch length, width, and mesiodistal width of the upper maxillary incisors. The standard values of these indices are then statistically correlated and compared with the actual values of the individual case. Certain diagnostic and prognostic indications such as deviation in transverse development of the arch widths and anteroposterior position of incisors can be gained by comparison of the actual and standard values.

In the 1840s the Swedish physician, Anders Retzius, developed one of the most influential cranio metric techniques, the Cephalic Index which measures the ratio between the width and length of the head. Generally he classified people as having one of the three types of head shapes – brachycephalic, dolichocephalic or mesocephalic(6).

The objectives of this study were to establish the dental arch indices, cephalometric measurements
and how these are correlated to one another, and to validate the dental arch indices in this study population.

Materials and Methods

Bootstrap statistics based on 1000 simulated means of sum of four maxillary incisors ($S_{IU}$) estimated a standard deviation of 2.3mm. This SD was used to calculate a sample of 85 subjects required to estimate $S_{IU}$ with a precision of +/- 0.5 mm at 95% confidence interval.

Preliminary screening procedures were conducted among students from Teachers Training College, Kota Bharu. Subjects whose age range between 20 and 35 yr, and has Malay parents and Malay grand parents from both the paternal and maternal sides were selected for this study. Subjects with maxillary dental arch irregularities and missing teeth, and whose first-degree relatives were selected

Figure 1a: Diagram showing the measurement of maximum skull length (g-op)

Figure 1b: Diagram showing the measurements of maximum skull breadth (eu-eu), and maximum face width (zy-zy).
for this study were excluded. Among those who were eligible, 85 subjects were consecutively selected. After a brief self-administered questionnaire session, head measurements and maxillary casts were taken. The head measurements made on the subjects were (i) maximum skull length (g-op), distance from opisthocranion (op) to glabella (g); (ii) maximum skull breadth or bieuryonic diameter (eu-eu), distance between the most lateral point of the skull(euryion); (iii) bizygomatic diameter (zy-zy), distance between two zygomatic prominences (zygion); (Fig-1b)

The following measurements were done on the dental casts. (i) maximum mesiodistal distance of each of the four maxillary incisors; (ii) anterior arch width (AAW), that is the distance between the lower-most points of the transverse fissure of the upper first premolar teeth, the reference points for (AAW); (iii) posterior arch width (PAW), the distance between the point of intersection of the transverse fissure with the buccal fissure of the upper first permanent molar teeth (the reference points for PAW); (iv) anterior arch length L_{u}, which is perpendicular from the most anterior labial surface of the central incisors to the connecting line of the reference points of AAW; (Fig-2c). All measurements were in millimeter to the nearest 0.1 mm.

**Data Analysis**

STATA 7.0 (7) was used to summarize the data and validate the indices using correlations and regression statistics. From the measurements made on dental casts, the sum of upper incisor mesiodistal distances (SI_{u}) was first computed and this measure was used to estimate the values of arch widths, using Pont’s Index, for AAW and PAW. Values of arch length (L_{u}) were computed using Korkhaus’ Index. These index values (standard values) were calculated
Table 1: Dental arch measurements among Malay men and women.

<table>
<thead>
<tr>
<th>Dental Arch Measurements</th>
<th>Gender</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n = 28</td>
<td>Female n = 57</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>SI_a</td>
<td>32.20</td>
<td>2.32</td>
</tr>
<tr>
<td>AAW</td>
<td>35.93</td>
<td>2.32</td>
</tr>
<tr>
<td>AAW*</td>
<td>37.89</td>
<td>2.73</td>
</tr>
<tr>
<td>PAW</td>
<td>48.98</td>
<td>2.46</td>
</tr>
<tr>
<td>PAW**</td>
<td>49.54</td>
<td>3.57</td>
</tr>
<tr>
<td>L_a</td>
<td>17.67</td>
<td>2.48</td>
</tr>
<tr>
<td>L_a***</td>
<td>20.13</td>
<td>1.45</td>
</tr>
</tbody>
</table>

SI_a = sum of four upper incisors
AAW = anterior arch width measured from the cast
AAW* = anterior arch width based on Pont’s index: AAW = SI_a * 100/85
PAW = posterior arch width measured from the cast
PAW** = posterior arch width based on Pont’s index: PAW = SI_a * 100/65
L_a = arch length measured from the cast
L_a*** = arch length based on Korkhaus’ index: L_a = SI_a * 100/16

All measurements are not significantly different between males and females (independent t-tests p > .05)
Table 2: Dental arch measurements (observed values) versus (index values) using Pont’s and Korkhaus’ Indices.

<table>
<thead>
<tr>
<th>Dental Arch Measurements</th>
<th>Mean (mm)</th>
<th>Beta-coefficient (p-value)</th>
<th>Correlation Coefficient (p-value)</th>
<th>Beta-coefficient (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(DAM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAW (observed)</td>
<td>35.54</td>
<td>0.355 (&lt;0.01)</td>
<td>0.296 (&lt;0.01)</td>
<td>0.42 (&lt;0.01)</td>
</tr>
<tr>
<td>AAW* (Pont’s)</td>
<td>37.71</td>
<td>1.0 (&lt;0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAW (observed)</td>
<td>-48.1</td>
<td>0.215 (&lt;0.05)</td>
<td>0.255 (&lt;0.02)</td>
<td>0.33 (&lt;0.05)</td>
</tr>
<tr>
<td>PAW ** (Pont’s)</td>
<td>-49.3</td>
<td>1.0 (&lt;0.01)</td>
<td>1.5 (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>L_a (observed)</td>
<td>17.8</td>
<td>0.779 (&lt;0.001)</td>
<td>0.478 (&lt;0.001)</td>
<td>0.46 (&lt;0.01)</td>
</tr>
<tr>
<td>L_a*** (Korkhaus’s)</td>
<td>20.03</td>
<td>1.0 (&lt;0.01)</td>
<td>0.63 (&lt;0.01)</td>
<td></td>
</tr>
</tbody>
</table>

Independent t-tests showed that predicted dental arch measurements were significantly greater than the observed measurements at p < 0.01.

Table 3: Differences between observed and predicted Dental Arch Measurements

<table>
<thead>
<tr>
<th>Dental Arch Measurements</th>
<th>Differences in mm (Observed vs Predicted)</th>
<th>% within +/- 1 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>AAW vs AAW*</td>
<td>-17</td>
<td>+8.4</td>
</tr>
<tr>
<td>PAW vs PAW **</td>
<td>-10.3</td>
<td>+12.0</td>
</tr>
<tr>
<td>L_a vs L_a***</td>
<td>-2.4</td>
<td>+8.4</td>
</tr>
</tbody>
</table>

AAW = anterior arch width measured from the cast
AAW* = anterior arch width based on Pont’s index: (AAW = SI_a*100/85)
PAW = posterior arch width measured from the cast
PAW ** = posterior arch width based on Pont’s index: (PAW = SI_a*100/65)
L_a = arch length measured from the cast
L_a*** = arch length based on Korkhaus’ index: (L_a = SI_a*100/160)
Figure 3: Correlations of observed and expected values of anterior arch width and sum of incisor widths

Predicted values of anterior arch width by regression of sum of incisor widths on Pont’s Index.

Predicted values of anterior arch width by regression of sum of incisor widths on observed anterior arch width.

Figure 4: Correlations of observed and expected values of posterior arch width and sum of incisor widths

Predicted values of posterior arch width by regression of sum of incisor widths on Pont’s Index.

Predicted values of posterior arch width by regression of sum of incisor widths on observed posterior arch width.
and then validated against the actual measurements made on the casts. Linear regression analysis was done between these values by fitting the regression lines, further strengthened the validity tests performed earlier.

Cephalic Index (CI) was calculated by taking the ratio between maximum skull breadth and maximum skull length. Finally, correlation between dental arch and cephalic measurements were tested.

**Results**

The sample comprised of 28 (33%) males and 57 (67%) females of Malay ethnicity. Their mean ages were 23.9 yr and 23.2 yr respectively, and were not significantly different (p > 0.05). Mean $SI_u$ was 32mm (+/- 2.3mm). Table 1 shows mean and SD of the dental arch measurements made directly from the casts and those derived from $SI_u$ values plugged into Pont’s and Korkhaus formulae as shown previously. Except for $L_u$, males had slightly larger values than females for all the measurements, but neither of them was statistically significant. In Table 2, the dental arch measurements, as predicted by the indices, were significantly greater than those measured directly on the casts (p <0.01). Correlation coefficients between the two measurements were also very weak (correlation coefficient ranged from 0.26 to 0.48). The indices, which formulae depend directly on the variation of $SI_u$, produced perfect correlation, where as observation of AAW, PAW and $L_u$ on the dental casts of subjects showed very weak correlation with $SI_u$. Pont’s Index predicts that AAW increases by 1.2 mm for every 1mm increase in $SI_u$; but our study showed an increment less than 0.5mm. The discrepancy for PAW was 1.5mm vs. 0.3mm. This indicates that the increase in the mesiodistal distances of the maxillary incisors in this study did not necessarily increase the size of the dental arch measurements proportionately. Table 3 depicts the distribution of differences between observed and expected dental arch measurements. Figures 3 to 5 illustrate the comparisons of the regression coefficients between summation of incisors and dental arch measurements (observed vs expected). The regression lines predicted from the Pont’s and Korkhaus’ Indices were highly correlated with the sum of incisor widths whereas the observed dental arch measurements was not in proportion to the incisor widths.

In Table 4, head measurements were seen to be significantly larger among males by a difference of 3mm to 6mm (p <.01). The mean cephalic index (CI) of the study subjects was found to be 86.4%.
and the mean CI of female subjects was slightly higher than the males (86.6 vs. 85.9), but were statistically not significant. Correlation between bizygomatic diameter(zy-zy) or face width and Anterior Arch Width (AAW) was done to test the relationship of head form and arch form. They were 0.01, 0.22, and 0.18 for male, female and in total, respectively. This showed a weak correlation and all r-values were not statistically significant.

**Discussion**

A similar study conducted on a group of ethnic Chinese subjects reported mean anterior arch width of 35.74 (+/- 2.17mm), and mean of SI value of 8.85 (+/- 0.59mm) (8). These findings indicate that Chinese people seemed to have bigger tooth size than the Malays as shown in this study. Very few studies have been done to measure the dental arch and most of these studies focus on the effects of craniofacial anomalies and surgical procedures on dental arch measurements (9,10). Some studies simply describe the racial and hereditary influences on these measurements (1,11). Since our study included adults of pure Malay ethnicity, matured with no dental abnormalities, the parameters obtained may represent ethnic Malays who share the same geographical environment as our sample.

The usefulness of Pont’s Index is controversial. In a study aimed to evaluate Pont’s Index in the untreated, non-crowded samples of Australian Aborigines, Indonesians, and White, a considerable individual variability was noted in each population with regard to the difference between observed values and Pont’s estimates, ranging from -5.9 mm to +6.2 mm (AAW) and -6.1 mm to +12.7 mm (PAW) (12) which were comparable with our results shown in Table 3. None of the subjects displayed ideal arch dimensions predicted by the Index, but values were within +/- 1.0 mm for 17.5% of the Indonesian sample, 20.6% of the Aboriginal sample, 30.8% of the White sample (12), and 19.5 to 20.7% in the Malays of this study. Dental arch width was generally underestimated by the Index in Indonesians who tended to display relatively small tooth size and large arch width. A more even
distribution of estimates was noted in Australian Aborigines and White subjects, with the Aborigines showing large tooth sizes and broad dental arches, and the White subjects displaying smaller tooth size and narrow arches (12). Correlation coefficients computed between observed and expected values were low in all three populations studied (range r = 0.01 to r = 0.56). (12) These findings are comparable with this study results as shown in Table 1 and 2, and Figures 3, 4 and 5. As seen in Table 3, the Pont’s indices consistently over-estimated the dental arch widths whereas Korkhaus’ Index under-estimated the arch lengths of our population. The existence of negative correlation between arch width and arch length was not supported by the results of our study. In this study, subjects’ arch widths did not increase proportionately with the increasing size of incisors. In a similar study, maxillary arch dimensions conducted on Chinese adult subjects revealed poor correlation between tooth size and arch width.

It is concluded that this variation could be attributed to differences in the genetic inheritance of the different races. (13).

Regarding the Cephalic Index, our study subjects were found to be brachycephalic (86.4%) with no significant gender difference. It was consistent with findings of Diament and Rodrigues, 1976 (14). The reference values for cephalic index were <76% dolichocephalic, 76-80.9% mesocephalic, 81–85.4% brachycephalic and >85.5% hyperbrachycephalic. (15) Generally, Chinese, Japanese, Koreans and Filipinos were characterized by having longer lateral and smaller anteroposterior dimensions relative to the Caucasians (16). The information gathered about the trend of CI over time by doing a cohort analysis of CI data of a country by ages may be used for evidence of the effect of environment on the anthropometric dimensions of a population. This fact was observed in one study which showed that the CI among Jordanians changed with the economic condition that prevailed when the person was born. (17).

There was a weak correlation between the bizygomatic width (face width) and the maxillary anterior arch width. This finding was not consistent with that reported by Sergl et al, 1944 (18) where they found a strong correlation between the zygomatic width and the maxillary dental arch width. However, the analysis was based on the data obtained from the models and anthropological measurements of 50 adult German subjects with fairly eugnathic dentition, and their dental arch widths showed a perfect correlation with Pont’s Indices.

In conclusion, the results for the dental arch measurements and its relationship to the head form obtained from this study should be further verified and compared with those of other ethnic groups in Malaysia.

Acknowledgement

This study was supported by the Malaysian Government through the short-term grant (No.304/PPSP/6131236). The authors would like to thank the School of Medical Sciences, and the School of Dental Sciences, Universiti Sains Malaysia for the facilities provided and finally to the staff and students from the Teachers’ Training School, Kota Bharu, for without their assistance and participation, this study would not have been conducted.

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References


17. A. Abu Dalou. *Head shape of adult males as a possible indicator of economic changes in northern Jordan (1900-1978).* Dept.of Anthropology, University of Missouri-Columbia, Columbia, MO, 65211, USA. 2000. (GENERIC) Ref Type: Electronic Citation.

Perceived breathlessness played an important role in guiding treatment in asthma. We developed a simple, user-friendly method of scoring perception of dyspnoea (POD) using an incentive spirometer, Triflo II (Tyco Healthcare, Mansfield, USA) by means of repetitive inspiratory efforts achieved within three minutes in 175 normal healthy subjects and 158 asthmatic patients of mild (n=26), moderate (n=78) and severe (n=54). Severity was stratified according to GINA guideline. The mean POD index in normal subjects, and asthmatic patients of mild, moderate and severe severity were: 6 (4-7) 16 (9-23), 25 (14-37), and 57 (14-100) respectively (p<0.001 One-Way ANOVA). Based on 17 asthmatic and 20 normal healthy subjects, intraclass correlation coefficients for POD index within subjects were high. In 14 asthmatic patients randomized to receiving nebulised b₂-agonist or saline in a crossover, double-blind study, % FEV₁ change correlated with % changes in POD index [r = -0.46, p=0.012]. Finally, when compared with 6-minutes walking test (6MWT) in an open label study, respiratory POD index correlated with walking POD index in 21 asthmatic patients [r = 0.58 (0.17 to 0.81) (p=0.007] and 26 normal subjects [0.50 (0.13 to 0.75) (p=0.008)]. We concluded that this test is discriminative between asthmatic patients of varying severity and from normal subjects, is reproducible, responsive to bronchodilator effect, and comparable with 6MWT. Taken together, it has the potential to score disability and POD in asthma effectively and simply.

**Key words**: Perception of dyspnoea, asthma, normal subject, incentive spirometer, six-minute walking test

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**Introduction**

Dyspnoea can be defined as an appreciation of increased effort and discomfort associated with the act of breathing (1). These symptoms are best appreciated during exercise by placing the cardiorespiratory system under the stress of increased oxygen requirement and carbon dioxide output. Thus, quantification of exercise capacity provides an objective evidence of the degree of dyspnoea, especially when resting cardiopulmonary test results are disproportionately ‘normal’ for the level of complaint reported (2).

The global increase in asthma morbidity and mortality is worrying (3). One important factor that contributes to this increase is the lack of appreciation of the disease severity by both the patients and healthcare professionals (4, 5, 6). Furthermore, there is evidence that perception of dyspnoea in some asthmatic patients may be impaired resulting in fatal and near-fatal asthma attacks, and increased hospitalization (7, 8, 9).

The most widely used exercise testing employ workloads that are progressively increased by a standardized amount at intervals of time, until the subject is unable to continue or reaches a predetermined end-point. The treadmill and cycle ergometer are commonly used for this purpose and a variety of non-invasive (e.g. ventilation and heart rate) or invasive (e.g. arterial and central venous catheterization) measurements can be carried out during this time to enable documentation of cardiorespiratory adaptation from rest to capacity (2). However, these tests are cumbersome to perform,
time-consuming and not readily accessible in the majority of places. As a result, simpler tests such as the six-minute walking test that measure disability and dyspnoea provide a useful alternative. They have the attractiveness of ease, better tolerability, and ‘low technical requirement’ to perform in large-sample population studies, disabled individuals and elderly subjects (10, 11, 12).

In keeping with the concept that exercise test is preferable to measurements done at rest in assessing disability and perception of dyspnoea, and that a test that is cheap and easy to perform has many advantages, we developed the idea of using an incentive spirometry in asthmatic patients to score the maximal breathing capacity (MBC) by repetitive inspiratory efforts within a set time (3 minutes), and breathlessness perceived at the end of the inspiratory exercise- converted into a score dependent on MBC- as an index of perception of dyspnoea (POD). The objective of this present study was to provide preliminary validation of this tool (Three-Minute Respiratory Exerciser Test, 3MRET) for use in asthmatic patients with regards to its reliability to differentiate between disease severities, its correlation with Forced Expiratory Volume in One Second (FEV₁), its reproducibility, and its responsiveness to the effect of bronchodilation, and finally, in how well it compared with the Six-Minute Walking Test (6MWT). Normal healthy subjects were added for comparison.

Subject and Methods

Subjects

Between January and July 2003, a convenient sample of 158 asthmatic patients followed up in chest clinic of an 800-bed urban-based teaching hospital was recruited for the study. Asthma diagnosis was made according to international guidelines (3) and all severity of asthma was accepted. Over the same period of time, 175 normal healthy subjects, defined as having no chronic respiratory symptoms or any other known medical conditions, were recruited from the public and hospital staff. Normal healthy subjects were not current cigarette smoker and if previously had do so, smoked < 10 pack years.

Socio-demographic and asthma-related variables were recorded using a standard questionnaire. All patients completed a selfanswered, health-related quality of life questionnaire, St Georges’ Respiratory Questionnaire (SGRQ) and underwent a three-minute respiratory exerciser test. Patients who had respiratory tract infections or asthma exacerbations postponed their study until at least 6 weeks after the complete resolution of symptoms. The study was approved by the local university research and ethics
committee. Written informed consent was obtained from all patients.

Three-minute respiratory exerciser test (3MRET)

This test scored the maximal breathing capacity (MBC) by repetitive inspiratory efforts within three minutes, based on an incentive spirometry, Triflo II (Tyco Healthcare, Mansfield, USA) [Picture A] that was routinely used in our hospital for the purpose of chest physiotherapy, and scored the perception of dyspnoea (POD) at the end of this three-minute period. By repeated inspiratory effort, patients were asked to get as many balls as possible to reach the top of each of the three columns (A, B and C) within three minutes. Columns A, B and C required flow rates of 600 ml/min, 900 ml/min and 1200 ml/min respectively to bring the balls to reach the top, and as such, required the generation of sufficient inspiratory effort on the part of the subject to achieve this. When all three balls reached their column tops, it indicated that the subject was able to generate inspiratory airflow of 1200 ml/min. When only two balls reached their column tops, it indicated that the subject could only generate 600 ml/min. The cumulative times of three balls, two balls or one ball reaching the column top became an index of maximal breathing capacity (MBC score), and the formula used was as follows: MBC score = (number of times that all three balls reached the top of columns) x 2 + (number of times that two balls reached the column top) x 1.5 + (number of times that only one ball reached the column top) x 1.

Immediately after the test, the patient placed a score on a visual analogue scale (VAS) between zero (not breathless at all) and ten (worst imaginable breathlessness) with regards to his POD. The POD index was calculated by dividing the POD VAS score with the MBC score, and then multiplying it by 1000. To standardize, POD VAS score of zero was replaced by a standard value of 0.125, to enable the calculation of the POD index using the equation described. Higher POD index indicated greater perception of breathlessness.

Reproducibility study

From the original cohort of recruited subjects, 17 asthmatic and 20 normal subjects, matched for sex and age, were randomly recruited to undergo a
study of the repeatability of MBC and POD measurements, where they underwent another 3MRET on a separate occasion at least one week after.

**Study on the bronchodilator effects on 3MRET**

From the original cohort of asthmatic patients, 14 were randomly invited to undergo a randomized, placebo-controlled, double-blind, crossover study. In two visits separated by at least one week, each patient was nebulised with either 10mg bricanyl (active) or normal saline (placebo), and 3MRET preceded by measurement of FEV1 was performed before and after each nebulisation.

**Study comparing 3MRET and 6MWT**

23 asthmatic and 26 normal subjects, matched for age and sex, randomly chosen from the original cohort of recruits were invited to participate in this open-label study whereby the subjects attended a separate visit at least one week after for a 6MWT. During this visit, the subjects were required to walk to and fro along a flat corridor as fast as they could within 6 minutes. The distance walked within this time was recorded as ‘walking distance’, considered equivalent to MBC by 3MRET. Immediately after the completion of 6MWT, the subjects were asked to place a score on a visual analogue scale (VAS) between zero (not breathless at all) and ten (worst imaginable breathlessness) with regards to their POD. Similar to the way POD index was calculated in 3MRET, walking POD index was produced by dividing POD VAS score by walking distance, and then multiplying it by 1000. Like before, to standardize, POD VAS score of zero was replaced by a standard value of 0.125, to enable the calculation of the POD index using the equation described.

**Statistical analysis**

Descriptive statistics were used to study the clinical variables of asthmatic and normal subjects. The values were expressed in mean and 95% confidence interval, or percentage. Differences in
means or percentages of variables between groups were assessed using One-Way ANOVA for continuous data and Chi-Square test for categorical data. MBC score and POD index were expressed as means and 95% confidence interval, and their differences between groups were first assessed by One-Way ANOVA followed by post-hoc analysis with unpaired t tests if significant difference was found. Correlations between FEV₁ % predicted normal with MBC score and POD index in asthmatic and normal subjects were assessed by Spearman rank tests. Intra-subject reproducibility of MBC score and POD index was assessed by intraclass correlation coefficients (13). Responsiveness of the 3MRET to

Figure 2: Correlations between Forced Expiratory Volume in One Second (FEV₁) with (A) Maximal Breathing Capacity (MBC) score and (B) Perception of Dyspnoea (POD) score in asthmatic (n=175) and normal subjects (n=158). Symbols (∑ and _) denote individual asthmatic and normal subjects. Correlation coefficients (Spearman rank test) between MBC score and FEV₁ in asthmatic and normal subjects were 0.27 (p<0.001) and 0.09 (p=0.19) respectively. Correlation coefficients between POD index and FEV₁ in asthmatic and normal subjects were –0.05 (p= 0.528) and –0.04 (p=0.537) respectively. Scales are log transformed for sake of clarity.
bronchodilator effects on asthmatic patients was studied by assessment of correlations (by Spearman rank tests) between percentage FEV<sub>1</sub> change and percentage change in MBC score and POD index before and after bronchodilation. Finally, correlations between measurements on disability (MBC for 3MRET and walking distance for 6MWT) and POD (POD index by 3MRET and 6MWT) were compared by Spearman rank tests for strength of associations and significance.

**Results**

**Subjects**

The clinical features of recruited subjects are listed in Table 1. Except for gender, the clinical variables that were statistically different between the groups were consistent with the severity of asthma in that patients with more severe disease were older, required more controller medication such as theophylline, had more severe exacerbations, exhibited greater airflow limitation, and reported poorer quality of life.

**MBC and POD score in asthmatic and normal subjects**

The mean MBC score in normal healthy subjects was the highest [mean (95% CI): 202 (191-214)] across all groups. The mean scores were progressively lower with increasing disease severity [mild: 168 (145-192); moderate: 153 (136-169); severe: 125 (109-142)]. Statistically significant differences were observed between normal subjects and moderate asthma patients [mean difference (95% CI): 49 (23-76), p<0.001] and between normal subjects and severe asthma patients [77 (49-104), p<0.001], and between mild and severe asthma patients [42 (4-81), p=0.004] [Figure 1].

Mean POD index was lowest in normal subjects across all groups [mean (95% CI): 6 (4-7)]. The mean scores were progressively higher with increasing disease severity [mild: 16 (9-23); moderate: 25 (14-37); severe: 57 (14-100)]. Statistically significant differences were observed between normal subjects and mild asthma patients [mean difference (95% CI): 10 (1-19), p=0.004] and between normal subjects and moderate asthma patients [19 (4-34), p=0.001] [Figure 1]. It is noteworthy that unlike MBC, the spread around mean POD index increased dramatically with the increase of asthma severity. In severe asthmatic patients, the 95% confidence interval of mean POD index was between 14 and 100.

The only correlation of FEV<sub>1</sub> (% predicted normal) with MBC score in asthmatic patients was significant \( r_s (95\% CI) = 0.27 \) (0.118-0.416); \( p<0.001 \). There was no correlation between POD score and FEV<sub>1</sub> in asthmatic and normal subjects, or between MBC score and FEV<sub>1</sub> in normal subjects [Figure 2].

**Reproducibility of MBC score and POD index**

17 asthmatic patients [mean (95% CI): age, 44 (35-52) yrs, FEV<sub>1</sub> % predicted normal, 64 (56-71); 41% male] and 20 normal healthy subjects [age,
Figure 3: Correlations between percentage changes in FEV$_1$ and percentage changes in (A) Maximal Breathing Capacity (MBC) score, and (B) Perception of Dyspnoea (POD) index, in asthmatic subjects (n=14) in response to bronchodilator effect. Symbols (Σ and _) denote respective measurements during nebulisation with active (i.e. short acting b$_2$-agonist) and placebo (normal saline), in a randomized, double-blind, crossover study. Correlation coefficients, r (95% confidence interval) between FEV$_1$ and MBC score and POD index were 0.49 (0.14 to 0.74) (p<0.001) and −0.46 (-0.72 to −0.10) (p=0.012) respectively (Spearman rank test).
**Figure 4**: Correlations between measurements based on six-minute walking test and three-minute respiratory exercise test in asthmatic (n=21) and normal subjects (n=26). MBC = maximal breathing capacity (of three-minute respiratory exercise test); POD = perception of dyspnoea. Correlation coefficients, $r_s$ (95% CI) between MBC score and walking distance in asthmatic and normal subjects were 0.47 (0.02 to 0.76) [$p=0.03$] and 0.53 (0.16 to 0.76) [$p=0.005$] respectively. Correlation coefficients, $r_s$ (95% CI) between breathing and walking POD index in asthmatic and normal subjects were 0.58 (0.17 to 0.81) [$p=0.007$] and 0.50 (0.13 to 0.75) [$p=0.008$] respectively. Scales are log transformed for clarify.
Intracorrelation coefficients of measurements within subjects for both MBC score and POD index performed on two separate occasions were generally high and all were statistically significant [Table 2]. Normal subjects had overall higher intra-subject consistency in both measurements, compared with asthmatic subjects. The lowest consistency (r = 0.64) and widest 95% confidence interval (between 0.33 and 0.87) is found in the measurement of POD index in asthmatic subjects.

**Responsiveness of MBC score and POD index to bronchodilation**

14 asthmatic patients [mean (95%): age, 39 (27-50) yrs, FEV₁, 63 (57-68)% predicted normal; male, 42%] successfully completed the randomized, double-blind, placebo-controlled, crossover study. The mean percentage (95% CI) FEV₁ change after nebulised short acting β₂-agonist and normal saline were +23 (+8 to +38) and 0 (-6 to +5) respectively. Correlations, r (95% CI) between changes in % FEV₁ and those in MBC score and POD index were 0.49 (0.14 to 0.74) (p<0.01) and –0.46 (-0.72 to –0.10), (p=0.012) respectively.

**Correlations between three-minute respiratory exercise test and six-minute walking test**

Apart for 2 asthmatic patients who were withdrawn following development of clinically important wheezing during the 6MWT, 21 asthmatic patients [mean (95% CI): age, 42 (32-51) yrs, FEV₁ % predicted normal, 69 (60-77); male, 19%] and 26 normal healthy subjects [age, 40 (32-49) yrs; FEV₁ % predicted normal, 89 (86-93); male, 26%] successfully completed the study. Correlation coefficients, r (95% CI) between MBC score and walking distance in asthmatic and normal subjects were 0.47 (0.02 to 0.76) [p=0.03] and 0.53 (0.16 to 0.76) [p=0.005] respectively. Correlation coefficients, r (95% CI) between respiratory and walking POD index in asthmatic and normal subjects were 0.58 (0.17 to 0.81) [p=0.007] and 0.50 (0.13 to 0.75) [p=0.008] respectively.

**Discussion**

We have shown that 3MRET can differentiate between asthma severities, and MBC score in asthma patients correlated with FEV₁. Furthermore, we showed that the scores by 3MRET are reproducible, responsive to the effect of bronchodilation, and comparable to that of 6MWT.

This exercise test requires repetitive inspiratory effort by the subject to achieve as high score as possible from the number of balls reaching the column tops of an incentive spirometry (Triflo II by Tyco Healthcare). It is simple for the subject to perform and easy for the assessor to score. The apparatus is cheap (about US$ 4.00 each), washable and reusable up to at least 50 times. The time limit of three minutes was arbitrarily chosen based on the preliminary observation that most normal healthy subjects experience some degree of breathlessness after this time (data not shown). Also in this study, subjects were also routinely asked whether they could differentiate between ‘breathlessness’ and ‘tiredness’ for the purpose of scoring POD on the VAS. All except one normal healthy subject responded in affirmation, suggesting that differentiation between dyspnoea and fatigue is unlikely to be a major problem for most subjects. This can be important as dyspnoea and fatigue may be considered as separate variables of outcome measures in exercise test including the 6MWT. It is safe in that no subjects, normal or asthmatic, reported any adverse events, for example, severe wheezing or fainting, from the 3MRET test. However, two asthmatic patients were not able to complete the 6MWT due to development of clinically significant wheezing during walking.

Like other dynamic lung function test like spirometry, the 3MRET is a volitional test in that it is dependent very much on the effort put in by the subject. Thus, it requires proper supervision and adequate motivation. Low effort by the subject will result in low MBC score that may not cause much sense of breathlessness. For this reason, POD was scored by dividing MBC score over VAS on POD, naming it as POD index. As a result, a subject who has a low MBC score resulting in a low VAS on POD, would produce a similar POD index to another subject who has a high MBC score resulting in a high VAS on POD. However, a subject who truly has breathlessness beyond that which is normally experienced, would have a low MBC score but high VAS on POD, leading to a high POD index. Conversely, a subject who is capable of a high MBC score but has little breathlessness, would have a very low POD index. Therefore, POD index makes correction for the breathing workload capable of an individual. Our findings of relatively narrow 95% confidence intervals (CI) in POD index of normal and asthmatic subjects of mild and moderate severity, but relatively wide 95% CI in severe asthma
patients, provided some validity that such index is meaningful, since if this approach is inappropriate, 95% CI would be large and similarly so in all groups of asthma severity and in normal subjects. Another important consideration is whether our test results are subjected to bias by intra- and inter-observer error. The assessor, due to the speed by which balls hit the top, may misjudge the number and frequency of balls reaching their column tops. This had not been formally investigated in our study and should be further explored.

The mechanics in POD is complex in health and disease (14). Contribution of POD in how certain diseases like asthma is managed is huge because the treatment is principally aimed at alleviating and controlling symptoms (1 & 15). POD does not always correlate with standard measurement of lung function such as FEV\(_1\) (16, 17, 18), and may have major influence on morbidity and mortality if the patient under-perceives his symptoms or the clinician underestimates the disease severity (7, 8, 9). To enable some scoring of disability and POD, the conventional six-minute or twelve-minute walking test offers much advantages over technically cumbersome exercise tests using treadmill and cycle ergometer in that it is simple to perform, requires no equipment, and provides a summative disability score that allows monitoring of disease progression and therapeutic intervention. We developed on this concept further by limiting the assessment of disability and POD on breathing capacity alone using a type of incentive spirometer widely available in our local healthcare market. The test obviates the need of walking along a hospital corridor and measurement of walking distance. It takes shorter time and can be carried out across the consultation desk by the clinician himself.

Importantly, we showed that while the 95% confidence intervals in MBC scores in normal and asthmatic of different severity were relatively similar, the intervals in POD index were progressively widened with increasing asthma severity. This indicates that in patients with more severe asthma, the POD can vary considerably for the same degree of MBC. In general, it has been shown that diminution or impairment of POD can occur in asthmatic patients with more severe disease in terms of lower baseline FEV\(_1\) (19, 20, 21), greater airway hyperresponsiveness (19, 20, 21), persistence of airway inflammation (23). In 'exercise', as in our test, we showed that the variation of POD also increased, most prominently in severe disease, providing support for the notion that in severe disease, there are over- and under-perceiver of dyspnoea. Our observation that POD index correlated poorly with FEV\(_1\), in asthmatic patients provides further support that change in FEV\(_1\) alone in asthmatic patients does not explain their perceived breathlessness (2), and therefore, other factors such as age (24), hyperinflation (25), and treatment with inhaled corticosteroids (26), may also have an influence on POD.

The reproducibility of these measurements by 3MRET is important as it supports the validity of the results of a crossover study. While statistical significance was observed in all outcome measures for both the reproducibility and randomized controlled study, it is worth noting that POD score generally fared poorer than MBC score, reflecting the wider variability in POD between and within subjects when compared with MBC. The converse however is true when compared with 6MWT where POD score calculated at the end of maximal exercise, whether by repetitive inspiratory effort or walking, correlated at a greater significance level than the degree of maximal exercise capable of the individual. This seems to suggest that regardless of the forms of symptoms limiting exercise, scoring of POD can be reliably carried out using our simple ratio of perceived breathlessness over maximal workload capable of the individual. It may be that the 3MRET is superior in that the exercise is confined to respiratory effort, and not complicated by other physiological constraints such as leg muscle fatigue when walking or cycling is involved.

Our preliminary study suggests that the 3MRET has discriminative value between normal healthy subjects and asthmatic patients of varying severity. Its reproducibility and responsiveness to bronchodilator effect, and correlation with 6MWT suggest that it has evaluative value for assessing disease progression and therapeutic interventions. The full potential for this test requires testing in larger sample of subjects and patients with other chronic respiratory diseases such as COPD and pulmonary fibrosis. The possibility of a tool like 3MRET that reliably scores POD in such convenient and user-friendly manner may provide insight into the research on nature of POD in various disease states, and the study of asthmatic patients who under- or over-perceive breathlessness.

Acknowledgements

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References

1. Killian KJ. The objective measurement of breathlessness. *Chest* 1985; 88: 84S-90S.
Even though extensive studies have been conducted on the effect of noise exposure on hearing apparatus / auditory system, information on the effect of noise on other body functions is sparse. The present study examined the effect of exposure of albino rats to acute and chronic noise stress on two important interlaced endocrine levels. In acute experiments the animals were exposed to 120 dB noise for a duration of 1, 2, 3 hrs. In chronic experiments the animals were exposed to noise for one hour daily for 30, 60 and 90 days. Plasma corticosterone and leptin levels were measured in these animals. There was significant elevation in the levels of corticosterone and leptin after exposure to noise stress. The elevation in corticosterone level after noise stress is in agreement with earlier reports. So noise acts like a stressor and elevates the secretion of the corticosterone, the stress hormone and leptin, the product of the ob gene there is an elevation in leptin levels after noise stress.

Key words: Leptin, corticosterone, noise stress, Wistar rats

Introduction

Noise is considered a kind of stress, which produces significant physiological and biochemical changes in animals as well as in humans (1). The damaging effect of noise on hearing has been extensively studied (2, 3). However, very little information is available regarding the effect of noise on other body functions. Similar to other types of stress, noise stress has also been shown to increase levels of stress hormones like corticosterone and norepinephrine (4, 5).

Recent studies indicate that corticosterone can stimulate the secretion of adipose-derived hormone, leptin (6, 7). Leptin is an important hormone concerned with food intake, metabolism and reproduction (8). As noise increases corticosterone secretion it may be proposed the exposure to stressors like noise could induce alterations in serum leptin levels. Such a possibility has not been reported in the literature. In the present study an attempt has been made to monitor serum leptin and corticosterone levels in male albino rats after exposure to acute and chronic noise stress.

Materials and Methods

Animals:

Male Wistar-Kyoto rats weighing 100 – 120 g were housed in polypropylene cages with access to laboratory chew and water ad libitum. They were maintained in an animal experimental laboratory where night and day temperatures varied from between 24° C to 32° C with a relative humidity of 70 to 80% and a 12-hour light and dark cycle.

Animal groups:

The animals were randomly grouped into control and experimental groups. Each group had acute and chronic subgroups. The rats of the acute subgroup were exposed to noise of 120db for 1, 2 and 3 hours. The rats of the chronic group were exposed to noise of 120 dB of one hour duration every day for 30, 45 or 60 days. Each subgroup had 6 animals. Control groups were treated like the experimental group, but not exposed to noise.
Induction of noise:
The animals were exposed to noise stress of 120 dB, which was generated by a noise generator (Suguna 100 M HP 2). The generator was connected to an amplifier (Inkel, Korea) to amplify the sound at an intensity of 120 dB. A decibel meter (C. R 303 Philips, India) with a measurement range of 35 to 135 dB was used to measure the sound level.

Blood collection and analysis:
Immediately after the experiments were completed blood was collected by retro orbital puncture. Serum was stored at -20° C until assayed for leptin and corticosterone. Leptin concentration in serum was determined using leptin colorimetric EIA Kit (Assay Design Inc., U S A). Serum corticosterone was estimated using commercially available RIA kits.

Table 1 : The effect of noise on serum corticosterone level

<table>
<thead>
<tr>
<th>Time of exposure to noise</th>
<th>Serum corticosterone m.mol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>Experimental (n=6)</td>
</tr>
<tr>
<td>Acute</td>
<td></td>
</tr>
<tr>
<td>1 Hour</td>
<td>390 ± 6.50</td>
</tr>
<tr>
<td>2 Hours</td>
<td>400 ± 7.20</td>
</tr>
<tr>
<td>3 Hours</td>
<td>396 ± 5.0</td>
</tr>
<tr>
<td>Chronic</td>
<td></td>
</tr>
<tr>
<td>30 Days</td>
<td>401 ± 8.8</td>
</tr>
<tr>
<td>45 Days</td>
<td>410 ± 15.1</td>
</tr>
<tr>
<td>60 Days</td>
<td>392 ± 8.50</td>
</tr>
</tbody>
</table>

Each value represents mean ± sem of 6 observations. * p<0.05 and ** p<0.01

Statistical Analysis:
Results were analyzed using ANOVA followed by Dunnet’s test and a ‘P’ level of <0.05 was considered statistically significant.

Results

Changes in serum corticosterone levels after exposure to noise
Exposure of rats to acute noise stress for 1, 2 and 3 hour periods resulted in a significant elevation in the level of serum corticosterone compared to the control animals (Table 1). Corticosterone was significantly higher in rats exposed to noise for 2 and 3 hours compared to its level in rats exposed to noise for 1 hour. Serum corticosterone levels were not significantly different between rats exposed to noise for 2 and 3 hours.

Similar to acute exposure, a significant increase in serum corticosterone level was observed
in animals that were exposed to noise stress for 30, 45 and 60 days compared to the respective control values. A maximum increase was noted after 45 days and there appears to be a downward trend in the elevation of serum corticosterone at 60 days.

Changes in serum leptin levels after exposure to noise.

Serum leptin concentrations were significantly higher in animals that were exposed to noise stress for 1, 2 or 3 hrs when compared to the control animals. There was no significant difference in serum leptin levels between animals exposed to different durations of acute stress (Table 2).

Significantly elevated serum leptin levels were also evident in animals that were exposed to chronic noise stress for 30, 45 and 60 days. Serum leptin levels were significantly higher in rats exposed to stress for 45 days compared to those exposed to stress for 30 days. However, no significant difference was evident in serum leptin levels of rats exposed to stress for 45 days and those exposed to stress for 60 days.

Discussion

The results of the present study indicate significantly higher levels of serum corticosterone and leptin after exposure to both acute and chronic noise stress. Raised serum corticosterone levels following noise stress in rats have been reported before (9, 10, 11). In addition, elevation of glucocorticoid levels following many types of stressors is also well known. The precise mechanism for this remains unclear but it may be related to altered activity of the hypothalamic-pituitary-adrenal axis secondary to the noise stress and may involve alterations in the secretion of corticotropin releasing hormone (CRH), ACTH and proopiomelanocortin (POMC) gene expression. The increase in glucocorticoid secretion during stress appears to be important for the appropriate defense mechanism to be put into place.

Significantly higher levels of corticosterone were also evident in rats exposed to chronic stress for 30-60 days indicating poor or absent adaptation of the rats to noise stress. This is in contrast to what has been observed before by others where a somewhat decreased corticosterone response to noise was observed on chronically stressed rats (11, 12). The reason for this difference is unclear.

There are several reports indicating that glucocorticoids are capable of stimulating the synthesis and secretion of adipocyte-derived leptin (6, 13, 14), which regulates food intake and energy expenditure. Leptin secretion is under the influence of hormonal and neural control. (6, 12, 15). The results indicate significant elevation in leptin levels.
both after acute as well as chronic exposure to noise stress. Heimen et al (1997) (16), in an earlier study examined the influence of exogenous administration of leptin on plasma corticosterone and ACTH in animals subjected to restraint stress. They reported that leptin was able to inhibit the release of corticotrophin releasing hormone from the hypothalamus in vitro and also blunted the plasma ACTH and corticosterone elevation due to restraint stress. They also speculated a possibility for reduction of leptin level during acute and chronic stress and thus facilitating the responsiveness of hypothalamic-pituitary-adrenal axis. However they failed to demonstrate any reduction in leptin levels in their study on restraint stress and thus the speculation remains unsubstantiated. In fact the data indicated an elevation of serum leptin levels after restraint stress though the levels were not statistically significant. In another study chronic subcutaneous leptin infusions have been shown to diminish responsiveness of the hypothalamic-pituitary-adrenal axis in female rhesus monkeys (17). It therefore seems that there is a significant interplay between leptin and the hypothalamic-pituitary-adrenal axis. The results of the present study in rats subjected to acute and chronic noise stress clearly indicate simultaneous elevation of corticosterone and leptin levels during both acute and chronic stress. It appears that the inhibitory effect of leptin on corticosterone secretion was somewhat absent during noise stress in this study. The reason for the variation between our observation and that in the mentioned studies is unclear but may be due to species variation or the different nature of stress. Nevertheless our study suggests that one arm of the hypothalamic-pituitary-adrenal-leptin axis appears disabled during noise stress, which permits for increase corticosterone secretion during stress.

Table 2: The effect of noise on serum leptin level

<table>
<thead>
<tr>
<th>Time of exposure to noise</th>
<th>Serum leptin Pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=6)</td>
</tr>
<tr>
<td></td>
<td>Experimental (n=6)</td>
</tr>
<tr>
<td>Acute</td>
<td></td>
</tr>
<tr>
<td>1 Hour</td>
<td>5.7 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>7.1 ± 0.20 **</td>
</tr>
<tr>
<td>2 Hours</td>
<td>5.9 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>7.2 ± 0.20 **</td>
</tr>
<tr>
<td>3 Hours</td>
<td>6.1 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>7.4 ± 0.30 **</td>
</tr>
<tr>
<td>Chronic</td>
<td></td>
</tr>
<tr>
<td>30 Days</td>
<td>6.0 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>8.2 ± 0.17 **</td>
</tr>
<tr>
<td>45 Days</td>
<td>6.1 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>9.4 ± 0.11 **</td>
</tr>
<tr>
<td>60 Days</td>
<td>6.3 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>9.1 ± 0.20 **</td>
</tr>
</tbody>
</table>

Each value represents mean± sem of 6 observations.* p<0.05 and p<0.01.
In conclusion, the present results clearly indicate that sustained exposure to noise stress results in a significant elevation of corticosterone and leptin. These two hormones have wide ranging effects on metabolism, growth and reproduction (8, 18). The elevated levels of leptin and corticosterone even after exposure to continuous noise stress for a period of 90 days, indicates that the expected adaptation is absent. Hence continuous exposure to noise stress may have many adverse effects on some of vital physiological functions (19, 20, 21) in which the alteration in the levels of these two hormones may play a significant contributory role.

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References

2. Smith A.W. Noise can damage your hearing. World Health organization. 1996; 5 : 10-11


ANXIETY, DEPRESSION AND PSYCHOSOCIAL STRESS IN PATIENTS WITH CARDIAC EVENTS

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Stress tends to worsen the prognosis of patients with coronary heart disease. The aim of the study is to determine the relationship between stress related psychosocial factors like anxiety, depression and life events and temporally cardiac events specified as acute myocardial infarction and unstable angina. 65 subjects with confirmed myocardial infarction or unstable angina were interviewed using 2 sets of questionnaire, the Hospital Anxiety and Depression Scale (HADS) and Life Changes Stress Test, a segment of the Rahe’s Stress and Coping Inventory first at time of occurrence of their cardiac event and the second time was 6 months later. Anxiety, depression and life events scores were calculated for both and recurrence of cardiac event for the 6 month duration was also recorded. Patients who had significant levels of depression and or life events were ten times more likely to have recurrence of cardiac events as compared to those without risk for either of these psychological symptoms. Anxiety, depression and stress levels are significantly increased after the onset of ischemic heart disease and could be contributing or predisposing factors for the recurrence of cardiac events for these patients.

Key words: Acute myocardial infarction, unstable angina, stress, life events, depression

Introduction

Psychological factors can be as detrimental to the recovery of disease among patients with confirmed cardiac events as to the disease development (1). Stress in particular if not managed with proper intervention in the aftermath of myocardial infarction predicts 1-year mortality and rehospitalization (2, 3). Many cardiovascular patients with high levels of stress continue to show increase in morbidity as stress affects mechanisms related to cardiac events especially clustering of traditional cardiovascular risk factors, endothelial dysfunction, myocardial ischemia, plaque rupture, thrombosis and malignant arrhythmias (4).

Numerous studies have also associated the presence of depression with the worsening of the prognosis of patients with cardiac events (5). One of these studies further stresses that depression clearly affects the condition of patients with established coronary artery disease (CAD) but its role in the initial development of coronary disease is less evident (5). Studies done on psychiatric patients also showed higher rates of heart disease among depressed patients (6) and lithium treatment for two years have reduced the mortality from cardiovascular disease in these patients (7). Studies have also linked other treatment of depression with reduced risk of myocardial infarction among depressive patients (8, 9).

The paper aims to determine the various associations between stress symptoms as determined by the Hospital Anxiety and Depression Scale and Life Changes Stress Test and temporally cardiac events specified as acute myocardial infarction and unstable angina. The questions we seek to answer are whether anxiety depression and life events considered as higher risks for cardiac events...
notwithstanding the existence of other physical and psychosocial variables in these patients.

**Methodology**

**Subjects**

The subjects comprised of 65 patients with either first episode unstable angina or myocardial infarction. These patients drawn from all the patients admitted in the cardiac care unit and in Hospital Universiti Kebangsaan Malaysia, an urban government hospital were selected from the following criteria: 1) was admitted within a few days and diagnosed with either unstable angina or myocardial infarction, 2) their cardiac events was confirmed as a first episode and 3) they gave signed consent to participate in study. Cardiologists determined presence of either cardiac event based on typical clinical symptomatology, ECG evidence and typically elevated serum levels of myocardial enzymes. The 65 consenting patients with 30 men (46%) and 35 women (53%) ranging from 22-86 years had a mean age of 60 and consisted of 44% Malays, 29% Chinese, 26% Indian. 21% of the subjects were below 50 years while the majority were in the 50-65 age group (45%). 30% were patients with myocardial infarction while the remaining had unstable angina. There were no fatalities among the 65 patients and all returned for second screening.

**Measures**

Demographic data gathered were gender, age and recurrence of cardiac event during the six months after hospital admission. The psychological measures were done using 2 questionnaires, the Hospital Anxiety and Depression Scale and Life Changes Stress Test. Both questionnaires were designed to be self-rated, however due to the low literacy rate among the participants; the questionnaires were interview-aided. Only one interviewer administered the questions to avoid bias. The Hospital Anxiety and Depression Scale (HADS) identifies risk for both anxiety and depression separately by having scores divided into cut-off points which indicates ranges from normal to severe. The normal rate is below 8, while 8-10 indicates mild symptoms, 11-14; moderate and 15-21 points to a severe state of depression/anxiety. It was chosen as the sample size was small and it has undergone validation for usage in non-psychiatric units. During the interview, participants are required to give prompt answers and not to dwell too long on each question. Immediate answers are integral as HADS screens for severity of both these conditions during that instant. The Life Changes Stress Test, the first part of the Stress Coping Inventory had undergone reliability testing on a sample of 1772 individuals (11). It comprised of a 50-item questionnaire, which identifies life events for the past six months in five different categories; health, work, finance, personal.
and social as well as home and family. However, questions on health was excluded in this study to avoid inclusion of factors due to cardiac events. The scores for each group were totaled as Life Changes Unit with several cut-off points to indicate susceptibility to illnesses. The grand totals between 201 and 300 connote a moderate risk and grand totals of 300 and 450 signify an elevated risk to illnesses, although not specifically cardiac diseases.

Procedure

The questionnaires were administered twice, first during hospital admission at the initial diagnosis of cardiac events (1st screening). A follow up was conducted six months later. The recurrence of cardiac event during that period was also recorded. Paired t test were applied to the group to obtain differences. Odds ratio was calculated to determine the odds of an individual to have a recurrent cardiac event if they are at risk of anxiety, depression, or life events.

Results

Anxiety and depression was totaled separately from HADS and life events was totaled from the Life Changes Stress Test. Each bar represents total patients with scores ≥8 (anxiety and depression risk) and scores ≥201 (life events risk). Values above each graph were recurrence rate (new cases rate) for second screening. Rates for recurrence was calculated for second screening based on total from 1st screening and rates for new cases from total of 2nd screening.

Comparisons were done using paired sample t test. *P<0.01. *The smallest value is shown as multiple modes exist.

Characteristics of sample population

Both men and women when considered separately had an almost similar mean score. All three ethnic groups scored similar mean scores for depression and life events although a slight difference was observed in anxiety scores for Indians. There was also no difference between patients with unstable angina and myocardial infarction. The remaining results sections were analyzed using all 65 patients regardless of ethnic group, gender, or type of cardiac event.

Anxiety

Most participants had normal range of anxiety levels with only 26% and 32% of them scoring above normal levels during the first and second screening. However, despite a small percentage of patients scoring 8 and higher, a mere 6-month duration from first and second screening saw a 6% increase. A detail analysis indicated all patients except one had a recurrence of higher than normal anxiety levels (rate of 0.94) (Chart 1). Comparison of scores indicated a significant increase (P<0.001) for second...
screening (Table 1) and this is mostly caused by increases in scores for the normal range than the increases in the scores for the risk range. Another indication would be that the mod values for both interviews were in the normal range. However, patients with anxiety scores 8 and higher were as likely as normal patients to have a recurrent cardiac event six months later (Table 2).

**Depression**

Participants with depression scores of 8 and above (31% for first screening and 42% for second screening) for both screenings were more than those with similar anxiety scores. However, for the second screening, there were 9% more patients for depression risk compared to anxiety risk than the first screening which saw a 5% increase. Individually, only 40-41% of those who had depression risk also had anxiety risk for both screenings. Like anxiety, all patients except one experienced a recurrence of depression risk (0.95) (Chart 1). However, unlike anxiety, the patients with decreased depression scores from risk range to normal range had normal anxiety scores for both screenings. The earlier described patient with decreased anxiety scores had depression scores above 8 for both screenings.

Comparison of scores indicated a significant increase (P<0.001) for second screening (Table 1). For depression, the mod values indicated that the most frequent score was within the normal range for the first screening but this changed during the second screening in which the most frequent score was in the risk range (Table 2). This difference from anxiety could be a factor for the ten-time likelihood (odds ratio≈10.082) (Table 2) for those scoring 8 and higher to have a recurrent of cardiac event compared to those scoring less than 8.

**Life Events**

Only 25-26% of the patients had life events score of 201 and above for both screenings. There were a small increase of new cases (rate of 0.06) (Chart 1) and all patients at risk in the first screening remained in the risk range for the second interview. This was expected as life events scores was calculated based on events for duration of six months prior to the interview and not like the HADS scale that recorded most recent anxiety and depression conditions. Although changes in the number of risk cases have not increased, there were significant changes in the individual scoring (P<0.001) (Table 1). Mod values were not any indication for life events as there were more than a few different values. Life events like depression had a ten times odds (odds ratio=9.778) (Table 2) for those scoring in the risk range to develop a recurrent cardiac event than those scoring in the normal category.

**Discussion**

This prospective study had good representation of all three ethnic groups and had an equal distribution of both sexes. A study done on Malay, Chinese and Indian residents in Singapore found strong ethnic differences in myocardial infarction (MI) event with Indians having the highest MI rates and Malays with the highest case-fatality (13). Several factors likely to cause the inter-ethnic differences were probably caused by environmental factors or genetic factors but these explanations were still inconclusive (14). As the sample size in our
study was small, we grouped all races together as studies have indicated that the risk factors for cardiovascular diseases bring about adverse effects in all races, although the relative importance of these factors may vary across populations (15).

For this study, risk for either anxiety or depression was set at scores 8 and above which indicated mild to severe symptoms while for life events, risk of susceptibility to illness was set at moderate to severe which were scores 201 and above. Recurrent of cardiac events are common especially after an acute myocardial infarction (AMI) (16). The results indicated that the likelihood of recurrent cardiac events is much more linked to depression and life events compared to anxiety. Carney et al reported 16-22 % of post-MI patients having major depression and 45% of them having some form of unipolar depression (17,9). The absence of severe risk cases for depression and no deaths among the patients for the 6-month follow up was similar to another study that found an association between major depression and mortality in the first six months after an acute MI (3). It was not unusual for patients who reported no risk of depression during the first screening having higher scores consequently 6 months later as 1 in 3 develop major depression 12 months after an acute MI (17).

Depression also has a strong relationship with other ischaemic heart diseases besides myocardial infarction. One study in particular found that patients who developed depression after an episode of unstable angina had an increased risk of major cardiac events during the following year (18). During exercise testing, incidence of angina develops sooner and lasts longer in depressed patients (19). The stronger association of depression and coronary heart disease exceeds even the one between the cardiac diseases and type A behavior, which was the most prominent psychological risk factor. The association between depression and coronary heart disease is explained through numerous mechanisms. One possible explanation is that depressed individuals normally adopt unhealthy behaviors such as smoking, alcoholism, tend to live a sedentary lifestyle or eat an unhealthy diet (20). Depression and coronary risk could also be linked via personality characteristics such as hostility (21).

A correlation between depression and coronary risk can also be linked with life events. Psychosocial factors such as low socioeconomic status can lead to depression. Prolonged exposure to the stressors like these life events can ultimately lead to vital exhaustion, a state of fatigue. This condition is often present in the weeks prior to a myocardial infarction. Cortisol reactivity is also known to occur during stressful circumstances (22). Another theory claimed that depression may induce hypothalamic-pituitary-adrenal axis hyperactivity (23) independently without these associated factors. Depression can also influence other biochemical and physiological changes like the sympathoadrenal hyperactivity, diminished heart rate variability, ventricular instability and myocardial ischaemia (24).

The association of anxiety in myocardial infarction (MI) and unstable angina is similar to another study that found no relationship between phobic anxiety and non-fatal MI (25). However, an association exists between anxiety and fatal coronary heart disease (26). This suggests a different mechanism involved for anxiety and coronary heart disease than for depression and coronary heart disease. Sudden cardiac death, which is death due to non-cardiac causes and occurring 1 hour after onset of symptoms, is less frequently associated with acute myocardial infarction (27). This indicates different risk factors for sudden cardiac death and AMI (28). Anxiety disorders are associated with hyperventilation that increases the susceptibility to arrhythmias, one of the causes for cardiac death (29).

Conclusion

Recurrence of cardiac events for ischemic heart patients is resulted from traditional risk factors as well as predisposing factors. Although levels of blood cholesterol, elevated blood pressure, diabetes mellitus and advancing age are unchangeable, factors such as diet, physical activity and psychosocial factors can be managed if not treated. Singapore in a recent nationwide 10–year study suggested the implementation of therapeutic programs the reason for a significant fall in mortality from MI (30). A simple change of life style incorporating basic stress management programs or relaxation techniques could lead to a significant improvement in the morbidity and mortality of ischemic heart patients. Any program fitted into a daily schedule might at best improve the chances of survival or the very least upgrade the quality of life of these patients.

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References

1. Rutledge T, Linden W, Davies RF. The Canadian
Amiodopine/Atenolol in Silent Ischemia Study
(CASIS) investigators. Psychological risk factors may
moderate pharmacological treatment effects among
ischemic heart disease patients Psychosomatic Med
1999; 61: 834-841

2. Denollet J, Sys SU, Brutaert DL. Personality and
mortality after myocardial infarction in men
Psychosom Med 1995; 57: 582-591

3. Frasure-Smith N, Lesperance F, Talajic M. Depression
following myocardial infarction JAMA 1993; 270:
1819-1825

KG, Salerno JW, Scheider RH. Psychological stress
and cardiovascular disease: Pathophysiological links

5. Barefoot JC, Schroll M. Symptoms of depression, acute
myocardial infarction, and total mortality in a

6. Dreyfuss F, Dasberg H, Assael MI. The relationship of
myocardial infarction to depressive illness
Psychother Psychosom 1969; 17: 73–81

7. Ahrens B, Mueller-Oerlinghausen, Schou M, Wolf T,
Alda M, Grol E, Grol P, Lejiz G, Simhandl C, Thau K,
Vestergaard P, Wolf R, Moeller H., Cardiovascular and
suicide mortality of affective disorders may be reduced

8. Avery D, Winokur G. Mortality in depressed patients
treated with electroconvulsive therapy and
antidepressants Arch Gen Psychiatry 1976; 33: 1029-
1037.

9. Carney RM, Freedland KE, Jaffe AS. Insomnia and
depression before myocardial infarction Psychosom
Med 1990; 52: 603–609

MC. Psychometric evaluation of the Hospital Anxiety
and Depression Scale (HADS) among female cardiac
patients Br J Health Psychol 2001; 6(4): 373-383

TL, Bryson S. A novel stress and coping workplace
program reduces illness and healthcare utilization
Psychosom Med 2002; 64: 278-286

12. Mak KH, Chia KS, Kark JD, Chua T, Tan C, Foong
BH, Lim YL, Chew SK. Ethnic differences in acute
myocardial infarction in Singapore European Heart

13. Ounpuu S, Yusuf S. Singapore and coronary heart
disease: a population laboratory to explore ethnic
variations in the epidemiological transition. European
Heart Journal 2003; 24: 127-129.

14. Gotto AM. Triglyceride as a risk factor for coronary
artery disease American Journal of Cardiology 1998; 82:
22-25

15. Fowles RE. Myocardial infarction in the 1990s. The
importance of early thrombolytic therapy Postgrad

16. Schleifer SJ, Macari-Hinson MM, Coyle DA, Slater
WR, Kahn M, Gorlin R, Zucker HD. The nature and
course of depression following myocardial infarction
Arch Intern Med 1998; 149: 1785-1789

17. Lesperance F, Frasure-Smith N, Talajic M. Major
depression before and after myocardial infarction: its
nature and consequences Psychosomatic Medicine
1996; 58(2): 99-110

P. Depression and 1-year prognosis in unstable angina

19. Krittayaphong R, Light KC, Golden RN, Finkel JB,
Sheps PC. Relationship among depression scores, beta-
endorphin and angina pectoris during exercise in
patients with coronary artery disease Clin J Pain 1996;
12: 126-133

20. Ruuskanen JM, Ruoppila I. Physical activity and
psychological well-being among people aged 65 to 84
years Age ageing 1995; 24: 292-296

21. King KB. Psychologic and social aspects of
cardiovascular disease Ann Behav Med 1997; 19:
264-270

22. van Eck M, Berkhof H, Nicolson N. The effects of
perceived stress, traits, mood states and stressful daily
events on salivary cortisol Psychosomatic Medicine
1996; 58: 447-448

23. Siever LJ, Davis KL. Overview: toward a dysregulation
hypothesis of depression Am J Psychiatry 1985; 142:
1017-1031

24. Musselman DL. Evans DL, Nemeroff CB. The
Relationship of Depression to Cardiovascular Disease:
Epidemiology, Biology, and Treatment Arch Gen
Psychiatry 1998; 55: 580-592


GALLIUM SCAN IN DIAGNOSING OCULAR SARCOIDOSIS

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A 40-year-old man presented with floaters and painless progressive blurring of vision in the right eye for one month duration. Visual acuity in the right eye was 6/24. There was mild anterior chamber reaction and vitritis. The optic disc was swollen and elevated with presence of granulomatous lesion in the optic disc head. Blood investigations were unremarkable. Serum angiotensin converting enzyme (ACE) was normal and conjunctival biopsy showed presence of inflammatory cells. B-Scan ultrasound revealed an echo-dense lesion in the optic nerve head. There was increased uptake of the right lacrimal gland and presence of ‘Panda sign’ with Gallium scan. A diagnosis of right ocular sarcoidosis was made base on the clinical features and Gallium scan.

Key words: Ocular sarcoidosis, Gallium scan

Introduction

Ocular sarcoidosis may present with a wide variety of ocular symptoms in all parts of the eye. The diagnosis may be difficult owing to the absence of diagnostic criteria and the variety of presentations. The diagnosis usually is suggested by a combination of clinical, radiology and laboratory findings and supported by a tissue biopsy showing non-caseating granulomas (1). Gallium scan has been shown to be useful in aiding the clinical diagnosis of ocular sarcoidosis in patients with either normal or equivocal chest radiographs.

Case report

The patient is a 40-year-old Malay male who presented with a history of painless progressive reduced vision in the right eye for one month duration. The central vision was affected and

Figure 1: Fundus photograph shows optic nerve head granuloma in the right eye
decreased vision was associated with floaters. Vision in the left eye was good. There was no history of joint pain, backache, skin rash or nodule, mouth or genital ulcer, haemoptysis and loss of weight or appetite. He denied other medical illness before.

The visual acuity in the right eye was 6/24 and not improved with pinhole. Vision in the left eye was 6/6. There was relative afferent pupillary defect in the right eye. The other optic nerve function tests such as colour vision and light brightness were also impaired. There was no enlargement of the lacrimal gland or eyelid nodule noted.

Anterior segment examination of the right eye revealed a clear cornea and normal conjunctiva. There was mild anterior chamber reaction and normal iris texture. The intraocular pressure was normal. The anterior segment examination of the left eye was unremarkable.

The right posterior segment revealed moderate vitritis with presence of few snowballs and vitreous strands inferiorly. The optic disc was swollen and elevated with presence of granuloma in the optic disc head (Figure 1). The vessels were dilated and tortuous with sheathing of superior branch of retinal vein. There were presence of multiple discrete yellow-white choroidal lesions at the superotemporal area of peripheral retina. The macula was normal. The fundus examination of the left eye was unremarkable.

Visual field examination showed an enlarged blind spot with central scotoma in the right eye and normal field in the left eye (Figure 2). Systemic physical examination was unremarkable. There was no lymphadenopathy, organomegaly or neurological deficit noted. No sign of chronic inflammatory disease was elicited.

Investigation

Blood investigations were unremarkable. The Mantoux test was normal (10mm). The Chest X-ray finding was also normal without hilar opacity seen. Both serum and urine calcium levels were within normal range.

The angiotensin converting enzyme level was 53 U/L (normal range: 40-140U/L). Conjunctival biopsy specimen from the right eye showed evidence
of chronic inflammatory condition with no granuloma seen. Gallium-67 citrate showed increased uptake of the right lacrimal gland and presence of ‘Panda sign’ (Figure 3)

**Diagnosis and Treatment**

Based on the clinical features and Gallium scan finding, the patient was diagnosed to have ocular sarcoidosis of the right eye. He was treated with oral prednisolone 1.5 mg/kg daily and topical dexamethasone every 2 hourly. The ocular features were monitored closely including visual acuity and visual field. His visual acuity gradually improved to 6/6 over 10 days of oral prednisolone.

Review of the fundus showed improving vitritis with less vitreous strands. The optic disc was still elevated but with clearer margin. Sheathing of the superior branch of retinal vein had disappeared. The choroidal lesions became less prominent and there was no new lesion noted. The oral prednisolone was tapered down after two weeks and subsequently off after six weeks.

The visual field gradually improved back to normal following the above treatment. The size of the granuloma of the optic nerve head became smaller and the vitreous became clear. There was presence of a chorioretinal scar inferiorly with no more vitreous strand seen.

**Discussion**

Gallium scanning has been used extensively for diagnosing sarcoidosis and other inflammatory process (2,3,4). The 67-gallium molecule probably blinds to the T lymphocyte and macrophages, representing a regional inflammatory response. Combined lacrimal, parotid, and submandibular gland uptake has been termed the ‘panda sign’, whereas the triad of right paratracheal and bilateral pulmonary and mediastinal uptake is the ‘lambda sign’, highly suggestive of sarcoidosis (5,6).

In the above patient, gallium scanning of the orbit, head and neck demonstrated significantly increased 67-gallium uptake in the right lacrimal gland. There was also increased 67-gallium uptake in the left lacrimal gland and parotid glands that gave an appearance of ‘Panda sign’.

Depression delayed-type hypersensitivity is one of the immunological changes in sarcoidosis. Increased proportion of circulating suppressor cells have been demonstrated in sarcoidosis (7). Positive tuberculin reactivity in sarcoid patient is suggestive of increased helper-cell activity (8).

Serum ACE and serum lysozyme are found to be elevated in sarcoidosis. The source of the ACE is probably the giant cells. Baarsma et al (9) reported that in patients with uveitis who had serum ACE level above 50 u/L, the sensitivity of the test was 84% and the specificity was 95% in the diagnosis of ocular sarcoidosis.

The combination of positive gallium uptake and an elevated serum ACE was a specific and sensitive tool for diagnosing patients suspected of having ocular sarcoidosis but had normal chest radiographs. Power et al (10) reported that the specificity for diagnosis was 100% and sensitivity 73% when there was a combination of elevated serum ACE level and a positive 67-Gallium scan present.

Serum ACE activity reflects overall systemic inflammatory activity, whereas Gallium scanning assessing localized sites of inflammation (10). The most useful non-invasive test which should be performed in helping to confirm the diagnosis of sarcoidosis are serum ACE and Gallium scan of the lacrimal gland.
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References

ACTINOMYCOSIS OF THE KNEE

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We report a case of actinomycosis presenting as a knee swelling in a 34 year-old man. Knee actinomycosis poses a diagnosis challenge to clinicians as it is rare, often mimics knee tuberculosis and culture of the causative microbes is technically difficult. The classic microscopic appearance of this Gram-positive bacteria often forms the basis of diagnosis.

Key words: Actinomycosis, knee

Introduction

A 34 year old teacher presented with progressive right supra patella knee swelling for 2 months. It was not associated with fever or limitation of joint movement. He started to have intermittent painless swelling of the knee for the past 4 years, following his daily activities. However it was not associated with fever, locking or instabilities. No recent trauma, loss of weight or appetite were noted.

Local examination revealed painless gross swelling of his right knee. There was no sinus or discharge noted. The synovium was thickened and range of movement was normal.

Hemoglobin and total white counts were normal. Erythrocyte Sedimentation Rate (ESR) was

Figure 1: Histopathology of the synovium (H&E) shows central area of sulphur granule containing aggregate of branching, pleomorphic rods, surrounded by intense reaction of leucocytes.
above 100mm/hr, C-Reactive Protein (CRP) was more than 24 mg/L and alkaline phosphatase was 176 Iu/L. MRI shows cystic lesion in the anterior compartment of the lower thigh with thickened synovium in the suprapatella bursa.

Total synovectomy was done. Intraoperative findings revealed total cartilage erosion over the medial femoral condyle and inferior patella. Synovial cultures for both aerobic and anaerobic were negative. No organism seen (including Acid Fast Bacilli) were seen on microscopy. However synovium histopathology examination was consistent with actinomycosis. No tuberculous granuloma was noted. (Figure 1)

He was treated with Bactrim and Amoxycillin for a period of six months. He responded well with the treatment. Two and half years after the treatment, he is almost pain free. However the movement of the affected joint was limited due to articular surface damage secondary to the infection.

Discussion

Actinomycosis is a chronic disease involving mainly cervicofascial, thoracic and abdominal regions. The infection is characterised by abscess formation, draining sinuses and tissue fibrosis. Main pathogens are Actinomyces israelii or A. naeslundii. Actinomycetes sp is strictly a commensal of the oropharynx, gastrointestinal and female genital tracts therefore Actinomycosis is usually secondary to trauma to these area. Intraterine device may predispose a patient to Actinomycosis of the genital tract. A case of infected total knee replacement secondary to Actinomyces naeslundii had been reported. However other forms of actinomycosis is usually visceral in nature.

Actinomycosis involving a joint is very rare but it may occur in any joint, especially in an immunocompromised patients. Hematogenous spread of an actinomycotic granule to the hip joint had been reported in a woman under immunosuppressive treatment. Actinomycosis of the ankle in an elderly diabetic patient had also been reported. Synovial actinomycosis of the knee had been reported by Bose. However, the pathogenesis in these two patients remains unexplained as they are not immunosuppressed.

Tuberculous arthritis may mimic knee Actinomycosis, especially in countries where tuberculosis is endemic. High ESR more than 100 mm/hr is typical and tuberculous arthritis is far more common.

Actinomycosis of the knee can also be mistakenly diagnosed as soft tissue tumor. Actinomycosis of the thigh presented like a neoplasm has been reported.

Actinomycosis of a knee is rare. Therefore high index of suspicion and proper investigations including tissue culture and histological examination should be routine in certain situations.

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References